Original article

Retinal development in the lobster *Homarus americanus*

Comparison with compound eyes of insects and other crustaceans

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Abstract. Pattern formation and ommatidial differentiation were examined in the developing retina of the lobster *Homarus americanus* using light and electron microscopy. In the lobster the retina differentiates from the surface ectoderm that covers the optic primordia. Initially a single band of proliferation moves across this surface ectoderm. Immediately following this wave of proliferation, rows of ommatidial cell clusters appear. The earliest cell clusters are often seen adjacent to dividing cells of the proliferation band. The changing organization of the first seven rows of ommatidial clusters, visible at the surface of the retina, reveals events in early ommatidial differentiation. A rosette-like cluster of 18 cells forms the first row. Each stage following the rosette clusters occurs in a separate staggered row. Developing ommatidia have a central cluster of retinula cells, whose organization changes at each stage. Four cone cells enclose the retinula cells in each cluster and extend to the surface. In the seventh row, rhabdome formation begins and the retinula cells recede, leaving only cone cells visible at the retinal surface. This change initiates the two-tiered organization of the adult ommatidium. In 70% embryos, asymmetries in the position of the R8 axon around R7 create an equatorial line separating the dorsal and ventral halves of the retina. Possible mechanisms for the formation of these asymmetries are discussed. Postembryonic growth of the retina continues in stage VI juvenile animals along the ventral edge of the retina.

Key words. Lobster - Retina - Development - Equator - Ommatidium
Introduction

One of the insights resulting from gene expression studies in a variety of developing systems is that certain developmental mechanisms are conserved in animals from a wide range of different phyla. One example, with relevance to the eye and nervous system, is the expression of the *Pax 6* gene (Callearts et al. 1997). This gene is expressed in the eyes and developing nervous system of animals ranging from Cephalopods to Arthropods and mice to man (Callearts et al. 1997). In some of these animals, such as *Drosophila melanogaster* (Insecta, Diptera, Brachycera), interpretation of gene expression data in the eye anlage has been enhanced by an understanding of the basic process of pattern formation and differentiation in the retina. In order to focus the present broad comparisons of gene expression, we must expand the numbers of animals within a particular group or phylum in which these types of studies are done. Such investigations will help to identify parallelisms and convergently similar features within morphological regions such as the eye and brain (Wray 2000). It has also been pointed out that the selection of representative organisms should be either for similarities in their structure and/or differences in their developmental mechanism (Hughes and Kaufman 2000).

This study of retinal development in the lobster *Homarus americanus* (Malacostraca, Decapoda, Homarida), along with our previous work on retinal development in the crayfish *Procambarus clarkii* (Malacosteaca, Decapoda, Astacida: Hafner and Tokarski 1998), establishes a basic understanding of the process of retinal development and ommatidial differentiation in two species of decapod crustaceans. These two representatives were selected because of their similar retinal morphologies and different developmental histories. The stalked eyes of *H. americanus* and *P. clarkii* are nearly identical in appearance and organization of the ommatidia that make up their retinas. In *H. americanus*, like *P. clarkii*, a single ommatidium is composed of five different cell types and a total of at least 16 cells (Parker 1890; Hafner and Tokarski 1998). These cells include two corneagenous cells, four cone cells, two distal screening pigment cells, eight retinula cells, and an undetermined number of proximal accessory pigment cells per ommatidium.

In contrast to their many similarities, the two species have significant differences in their mode of development. The development of *H. americanus* differs from *P. clarkii* in that it is longer and contains several free-swimming larval stages before a final metamorphic molt gives rise to the bottom-dwelling adult form (Helluy and Beltz 1991). In general, crayfish embryonic development is half as long as that of *H. americanus* and more direct. The three postembryonic stages resemble the adult body form but contain a significant yolk deposit that is rapidly consumed (Helluy et al. 1993). After the third postembryonic molt, individuals independent of the mother are present (Sandeman and Sandeman 1991). In both cases, percent staging systems which describe embryonic development as a percentage of the total embryonic period have been proposed (Helluy and Beltz 1991; Sandeman and Sandeman 1991).

In *H. americanus*, optic primordia appear between 5% and 8% development. The first eye pigmentation (E13%) appears at approx. day 21 as a crescent line at the posterior border of the optic lobe (Helluy and Beltz 1991). Pigmentation of the retina progresses in an anterior-medial direction and becomes oval in shape at 40% development (Helluy and Beltz 1991).

