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Mechanisms of eye development and evolution of the arthropod visual system: The lateral eyes of myriapoda are not modified insect ommatidia

Steffen Harzsch[a,*], Roland R. Melzer[b], Carsten H.G. Müller[c]

[a]Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Beutenberg Campus, Hans-Knöll-Str. 8, 07745 Jena, Germany
[b]Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 München, Germany
[c]Allgemeine und Systematische Zoologie, Institut für Biowissenschaften, Universität Rostock, Universitätsplatz 2, 18051 Rostock, Germany

Abstract

The lateral eyes of Crustacea and Insecta consist of many single optical units, the ommatidia, that are composed of a small, strictly determined and evolutionarily conserved set of cells. In contrast, the eyes of Myriapoda (millipedes and centipedes) are fields of optical units, the lateral ocelli, each of which is composed of up to several hundreds of cells. For many years these striking differences between the lateral eyes of Crustacea/Insecta versus Myriapoda have puzzled evolutionary biologists, as the Myriapoda are traditionally considered to be closely related to the Insecta. The prevailing hypothesis to explain this paradox has been that the myriapod fields of lateral ocelli derive from insect compound eyes by disintegration of the latter into single ommatidia and subsequent fusion of several ommatidia to form multicellular ocelli. To provide a fresh view on this problem, we counted and mapped the arrangement of ocelli during postembryonic development of a diplopod. Furthermore, the arrangement of proliferating cells in the eyes of another diplopod and two chilopods was monitored by labelling with the mitosis marker bromodeoxyuridine. Our results confirm that during eye growth in Myriapoda new elements are added to the side of the eye field, which extend the rows of earlier-generated optical units. This pattern closely resembles that in horseshoe crabs (Chelicerata) and Trilobita. We conclude that the trilobite, xiphosuran, diplopod and chilopod mechanism of eye growth represents the ancestral euarthropod mode of visual-system formation, which raises the possibility that the eyes of Diplopoda and Chilopoda may not be secondarily reconstructed insect eyes.
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Introduction

In discussions about the phylogenetic relationships of Arthropoda, the structure (Paulus 1979, 2000; Spies 1981; Melzer et al. 1997a; Müller et al. 2003; Bitsch and Bitsch 2005) and development of the lateral eyes (Melzer et al. 2000; Hafner and Tokarski 1998, 2001) and optic ganglia (Melzer et al. 1997b; Harzsch and Walossek 2001; Harzsch 2002; Wildt and Harzsch 2002; Sinakevitch et al. 2003; Strausfeld 2005) have always played pivotal roles. Despite this extensive body of literature, the mechanisms of eye growth have not yet been explored systematically across the Euarthropoda. In the present study, we therefore analysed the formation of new visual units and their integration into the eyes in

*Corresponding author.
E-mail address: sharzsch@ice.mpg.de (S. Harzsch).

four representatives of the Myriapoda and compared these mechanisms with those in other Euarthropoda representing different types of eye architecture.

Limulus polyphemus Linnaeus, 1758 is a representative of the Chelicerata, in which lateral eyes composed of several similar optical units, the ommatidia, are still present (reviewed by Fahrenbach 1975). Each ommatidium has a corneal lens and is composed of a variable number of more than 300 cells. Other Chelicerata have varying numbers of lateral single eyes that have been suggested as derived by modification from lateral facetted eyes (Paulus 1979; Schliwa and Fleissner 1980; Spreitzer and Melzer 2003). Most Progoneata and Chilopoda (Myriapoda) have lateral eyes composed of several similar subunits generally named lateral ocelli (that will be termed "ocellar ommatidia" in the following), but in many aspects the architecture of these subunits is different from that in *L. polyphemus* (Paulus 1979, 2000; Müller et al. 2003; Müller and Meyer-Rochow 2005). The ocellar ommatidia of most Diplopoda are composed of a high and varying number of contributing cells, their rhabdomeres are usually made up of more than 60 retinula cells (Spies 1981; Paulus 2000). All chilopod eyes have in common a dual-type retinula that is arranged in either two or numerous sometimes disintegrated horizontal layers (Bähr 1974; Paulus 1979; Müller et al. 2003; Müller and Meyer-Rochow 2006a,b; Müller and Rosenberg 2006).

Eye design in Scutigeromorpha (Notostigmophora), which presumably are basal representatives of the Chilopoda (Edgecombe 2004; Edgecombe and Giribet 2004) in many respects is different from that in other Chilopoda (Bähr 1974; Paulus 1979). Scutigeromorpha have ommatidia composed of between 39 and 46 cells, and a crystalline cone built by four or (rarely) five cone cells (Müller et al. 2003). The principal cell types in the eyes of Scutigeromorpha, in particular the crystalline cone cells, can be homologised with those in Hexapoda and Crustacea (Müller et al. 2003). However, the ocellar ommatidia of pleurostigmophoran centipedes (including Lithobiomorpha and Scolopendromorpha) lack crystalline cones and other scutigeromorph features such as interommatidial pigment cells and primary pigment cells. They may be encircled by external pigment cells (discussed in Paulus 2000; Müller and Meyer-Rochow 2006a,b; Müller and Rosenberg 2006). A survey of eye growth patterns in diplopods has been given in Enghoff et al. (1993), indicating a moult-related row growth.

The Insecta and Crustacea have compound eyes with many similarly structured ommatidia. However, contrary to the Chelicerata and Myriapoda, their optical units are composed of a small, strictly determined and evolutionarily conserved set of cells. Recent data suggest that many aspects of retinal pattern formation, ommatidial differentiation and optic stem cell proliferation are similar between representatives of Crustacea and Insecta

(Harzsch et al. 1999; Melzer et al. 2000; Hafner and Tokarski 1998, 2001; Harzsch and Walossek 2001; Harzsch 2002; Wildt and Harzsch 2002). Melzer et al. (2000), Paulus (2000), Dohle (2001) and Richter (2002) suggested that in the ground pattern of these two taxa each ommatidium is composed of a small, constant number of cells: two corneagenous cells, four crystalline cone cells, eight retinula cells, and several pigment cells. This fixed architecture is supposed to be a synapomorphy of a taxon formed comprising these two groups for which Dohle (2001) proposed the name "Tetraconata" in reference to the tetrapartite crystalline cone. We will therefore refer to the ommatidia of Crustacea and Hexapoda as "tetraconate ommatidia" in the following.

