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SHORT COMMUNICATION

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Walking on insect paths? Early ommatidial development in the compound eye of the ancestral crustacean, *Triops cancriformis*

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Ommatidia (the compound eye's functional units) in insects are formed by the recruitment of undifferentiated cells under the control of signalling factors. During this process, a sequence of "preclusters" composed of specifically arranged precursor cells is followed. In the growth zone of the eye of *Triops*, an ancestral crustacean, we observed a patterning process that corresponds well with that of insects. In both taxa, clusters with arc-like, five-cell and eight-cell patterns are found, and the sequence in which the photoreceptor or R-cells of each ommatidium become identifiable is basically the same. The first to appear are R8-like and R2/5-like cells, second are R3/4-like, and third are R1/6- and R7-like cells (if the fly's cell-numbering system is used). Thus, the morphogenetic steps during which the cell identities and the cellular architecture of the ommatidia develop appear to be conserved between these arthropod groups. Furthermore, the individual cells and cell pairs which build an insect ommatidium seem to have their homologues in crustaceans. In the evolution of developmental processes, intercellular recruitment seems to be a mechanism operating on the level of single cells even in distantly related species.

The fundamental feature of the arthropod compound eye is its composition of regularly arrayed subunits or ommatidia. Within these units the photoreceptor or retinula (R-) cells exhibit a highly specific cellular pattern (Paulus 1979), in many taxa composed of two single cells (R7 and R8) and three symmetrical cell pairs (R1/6, R2/5, R3/4) (Meinertzhagen 1991; Melzer et al. 1997; Fig. 1E, E′). The identity of each cell is well-defined within this pattern and retained in every ommatidium throughout the eye. Instead of cell lineage, the determination and exact positioning of the cells in *Drosophila* is carried out by the sequential recruitment of photoreceptor precursors via cell–cell contacts, based on a cascade of gene products operating as signalling factors (Ready et al. 1976).

During this process, the immature ommatidium passes through several "precluster" stages (Fig. 1A–D), each characterized by its own distinct cellular pattern (Tomlinson 1985; Wolf and Ready 1993). Among insects, the basic sequence of morphogenetic steps leading to the architecture of the mature ommatidium appears to be strongly conserved (Ready 1989; Meinertzhagen 1991; Friedrich et al. 1996). To find out whether developmental paths homologous with those of insects are used to form the compound eye in crustaceans, the second large arthropod group with this eye type, we studied ommatidial development in the tadpole shrimp, *Triops cancriformis* (Notostraca), a phyllopod generally thought to possess ancestral features.

Immature *Triops* individuals pass through numerous ecdyses before they reach the adult stage (Fryer 1988). During this, the compound eyes located dorsally on the cephalothorax are continuously enlarging. New ommatidia are formed within a growth zone at the medial and anterior borders of the eye. Here, three regions representing different steps of ommatidial differentiation can be distinguished (Fig. 1E, G).

1. Medially, undifferentiated embryonic cells are found which provide the cellular material for the ommatidia to be built.
2. More laterally, i.e. closer to the compound eye, the initial steps of ommatidial differentiation occur, i.e. the R-cell precursors are sequentially recruited within preclusters. This zone looks like a furrow with densely packed precursor cells which possess pronounced apical microvilli portions and are connected by large junctional complexes.
3. Next to the compound eye, the protoommatidia have the full cellular equipment with eight R-cells and patterns similar to those of the mature units. Here the crystalline cone, screening pigment and the rhabdom of the photoreceptors are formed.

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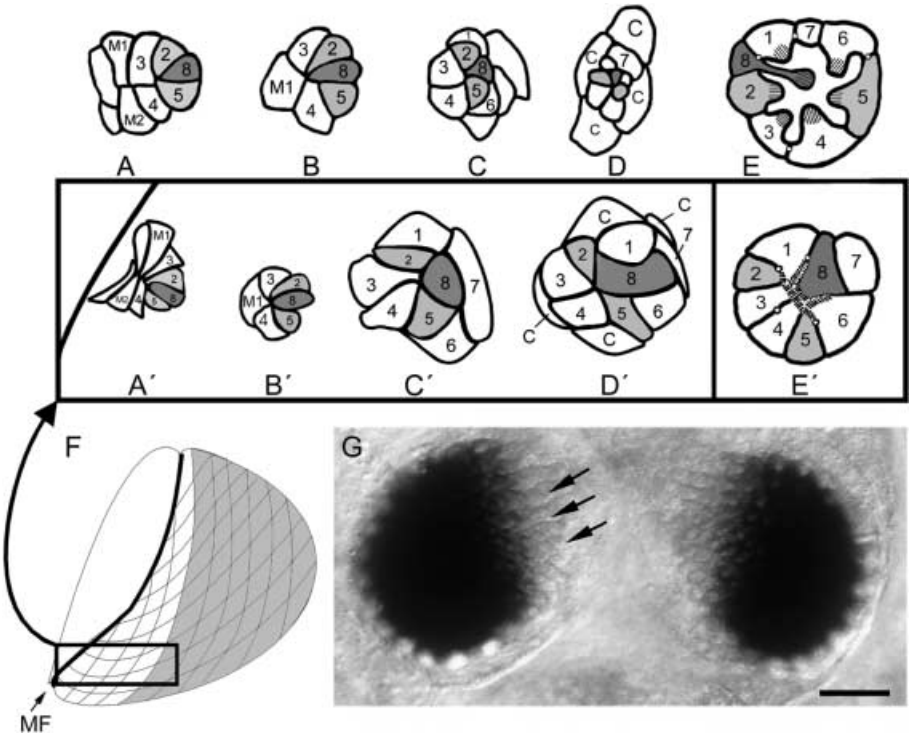

