Genetic Regulation of Early Eye Development in Non-dipteran Insects

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Comparative analyses of eye development in *Drosophila* and distantly related phyla have fundamentally changed the way we think about the evolution of animal eyes today. On the one hand, it is clear that select eye-patterning mechanisms have deep evolutionary roots, such as the involvement of Pax6 and an ever-extending catalogue of additional transcription factors with selector gene-like functions in development (Donner and Maas 2004; Gehring 2002; Kozmik 2008; Pichaud and Desplan 2002). On the other hand, the diversity of distinct eye types in extant animals implies the evolution of lineage-specific patterning processes, superimposed onto the ancient gene interactions inherited from the prototype eye at the dawn of animal evolution (Lamb 2011; Nilsson 1996; Salvini-Plawen and Mayr 1977; Zuker 1994). Therefore, an important question to consider is how far back the regulatory program organizing the development of the compound eye in *Drosophila* can be traced to arthropod evolution.

Elaborate compound eyes are found in living representatives of all arthropod phyla, namely Crustacea, Chelicerates, and Myriapods, in addition to the insects (Buschbeck and Friedrich 2008; Fahrenbach 1969; Müller et al. 2003). The earliest fossils of advanced compound eye design have been discovered in deposits of the early Cambrian, which dates 515 million years before present (Lee et al. 2011;

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Paterson et al. 2011). This implies that the regulatory program patterning the *Drosophila* compound eye retina is hundreds of millions of years of age. Comparative analysis in arthropods, therefore, offers unique opportunities to dissect the conserved and evolutionary younger components in the genetic control networks which pattern the *Drosophila* eye. To this end, a number of gene-specific studies have been carried out in representatives of other arthropod phyla, such as crustaceans and the horseshoe crab *Limulus polyphemus*, the only extant chelicerate with compound eyes (Blackburn et al. 2008; Duman-Scheel et al. 2002; Smith et al. 1993). Also, the cellular organization of growth and differentiation of the visual system has been studied in non-insect arthropods (Hafner and Tokarski 1998, 2001; Harzsch and Walossek 2001; Melzer et al. 2000). However, the most comprehensive comparative molecular studies of compound eye development have focused on non-dipteran insect species up to this point.

Here, I introduce the satellite model organisms in current comparative genetic studies of insect compound eye development and their phylogenetic relationships. This is followed by a systematic review of the molecular findings that concern the patterning of the retinal precursor tissues in these organisms, which, at this point, are based on gene expression pattern analysis and lack-of-function analyses by RNA interference (RNAi)-mediated gene knockdown. The cellular assembly of retinal precursor cells in the differentiating retina is strongly conserved in arthropods and has been previously reviewed in depth (Buschbeck and Friedrich 2008; Friedrich et al. 2006). It will not be further explored here. I will conclude pointing out broader insights and the most important pending questions regarding the developmental evolution of the *Drosophila* compound eye, a story of profound sensory organ primordium reorganization.

The Phylogenetic Framework

Against the backdrop of insect diversity, the number of non-dipteran species that have been studied with comparative questions regarding the developing eye is dwindlingly small (Fig. 1). Besides studies looking at the morphogenesis of very unusual visual systems, such as stalk-eyed flies or the enigmatic Strepsiptera (Buschbeck 2005; Buschbeck et al. 2001), molecular work boils down to five species. Two of these belong to the same basal order of hemimetabolous insects. This refers to the bispotted cricket *Gryllus bimaculatus* and the American desert locust *Schistocerca americana*, both of which are members of the Orthoptera, although of distantly related subgroups. *G. bimaculatus* belongs to the suborder Ensifera while *S. americana* is part of the second orthopteran suborder, the Caelifera.

The insect order Orthoptera is one of the 22 currently recognized direct-developing insect orders. The latter refers to the direct development of most adult body structures in the embryo, which continue to gain size during the postembryonic growth stages of the nymphs. Except for wing and genital appendages, the nymph disposes over all essential body structures of the future adult form (Truman and Riddiford 2002). The ancestral lack of wings distinguishes ametabolous direct-developers from

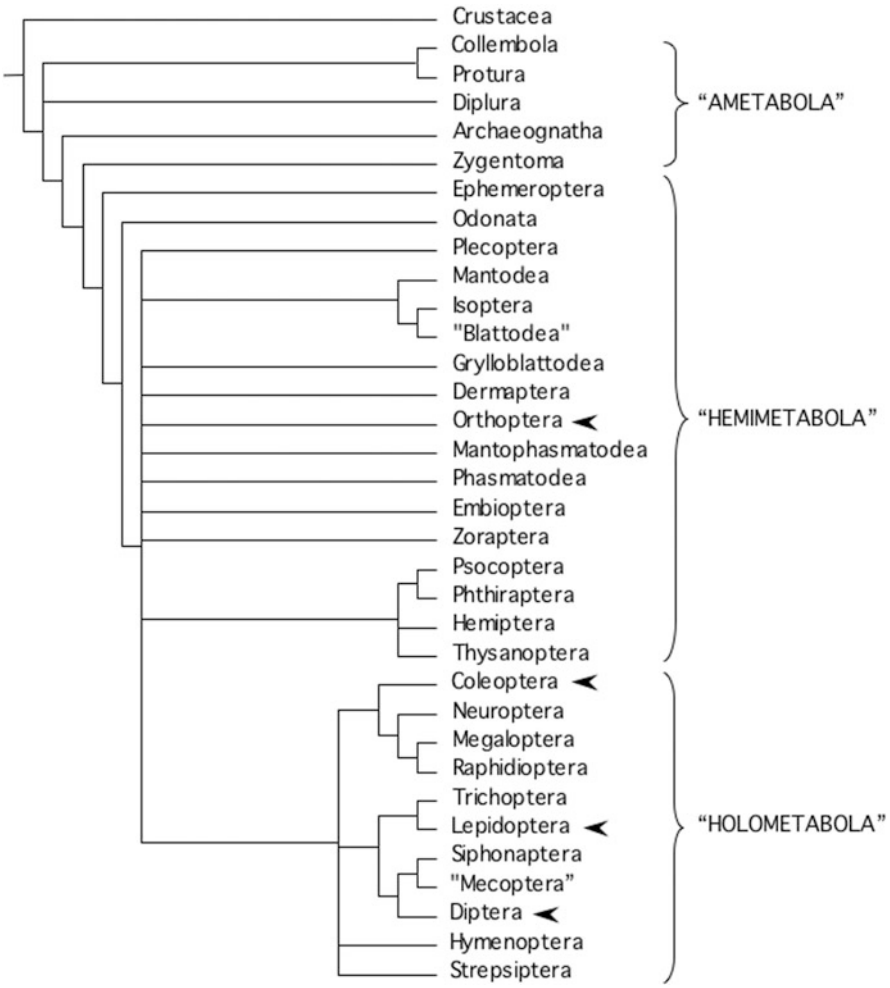

Fig. 1 Phylogenetic framework. *Arrowheads* indicate groups that include model system used in studies of insect eye development. *Quotation marks* indicate paraphyletic groups. Ametabolous insects are primitively wingless and undergo less postembryonic changes than hemi- and holometabolous forms. (Adapted from Friedrich et al. 2006)

hemimetabolous direct developers like orthopterans due to the final differentiation of the wings in the transition from the last nymphal growth instar to the adult. The Orthoptera are considered to have split at least 350 million years ago from the lineage that eventually gave rise to the ancestor of the large superclade of endopterygote or holometabolous insects, which transition through a larval growth stage and the pupal-resting stage before acquiring adult morphology (Beutel et al. 2011; Kristensen 1999; Figs. 1 and 2).

Besides *Drosophila*, holometabolous insects include three further significant models of insect eye development: the flour beetle *Tribolium castaneum*, the silk moth *Bombyx mori*, and the tobacco hornworm *Manduca sexta*. As a representative of the Coleoptera (beetles), *Tribolium* represents one of the oldest orders in the Holometabola, while the silk moth and tobacco hornworm, as representatives of the order Lepidoptera, are more closely related to the dipteran order (Beutel et al. 2011; Kristensen 1999; Wiegmann et al. 2009).

Comparing Drosophila Adult Eye Development with Direct-Developing Species: Continuous Versus Biphasic Visual System Development

The comparison of compound eye development between direct-developing species and the holometabolous *Drosophila* requires the pointing out of homology relationships between specific phases of eye development, which are not obvious at first glance (Fig. 2). In direct-developing species, a significant part of the adult compound eye differentiates already in the embryo. As a result, about 20 % of the posterior adult compound eye is of embryonic origin. The remaining anterior portion is added on during postembryonic development (Friedrich 2006). This mode of compound eye development is typical of direct-developing insects where larval and adult form shows relatively mild body plan differences.