Optic primordia in the crayfish *Cherax destructor* (Malacostraca, Decapoda, Astacida) first appear at E35%. Eye pigmentation first appears at approx. day 29 (E65-70%) at the lateral border of the optic primordia. As development proceeds, pigmentation increases within the eye, and at hatching a band covering approximately one-quarter of the retina is present (Sandeman and Sandeman 1991; Helluy et al. 1993). Hafner et al. (1982) have made similar observations in *P. clarkii*.
Retinal development in *H. americanus* was first described by Parker (1890). He found that the optic primordia appeared as two oval disks on the ventral surface of the embryo at its anterior end. Within these regions both the retina and neuropsils of the eyestalk develop. The retina forms from the surface ectoderm at the lateral edge of the disk. As the retina and associated neuropsils grow and differentiate, the eye disks shift from their ventral facing orientation to a dorsal-lateral position so that the lateral edge of the disk is now posterior and the medial side of the disk is more anterior. Also, with the growth of the neuropsils, the optic disks elongate, taking on the cylindrical shape of the adult eyestalk. Each eyestalk extends anteriorly and medially toward the rostral end of the embryo and connects with the brain via the protocerebral tract. The retina forms at the distal or posterior end of the stalk.

Many of Parker’s observations are similar to those reported for *C. destructor*, by Sandeman and Sandeman (1991), and for eye development in *P. clarkii*, by Hafner et al. (1982; Hafner and Tokarski 1998). This study focuses on the process of retinal formation and ommatidial differentiation in the lobster *H. americanus*. We compare our findings with previous work on the freshwater crayfish *P. clarkii*, the fruitfly *D. melanogaster*, and other Arthropods where details of these processes have been reported.

**Methods**

Embryos of the North American lobster, *H. americanus*, were collected and fixed in the laboratory of Dr. B. Beltz, Department of Biology, Wellesley College, Wellesley, Mass. A variety of embryonic stages were examined, beginning at 46% development and extending through 95%. No larval stages were examined, but stage VI juveniles were studied for their adult characteristics. The staging of all embryos is based on the percent development criteria of Helluy and Beltz (1991) for *H. americanus*. Embryos were fixed for light and electron microscopy using the protocol of King (1976). The fixative consisted of 2.5% glutaraldehyde and 1.0% paraformaldehyde in 0.2 M Millonig’s phosphate buffer, pH 7.4, plus 0.14 M NaCl. Dissected embryos were placed in fixative at room temperature for 1-2 h with agitation. After the initial fixation, the tissue was rinsed for 10 min in 0.2 M phosphate buffer containing 0.3 M NaCl. Animals were postfixed on ice for 1 h in 1% OsO₄ in 0.1 M phosphate buffer, pH 6.0, plus 0.38 M NaCl. Osmicated blocks were stained in aqueous 2% uranyl acetate, dehydrated in a cold acetone series to propylene oxide, and embedded in Spurr. Thick sections were stained with 1% azure II and 1% toluidine blue in sodium borate. Thin sections were stained with lead citrate and uranyl acetate and examined on a Siemens 101 electron microscope.

**Results**

**Light microscopy**

In the earliest embryonic stage examined (46%), the eyes were elongated and extended from the medial-anterior end of the embryo dorsally and laterally. At this stage, the ommatidia in the retina exhibit a nearly complete range of differentiation, from the earliest ommatidial clusters to small but mature-looking ommatidia. The more mature ommatidia are found in the posterior-lateral region of the retina and the degree of differentiation decreased in an anterior-medial direction. Thus, in almost any embryonic stage, a complete or nearly complete spectrum of ommatidial development can be found.

Many of the light-microscopic observations of Parker (1890) were confirmed by this study. Figure 1 is a longitudinal section of the eye from a 46% embryo. Here, the earliest changes in retinal development can be followed along the ventral ectodermal surface. The earliest recognizable event is an initial wave of mitotic activity, indicated here by a single mitotic figure (small arrow in Fig. 1). Initially, in the
ectoderm layer, cells elongate and, during the mitotic cycle, round up at the surface and divide. In serial thick sections, the mitotic band within the surface ectoderm is semicircular, extending dorsally to ventrally over the developing retina. This single band of mitotic activity was first identified in *H. americanus*, by Harzsch et al. (1999), using bromodeoxyuridine (BrdU) staining of dividing cells.
Fig. 1. Light micrograph of a 46% embryonic eye showing developing retina and neuropils. The earliest stages in retinal development occur along a thin surface ectoderm layer that covers the optic primordia. The initial event in retinal formation is a proliferation band within the ectoderm. This band is indicated here by a single mitotic feature (small arrow). At the retinal surface, immediately adjacent to the proliferating cells, several rows of ommatidial cell clusters can be identified (region between arrowheads). The region of the retina where intermediate-stage ommatidia were examined is shown by the large arrow. Bar 20 µm

The band of mitotic activity is followed by a gradual increase in the thickness of the ectodermal layer (Fig. 1). Initially, the increase in thickness is due to the elongation of the postmitotic cells, which extend from the basement membrane to the surface. The earliest ommatidial clusters are often seen adjacent to dividing cells of the mitotic band. As the ommatidial clusters continue to differentiate, the cells in each cluster separate into two layers, which further increases the thickness of the retina. In Fig. 1 this elongation and separation is reflected in the increasing thickness of the retinal layer behind the mitotic zone.