Paulus (1986, 2000) has suggested an evolutionary scenario to explain the relationships of these different eye types among the Euarthropoda. According to his model, compound eyes with ommatidia like those of recent Crustacea, Hexapoda or Scutigeromorpha may represent the ancestral eye type of Mandibulata. From this plesiomorphic character state, the compound eyes disintegrated into single ommatidia. Then, by fusion of several ommatidia and/or increase of cell numbers in the ommatidia, multicellular ocelli (fusion stemmata) like those of Progoneata and Chilopoda emerged. Harzsch et al. (2005) recently challenged this hypothesis and instead suggested an evolutionary scenario following the opposite direction. They proposed the multicellular eye subunits of Chelicerata/Xiphosura with their high and variable cell number to be plesiomorphic for the Euarthropoda. Some taxa of Progoneata and Chilopoda (genera *Scutigera*, *Polyxenus*) have reduced the number of cells of which each eye subunit is composed, and some cell types occur in constant numbers. In the new model of Harzsch et al. (2005), these taxa represent an intermediate on the path towards the Tetraconata in which the eye subunits have a fixed architecture with a relatively low, constant cell number.

Despite the potential of developmental studies to add new aspects to this controversy, information on eye development is not available for the Chilopoda. However, in the Progoneata, eye growth has been analysed in correlation with growth of the animals (e.g. Vachon 1947; Saudray 1952; Sahli 1955; Peitsalmi and Pajunen 1991, 1992). For a long time, myriapodologists found it hard to determine the age and/or developmental stage of an individual diplopod when looking at the number or the way of accretion of new trunk diplosegments. Exploring diplopod eye development has shown that the actual number of moults can be determined by looking at the number of ocellus rows, as with each moult a new row of ocellar ommatidia or a single new ommatidium is added to the eye field laid down earlier in development (reviewed in Blower 1985; Hopkin and Read 1992; Enghoff et al. 1993). One would expect that some residual evidence of the evolutionary pathway

suggested by Paulus (1986) should be revealed by the developmental program by which the generation of the eyes in recent Myriapoda is governed. However, such evidence is lacking so far. In order to gain new insight into the evolutionary relationship of myriapod eyes with respect to the competing scenarios, the present study explores eye growth in four representatives of the Myriapoda: the two diplopods *Cylindroiulus truncorum* (Silvestri, 1896) and *Archispirostreptus gigas* (Peters, 1855), and the two chilopods *Scolopendra oraniensis* Lucas, 1846 and *Scutigera coleoptrata* (Linnaeus, 1758). Specifically, we mapped the pattern in which new visual units are added to the existing eye field. Furthermore, the arrangement of proliferating cells in the developing eyes was monitored by in vivo labelling with the mitosis marker bromodeoxyuridine (BrdU) (Harzsch et al. 1999; Harzsch and Walossek 2001; Wildt and Harzsch 2002).

Material and methods

Animals

Juvenile and adult centipedes of the species *S. oraniensis* Lucas, 1846 (Chilopoda, Scolopendromorpha) and *S. coleoptrata* (Linnaeus, 1758) (Chilopoda, Notostigmophora) were collected on the Balearic Island of Ibiza, Spain. Millipedes *A. gigas* (Peters, 1855) (Diplopoda, Spirostreptida, Spirostreptidae) were purchased from a commercial supplier (B.T.B.E., Born To Be Eaten, Schnürpflingen, Germany; ⟨http://www.btbe.de⟩). Numerous individuals of *C. truncorum* (Silvestri, 1896) (Diplopoda, Julidae) representing various stages and moults were collected from a compost heap in the garden of the Zoological Institute in Munich, Germany. On 60 animals of this species, the number of ocellar ommatidia and the shape of the eye field were examined and/or photographed, either under a Wild stereo microscope or a Zeiss Axioplan photomicroscope with brightfield illumination.

BrdU labelling

Proliferation of cells was monitored by in vivo labelling with the s-phase specific mitosis marker BrdU (Harzsch et al. 1999). Because we assumed eye growth to proceed rather slowly in the juvenile animals we provided several pulses of BrdU labelling reagent during a period of 2–3 months. On December 10th 2004, juvenile centipedes *S. oraniensis* and millipedes *A. gigas* were anaesthetized with CO_2, subsequently cooled to 4 °C, then injected with 20 µl BrdU labelling reagent (Amersham, Cell Proliferation Kit RPN 20) using a 0.4 mm syringe. The animals received a second pulse of BrdU into the haemocoel on January 18th 2005. All attempts at injecting centipedes *S. coleoptrata* resulted in the death of the specimens. On January 20th (for *S. coleoptrata*), January 25th (*A. gigas*), and March 3rd (*S. oraniensis*), respectively, the animals were anaesthetized with CO_2 and subsequently cooled to 4 °C. Their heads were cut off and incubated in BrdU diluted in locust ringer pH 6.8 (140 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl$, 5 mM $NaHCO_3$ and 5 mM NaH_2PO_4) to a concentration of 0.2 mg/ml for 12 h at 18 °C. Whole mounts of the dissected eyes were processed immunohistochemically as described by Harzsch et al. (1999). Briefly, specimens were fixed overnight in 4% paraformaldehyde, then washed in phosphate-buffered saline (pH 7.4). Incubating in commercial bleach for 60 s degraded the photopigment. Specimens were then incubated for 2.5 h in a primary anti-BrdU mouse (1:100, Amersham, Cell Proliferation Kit RPN 20), and afterwards for 1 h in a peroxidase-coupled goat anti-mouse secondary antibody (1:70). The enzyme label was visualized by reacting with diaminobenzidine and peroxide (Harzsch and Dawirs 1994). The whole mounts were dehydrated, mounted and viewed with a Zeiss Axioskop fitted with a CCD-1300B digital camera (Vosskühler GmbH). Digital images were processed with the Lucia 4.82 software package (Laboratory Imaging Ltd.) and Photoshop Elements (Adobe). Stacks of images taken from the same sample at different focal levels were either transformed into sharp 2D photomontages or into blue–green coded 3D reconstructions by the Lucia software.