Importantly, although the embryonic phase of eye development contributes to structures of the adult eye in direct-developing species, this developmental process is not homologous to the development of the adult eye in the *Drosophila* eye disc. The latter corresponds, instead, specifically to the postembryonic phase of compound eye development in direct-developing insects (Fig. 2), while the embryonic phase of compound eye development in direct-developing species is homologous to the embryonic development of the larval eyes of holometabolous insects such as the *Drosophila* Bolwig organs (see associated Chap. 12). These homology relationships follow from comparative morphogenetic and molecular evidence (Friedrich 2006, 2008) and, as will emerge later, have important consequences regarding the comparison of retinal primordium-patterning mechanisms.

The postembryonic phase of eye development in direct-developing insects is, thus, the closest evolutionary reference point for comparisons with the development of the *Drosophila* compound eye. Notwithstanding this, it remains a meaningful and evolutionarily significant question to ask whether and to which extent mechanisms regulating the commitment and differentiation of retinal precursor cells during the embryonic phase of eye development in direct-developing insects are recapitulated in the de novo development of the retinal primordium of *Drosophila* eye disc.

Direct-developing insects also differ from holometabolous insects with respect to the transition from embryonic to postembryonic visual development. In direct-developing insects, this transition proceeds with continued retinal differentiation.

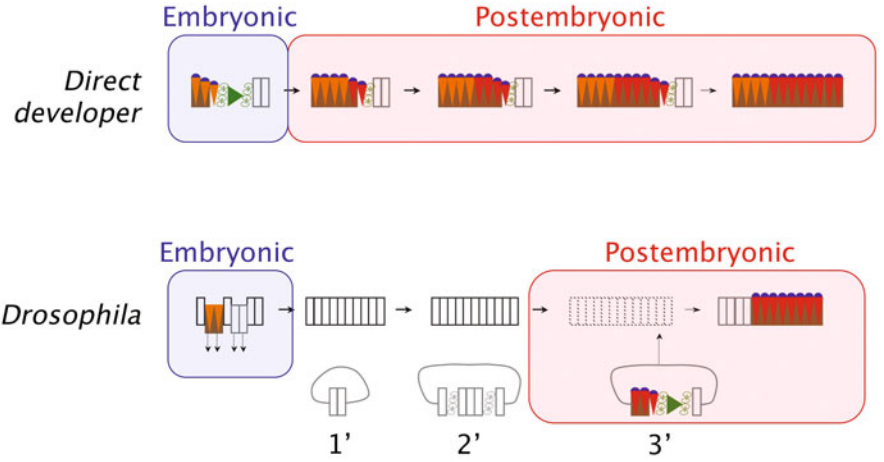

Fig. 2 Homology of embryonic and postembryonic visual system development between direct-developing species and *Drosophila*. Conceptual alignment of homologous phases of visual system development in the direct-developing species and the holometabolous *Drosophila*. In direct-developing species, ommatidia develop during both embryogenesis (*blue* backdrop shade) and postembryogenesis (*red* backdrop shade). Ommatidia of both embryonic (*orange* cell bodies) and postembryonic (*red* cell bodies) origin become part of the adult eye. In *Drosophila*, the development of the visual system is split in two discrete phases. The embryonic phase produces larval eyes, which are not integrated into the adult eye. The postembryonic phase begins with the initiation of retinal determination and differentiation in the eye–antennal imaginal disc of the third (3') larval instar. As a result, the adult *Drosophila* eye consists entirely of postembryonic ommatidia. The eye–antennal disc precursor disc separates from the larval epidermis during embryogenesis and experiences continued growth during the first (1') and second (2') larval instar. During metamorphosis, the eye–antennal imaginal disc derivatives completely replace the larval epidermis during pupation. Apoptosis of larval epidermis is indicated by *dotted outlines*. Color code of cellular components: *gray* = epithelial cells which persist from the embryo into adult, *black* = epithelial cells which are disposed during postembryogenesis, *dark blue* = cone cells, *brown* = pigment cells, *orange cones* = embryonic photoreceptor cells, *red cones* = postembryonic photoreceptor cells, *green* = mitotic cells. Progressing front of retinal differentiation is represented by forward pointing *green arrowhead*

In holometabolous insects, however, larval and adult eye development are temporally and spatially separate processes (Fig. 2). It has been hypothesized that the developmental evolution of this separation began with the transient arrest of retinal differentiation (Dong and Friedrich 2010). In support of this, a transient arrest of retinal differentiation can be enforced by the specific manipulation of eye developmental regulators in direct-developing insects like grasshopper (Dong and Friedrich 2010). Of note, the transient arrest model of biphasic eye development evolution is also consistent with the intermittent developmental arrest of other organs such as the leg appendages in the larval stage of holometabolous insects (Singh et al. 2007; Suzuki et al. 2009).

The American Desert Locust *Schistocerca americana*

The American desert locust and closely related grasshopper species, including the African desert locust *Schistocerca gregaria*, have a long history of serving as experimental models in developmental and neurobiological research due to the accessibility of neural elements in both the embryo and the adult form (Moreaux and Laurent 2007; Rogers et al. 2010; Sanchez et al. 1995). More recently, the grasshopper system has been adopted for the comparative developmental analysis of insect segmentation (Dearden and Akam 2000), appendage development (Mahfooz et al. 2004), and the development of the peripheral visual system (Dong and Friedrich 2005, 2010).

Organization of the Grasshopper Retina

Desert locusts are famous for their voracious food consumption, large body size, and coordinated long distance flights, translating into their economic importance as major pest species (Lomer et al. 2001). These features are supported by an enormous visual system. First instar grasshopper nymphs hatch with compound eyes of close to 2,500 ommatidia (Anderson 1978). This number increases to approximately 9,400 in the adult eye by the addition of new ommatidia at the anterior margin of the eye during the total of 5–6 nymphal intermolt stages (Dong and Friedrich 2010). Grasshopper ommatidia contain a conserved set of 8 photoreceptor cells, 4 cone cells, and 2 primary pigment cells, surrounded by 16 secondary pigment cells (Wilson et al. 1978). The photoreceptor cells exhibit three morphological subtypes. There are two photoreceptors with proximally restricted rhabdomeres, five photoreceptors with rhabdomeres extending along the entire proximodistal axis of the ommatidium, and a single photoreceptor with a distally restricted rhabdomere that corresponds to the *Drosophila* R7 cell (Wilson et al. 1978). Electrophysiological data suggest the presence of green-sensitive, blue-sensitive, and UV-sensitive photoreceptors (Bennet et al. 1967; Vishnevskaya et al. 1985). However, the spatial patterns of opsin gene expression have not yet been investigated, despite the isolation of green-sensitive and UV-sensitive opsin gene family paralogs (Towner et al. 1997). So, it is not yet known whether the grasshopper retina is subdivided into specialized subcompartments. There is, however, a detailed analysis of the retinal organization of the distinct dorsal rim area (DRA) at the dorsal margin of the eye that is populated with anatomically specialized photoreceptor cells (Homberg and Paech 2002). The DRA is a polarized light-sensitive compartment of the insect eye, which is found with varying outlines including the DRA in *Drosophila* (Labhart and Meyer 1999).

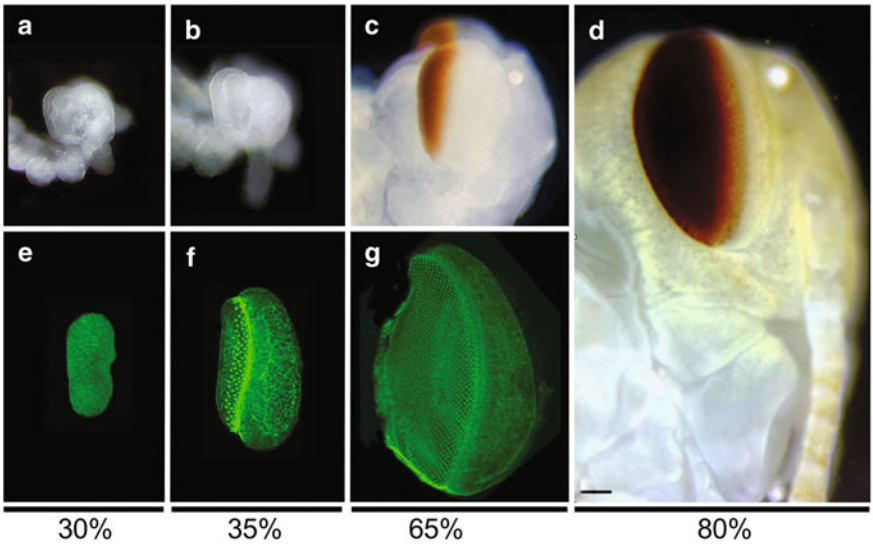

Fig. 3 Embryonic eye development in the grasshopper *S. americana*. **a**–**d** Lateral stereomicroscopy view of embryonic head at 30 % (**a**), 35 % (**b**), 65 % (**c**), and 80 % (**d**) of embryonic development. **e**–**g** Laser-scanning confocal images of differentiating embryonic retina labeled with phalloidin, which highlights cell morphogenesis by binding to f-actin, at respective stages of development. A morphogenetic furrow-like differentiation front can be seen starting from 35 % of development (**f**)