Fig. 1 Survey of the ommatidial development in *Drosophila* (**A–E**, after Wolf and Ready (1993), and *Triops* (**A′–E′**, **F** and **G**). **A–D** and **A′–D′** are likely to be evolutionarily conserved preclusters, **E** and **E′** show the mature retinula cell patterns, both numbered here after Dietrich's (1909) scheme, which allows to specify each ommatidial cell with respect to its position and symmetry. However, note that insect and crustacean R7 and R8 are reversely named in most studies (Melzer et al. 1997). **F** surveys the composition of the compound eye and its growth zone, **G** is a light microscope photograph of the two eyes and the immature ommatidia (*arrows*) of an 8-day-old *Triops* (dorsal view of whole mount). *1-8* and *M1*, *M2* are corresponding retinula and "mystery" cells having the same relative positions in both *Drosophila* and *Triops* clusters; *MF* morphogenetic furrow, *C* cone cell

The preommatidia are arranged in 1–4 rows which follow the axes of the mature ommatidial lattice. A few units, however, exhibit mirror symmetry with respect to the others, which also holds true for the mature part of the eye. This composition of the growth zone is the same as in insects, especially hemimetabolans (Meinertzhagen 1973; Wolf and Ready 1993). In previous studies on crustaceans, actually groups with advanced features only (Hafner and Tokarsky 1998), an "initialization" area with clusters earlier than those of the 8 R-cell stage has not been observed.

A closer look at the ommatidial preclusters reveals a high degree of correspondence with those of insects (Tomlinson 1985; Tomlinson and Ready 1987; Wolf and Ready 1993; Friedrich et al. 1996). Usually they can even be corroborated cell by cell (Fig. 1A–D, A′–D′). The earliest clusters we observed are composed of about 10 cells surrounding a "core" made of three symmetrically arranged cells, a single cell at the main axis of the cluster's cross-section that is flanked by a cell pair exhibiting mirror symmetry. In slightly older clusters, further cell pairs are incorporated into the core region. These are very similar to *Drosophila*'s "closing" arc stage. The "core" is made of seven cells, with the single cell now positioned in the vertex of the cluster's main axis, and three cell pairs arranged symmetrically (Fig. 1A′; 2A, B). In older clusters, the third cell pair loses its mirror symmetry: One of the pair is relocated into a central position having contact with the remaining cells (Fig. 2C, D). This process corresponds with the fate of the *Drosophila* "mystery" cells that are also rearranged at an early stage of differentiation. Hence we find five-cell preclusters composed of the vertex cell, two symmetrical cell pairs and the central mystery cell (Fig. 1B′; 2E). Genuine five-cell preclusters in which the latter cell is missing are also seen. The next stage is similar to *Drosophila*'s immature eight-cell clusters. Here the five cells already mentioned plus three newly recruited cells are found: with one single cell lying close to the former vertex cell which is now in the core of the cluster, and a third cell pair positioned at both sides of the two single cells (Fig. 1C′; 2E). The last stage consists of four cone cell clusters composed of the eight R-cells and two pairs of cone cells (Fig. 1D′; 2F).

Thus, the precursor cell patterns and their sequence seem to be highly conserved between insects and *Triops*. Moreover, if one traces the individual cells through the developmental stages, one finds corresponding cell identities as well (Fig. 1A–E′). In both taxa, the cell which will become the larger of the two single cells in the mature ommatidium [named R8 in many insects, but named R7 in many crustaceans (see Melzer et al. 1997)] is the vertex cell as already seen in the earliest clusters. Conversely, the second single cell that will later form a distal rhabdomer (named R7 in many insects, but R8 in many crustaceans) appears late during development in both groups, and its position is at the opposite border of the cluster with respect to the mystery cells. The decision which of the two cells is to be named R7 and which R8 is best made using the criterion of their homologous developmental origin (Friedrich et al. 1996). Furthermore, the remaining six cells occur during ontogeny as pairs recruited in a consorted manner as in insects. They have the same relative positions within the clusters, and the sequence in which