Results

Mapping growth of the eye field in the diplopod *C. truncorum*

The lateral head capsule with the eye field was examined in 60 juvenile *C. truncorum* representing various developmental stages (Fig. 1). Fig. 2 summarizes counts of ocellar ommatidia and mappings of their arrangement, as well as the eye-field orientation. In this figure the specimens are arranged in ascending order according to the number of ocellar ommatidia (units) in their eye fields. In addition, the moult stage of the studied specimens is indicated according to Enghoff et al. (1993). Furthermore, the number of individuals (n) with each observed pattern is indicated. The eye fields of the 60 individuals examined were composed of 1–39 units that were arranged in 1–9 rows of ocellar ommatidia (RO, 'rangée d'ocelles'). In those individuals with 3–7 RO, different unit numbers and arrangements were recorded. For example, the fourteen 7-RO specimens fell into four different classes with 25–28 ommatidia (Fig. 2). The eye fields of the 60 specimens screened fell into 17 different classes.
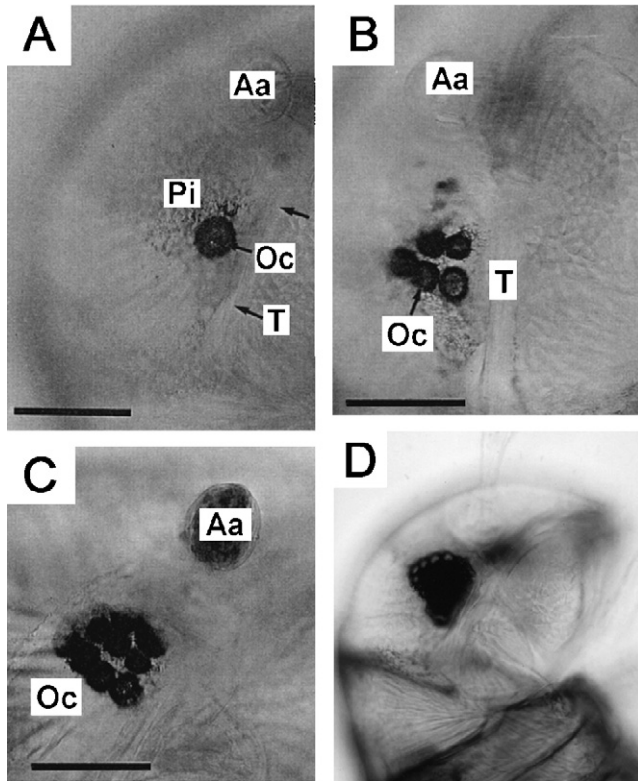
Fig. 1. Light micrographs of lateral head capsule with eye field of juvenile *Cylindroiulus truncorum* in various developmental stages, respectively, comprising one (A), five (B), and ten (C) ocellar ommatidia; (D) overview. Abbreviations: Aa = first antennal article, Oc = ocellus, Pi = pigmented area from which new ocelli develop, T = tentorium. Scale bars: 100 μm.

From our counts it is possible to gain insight into how the eye field grows. Correlated to the moults are waves of differentiation of new rows of ocelli, beginning with individuals that possess only a single ocellus. Successively, one finds 2 RO in the second stage, 3 RO in the third, and so on, until the maximum of 7 rows is attained at stage seven. The number of ocelli added within each row shows some individual variation that brings about different subtypes of the RO classes; e.g., in the 3RO eye fields one finds individuals with either 2 or 3 ocelli in the newest row, and in 4-RO eye fields the newest row is composed of either 3 or 4 ocelli (for details and the other RO classes see Fig. 2). One can conclude that in *C. truncorum* there is a moult-related regular accretion of new rows of ocelli at the antero-dorsal edge of the eye field, a feature that closely fits the pattern of eye growth in other diplopods (Enghoff et al. 1993). Furthermore, we observed that in the 1RO1 to 4RO individuals the oldest ocellar ommatidium, i.e., the single unit found in the 1-unit specimens, is slightly larger than the units that form later, and is located at the ventro-caudalmost tip of the eye field.

Analysis of eye growth in the diplopod *A. gigas* by BrdU labelling

In order to obtain additional insight into where the new cellular material of the new units that are added to the eye field is generated, we monitored neurogenesis applying the proliferation marker BrdU. Fig. 3 shows the eye fields of two exemplary juvenile *A. gigas* with 36 ocellar ommatidia (arranged in 8 rows, with row eight
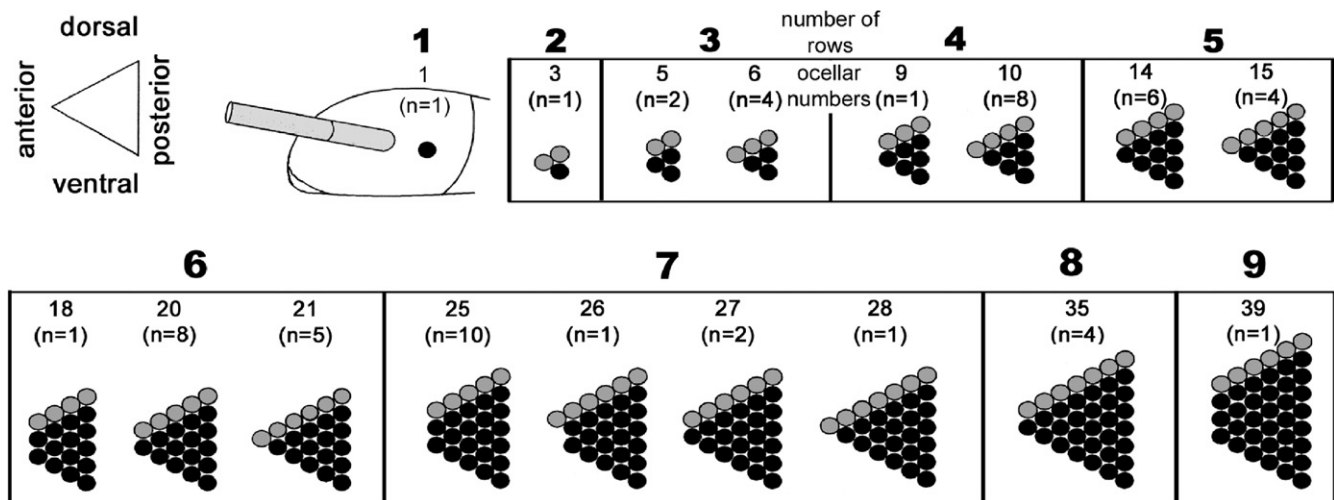