Ommatidial cluster formation

Tangential sections cut just below the surface of the retina reveal the emerging pattern of ommatidial formation. Figure 2a is a low-power electron micrograph cut tangential to the surface in the region marked by the arrowheads in Fig. 1. This section shows a portion of the retina beginning at the mitotic band and extending posteriorly toward the most mature region of the retina (see Fig. 1). Using higher-magnification montages of this same region, specific cluster types were identified and their
location mapped on this section (Fig. 2a). The cluster map shows very little space between the mitotically active region and the first identifiable ommatidial clusters. The clusters are arranged in rows that run parallel to the dorsal-ventral axis of the eye. The clusters in each row are staggered with respect to the rows on either side. Also, all the clusters in a row have a similar organization and thus represent a specific stage in the differentiation of an ommatidium. Because of the curvature of the retina, it is difficult to determine in an individual row whether there is any variation in the cluster organization between the center and edge. Two rows containing the same type of organization are also present. It is possible that there are differences in these cluster rows that are too subtle for this level of analysis.
Fig. 2. a Low-power electron micrograph from a tangential section through the region indicated by the arrowheads in Fig. 1. The pattern of ommatidial cell clusters were identified from higher-power montages and marked on this section. Dividing cells indicate the mitotic band (m) at the bottom. The first identified row of ommatidia contains scattered rosette clusters (Ro) followed by symmetrical (S), transitional (T), and block (B) rhabdome containing retinula cells (R) and cone clusters (C). Note that the clusters in each row are staggered in relation to the row ahead and behind. Beyond row C, retinula cells recede from the surface. The posterior-distal direction is toward the top. Bar 10 µm. b Line drawings of the cellular organization in each row of developing ommatidia in a. Each drawing was traced from an electron micrograph taken at or just below the surface of the retina and represents a distinct stage in ommatidium differentiation. In the rosette cluster (Ro), the specific identity of the
The earliest cluster stage has a rosette organization (Ro in Fig. 2a). These clusters are located within or immediately adjacent to the mitotic band and form a broken or irregular row. Each rosette cluster has a central cell process, which is surrounded by two additional rings of cells (Fig. 2b). The inner ring contains seven cells that contact the central cell. The outer ring contains 10 or 11 cells, all contacting one or two of the cells in the inner ring. When the changes in the ommatidial clusters are followed across the retina, it appears that the central group of eight cells in the rosette cluster are the retinula cells (R). The outer ring of cells potentially represent cone, corneagenous, and screening pigment cells, though their specific identities are less certain.

The numbering of cells within the ommatidial clusters is based on the system used in the adult ommatidium of *H. americanus* by Parker (1890). Here the asymmetric retinula cells are R7 and R8. Parker considered R8 rudimentary. R7 lies on the posterior side of the main rhabdome and can be traced back to the central position in the rosette cluster. R7 and R8 are found next to one another in early differentiating clusters. The remaining six retinula cells form pairs which border the main rhabdome. R3 and R4 lie opposite R7 on the anterior side of the ommatidium. R1 and 2 are located opposite cells R5 and R6 on the other two sides of the main rhabdome.

The organization and distribution of the cluster stages following the rosette clusters are mapped in Fig. 2a and shown in detail by line drawings taken from electron micrographs in Fig. 2b. The distance over the retina, occupied by all these cluster rows, beginning at the mitotic zone, is approximately 70 µm (arrowheads in Fig. 1). In the two rows of symmetrical clusters (S in Fig. 2a, b) each has the central R7 cell process completely enclosed by neighboring cells R2 and R5. In the second row of symmetrical clusters there is a slight shift of R7 in the posterior direction in some but not all clusters. Next is a row of transitional clusters (T in Fig. 2a, b) in which R7 has partially migrated posterior between R1 and 6. The transitional row is followed by two rows of block clusters (B in Fig. 2a, b). In these clusters the completed migration of R7 between R1 and 6 forms a row of three cells adjacent to R8 on the posterior side of the cluster. Also in this cluster, R2 and R5 as well as R3 and R4 pair up to contribute to the roughly rectangular block-like organization of this cluster. An anterior-posterior axis of bilateral symmetry passes through R7 and between R3 and 4 on the opposite side of the cluster. Unlike the earlier clusters, the retinula cells in the block cluster are completely surrounded by four cone cells. In the next row (R in Fig. 2a, b), the changes in the retinula cells begin distally and progress proximally. Near the surface, the retinula cells taper toward the center and border a small central extracellular space. Microvilli begin to form along their central surface and extend into the common central space. As each R-cluster differentiates, microvilli that will form the adult, light-receptive rhabdome develop first distally and progress proximal. In older clusters sectioned below the level where microvilli are present, the retinula cell organization appears more like the block type.