Embryonic Phase of Grasshopper Eye Development

The embryonic development of grasshopper species like *S. gregaria* takes about 20 days, which means that development advances by approximately 5 % per day (Bentley et al. 1979). At about 20 % embryogenesis, the grasshopper embryo has formed a distinct head region with two prominent lateral extensions, i.e., the head lobes. The posterior region of the head lobes will then transform to produce a secondary set of lobe-like compartments that are exclusively occupied by precursor tissue of the visual system. These compartments are the eye lobes (Fig. 3a; Dong et al. 2003; Roonwal 1936). The outermost epithelial layer of the eye lobes represents the precursor tissue, i.e., primordium of the retina. In addition, the optic lobes house the developing outer and inner optic neuropiles: lamina, medulla, and lobula (Dong et al. 2003).

Retinal differentiation initiates between 30 and 35 % of development, leading to the formation of a morphogenetic furrow-like front of differentiation, which travels across the eye lobe ectoderm from posterior to anterior (Fig. 3b, f). Of note, the nonhomology of embryonic eye development in direct-developing insects and the *Drosophila* eye–antennal imaginal disc implies that the similarity of the *Drosophila* morphogenetic furrow and the differentiation front in the grasshopper embryonic eye lobe ectoderm reflects generic cell morphological consequences of neurogenesis in cellular epithelia.

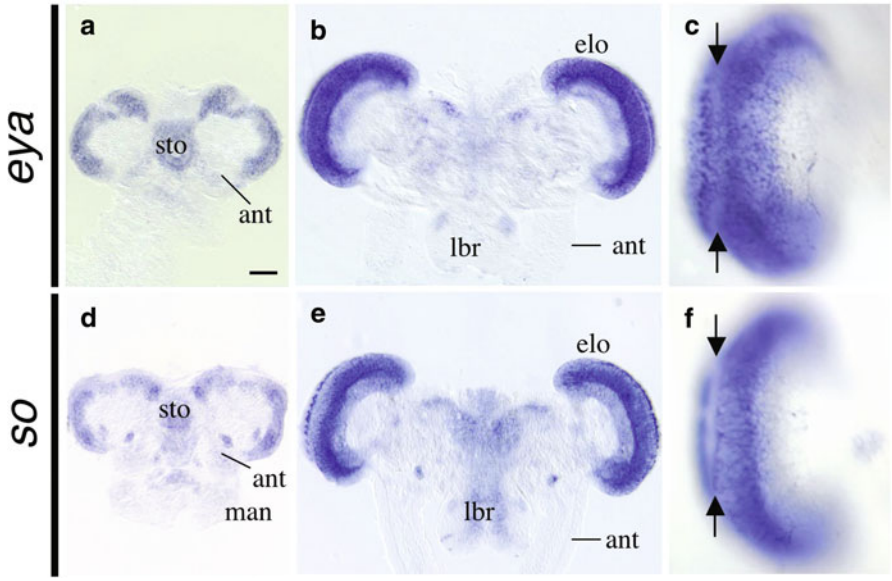

Fig. 4 Expression of *eya* and *so* in the grasshopper eye lobes. **a**, **b**, **d**, **e** Frontal view of grasshopper embryonic head. Dorsal *up*. **c**, **f** Optical section of eye lobe from a lateral perspective at the level of the peripheral ectoderm. Specimens labeled by whole mount in situ hybridization for transcript detection of *eya* (**a**–**c**) and *so* (**d**–**f**). *Black arrows* indicate retinal front of differentiation. Dorsal *up* and anterior to the *right*. *ant* antenna, *elo* eye lobe, *lbr* labrum, *man* mandible, *sto* stomodeum

Coexpression of so *and* eya *in the Grasshopper Embryonic Eye Lobes*

The transcription factor genes *eyes absent* (*eya*) and *sine oculis* (*so*) represent the earliest markers of the visual anlage in the *Drosophila* embryo, a neuroectermal field in the median head that contains the precursor cells of the entire visual system (Chang et al. 2001). Consistent with a conserved function of *eya* and *so* in the specification of the embryonic visual anlagen, the grasshopper orthologs of *so* and *eya* are coexpressed in the periphery of the head lobes and, thus, soon after grastrulation (Dong and Friedrich 2005; Fig. 4a, d). As the optic lobes emerge, *eya* and *so* continue to be strongly coexpressed in the retina, lamina, and medulla tissue layers (Fig. 4b, c, e, f).

After the initiation of retinal differentiation, *eya* and *so* are detected throughout the differentiating retina and the morphogenetic furrow as well as extending into a wide area of the undifferentiated neuroectoderm ahead of the morphogenetic furrow (Fig. 4d, f). The *eya* and *so* expressing field ahead of the furrow is limited to a range defined by its distance to the morphogenetic furrow. This observation, and the gradient-like decrease of the *eya* and *so* expression levels toward the anterior margin of their coexpression domain, have been taken as circumstantial evidence

that the expression of *eya* and *so* may be primarily transcriptionally activated by signals emanating from the morphogenetic furrow in a manner comparable to the induction of the preproneural (PPN) field in the *Drosophila* eye disc (Bessa et al. 2002; Dong and Friedrich 2005; Greenwood and Struhl 1999).

In *Drosophila*, the PPN field is activated through the long-distance signaling impact by the Transforming Growth Factor β homolog *decapentaplegic* (*dpp*; Heberlein et al. 1993), which is associated with the strong and specific expression of *dpp* in the morphogenetic furrow. In the grasshopper, however, *dpp* is not expressed in the morphogenetic furrow (Friedrich and Benzer 2000). Instead, a low transcript level of *dpp* is detected throughout the anterior eye lobe ectoderm ahead of the morphogenetic furrow (Fig. 8). While *dpp* may function in this domain as a growth activating factor, this pattern rules out a similar furrow movement organizing function as in the *Drosophila* eye–antennal disc. That leaves the signaling factor *hedgehog* (*hh*) as a candidate inducer of the PPN expression domain in the grasshopper based on the *Drosophila* paradigm (Heberlein et al. 1993; Ma et al. 1993). The expression of *hh* in the grasshopper eye lobe remains to be explored, but this scenario is supported by the reported expression of *hh* in crickets (see further text; Niwa et al. 2000).

Expression and Function of wg

The investigation of the complex expression patterns of the signaling factor *wingless* (*wg*) in the grasshopper has produced evidence that *wg* functions as an antagonist of *eya* and *so* transcription at the anterior poles of the embryonic eye lobes, very similar to the situation in the anterior eye–antennal disc of *Drosophila* (Dong and Friedrich 2005; Pichaud and Casares 2000). In the embryonic eye lobe, *wg* is expressed in two prominent polar domains (Friedrich and Benzer 2000; Liu et al. 2006). In these areas, *eya* as well as *so* expression seems to be nonoverlapping with *wg* (Fig. 5).

The suggested repressive effect of *wg* in retinal specification and differentiation was tested by LiCl incubation experiments with cultured embryonic eye discs (Dong and Friedrich 2005). Through its inhibition of glycogen synthase kinase 3β, LiCl application is known to stimulate Wg signaling (Stambolic et al. 1996). In cultured eye lobes, the addition of LiCl caused a stalling of retinal differentiation. This was associated with a strong increase of cell division anterior to the morphogenetic furrow and strong increase of cell death, specifically posterior to the morphogenetic furrow (Dong and Friedrich 2005). These findings are consistent with the role of *wg* as a growth activator in the anterior *Drosophila* eye disc and its impact on differentiation in the posterior *Drosophila* eye disc (Baonza and Freeman 2002; Lee and Treisman 2001; Treisman and Rubin 1995), suggesting deeply conserved functions of *wg* in the control of retinal patterning.