Fig. 2. Summary of ocellar counts and mappings of their arrangement, and eye-field orientation in 60 juveniles of *Cylindroiulus truncorum* in various stages of development; specimens arranged in ascending order according to number of ocellar ommatidia (units) in eye fields; *n* = number of individuals with respective pattern; grey circles indicate units that are new compared to the previous pattern. Eye fields were composed of 1–39 units arranged in 1–9 ocellar rows (RO); observed unit numbers were 1, 3, 5, 6, 9, 10, 14, 15, 18, 20, 21, 25, 26, 27, 28, 35, and 39, so that eye fields fell into 17 classes. Moult stage (1–9) for each RO type given according to Enghoff et al. (1993).
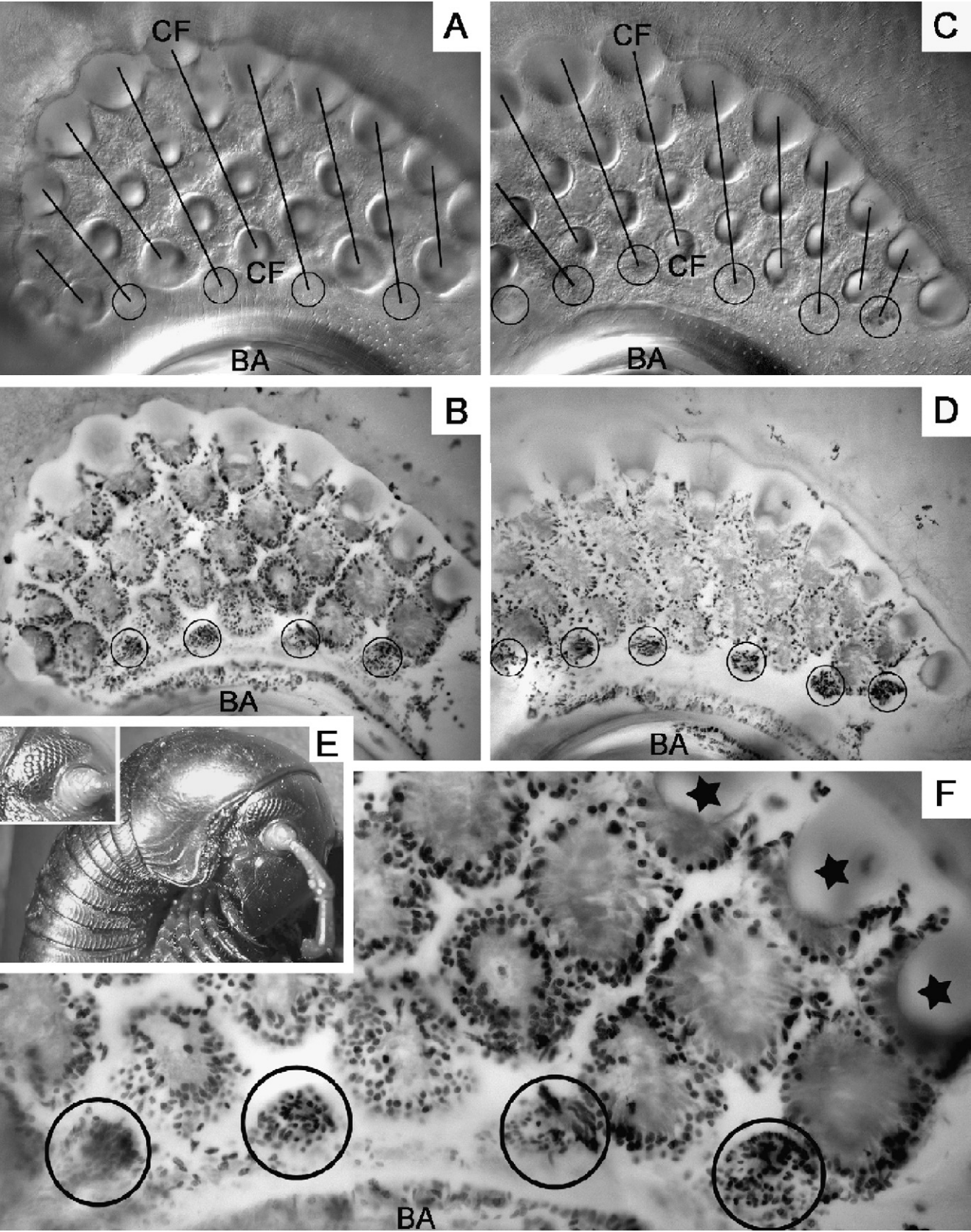
comprising the newly emerging units), viewed with Nomarski interference contrast (Figs. 3A and C) to show the surface of the eye field, and with brightfield illumination (Figs. 3B and D) to show the BrdU-labelled nuclei. An older animal is shown in Fig. 3E to provide the orientation for figures 3A–D. As in *C. truncorum*, in addition to being organized in 8 rows, the eye field at this stage can be viewed as comprising a central file with 4 mature ocellar ommatidia which on both sides is flanked by a file composed of 3 mature ocellar ommatidia but in addition a protommatidium (circle; black lines connect the units of one file). Towards the sides, next there is a file with 3 units, then a file with two units plus a protommatidium, etc. (note that in Figs. 3A–D not the complete eye fields are shown). With brightfield optics, the protommatidia appear as clusters of dozens of black-labelled nuclei (circles in Figs. 3B and D), which at a higher magnification all appear to be of a similar size (Fig. 3F). As in *C. truncorum*, the new row of protommatidia is added to the eye field from the side where the base of the antenna adjoins the eye. In addition to the protommatidia, BrdU labelling reveals that every mature ocellar ommatidium at its base is surrounded by a broad rim of mitotic nuclei, suggesting a persistent intercalary growth. Scanning the whole mounts at different focal planes and comparison with published information on the architecture of diplopod eyes (Paulus 1979; Spies 1981) revealed that this rim of labelled cells wraps around the base of the cornea laterally and proximally, suggesting these proliferating cells to be located within the layer of retinula cells. This is supported by analysis of ommatidia at the side of the eye field, which are slightly tilted, thus providing a side view (asterisks in Fig. 3F).