In cluster rows S, T, B, and R, the central cluster of eight retinula cells is partially and then completely enclosed by four cone cells. Cone cells are numbered in a clockwise direction, beginning with the cone cell adjacent to R8. Associated with each cluster are two corneagenous cells and two distal pigment cells. The corneagenous cells are consistently located on the posterior side of the cluster adjacent to C1. The distal pigment cells are located on the anterior side of the cluster adjacent to C3. At the retinal surface, ommatidial clusters in row C and beyond (Fig. 2b) are composed of the small distal ends of four cone processes surrounded by two corneagenous cells (C in Fig. 2b). At this stage and beyond, retinula cells have receded from the surface leaving the distal ends of the surrounding cone cells to close in over them. The withdrawal of retinula cells from the surface contributes to the progressive
thickening of the retinal layer seen in Fig. 1. In ommatidial rows beyond those labeled C in Fig. 2a, the nuclei of the cone cells lie progressively closer to the surface. In these ommatidia, sections passing just below the surface show a profile like that seen in D of Fig. 2b. At this level the four cone cells contain nuclei and are surrounded by the two corneagenous cells.

**Ommatidial differentiation beyond the early stages**

Beyond the initial six early cluster rows shown in Fig. 2a, ommatidia continue to elongate and form a two-tiered organization. Figure 3a is a line drawing of an older ommatidium beyond these early rows based on a sampling of sections cut both longitudinally and transversely through the region indicated by the large arrow in Fig. 1. At this stage, cone cells taper below their nuclear region down to the top of the main rhabdome. Surrounding the tapered crystalline cone are the cell bodies of the retinula cells (Fig. 3a). The cell bodies lie approximately 15 µm above the main rhabdome and are connected to it by thin processes, which form an irregular ring around each crystalline cone (Fig. 3b). There appear to be eight processes around each crystalline cone that corresponds to the number of retinula cells in an ommatidium. At the end of the crystalline cone, the retinula cell processes expand and form the rhabdome region characterized by microvilli that project from the central surface of each cell. In stage VI juvenile eyes, the cell bodies of the retinula cells surround the proximal end of the crystalline cone. This final location is a more proximal position than seen in Fig. 3a. Thus, as the ommatidium differentiates, the retinula cell processes between the cell body and the top of the rhabdome shorten as the cell body shifts proximally.
Fig. 3. **a** Line drawing of a representative intermediate stage ommatidium in the region of the retina indicated by the large arrow in Fig. 1. At the surface of the retina, corneagenous cells (Cor) surround the narrow distal ends of the four cone cells. More proximally the cell bodies of the retinula cells (R1-7) surround the crystalline cone and are connected to the rhabdome region by fine processes. The cytoplasm of the 8th retinula cell (R8) separates into four regions at the top of the main rhabdome. Each region contributes to the formation of the 8th cell rhabdome located at the distal end of the main rhabdome. *(Bm Basement membrane, DP distal screening pigment, AP accessory screening pigment).* **b-d** Sections through the levels indicated by planes A-C. **b** Electron micrograph at plane A in **a**. At this level the cone cells of the crystalline cone appear to twist creating a swirled pattern of their membranes. Surrounding the crystalline cone are several fine processes of the retinula cells (*arrows*) which extend from the cell bodies to the rhabdome region. Distal screening pigment cell cytoplasm containing dark pigment granules surrounds the crystalline cone and retinula cell processes. *Bar 1.8 µm.* **c** Electron micrograph taken at plane B in **a**. At this level, microvilli from the four 8th cell cytoplasmic regions (*8*) and from retinula cells (*1-7*) contribute to this very distal rhabdome structure. The crystalline cone has separated into the crystalline tracts (*c*) which are located between the retinula cells. *Bar 0.6 µm.* **d** Electron micrograph taken at plane C in **a**. Two ommatidia are surrounded by both proximal accessory screening pigment cytoplasm (*ap*) and distal screening pigment cell processes (*dp*). The unpaired R7 cell lies on the posterior side of each ommatidium. Opposite R7 on the anterior side of the ommatidium is the R3 and R4 pair of cells. Retinula cell pairs R1 and R2, and R5 and R6 lie along the dorsal-ventral axis of each ommatidium. The ommatidia show an asymmetry in the position of the R8 axon (*8*) on either side of R7. This change in symmetry occurs along an irregular equatorial line separating the dorsal and ventral regions of the retina. Retinula cells in each ommatidium are numbered 1-7 in relation to the change in symmetry. *Bar 1.4 µm*

The 8th cell rhabdome forms at the junction of the crystalline cone and main rhabdome (Fig. 3b). The cytoplasm of the 8th cell is separated into four lobes, as shown in Fig. 3c. Microvilli extend from these lobes to form the 8th cell rhabdome that in the mature ommatidium lies just distal to the main rhabdome. The section in Fig. 3c is at or just above the very top of the main rhabdome. The lobes of the 8th cell are located between the pairs of retinula cells. In the rhabdome, microvilli that arise from the lobes of the 8th cell overlap or interdigitate with microvilli from the main rhabdome. In this example, the incomplete separation of the R8 rhabdome from the main rhabdome may reflect the incomplete elongation of the ommatidium. Alternatively, it may be that the new 8th cell rhabdome is so thin that microvilli in the main and 8th cell rhabdomes are seen in the same section.