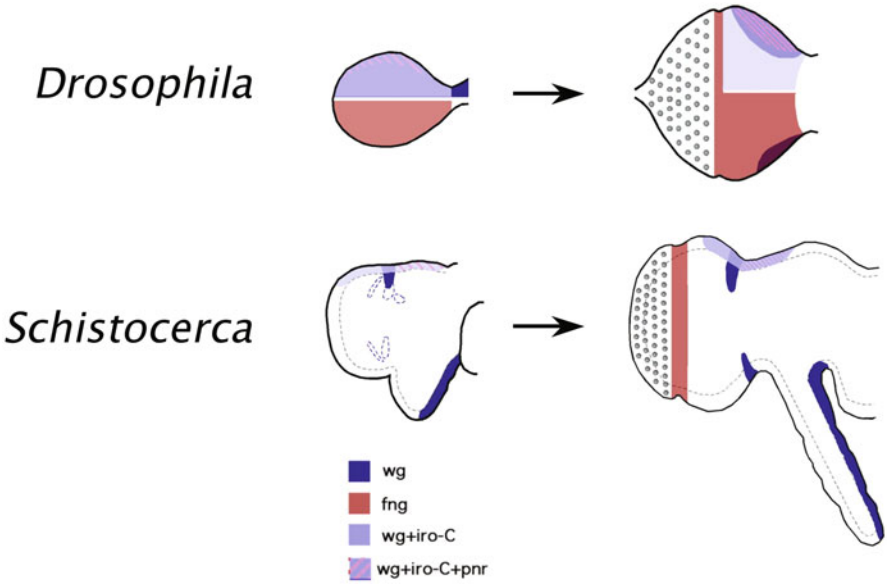

Fig. 5 Dorsoventral patterning gene expression in *Drosophila* and grasshopper. Schematic comparison of the expression domains of *wg* and *fng* as well as areas with overlapping expression of *wg* with *Iro-C* or *wg* with *Iro-C* and *pnr*. *Left column* shows the *Drosophila* eye disc and the grasshopper head hemisphere at an early developmental stage that precedes the onset of retinal differentiation (2nd larval instar eye–antennal imaginal disc in *Drosophila* and 30 % stage of *Schistocerca*). The *right column* compares the late 3rd larval instar eye imaginal disc of *Drosophila* with the *left* grasshopper head hemisphere at about 45 % stage of *Schistocerca* embryo. Dorsal *up* and anterior to the *right*. (Adapted from Dong and Friedrich 2005)

Dorsoventral Patterning

In *Drosophila*, the activation of focal Notch (N) signaling along the midline of the early eye disc is essential for stimulating the rapid expansion of the eye primordium by cell proliferation (Cho and Choi 1998; Dominguez and de Celis 1998; Dominguez et al. 2004; Kenyon et al. 2003; Papayannopoulos et al. 1998). In addition, the differential expression of N-signaling components in, precisely, the dorsal or ventral half of the eye disc anticipates the compartmentalization of the adult eye into dorsoventral compartments (Reifegerste and Moses 1999). Together with *wg*, the analysis of the expression of the grasshopper homologs of the N-signaling modifier glycosyltransferase *fringe* (*fng*), and the transcription factor genes *Delta* (*Dl*), *pannier* (*pnr*), and *Iroquois-C* (*Iro-C*) provided insights into the dorsoventral patterning organization of the grasshopper eye (Dong and Friedrich 2005).

Similar to the *Drosophila* situation (Cavodeassi et al. 1999, 2000; Maurel-Zaffran and Treisman 2000), *pnr* and *Iro-C* are expressed in dorsal cell populations of the

embryonic head. However, in contrast to *Drosophila*, the expression of *pnr* remains outside the eye lobes, representing an extension of the dorsal margin cells. Further, the expression of *Iro-C* extended only 10 % into the dorsal of the anterior embryonic eye lobe, consistent with a role in patterning the grasshopper DRA ommatidia but incompatible with a role in subdividing the retina field into a dorsal and ventral half. In combination, the data indicate conserved genetic mechanisms in DRA specification but divergence with regards to the dorsoventral patterning in the retina of grasshopper and *Drosophila* (Fig. 5). Also, in further support of the latter notion as well as the lack of a N-induced growth-promoting organizer in the embryonic grasshopper eye, the expression of *Dl* and *fng* shows no evidence of dorsoventral compartmentalization ahead of the morphogenetic furrow or prior to its initiation (Fig. 5; Dong and Friedrich 2005). Instead, the expression of these genes is associated with the initiation and progression of the morphogenetic furrow itself indicating roles in regulating the progress of neural differentiation.

Postembryonic Phase of Grasshopper Eye Development

During the transition from embryonic to postembryonic development, the retinal precursor cell population of the anterior eye lobe neuroectoderm transforms into a growth zone margin, outlining the anterior edge of the nymphal eye in direct-developing insects like *S. americana* (Figs. 2 and 6a, b; Dong et al. 2003; Friedrich 2006). The cellular organization of the growth zone, which is heavily enriched with mitotic cells, has been described in early histological and experimental papers (Anderson 1978; Bodenstein 1953). Today, it is interesting to note its organizational similarity to the ciliary margin region of the fish or amphibian eye (Perron et al. 1998; Raymond et al. 2006). Posterior to the proliferation zone, the transition into the fully differentiated retina is filled with intermediate stages of ommatidial development defining the differentiation zone (Fig. 6b; Anderson 1978; Dong and Friedrich 2010).

Unfortunately, the molecular organization of the grasshopper eye proliferation zone is still little investigated. Yet, RNAi-mediated gene knockdown experiments targeting *eya* and *so* produced first insights into the function of eye selector genes during postembryonic eye development in the grasshopper (Dong and Friedrich 2010). For both genes, a transient arrest of postembryonic retina differentiation was observed in nymphs which completed development into adult form, generating adult eyes with a pronounced vertical scar area (Fig. 6). These findings were interpreted as suggesting that the downregulation of *so* and *eya* does not irreversibly affect the organization of the mitotic activity in the growth zone (Dong and Friedrich 2010). Thus, *eya* and *so* have been proposed to act in a similar manner in the postembryonic grasshopper eye, as in the PPN zone of the *Drosophila* eye disc, by making cells responsive and competent to undergo retinal differentiation.

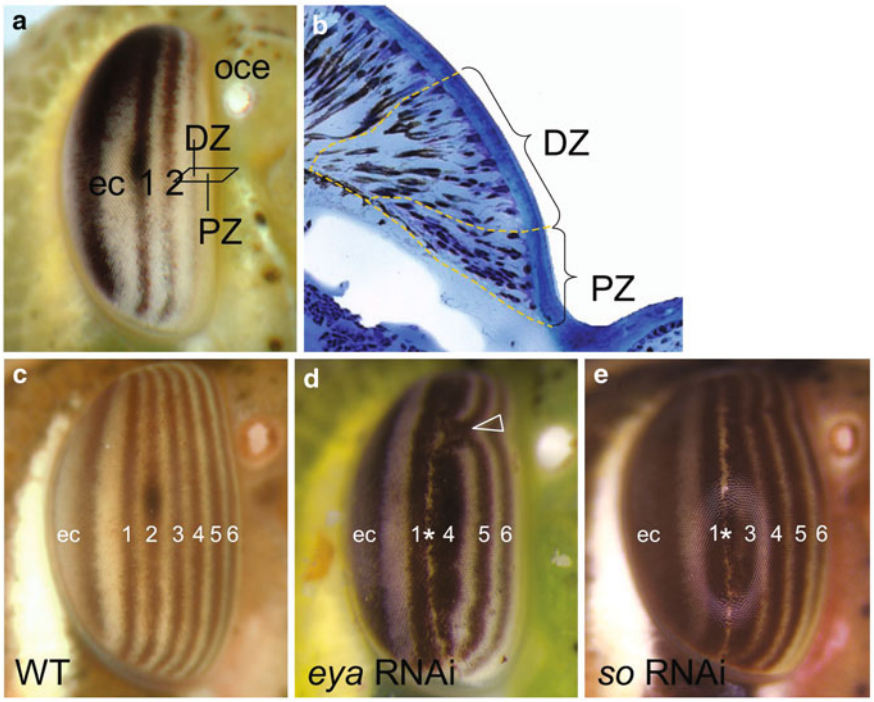

Fig. 6 Effect of *eya* and *so* knockdown on the postembryonic development of the grasshopper compound eye. **a** Frontolateral view of fourth instar grasshopper nymphal eye. Relative position of differentiation zone (*DZ*) and proliferation zone (*PZ*) are indicated and related to section plane of panel **b**. The posterior *dark* pigmented region of the eye that is generated in the embryo is labeled as the embryonic cap (*ec*). Numbers label pigment stripe areas formed during postembryonic retina differentiation in the first two nymphal instars. **b** Toluidine blue stained sagittal semithin section through the anterior compound eye of a first instar grasshopper nymph. Cells in the DZ elongate and accumulate pigment. Cells in the PZ are densely packed and indifferentiated. **c**–**e** Lateral view of the adult compound eye. **c** Untreated wild type animal. **d** Strongly affected *eya* knockdown animal. *Asterisk* in panel **d** indicates position of scar between stripes *1* and *4*. *Arrowhead* in **d** points at disrupted anterior stripe pattern. **e** Phenotypic *so* knockdown animal. *Asterisk* indicates position of scar between stripes *1* and *3*. In all panels anterior is to the *left* and dorsal *up*. Numbers identify specific lateral pigment stripes. *ec* embryonic cap, *gen* gena, *oce* ocellus. (Adapted from Dong and Friedrich 2010)