BrdU labelling in the chilopod *S. oraniensis*

The lateral eyes of *S. oraniensis* consist of only 4 ocellar ommatidia on each side of the head (Figs. 4A and B; only 3 units shown in B). Each optical element is composed of a corneal lens formed by 230–2200 cells, 560-1020 distal and 70–130 proximal retinula cells, 150–250 sheath cells, as well as numerous external pigment cells surrounding the entire eye cup (Müller and Meyer-Rochow 2006a). BrdU labelling in adult *S. oraniensis*, in which all 4 ocellar ommatidia are already present, revealed mitotic cells to be present within all parts of the unit, suggesting a persistent intercalary growth (Figs. 4C and D). The broad arrows in Fig. 4C mark spindle-shaped nuclei that closely resemble those of the corneagenous cells (Müller and Meyer-Rochow 2006a); the narrow arrows point to labelled nuclei belonging to the distalmost layers in the horizontally stacked system of distal retinula cells (note that the proximal and part of the distal rhabdom were torn off in this preparation). The narrow arrows in Fig. 4D point to mitotic nuclei located within the layer of the distal retinula cells that surrounds the distal rhabdom, an arrangement that resembles the localization of proliferating cells within the layer of retinula cells in the diplopod *A. gigas* as outlined above. Proliferating cells are also present in the layer of proximal retinula cells (data not shown).

BrdU labelling in the chilopod *S. coleoptrata*

In contrast to diplopod eyes, all ommatidia in the lateral eye of *S. coleoptrata* are of fairly similar size (Fig. 4E). These ommatidia are composed of a fixed cell array that shows little variation in numbers (Müller et al. 2003). BrdU labelling reveals that the growing eyes are entirely surrounded by a zone of mitotic cells (Figs. 4F–H). This proliferation zone is located laterally along the circumference of the eye and is the site where new optical units are generated. Analysing this proliferation zone with 3D images revealed that the proliferating cells seem to be clustered in a pattern that prefigures the outline of the protommatidia (Fig. 4H). Few or no labelled profiles where observed in the middle of the eye field where mature ommatidia are located.

Discussion

Growth of the lateral eyes in Diplopoda is similar to Xiphosura and Trilobita, and represents the plesio-

Fig. 3. Two examples of juvenile eye fields in the diplopod *Archispirostreptus gigas*, with 35 units labelled with the proliferation marker bromodeoxyuridine (BrdU); for orientation of images compare to adult specimen in Fig. E; BA = base of antenna. (A) and (C) Two different specimens viewed with Nomarski interference contrast, showing parts of surface of eye field (montages from stacks of 10 focal planes each); black lines connect ocellar ommatidia of one file; central file (CF) with four mature units flanked on both sides by a file composed of three mature ocellar ommatidia plus a protommatidium (circle; for further explanation see text). (B) and (D) Specimens as in (A) and (C) viewed with brightfield illumination (montages from stacks of 10 focal planes each), showing BrdU-labelled nuclei; circles identify protommatidia which appear as clusters of numerous black labelled nuclei; in both specimens, a new row of protommatidia added to eye field from side where base of antenna adjoins eye; furthermore, all existing units within eye field surrounded by distinct rim of mitotic cells. (F) Higher magnification of (B) (montage compiled from stack of 5 focal planes); circles identify row of protommatidia being added to side of eye field; at this stage, protommatidia are clusters of mitotic nuclei all of which appear to be of uniform size; note mitotic cells which surround base of existing ocellar ommatidia and are located within layer of retinula cells; asterisks identify ommatidia at side of eye field that are slightly tilted, thus providing a side view.
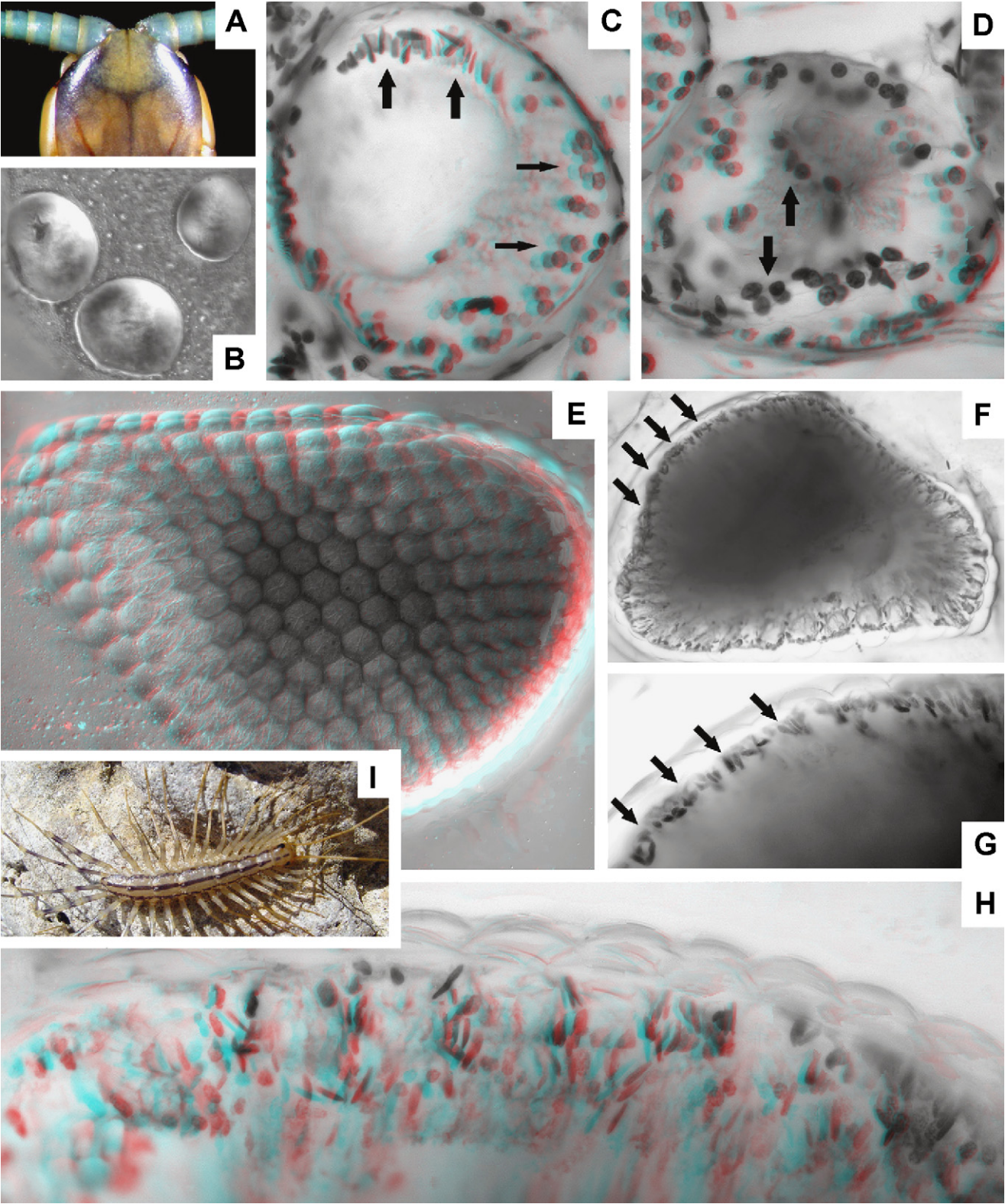
morphic euarthropod condition. It has been established firmly that during growth of the eyes of Trilobita new elements were formed in a generative zone that was arranged along the eye margin (Zhang and Clarkson 1990; Clarkson and Zhang 1991). From this generative zone, new lenses were added to the growing eye (Fig. 5A). For the xiphosuran *Limulus polyphemus* (Chelicerata) it has been suggested that the eyes grow by the addition of new elements at the anterior margin (Meadors et al. 2001; Smith et al. 2002). Confirmation for these data comes from recent BrdU labelling experiments in juvenile *L. polyphemus* (Figs. 5B and C; Harzsch et al. 2006). The pattern of eye growth in Diplopoda reported on here closely corresponds to that known from other diplopods in that new elements are added to the side of the eye field from an anteriorly/dorsally located proliferation zone, thus elongating the rows of earlier-generated optical units (Blower 1985; Peitsalmi and Pajunen 1991, 1992; Hopkin and Read 1992; Enghoff et al. 1993). Because this 'row-by-row' growth in Diplopoda closely corresponds to Xiphosura and Trilobita (Zhang and Clarkson 1990; Clarkson and Zhang 1991, Meadors et al. 2001; Smith et al. 2002), we propose that this mechanism represents the plesiomorphic state in the euarthropod ground pattern of how the visual system is generated. The gradient of the stage of differentiation of ocellar ommatidia within a single new row we observed needs further examination.