The close association of the rhabdomes of R8 and R1-7 is also indicated in Fig. 3c by the fact that there are 11 cell processes joined by zonula adherens junctions surrounding the rhabdome at this level. The 11 processes include the 4 lobes of the 8th cell and the 7 retinula cells. Only 1 of the 8th cell lobes will continue proximally through the retina as the 8th cell axon. Also at this level, the crystalline cone cells separate into four cone tracts that are located between the pairs of retinula cells. These tracts extend down to the basement membrane of the retina. The position of these tracts and the 8th cell axon are consistent markers of ommatidial symmetry. A crystalline tract is seen consistently between R1 and 2, between R3 and 4, and between R5 and 6 (Fig. 3c). In this example, the lobe of R8 between R6 and 7 forms the 8th cell axon and a cone tract is seen between R7 and 1.

Figure 3d is a section through the level of the main rhabdome of two ommatidia. The adult organization of the retinula cells is seen at this level. R7 occupies the posterior side of each rhabdome; R3 and R4 the anterior side. The rhabdome at this stage appears round, and large cisternae are adjacent to its edge. Crystalline tracts appear between pairs of retinula cells, and the 8th cell axon is seen between R7 and 6. The large area of cytoplasm surrounding the ommatidia is characteristic of the proximal accessory pigment cells. In the juvenile lobsters examined here, these cells extend upward...
from the basement membrane between the ommatidia. Their cytoplasm contains a tapetal-type reflective pigment. The smaller cell processes that contain developing, dark-screening pigment are the proximal ends of the distal screening pigment cells. In juvenile lobsters, these cells extend downward only as far as the end of the crystalline cone.

**Retinal equator**

The thick section of the retina (Fig. 4a) is taken from a 70% embryo. Using a low-magnification electron-microscopic (EM) montage of an adjacent thin section, the location of the R8 axon was mapped over a portion of the retina. Figure 4b shows more detail of this map, in which the rhabdome (round profile), R8 axon (filled profile), and R7 are outlined for each ommatidium. This map shows that the R8 axon is located on either the dorsal or ventral side of R7 in each ommatidium examined. In each case, on the opposite side of R7 there is a cone tract between R7 and the adjacent retinula cell. Mapping the changing position of R8 defines an irregular equatorial line separating the dorsal and ventral halves of the retina (Fig. 4a, b).
Fig. 4. a Section through the retina of a 70% embryo. Posterior is at the top, anterior at the bottom. The irregular line separating the dorsal (right) and ventral (left) halves of the retina is the equator determined by mapping the asymmetry of individual ommatidia on a low-power EM montage of an adjacent thin section. Bar 20 μm. b Low-power EM montage of 70% embryonic retina seen in a. The outlines of R7, the R8 axon (filled) and the rhabdome (circular profile) are traced for each ommatidium. The position of R8 on either side of R7 changes on either side of the equatorial line. Outlines of the complete retinula cell cluster reflect the asymmetry of the retinula cells following the numbering convention related to the position of R8.

Fig. 5. Light micrograph of a stage VI juvenile retina. Ommatidia in this retina generally have an adult configuration only if they are smaller. The more mature ommatidia exhibiting spindle-shaped rhabdomes (arrow) are on the dorsal (D) side of the retina. As one moves ventrally (V), the ommatidia become less mature, but the gradient is slight until you reach the ventral edge. At the ventral edge, ommatidia are smaller and more cylindrical. At the extreme edge, early ommatidial clusters are present. (P posterior, A anterior). Bar 100 μm.

Fig. 6. Electron micrograph of an early ommatidium at the ventral edge of a stage VI juvenile lobster retina. A cluster of seven retinula cells surrounds a central rhabdome region containing microvilli. Zonula adherens junctions are seen between the retinula cells (1-8). Bar 0.9 μm.

Fig. 7. Electron micrograph of an 8th cell rhabdome in the retina of a stage VI juvenile lobster. The 8th cell rhabdome (R8) is located between the crystalline cone (cc) and the distal end of the main rhabdome. The 8th cell rhabdome does not show the rhabdomere banding seen in the main rhabdome below it. The crystalline tracts (arrows) continue beyond the crystalline cone between the retinula cells down to the basement membrane. Bar 1.4 μm.