The Bispotted Cricket *Gryllus bimaculatus*

Driven by a major effort in developing tools for molecular analysis, including whole mount in situ hybridization, RNAi-mediated gene knockdown, and germline transformation, the cricket *G. bimaculatus* has evolved into a versatile and efficient model system for comparative development (Fig. 7; Mito and Noji 2008). With regards to vision-mediated behaviors, it is noteworthy that crickets are generally crepuscular and less prominent in the aerial insect fauna. Despite the fact that crickets do not

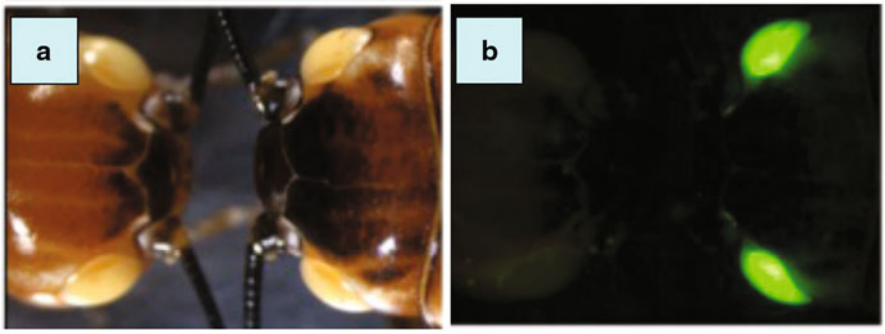

Fig. 7 Eye morphology of the cricket *Gryllus bimaculatus*. **a** Stereomicroscope view of dorsal head of white-eyed wild type (*left*) and transgenic (*right*) animal. **b** Epifluorescence image of the same, note strong EGFP expression in the compound eye of the transgenic animal. (Kindly provided by Dr. Sumihare Noji)

exhibit flight behavior under laboratory conditions unless artificially stimulated, female crickets are known for their extensive prereproductive flight dispersal, mostly at evening hours (Lorenz 2007).

Organization of the Cricket Retina

The eyes of adult *G. bimaculatus* consist of approximately 4,600 ommatidia (Labhart and Keller 1992). Like in the grasshopper, the *G. bimaculatus* eye includes a structurally and functionally distinct DRA, which is populated by blue-opsin and UV-opsin expressing photoreceptors (Blum and Labhart 2000; Henze et al. 2012). The recent analysis of opsin gene expression patterns in the cricket uncovered further compartmentalization in the retina (Henze et al. 2012). Accordingly, the *G. bimaculatus* main retina encompasses a blue-opsin and green-opsin expressing ventral area while the remainder of the retina expresses UV-opsin and green-opsin. The photoreceptor-specificity, as well as the ecological significance of these differential opsin expression patterns, awaits future study.

Patterning Gene Expression and Function During the Embryonic Phase of Cricket Eye Development

The early developing cricket visual system is organized in the same way as the eye lobe compartments in grasshoppers (Inoue et al. 2004). Likewise, in correspondence to the organization in the grasshopper, retinal differentiation is initiated in the posterior margin of the eye lobe ectoderm and a morphogenetic furrow-like front of

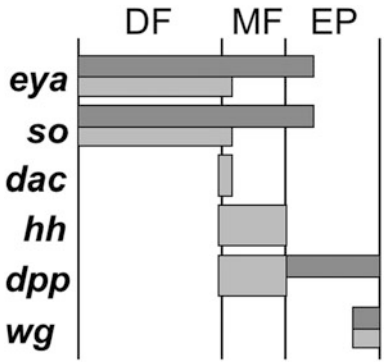

Fig. 8 Summary of eye developmental expression patterns in orthopteran species. *Gray* expression domain in cricket, *black* expression domain in grasshopper. *DF* differentiating retina, *EP* eye primordium, *MF* morphogenetic furrow

differentiation travels the cricket eye lobe neuroectoderm in posterior to anterior direction (Inoue et al. 2004; Takagi et al. 2012).

The available expression data on the cricket homologs of *wg*, *hh*, and *dpp* suggest that *wg* is expressed in the anterior margins of the eye lobe, while *hh* and *dpp* are expressed in different dorsoventral domains across the eye (Fig. 8; Niwa et al. 2000). *hh*, in particular, appears to be strongly expressed in the differentiating retina (Niwa et al. 2000). These data are prima facie consistent with conserved roles of *dpp* and *hh* in promoting eye development, and the grasshopper supported conserved role of *wg* as tissue growth-stimulating antagonist of retinal differentiation (Friedrich 2006; Liu et al. 2006).

At the transcription factor gene level, the expression of *so* and *eya* as well as *dachshund* (*dac*) has been studied in detail (Fig. 8; Inoue et al. 2004; Takagi et al. 2012). The expression of *dac* is detected in the eye lobe neuroectoderm prior to morphogenetic furrow initiation (Inoue et al. 2004). In the differentiating eye, *dac* transcript levels are concentrated in the morphogenetic furrow yet below detection level both anterior and posterior to the morphogenetic furrow (Inoue et al. 2004).

The *so* and *eya* orthologs of the cricket are strongly expressed in the nondifferentiated area of the eye lobes prior to the initiation of eye differentiation (Takagi et al. 2012). Thereafter, *so* and *eya* expression extends from the morphogenetic furrow uniformly across the differentiating retina in the posterior head lobe, much the same as in grasshopper. However, the expression of *so* and *eya* seems more confined anterior to the morphogenetic furrow raising the possibility of differences in the transcriptional organization of retinal induction between the two species (Fig. 8). Consistent with the predicted important function of *eya* in specification and differentiation of the eye during embryonic development, parental RNAi-mediated knockdown resulted in strong eye depletion phenotypes, including complete loss (Takagi et al. 2012).

Expression and Function of eya *and* so *During the Postembryonic Phase of Cricket Eye Development*

The role of *eya* and *so* has also been studied in the nymphal eye of *G. bimaculatus* (Takagi et al. 2012). This analysis revealed the presence of defined anterior proliferation and differentiation zones as in the nymphal eye of grasshopper. In situ hybridization analysis of the expression of *eya* revealed the differential accumulation of transcripts in the proliferation zone and posterior to it, in both differentiating and differentiated pigment cells (Takagi et al. 2012). The RNAi-mediated knockdown of *eya* or *so* by dsRNA injection into third instar nymphs resulted in highly informative phenotypes. In the strongest *eya* knockdown animals, the proliferation zone appeared completely missing in contrast to the preservation of the growth zone in the corresponding *eya* knockdown experiments with grasshopper. Moreover, the posterior retina region of the cricket, which had differentiated prior to injection, reorganized into a nonsensory head cuticle (Takagi et al. 2012).

While these data are consistent with the expected role of *eya* in specification and differentiation of the postembryonic cricket eye, the mechanism explaining its role in the maintenance of the differentiated state will require further investigation. In contrast to grasshopper, the data suggest that *eya* and *so* are not only essential for the differentiation of the nymphal retina but also for the maintenance of the proliferation zone. Before mechanistic conclusions can be drawn with confidence, it will be important to address whether these differences reflect differences in gene knockdown efficiencies, stage of the injected nymphs, or lineage-specific differences in regulatory mechanisms.

Comparing Drosophila Adult Eye Development with Other Holometabolous Species: Early Versus Late Eye Discs

The physical separation of the products of embryonic and postembryonic eye development in holometabolous species dominates the comparison of *Drosophila* to direct-developing species (Fig. 2). The comparison of eye development within holometabolous species attracts interest because of the dramatic differences in the morphogenetic organization of postembryonic eye primordium formation (Fig. 9). In the most ancestrally organized Holometabola, the retina differentiates in the lateral head epidermis of the adult-like head capsule of the eucephalic larva. Pending the size of the prospective adult eye, this can be associated with the formation of an eye disc during metamorphosis, i.e., the last larval instar and the pupa. This contrasts with the early formation of the *Drosophila* eye–antennal imaginal disc during embryogenesis.