This suggestion is important with regard to the competing scenarios concerning the evolutionary relationship of myriapod and insect eyes. According to the hypothesis of Paulus (1979, 1986, 2000), myriapod eyes are secondarily reconstructed insect eyes derived by distinct evolutionary modifications: insect compound eyes disintegrated into single ommatidia which then fused again to form the myriapod multicellular ocelli. Our data do not provide evidence in favour of this scenario, but rather suggest that the Diplopoda share the ancestral mechanism of eye growth as is present in other Euarthropoda. This raises the possibility that the eyes of Diplopoda may not be modified insect eyes.

Eye growth in pleurostigmophoran Chilopoda

The eyes of the scolopendromorph *S. oraniensis* that we examined comprise only four ocellar ommatidia, a fact that has been interpreted by many authors as a derived character state for the Chilopoda. This notion was based either on the assumption of scutigeromorph eyes to be 'pseudo-facetted' and hence containing ommatidia not homologous with those of the mandibulate type (Paulus 1979, 2000; Spies 1981) or, most recently, on the suggestion of scutigeromorph ommatidia as being plesiomorphic with respect to the Pleurostigmophora (Paulus 1979; Bitsch and Bitsch 2005; Müller and Meyer-Rochow 2006a,b; Müller and Rosenberg 2006). Our BrdU experiments suggest that there is a persistent proliferation within all major cell types of which the ocellar ommatidia are composed (intercalary growth), the corneagenous cells and the distal and proximal retinula cells. We assume that growth of the single ocellar ommatidia takes place during a long postembryonic period and perhaps persists as long as the animals grow and moult. In this respect, the intercalary eye growth in *S. oraniensis* resembles the continuing growth of the single ocellar ommatidia that we observed in the diplopod *S. gigas*. For the lithobiomorph *Lithobius forficatus*, with a larger eye field than in *S. oraniensis*, there is tentative evidence that new ocellar ommatidia are also generated in consecutive rows and in addition show an intercalary growth during subsequent moults (Andersson 1976). Furthermore, the number of cells contributing to a lithobiomorph ommatidium is not fixed (discussed in Harzsch et al. 2005) and always above the scutigeromorph level (e.g., 36–400 retinula cells in *Lithobius*; Müller and Rosenberg 2006). We therefore propose that a persistent ontogenetic increase in cell numbers of the ocellar ommatidia is another character in the euarthropod ground pattern.