Continuing growth of the retina in young adults

After hatching, lobster development progresses through three pelagic larval stages. The fourth stage begins pelagic but completes this stage by settling to the bottom and becoming benthic (Hulley and Beltz 1991). Each stage subsequent to this event is represented by a molt, so that a stage VI juvenile is two molts postlarval. Evidence that growth and differentiation of new ommatidia continue in the postlarval stages of the lobster was found in stage VI juvenile lobsters. These animals look like miniature adults and have stalked hemispherical eyes that are completely pigmented. Stage VI juvenile lobster eyes cut in a dorsal-ventral plane show a spectrum of maturity across their retinas (Fig. 5). At the ventral edge of the retina, early ommatidial cell clusters are found similar to those seen in the early embryonic retina (Fig. 5). Toward the dorsal side of the retina, ommatidia take on a more adult appearance characterized by a more spindle-shaped main rhabdome (Fig. 5). Indicative of increasing maturity is a clearly distinguishable 8th cell rhabdome, located between the crystalline cone and main rhabdome (Fig. 7). Figure 7 also shows two crystalline tracts extending between the retinula cells down toward the basement membrane of the retina.

Discussion

Lobster and crayfish: developmental history and retina formation

The process of retinal development and ommatidial pattern formation in the lobster H. americanus is very similar to retinal development in the freshwater crayfish P. clarkii, a closely related decapod crustacean (Hafner and Tokarski 1998). First, our observations of a single wave of mitotic activity in
the retina of *H. americanus*, confirm the earlier finding in the same species by Harzsch et al. (1999) using BrdU to label mitotically active cells. This result parallels a single proliferation zone observed in the developing retina of *P. clarkii* by Hafner and Tokarski (1998).

Second, in *H. americanus* no distinct transition zone is present between the dividing cells and the first ommatidial cell clusters. Dividing cells were observed juxtaposed to the earliest rosette ommatidial clusters and the tapered distal ends of elongated epithelial cells were interspersed between dividing cells. In addition, no observable surface feature marks the location of the transition region. In the transition zone of *P. clarkii*, the tapered distal ends of epithelial cells occupy a broader region. No clear cluster patterns are found in this area and no surface feature marks its location (Hafner and Tokarski 1998).

Last, the types and organization of the early ommatidial clusters in both *H. americanus* and *P. clarkii* (Hafner and Tokarski 1998) are strikingly similar. The presence of the rosette cluster in *H. americanus* appears to be the one difference between these two crustacean species. No preclusters such as those seen in insects have been identified in either species. Based on these morphological data, it appears that the different developmental histories of *H. americanus* and *P. clarkii* have little effect on the process of retinal formation or ommatidial differentiation. This may be a reflection of the conserved nature of the Arthropod ommatidial organization reviewed and discussed by Paulus (1979, 2000), Nilsson and Osario (1997), and Melzer et al. (1997).

**Retinal development and the evolution of the compound eye**

In a broader context, the present observations on retinal development in *H. americanus* add to a growing body of morphological and developmental data on the Arthropod compound eye. These data, in the view of some, suggest a common ancestral origin for the compound eyes of insects and crustaceans (Paulus 1979, 2000; Melzer et al. 1997; Nilsson and Osario 1997). With the limited data available, three elements of retinal development warrant discussion in this context: the presence of a proliferation zone, the morphogenetic furrow/transition zone, and ommatidial preclusters.

**The proliferation zone**

A single retinal proliferation band present in *H. americanus* and *P. clarkii* (Hafner and Tokarski 1998) is a consistent feature of visual system formation in those crustaceans so far studied. Using BrdU labeling, Harzsch et al. (1999) found a single retinal proliferation zone in three different crustaceans with different developmental histories: the spider crab *Hayas areneus* (Malacostraca, Decapoda, Brachyura); the lobster *H. americanus*; and the shrimp *Paleomonetes argentinus* (Malacostraca, Decapoda, Caridea). Harzsch and Dawirs (1995) also found a single proliferation zone in the retinas of *H. areneus* larva and juveniles. In addition, neurogenesis associated with the other visual neuropils was also similar in the three genera. More recently, Harzsch and Wolossek (2001) have extended their analysis of visual system neurogenesis to a more primitive taxonomic group the Entomostraca. Incorporation of BrdU into dividing cells of the visual system of *Triops longicaudatus* (Entomostraca, Branchiopoda, Phyllopoda, Calanostraca, Notostraca) revealed a single proliferation band in the metanauplius larval retina. The proliferation zones of the two visual neuropils in the head were also comparable with the other Malacostraca studied. These data from this small but growing sample of crustacean representatives point to a consistent process of cell addition to the developing retina.