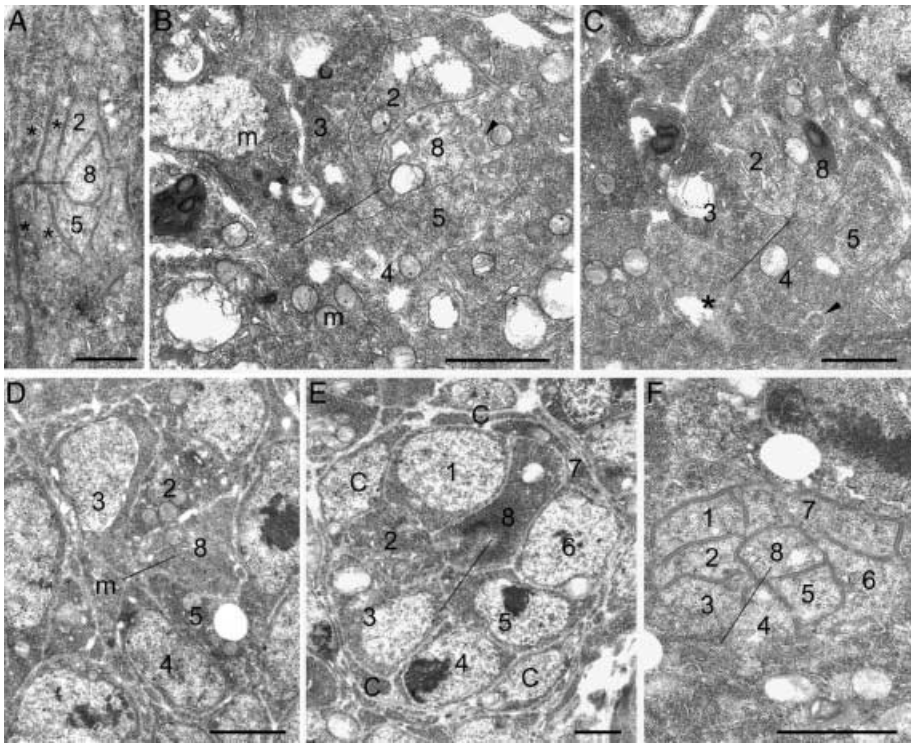

Fig. 2A–F. Ommatidial preclusters in *Triops* (transmission EM, transverse sections). **A** Closing arc-like precluster with future cell pair 2/5 surrounding the vertex cell (*8*) and two further cell pairs (*), sectioned at the apical junction level. **B** Well-developed closing arc stage, slightly older than **A**. **C** Early five-cell precluster with an *m*-cell (*) located in the main axis of the cluster's cross section. **D** Five-cell precluster with central position of *m*-cell. **E** Immature eight-cell cluster. **F** Four cone cell stage. *1-8* and *m* cells in *Triops* clusters have the same relative position as *Drosophila's* retinula cells R1–R8 and mystery cells M1 and M2. *C*, cone cells; *arrowheads*, centrioles indicating apical level of the sections. Note relatively low electron density of the "core" cells 8, 2 and 5. The *solid lines* are positioned at the main axes of the cluster's cross sections; *bars* represent 1 μm

they are incorporated into the respective pattern and its symmetry is likewise very similar. The pair that will have a R2/5-like position in the mature ommatidium is the first that can be identified, then the R3/4-like pair and finally a R1/6-like pair.

These similarities at the level of the ommatidial lattice, the composition of the growth zone and the sequence of cell recruitment within each precluster suggest that a considerable part of the patterning process is evolutionarily conserved between insects and *Triops*. It is reasonable to assume that homologous events at the molecular level, namely, a *Drosophila*-like signalling pathway (Freeman 1997) can also be found in crustaceans. In addition to the cellular patterns of mature ommatidia (Melzer et al. 1997), the cellular architecture and sequence of the ommatidial preclusters found in *Triops* reflect the "pairs and singles" theme (Meinertzhagen 1991) and indicate homology of the crustacean and insect compound eyes. This corresponds well with molecular trees indicating that the Insecta and the Crustacea are sister-groups (Friedrich and Tautz 1995) instead of the "Myriapoda" and Insecta, as is the traditional cladistic view. Findings on the patterns of stem cell formation and brain characters generally support this idea (Strausfeld 1998; Harzsch et al. 1999). Concerning the eyes, however, studies on their development in groups that have lateral ocelli instead of compound eyes will need to be undertaken. Otherwise, ancestral or unspecific features could be mistaken for new or specific ones (see also Nilsson 1996).

Most studies on the evolution of developmental processes in arthropods focus on the segmentation genes (Patel 1994; Damen and Tautz 1999) or on neuroblast differentiation (Reichert and Boyan 1997). Concerning eyes, few comparative studies dealing with the ontogeny of single cells and clusters composed of only a few cells have been done so far (Friedrich and Benzer 1997; Kobayashi et al. 1999). Our findings on a crustacean only distantly related to insects indicate that not only cell lineage, but also intercellular recruitment via cell-to-cell contacts may be evolutionary strategies that regulate developmental processes at the single-cell level, and may differentiate homologous eye structures by homologous developmental pathways.

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