Eye development in notostigmophoran Chilopoda and Tetraconata

In Crustacea, the formation of new ommatidia has been examined by classical histology (Melzer et al. 2000;

Fig. 4. Bromodeoxyuridine(BrdU)-labelled eye structures in chilopods *Scolopendra oraniensis* (A)–(D) and *Scutigera coleoptrata* (E)–(H); (I) Adult specimen of *S. coleoptrata*. (A) Lateral eyes of adult, dorsal view. (B) Surface view with Normarski optics (montage from stack of 12 focal planes), showing three of the four ocellar ommatidia of which the eye consists. (C) Ocellar ommatidium (colour-coded 3D montage from stack of 15 focal planes; for viewing, use red–blue glasses); eye viewed from inside of head, proximal and part of distal rhabdom torn off; broad arrows mark spindle-shaped nuclei of developing corneagenous cells, narrow arrows point to mitotic nuclei located within layer of distal retinula cells that surrounds distal rhabdom. (D) Ocellar ommatidium next to the one in (C) (colour-coded 3D montage); arrows point to mitotic nuclei located within layer of distal retinula cells that surrounds distal rhabdom. (E) Lateral eye, surface view (colour-coded 3D montage from stack of 18 focal planes; for viewing use red–blue glasses); all ommatida of fairly similar size. (F) and (G) Growing eye (photo montages) entirely surrounded by zone of mitotic cells located laterally along circumference of eye. (H) Proliferation zone viewed from inside of eye (colour-coded 3D montage from stack of 10 focal planes; for viewing use red–blue glasses); proliferating cells seem clustered in pattern prefiguring outline of protommatida.
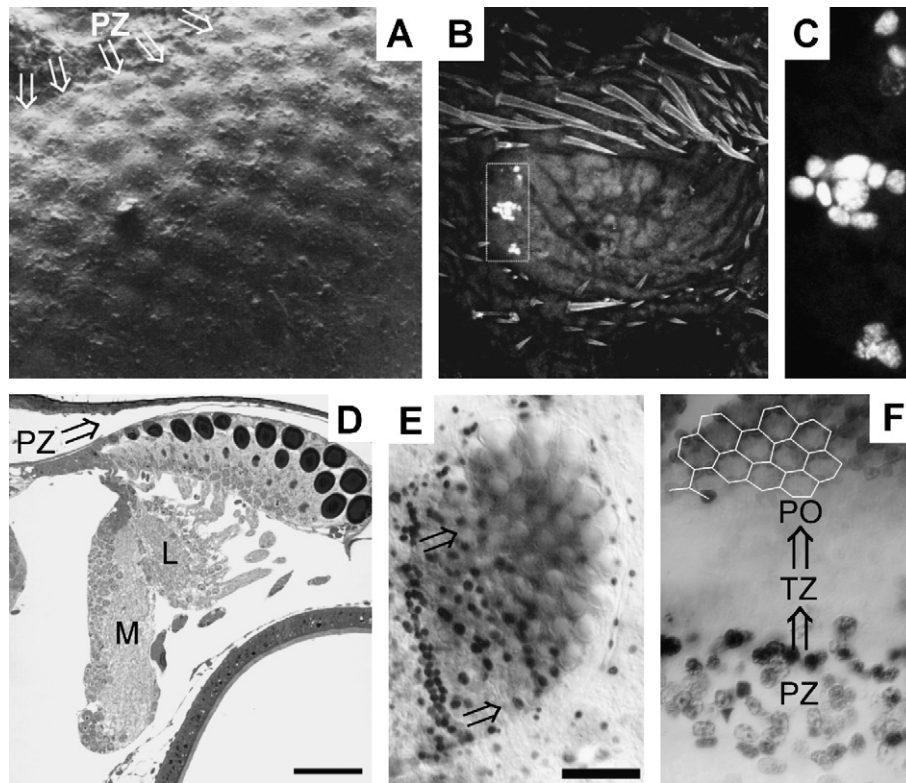
Fig. 5. (A) Lateral eye of juvenile trilobite, *Paladin eichwaldi shunnerensis* (modified from Clarkson and Zhang 1991); new elements are formed in proliferation zone (PZ) arranged along dorsal margin of eye, and new small lenses (arrows) added to growing eye. (B) and (C) Mitotic cells in developing eyes of a juvenile horseshoe crab, *Limulus polyphemus* (Chelicerata, Xiphosura), labelled with s-phase specific proliferation marker bromodeoxyuridin (BrdU; modified from Harzsch et al. (2006)); three clusters of labelled cells (boxed in B, enlarged in C) label site where new elements are formed which are then added to anterior side of growing eye. (D) Frontal section of visual system in advanced metanauplius of dinosaur shrimp, *Triops longicaudatus* (Crustacea, Branchiopoda); arrow indicates direction in which new tetraconate ommatidia are added to medial rim of developing eye; L = lamina ganglionaris, M = medulla externa; scale bar = 60 μm (modified from Harzsch and Walossek 2001). (E) Dorsal view of metanaupliar visual system in *Triops cancriformis* after 4 h incubation in proliferation marker BrdU and subsequent immunohistochemical detection; new cellular material is generated by proliferation zone (black labelled cells) and pushed towards rim of developing eye; scale bar = 30 μm (modified from Harzsch and Walossek 2001). (F) Mitotic cells in developing visual system of American lobster, *Homarus americanus* (Crustacea, Malacostraca), at 25% of embryonic development (4 h incubation in BrdU); new cellular material is generated in proliferation zone (PZ; black-labelled cells), in transition zone (TZ) it differentiates into protoommatidia (PO; labelled by network of white lines; colouration of these cells is from their own pigmentation) which are added to rim of developing eye (modified from Harzsch et al. 1999).

Hafner and Tokarski 1998, 2001) as well as by BrdU incorporation (Harzsch et al. 1999; Harzsch and Walossek 2001; Wildt and Harzsch 2002). In accordance with the principle described for the other euarthropod taxa, the cellular material that will form the new eye elements is generated in a proliferation zone along the rim of the developing eye (Fig. 5E). One difference is that this proliferation zone has the shape of a continuous band rather than that of a row of distinct mitotic clusters that mark the location of protommatidia, as shown for the Diplopoda. In Crustacea, the new protommatidia differentiate within the transition zone next to the proliferation zone (Fig. 5F) and are added to the side of the retina (Harzsch et al. 1999; Melzer et al. 2000; Hafner and Tokarski 1998, 2001; Harzsch

and Walossek 2001; Wildt and Harzsch 2002). The mechanisms of eye development in *Drosophila melanogaster* from an imaginal disc probably do not represent the plesiomorphic condition within the Hexapoda, due to the highly derived holometabolous developmental cycle of this species (discussed in Harzsch et al. 1999, Friedrich and Benzer 2000; Friedrich 2003). More information can be drawn from comparing eye formation in Crustacea and other Euarthropoda to holometabolous insects with external eye imaginal discs (Friedrich et al. 1996) and to hemimetabolous insects such as locusts and grasshoppers (Friedrich and Benzer 2000; Friedrich 2003) in which eye growth is strikingly similar to the crustacean visual system: new cells proliferate at the rim of the developing retina and aggregate

to form protommatidia which are added to the eye field (discussed in great detail by Hafner and Tokarski 1998, 2001; Harzsch et al. 1999; Harzsch and Walossek (2001; Friedrich and Benzer 2000; Friedrich 2003).