Among the insects, retinal development is best known in the holometabolic fruit fly *D. melanogaster*. In this case, two mitotic bands have been described (Wolff and Ready 1993). Based on mitotic activity related to early ommatidial cluster rows, Friedrich et al. (1996) have suggested that two bands of activity may also be present, in the more primitive holometabolic flour beetle *Tribolium casaneum*.
(Insecta, Coleoptera, Tenbrionidae). In the hemimetabolic grasshopper *Shistocerca americanus* (Insecta, Orthoptera, Saltatoria), two mitotic bands were also found (Friedrich and Benzer 2000). In other hemimetabolic insects such as the dragon fly *Aeschna mixta* (Insecta, Odonata), Mouze (1972) has shown that retinal development in the larval retina begins with cell proliferation in the surface ectoderm at the edge of the retina similar to the crustaceans. Adjacent to the proliferation zone is a region where new ommatidia form, but no details of cluster organizations have been reported. These data, from both crustaceans and insects, indicate that the process of cell addition during retinal development is represented by one or two bands of mitotic activity that progress across the surface ectoderm of the optic primordia. The differences between insects such as *D. melanogaster* and crustaceans could be reflected in the expression patterns of genes involved in the recruitment of cells into the early ommatidial clusters or preclusters. At present, little data exists on molecular events of retinal development among the crustacea. Because most is known about retinal development in the Malacostraca and Entomostraca, these groups are an obvious starting point for these investigations.

**Morphogenetic furrow/transition zone**

A second consistent character of compound eye retinal development is the presence of a distinctive morphological region between the proliferation zone and the earliest ommatidial cell clusters. In the insects such as *D. melanogaster*, the morphogenetic furrow extends in a dorsal-ventral plane across the eye/antennal imaginal disc. The region is marked by a depression in the surface of the retina and exhibits at the surface the narrow densely packed distal ends of postmitotic epithelial cells (Wolff and Ready 1993). Constriction of cells at the surface is also seen in confocal images of the furrow in the developing retina of *S. americanus* and *T. casaneum* (Friedrich and Benzer 2000). However, a surface depression was not reported in either eye (Freidrich et al. 1996). Friedrich et al. (1996) did report a basal indentation marking the morphogenetic furrow in *T. casaneum*. In both *P. clarkii* (Hafner and Tokarski 1998) and *H. americanus*, a transition zone is found with features generally similar to the morphogenetic furrow, but the surface furrow is absent. In the branchiopod *T. longicaudatus*, Melzer et al. (2000) and Harzsch and Wallossek (2001) both described a transition zone with densely packed cells. These data, from a limited number of insect and crustacean representatives, show a morphogenetic zone with generally similar morphology in all developing retinas so far studied.

**Ommatidial cell clusters**

The limited data on the process of ommatidial differentiation falls into two general classes. In one class, differentiation progresses through a number of precluster stages in which the number of retinula and cone cells are added to the cluster. In the second, the first clusters identified contain a complete compliment of retinula and cone cells. All of the insects studied at this level contain precluster forms (Wolff and Ready 1993) Friedrich et al. 1996. The malacostracans such as *H. americanus* and *P. clarkii* (Hafner and Tokarski 1998) belong to the second class, where a detailed precluster series has not been found. Interestingly, the branchiopod species *Triops cancriformes* (Entomostraca, Branchiopoda, Phyllopoda, Calanostraca, Notostraca) contains preclusters very similar to insects (Melzer et al. 2000). The absence of precluster stages in both *H. americanus* and *P. clarkii* may suggest the need for cell-marking methods that could reveal additional early stages. Nevertheless, the number of Arthropod groups about which we have an in depth knowledge of ommatidial cluster morphology is small and thus it is difficult to assess the significance of the variations seen. Taken as a whole, the similarities in these three structural features of retinal development in the compound eye tend to support the idea of a conserved ontogenetic process. However, there remains the need for more study of the morphological and molecular events in retinal and visual system development in these and other Arthropod groups.
Ommatidial asymmetry and the retinal equator

The present data show an apparent change in the symmetry of the ommatidia in the lobster retina. In early clusters, an anterior-posterior axis of bilateral symmetry extends through R7 and between R3 and 4. In cluster types T, B, and R, R8 lies along the axis of bilateral symmetry and outside R7. The change in symmetry seen in older ommatidia results when the R8 axon shifts its location from the dorsal to ventral side of R7. The shift of R8 is also accompanied by an apparent change in the position of the crystalline tracts that lie between the retinula cells. When mapped, these positional shifts and the associated identification of retinula cells create the appearance of a mirror image on either side of an irregular equatorial line separating the dorsal and ventral regions of the retina (Fig. 4a, b).