Correlated with this, there is a second fundamental morphogenetic difference between the ancestral late eye disc formation and the early eye disc development in *Drosophila*. In the first case, the eye disc is the growth-accommodating intermediate

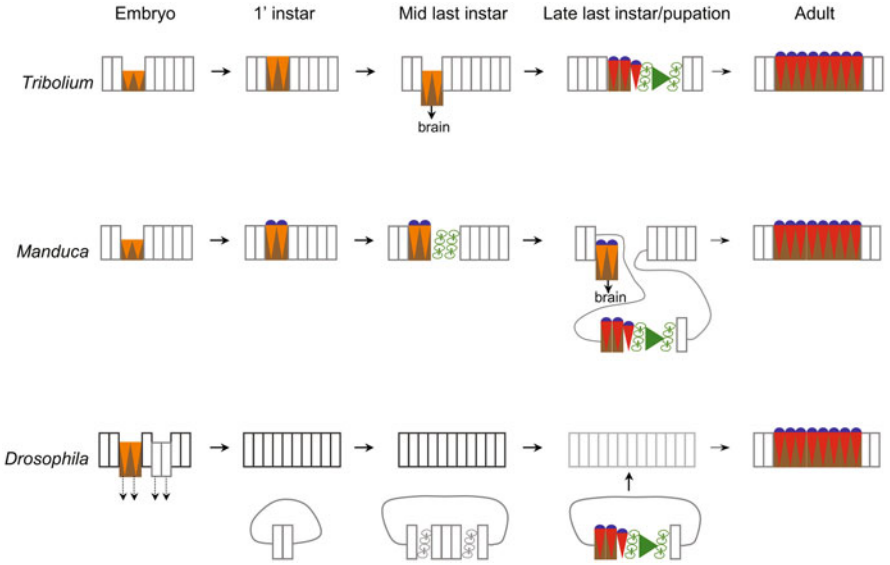

Fig. 9 Early and late eye disc formation in holometabolous insects. Cell body color-coding as in Fig. 2. Note the differentiation of photoreceptors with cone cells in *M. sexta*. In *Tribolium*, the adult retina differentiates in the lateral head epidermis without eye disc formation. In *Manduca*, a later eye disc is formed in the last larval instar and the pupa. The *Drosophila* eye–antennal imaginal disc is an example of early imaginal disc formation in the embryo

structure of single organ. In the second case, the eye–antennal imaginal disc functions as the precursor structures of many head cuticle structures and sensory organs (see also Fig. 15). This has the effect that organ-specific primordium have to be patterned via postembryonic regional specification in addition to their coordinated growth (for review, see Dominguez and Casares 2005). This compaction of head patterning processes into a single composite imaginal disc represents a derived state that emerged during the evolution of the acephalic morphology of the maggot-type larva (Melzer and Paulus 1989). The latter characterizes not only *Drosophila* and closely related flies but also one of the larger groups of the Diptera: the Cyclorrhapha. The early eye disc of *Drosophila* and other cyclorrhaphan flies, thus, represents an evolutionary novelty at the level of developmental precursor tissue organization.

The Red Flour Beetle *Tribolium castaneum*

The publication of the genome sequence in 2008 cemented the pivotal position of *Tribolium* in comparative evolutionary developmental biology (Klingler 2004; Richards et al. 2008). The recent surge in *Tribolium* research benefited profoundly from earlier genetic and population genetic studies exploring the biology of this major economic pest (Sokoloff 1972). The taxonomic significance of *Tribolium*

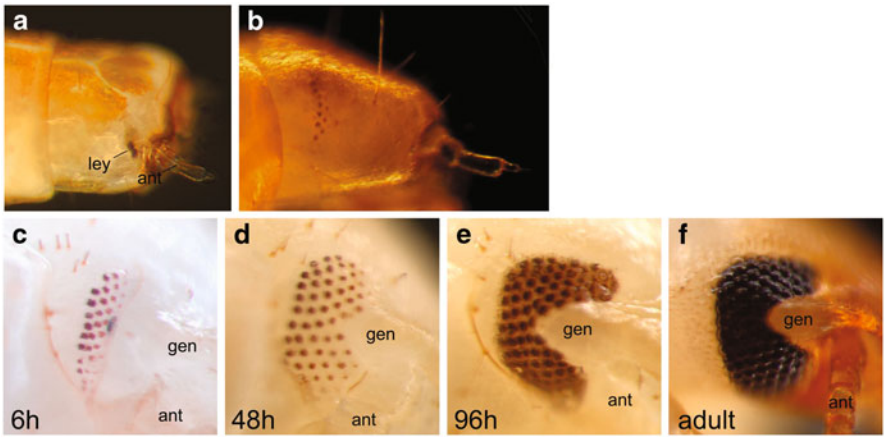

Fig. 10 Adult eye development in *Tribolium*. **a** Lateral view of last instar larval head before entering the resting stage. Note position of larval eyes (*ley*) posterior to the antenna (*ant*) and the gena (*gen*). **b** Lateral view of resting stage larva. The larval eyes have relocated from their antenna-associated position toward the brain (not shown). The first two rows of photoreceptors, visible by virtue of their pigment accumulation, have become visible in the posterior half of the lateral head capsule. **c**–**f** Lateral view of pupal (**c**–**e**) and freshly hatched adult (**f**) *Tribolium* head. (Adapted from Liu and Friedrich 2004; Yang et al. 2009b)

arises from representing the largest order of insects (Coleoptera) and the intermediate phylogenetic position between *Drosophila* and hemimetabolous insects (Fig. 1; Kristensen 1999; Savard et al. 2006; Wiegmann et al. 2009). These aspects and the short germband type of embryonic development have attracted considerable interest by comparative developmental biologists, leading to the development of refined and effective protocols for in situ hybridization, RNAi-mediated gene knockdown, transgenesis (Brown et al. 2009), and most recently, ectopic gene expression (Schinko et al. 2012). *Tribolium* has been used to gain insights into early embryonic patterning (Schroder 2003), segmentation (Maderspacher et al. 1998), appendage (Prpic et al. 2001), and head development (Posnien et al. 2010), including the visual system (Liu and Friedrich 2004).

Organization of the Tribolium *Compound Eye*

A first notable difference of the *Tribolium* eye to *Drosophila* is its smaller size: an average of 95 ommatidia in the *Tribolium* eye compared to the 800 ommatidia in the *Drosophila* eye (Fig. 10f; Friedrich et al. 1996). This size difference can be attributed to the crepuscular biology of *Tribolium*, which tends to spend much of its life span burrowed in nutritional substrate (Park 1934). However, recent studies document a previously underestimated frequency of flight-facilitated adult dispersal (Perez-Mendoza et al. 2011; Ridley et al. 2011). A second eye-catching difference

between the *Tribolium* and *Drosophila* eye is the midline notch at the anterior margin of the *Tribolium* eye, accommodating a posteriorly extended gena (Fig. 10e, f).

At the cellular level, the fused rhabdom formed by the *Tribolium* photoreceptor cells contrasts with the open rhabdom in *Drosophila* (Friedrich et al. 1996). Only two compound eye vision-related opsin genes are conserved in the *Tribolium* genome (Richards et al. 2008). This includes a green-sensitive opsin, which is expressed in all retinal photoreceptor cells, and a UV-sensitive opsin, which is specifically conserved in the *Tribolium* R7 photoreceptors (Jackowska et al. 2007). In combination, the *Tribolium* retina thus differs from *Drosophila* by the constitutive coexpression of opsin paralogs in all ommatidia. The functional consequences and gene regulatory mechanisms associated with this unique retinal opsin mosaic have not yet been investigated in detail.

Morphogenesis of the Tribolium *Compound Eye*

Like *Drosophila*, *Tribolium* develops a separate pair of lateral larval eyes in the embryo that are structurally very distinct from the adult compound eye. The larval eyes are situated close to the larval antenna from where they withdraw into the brain during metamorphosis (Fig. 10a, b; see Chap. 12 for further details; Liu and Friedrich 2004). The relative small size of the adult *Tribolium* eye allows for the differentiation of the retina in the lateral head epithelium without the detachment of the latter from the head cuticle (Figs. 9 and 10). Due to the early accumulation of retinal pigment granules in differentiating photoreceptor cells, the morphogenesis of the *Tribolium* compound eye can be conveniently followed by external observation (Fig. 9; Friedrich et al. 1996; Liu and Friedrich 2004). The first row of photoreceptors are recognizable at the end of the last larval instar (Fig. 10b), in preparation of pupation. At this point, the larvae enter a similar premetamorphic resting stage that is equivalent to the wandering stage of the *Drosophila* larva. In the case of *Tribolium*, however, the larvae simply remain motionless without food uptake (Parthasarathy et al. 2008).