As the present BrdU experiments reveal, eye development in the scutigeromorph chilopod *S. coleoptrata*, in particular the location of the growth zone, closely resembles the pattern in Tetraconata. This is in good accordance with the structural similarities of scutigeromorph and tetraconate ommatidia, specifically the presence of a four cone cells (Müller et al. 2003). Hence, we can now draw a direct evolutionary line from the proliferation zone in Trilobita, Xiphosura and Diplopoda (generation of ocellar ommatidia) to the proliferation zone in Scutigeromorpha, Insecta and Crustacea (generation of tetraconate ommatidia). Whereas the eyes of Scutigeromorpha were previously considered as secondarily reconstructed (Paulus 2000; see also review by Bitsch and Bitsch 2005), we take this developmental evidence as supporting the view held by Müller et al. (2003), that their eyes are true compound eyes and closely related to the eyes of Insecta and Crustacea. The fact that eye growth in Tetraconata and Scutigeromorpha conforms to a common pattern contradicts the traditional hypothesis (reviewed by Bitsch and Bitsch 2005) that the eyes of Scutigeromorpha are derived by modification from insect eyes.

Evolution of eye design within the Mandibulata

The similarities in eye development and structure between Scutigeromorpha and Tetraconata have a distinct impact on our understanding of eye evolution in Mandibulata. The Scutigeromorpha are traditionally considered as the most basal chilopod taxon, and as the sister group to the Pleurostigmophora which comprise more derived groups such as the Lithobiomorpha and Scolopendromorpha. This assumed sister-group relationship has been confirmed by recent cladistic analyses combining morphological and molecular data (Edgecombe 2004; Edgecombe and Giribet 2004; Giribet and Edgecombe 2006). If we accept this phylogenetic concept, there are two main lines of explaining the evolution of eye design in the ancestral lineage of the Mandibulata:

(1) Our preferred hypothesis is that the tetraconate ommatidium of Scutigeromorpha, with a constancy of many but not yet all cell types, not only represents the ancestral type for the Chilopoda, but also closely resembles the ancestral eye in the ground pattern of Mandibulata. In the Diplopoda and the pleurostigmophoran Chilopoda the developmental program then switched back to the plesiomorphic mode of generating ocellar ommatidia, a phenomenon called cryptotypism or "switchback evolution" (Oakley

2003). Hence, the eyes of the Lithobiomorpha and Scolopendromorpha are strongly modified ommatidia of the scutigeromorph type. In this scenario, the full constancy of retinal cell numbers (as opposed to not yet fully fixed cell numbers) has to be added to the list of autapomorphies defining the Tetraconata (Crustacea + Hexapoda).

(2) Alternatively, the constant development of a fixed set of cells composing an ommatidium (two corneagenous cells, four crystalline cone cells and eight retinula cells) already is part of the ground pattern of Mandibulata. This character is thus retained in the ommatidia of crustaceans and insects as symplesiomorphies. In this scenario, the varying numbers of the interommatidial pigment cells, corneagenous cells and distal retinula cells in scutigeromorph ommatidia (Müller et al. 2003) would result from an intermediate cryptotypism. The intercalary growth of ocellar ommatidia in Diplopoda and pleurostigmophoran Chilopoda would be the outcome of convergent switchback evolution.

Conclusions

Our results show that all Euarthropoda share a similar basic mode of eye growth: a proliferation zone generates new elements, which are added to the side of the eye field. Trilobita and Xiphosura have retained the plesiomorphic developmental mode from the euarthropod ground pattern in that their proliferation zone generates new rows of ocellar ommatidia which keep on growing continuously (intercalary growth). Scutigeromorpha, Insecta and Crustacea derive from this pattern in that their proliferation zone generates tetraconate ommatidia composed of a fixed cell array with a restricted cell number. In our view, the most parsimonious way to explain eye structure and development in Diplopoda and Chilopoda other than Scutigeromorpha is that these organisms, starting from a tetraconate ommatidium of the *Scutigera* type, have returned to the plesiomorphic mode of eye formation as represented by Trilobita and Xiphosura. These data provide evidence that myriapod eyes may not be secondarily reconstructed insect eyes as the prevailing hypothesis suggests.

Our traditional understanding of euarthropod phylogeny, that is, a sister-group relationship of the monophyletic taxa Crustacea and Tracheata (Hexapoda and Myriapoda) (e.g., Klass and Kristensen 2001; Bitsch and Bitsch 2004) has been challenged in recent years by studies in the field of molecular phylogeny. In fact, most of these reports have not supported the monophyly of the Tracheata but instead have favoured a close relationship of Hexapoda and Crustacea (recent contributions, e.g., Shultz and Regier 2000; Friedrich and

Tautz 2001; Hwang et al. 2001; Pisani et al. 2004; Mallatt et al. 2004; Hassanin 2006; Mallatt and Giribet, 2006; see also Schram and Koenemann 2004). Contrary to the view of Bitsch and Bitsch (2005), our findings on myriapod eye development are in line with a number of recent contributions that explored the architecture of the central nervous system with regard to arthropod phylogeny ('neurophylogeny'; e.g., Strausfeld 1998; Harzsch 2003, 2004; Fahrbach 2004; Fanenbruck et al. 2004; Harzsch et al. 2005; Loesel 2005; Strausfeld 2005; Harzsch 2006) in that they are compatible with or rather strongly support a sister-group relationship of Crustacea and Hexapoda, or even a position of Hexapoda within Crustacea.

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Note added in proof

While this paper was in the press a new contribution was published that reviews comparative aspects of visual system formation in Euarthropoda: Harzsch, S., Hafner, G., 2006. Evolution of eye development in arthropods: phylogenetic implications. Arthropod Struct. Dev. 35, 319–340.

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