An equatorial region in the retina has been reported in other crustaceans, including brachyuran crabs (Kunze 1968) and the crayfish Pacifastacus leniusculus (Malacostraca, Decapoda, Astacida; Nässel 1976). In P. clarkii, Hafner and Tokarski (1998) have found similar asymmetries in the location of the R8 axon within ommatidia of stage PO1 retinas. However, no convincing equatorial boundary could be demonstrated. In the primitive hemimetabolic flour beetle T. casaneum, Friedrich et al. (1996) were also unable to find any morphological indication of an equator. In addition, Diersch et al. (1999) have found variations in the position of cone tracts in the ommatidia of T. longicaudatus. These variations appeared to be randomly distributed within the retina. In contrast, numerous insect representatives have been shown to have some form of dorsal-ventral differentiation in their ommatidia (Arnett-Kibel and Meinertzhagen 1983; Bernard and Remington 1991; Menzel et al. 1991; Wada 1991; Wolff and Ready 1993).

The formation of a retinal equator in the compound eye has been studied most extensively in D. melanogaster and recently reviewed by Reifegerste and Moses (1999). Here, the establishment of the midline axis is determined in part by the anterior-posterior progression of the morphogenetic furrow and by the interaction of a family of tissue polarity genes that define the dorsal and ventral limits of the retina relative to the midline. This is followed by a midline out signal that effects the determination of R3 and R4 at the arc stage of ommatidial formation. Because R3 is the equatorial cell in each bilateral five-cell cluster and R4 the polar cell, the determination of R3 and R4 at this early stage establishes a polarity within the cluster relative to the midline of the retina. As the ommatidia differentiate, each cluster rotates in two 45° steps based on its earlier established polarity. After rotation, R7 in each cluster faces the equator and R3 is farthest from the equator in each ommatidium.

If one examines the ommatidial map of H. americanus in Fig. 4b, it is clear that a retinula cell cluster rotation similar to that in D. melanogaster does not occur, because the ommatidial axis of bilateral symmetry remains parallel to the anterior-posterior axis of the eye. How would it be possible to create the observed asymmetry? One hypothesis is that only R8 and the C1 cone cell shift their positions around R7. In early cluster stages such as stage R (Fig. 2b) and older, R8 and C1 are in tandem outside R7. As the clusters differentiate, these cells move to opposite sides of R7, creating the observed asymmetry. Melzer et al. (1997) has pointed out that the organization of retinula and cone cells in both insect and crustacean ommatidia are remarkably similar. The relation of R1-6 and three of the four cone tracts are impressive in their consistency. The main variation in this pattern is in the relation of R7, R8, and the fourth cone tract (identified here as C1). In retinas with an equator, the direction of the shift of either R8 or C1 could be influenced by a mechanism similar to the one that establishes the polarity of early clusters in D. melanogaster (Reifegerste and Moses 1999). Conversely, lack of some element in this mechanism could depolarize the ommatidium pattern. If our hypothesis is correct, the cone tracts between the retinula cell pairs 1-6 in each ommatidium would not change and the identity of the retinula cells should be the same on both sides of the equator. The asymmetry would be created by a change in the relation of R7, R8, and C1. Thus, ommatidia would not be numbered in relation to the shift of the cone tracts and the 8th cell as is seen in Figs. 3b and 4b. An analysis is in progress to
determine whether a bias in the position of either R8 or C1 can be detected in earlier ommatidial cell clusters.

**Continued growth of the retinal elements**

Crustaceans with direct development, as well as those with larval forms, all grow by means of a series of molts. The timing of these molts depends on a number of environmental factors. Several studies of crustaceans have shown that neurogenesis in the CNS, particularly the ventral nerve cord, all but ends slightly before or after the embryonic period (after the second metamorphosis in *H. areneus*: Harzsch and Dawirs [1994] and stage E90 in *H. americanus*: Harzsch et al. [1998]). The exception to these findings appears to be in a group of olfactory projection neurons in the brains of crabs, lobster, and crayfish (Harzsch and Schmidt [1996]; Schmidt and Harzsch [1996]). Here, neurogenesis persists, as determined by BrdU studies, well into adult life and seems to be correlated with the continued increase and renewal of sensory input from the antennules (Sandeman et al. [1998]). Investigations of the eyestalk neuropils by Harzsch and Dawirs (1995) in the crabs, *H. areneus* and *Carcinus maenas* (Malacostraca, Decapoda, Portunidae), as well as *H. americanus* (Harzsch et al. [1999]), indicate that proliferation of neurons also continues beyond the final metamorphosis into juvenile animals. These data support earlier studies in the retinas of brachyuran crabs (Harzsch and Dawirs [1994], [1995]), anomuran crabs (Meyer-Rochow [1990]), the crayfish (Tokarski and Hafner [1984]), and the lobster (Harzsch et al. [1999]), indicating that ommatidia increase throughout life. Growth of the eyestalk neuropils appears to be related to the addition of new sensory receptors/ommatidia similar to the olfactory system. The current observations of an immature ventral region of the juvenile eye of *H. americanus* containing very early ommatidial clusters further supports the concept of continued growth of the retina and visual neuropils beyond embryonic and larval stages into adulthood.

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