In the freshly hatched pupa, the number of photoreceptor columns extends in the anterior direction along the longitudinal body axis over the first 48 h after pupa formation (Fig. 10c, d; Liu and Friedrich 2004; Yang et al. 2009b). In the midline area, the progression of photoreceptor differentiation stalls earlier than in the dorsal and ventral halves (Fig. 10d, e). Investigations of cellular morphogenesis revealed that this process is associated with the split of the contiguous morphogenetic furrow in the midline region (Friedrich and Benzer 2000). About 96 h after pupa formation, the retinal field becomes homogeneously filled with dark color following the specification and differentiation of the pigment cells (Yang et al. 2009b).

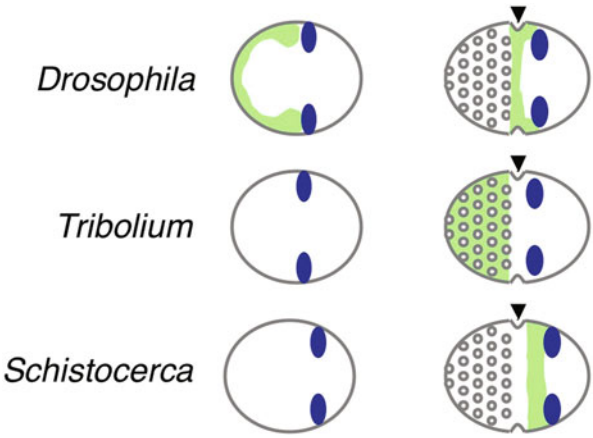

Fig. 11 Comparison of *wg* and *dpp* expression domains in *Drosophila*, *Tribolium*, and *Schistocerca*. *Left column* represents the eye field before the onset of retinal differentiation. The *right column* represents the eye field after the onset of retinal differentiation. *Arrowheads* point at the front of retina differentiation. Posterior to the right. Color code of gene expression domains: *green* = dpp, *blue* = wg. (Modified from Friedrich and Benzer 2000)

Signaling Factor Expression Patterns in the Developing **Tribolium** *Adult Eye*

The first molecular study of *Tribolium* eye development explored the expression patterns of *wg* and *dpp* (Fig. 11; Friedrich and Benzer 2000). Similar to the situation in grasshopper and *Drosophila*, *wg* is expressed in separate dorsal and ventral domains, consistent with evolutionary conservation of the repressive effect of Wg signaling on retinal differentiation in *Drosophila* and the grasshopper (Dong and Friedrich 2005).

The dorsoventral *wg* domains transform into a circumferential domain along the entire retinal field margin at about 36 h after pupal formation, thereby resembling the late expression of *wg* around the *Drosophila* eye (Friedrich and Benzer 2000). These data suggest that *wg* is also involved in eye margin patterning of the *Tribolium* eye, although this has not yet been functionally tested.

The expression of *dpp* in *Tribolium* is different from both grasshopper and *Drosophila* (Friedrich and Benzer 2000). At the onset of retinal differentiation, *dpp* is weakly expressed in the presumptive eye primordium (Fig. 11). After the initiation of retinal differentiation, *dpp* was detected through the entire differentiating retina in a pattern, which suggested the repression of *dpp* specifically in the differentiating photoreceptor cells.

Eye Selector Gene Expression in the Developing **Tribolium** *Adult Eye*

Following the candidate gene approach, the expression and function of *eya*, *so*, *dac*, and the Pax6 transcription factor genes *eyeless* (*ey*) and *twin of eyeless* (*toy*) have been studied in detail with respect to their role in *Tribolium* eye development (Figs. 12 and 13; Yang et al. 2009a, b). All of these genes are expressed in the

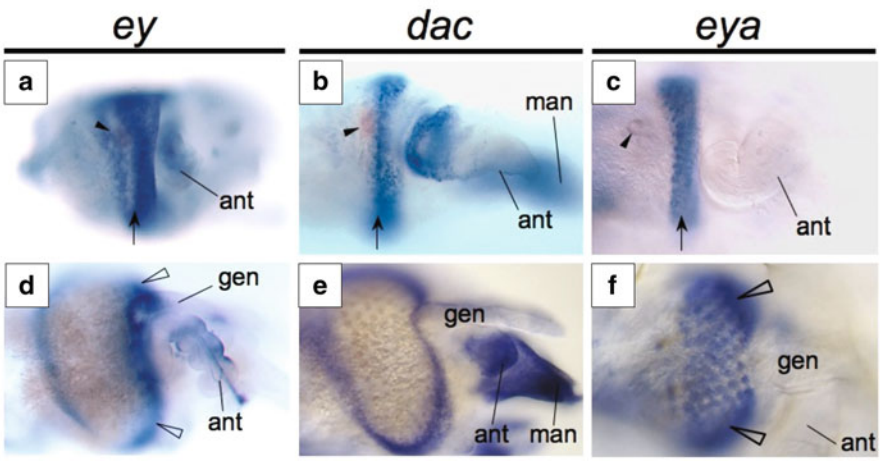

Fig. 12 Developmental transcription factor gene expression in the developing *Tribolium* compound eye. **a**–**c** Lateral view of dissected last instar larval head. **d**–**f** Lateral view of pupal head at approximately 48 h after pupal formation. Dorsal *up* and anterior to the *right*. *ant* antenna, *gen* gena, *man* mandible

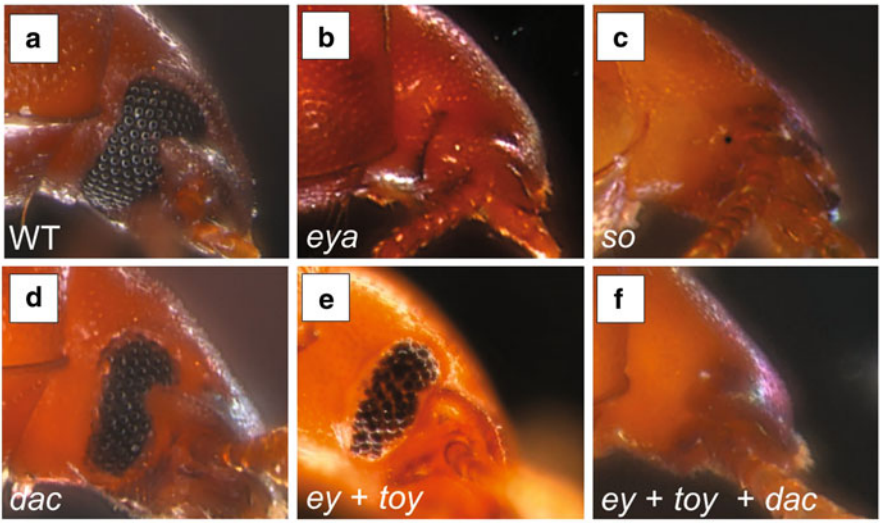

Fig. 13 Eye selector gene expression and function in *Tribolium* compound eye development. **a**–**f** Lateral view of adult head of wild type (**a**) and strongly phenotypic knockdown animals (**b**–**f**). See text for details. Dorsal *up* and anterior to the *right* (Adapted from Yang et al. 2009a, b)

undifferentiated eye primordium prior to retinal differentiation and subsequent to the initiation of differentiation ahead of the morphogenetic furrow, suggesting their coexpression in the early eye primordium (Fig. 12a–c). The extent of these expression domains, however, differs. The most restricted expression domain was detected for *eyg* (ZarinKamar et al. 2011). *eya* and *so* appear to be more specifically expressed in the retinal precursor tissue of the lateral head (Fig. 12c). *ey*, *toy*, and *dac*, by contrast, are characterized by wider expression domains, exceeding that of *so* and *eya*, suggesting broader roles in the patterning of the lateral head (Fig. 12a, b; Yang et al. 2009a).

Informative expression pattern differences were also observed in the differentiating retina. While *eya* and *so* continue to be expressed in the developing photoreceptor cells, *ey*, *toy*, and *dac* are downregulated as cells pass through the morphogenetic furrow. These expression dynamics are largely consistent with the expression and function of *eya* and *so* as early retina determination genes versus *toy* and *ey* as upstream specification genes in the *Drosophila* eye–antennal disc (Kumar 2009). Most noteworthy, perhaps, is the higher coordination of *dac* expression with *ey* and *toy* in *Tribolium* (Fig. 12d, e), considering the downstream position of *dac* in the *Drosophila* retina determination gene network.

These three genes are also coexpressed in a domain surrounding the late differentiating *Tribolium* retina, suggesting roles in eye margin patterning (Fig. 12d, e; Yang et al. 2009a, b).

Knockdown Analysis of Tribolium *Eye Development*

Lack-of-function analyses by RNAi have been very informative regarding the roles of *eya*, *so*, *ey*, *toy*, and *dac* in *Tribolium*. The strongest impact of larval RNAi-mediated gene knockdown was observed in the case of *eya* and *so*, which ranged from partial to complete depletion of the compound eye (Fig. 13b, c; Yang et al. 2009b). The analysis of *ey* and *toy*, however, revealed a first major difference of *Tribolium* from *Drosophila*. Knockdown of *ey* or *toy* individually or in combination leads to only a subtle, although significant, decrease in eye size as measured by number of ommatidia (Fig. 13e; Yang et al. 2009a). This result contrasts strongly with the sensitivity of adult head and eye development to the reduction of these genes in *Drosophila* (Kronhamn et al. 2002). However, the combinatorial knockdown of *ey* and *toy* in the developing embryonic head results in a high penetrance larval eye deletion phenotype (Yang et al. 2009a), suggesting similarly important functions of *ey* and *toy* in the developing visual system of *Tribolium* as in *Drosophila*.

In the adult eye, the knockdown of *dac* also yielded only partial reduction of the eye, although more dramatic in comparison to the average of 10 % eye reduction in *ey* and *toy* knockdown animals (Yang et al. 2009a). Most important, the combinatorial knockdown of *ey* and *toy* with *dac* leads to complete eye depletion phenotypes (Fig. 13f; Yang et al. 2009a). The model inferred from these data poses that the Pax6 genes *ey* and *toy* play roles in visual system specification during embryogenesis and

remain essential for eye primordium maintenance throughout the postembryonic phase of development in functional redundance with *dac* (Yang et al. 2009a).

An Unexpected Role of eyg *in the* Tribolium *Eye*

The second major deviation in gene function between *Tribolium* and *Drosophila* concerns the role of the Pax gene *eyegone* (*eyg*) (ZarinKamar et al. 2011). Reducing *eyg* levels in the *Drosophila* eye–antennal disc has strong eye depletion effects (Dominguez et al. 2004; Jun et al. 1998). In *Tribolium*, the knockdown of *eyg* leads to the opposite: a 5 % increase in eye size (ZarinKamar et al. 2011). Analysis of the morphogenetic origin of the *eyg* phenotype in *Tribolium* revealed that the morphogenetic furrow is not suppressed in the midline when approaching the introducing gena tissue. In this case, retinal differentiation in the median head appears to gain dominance over the developmental program involved in gena formation. The result is the differentiation of six surplus ommatidia on an average, in the median anterior *Tribolium* eye (ZarinKamar et al. 2011).

Given that *eyg* is not expressed in the gena, it is currently assumed that *eyg* functions as a competence factor that renders the anterior eye field sensitive to retina suppressing factors released by the developing gena (ZarinKamar et al. 2011). Such eye-antagonistic role of *eyg* is striking given the contrast to its facilitating role in the *Drosophila* eye, which leads to the idea that *eyg* may represent a functional homolog of the primordium growth-activating Pax6(a) isoform (Moses and Rodrigues 2004). A parallel investigation into the evolutionary origin of *eyg*, however, showed that *eyg* represents a deeply conserved Pax gene subfamily of its own (Friedrich and Caravas 2011).

The Tobacco Hornworm *Manduca sexta*

Compared to *Tribolium*, the tobacco hornworm *M.sexta* has thus far played a lesser role in the comparative analysis of visual system development. Early work described basic aspects of the differentiation of the retina, which align well with the events in the wake of the morphogenetic furrow in *Drosophila* and other species (Champlin and Truman 1998; Egelhaaf 1988; Friedrich et al. 1996). Even more significant is the body of work, which elucidated the mechanisms that regulate the postembryonic activation of the adult eye primordium (Champlin and Truman 1998; Truman et al. 2006), thereby coordinating eye disc development with other metamorphic events. In vivo and in vitro experiments revealed that the early initiation of the adult eye primordium occurs because nutritional signals mediated through the insulin signal pathway begin to overrule the differentiation-suppressing effect of juvenile hormone (Koyama et al. 2008; Truman et al. 2006).

As mentioned earlier (Fig. 9), *Manduca* is a significant point of comparison in insect eye development because of the late formation of an eye-specific imaginal disc

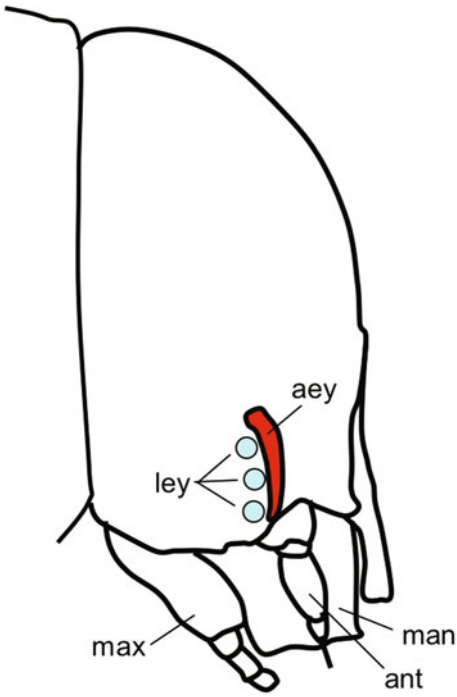

Fig. 14 Spatial organization of adult eye primordium initiation in relation to the larval eyes in *Manduca*. Drawing of lateral view on *Manduca* final instar larval head based on Allee et al. (2006). The adult eye primordium is initiated as a wedge of proliferating tissue anterior to the three ommatidia-like larval eyes (*turquoise*). Dorsal is *up* and anterior to the *right*. *aey* adult eye primordium, *ant* antenna, *ley* larval eye, *man* mandible, *max* maxilla

(Allee et al. 2006; Friedrich 2006; Truman and Riddiford 2002). It is reasonable to assume that the late-forming disc type of *Manduca* resembles an ancestral precursor stage toward the evolution of the *Drosophila* eye–antennal imaginal disc.

Early Development of the Manduca *Compound Eye Primordium*

The adult eye primordium of *Manduca* becomes detectable in the late final instar larva. Morphologically, it has been described as a half moon crest-shaped rim of compacted, proliferating tissue that begins to delaminate from the larval head capsule cuticle, thus forming the eye disc (Fig. 14; Allee et al. 2006; MacWhinnie et al. 2005; Monsma and Booker 1996). This position of the emerging eye disc is notable because it is consistent with the transient arrest model of the larval eyes in holometabolous insects. The latter predicts that the larval eye primordium is initiated as a continuation of larval eye development in the anterior direction (Fig. 2).

Unfortunately, no data are as yet available regarding the expression of head and eye determination genes during eye disc activation in *Manduca*. However, the expression and function of specific isoforms of the zinc finger transcription factor *broad* (*br*), which is a molecular signature of primordium commitment to the pupal state in holometabolous insects, have been studied in detail (Konopova and Jindra 2008; Parthasarathy et al. 2008; Suzuki et al. 2008; Uhlirova et al. 2003). The expression of *br* is specifically activated in the early *Manduca* eye primordium (Allee et al.

2006). Functional data regarding the role of *br* are not yet available in *Manduca*. However, *br* knockdown in *B. mori* and in *Tribolium* leads to an attenuation of eye development, demonstrating the importance of *br* for eye primordium commitment (Parthasarathy et al. 2008; Uhlirova et al. 2003).

Of note, in direct-developing insects *br* is expressed throughout the nymphal stages (Erezyilmaz et al. 2006), lending further molecular support to the homology of postembryonic eye development in the pupae of holometabolous species and the nymph of direct developers (Fig. 2; Erezyilmaz et al. 2006; Suzuki et al. 2008).

Eye Specification Across Insect Species: Summary and Perspectives

From both phylogenetic and developmental perspectives, the diversity of adult eye morphogenesis is enormous in insects, posing challenges to the experienced comparative biologist and the weathered *Drosophila* geneticist alike. Fortunately, some of the available molecular data allow for identifying shared ancestral themes in the early molecular development of the compound eye in both direct-developing and indirect-developing species. Arguably, the clearest example of this is the involvement of *eya* and *so* as facilitators of retinal precursor tissue determination and subsequent retinal differentiation (Figs. 4 and 12). A similar point may be made regarding *dac*, *ey*, and *toy*. These genes share broad expression patterns that include the retinal precursor tissue and are downregulated in the differentiating retina, pointing at a conserved role in implementing competence for retinal determination (Fig. 12). Taken together, these data are consistent with the roles experimentally ascribed to *eya*, *so*, *dac*, *ey*, and *toy* in *Drosophila* (Kumar 2009), which in this regard serves as a confirmed general model. The conserved expression of *eya* and *so* is further suggestive of a broad conservation of the PPN state of retinal commitment, at least at the transcription factor landscape level (Bessa et al. 2002; Dong and Friedrich 2005; Greenwood and Struhl 1999).

At the signaling gene level, the repressive effect of *wg* in the anterior developing eye field is a highly conserved aspect of compound eye patterning. It is reflected in the conservation of the polar domains in the anterior eye precursor field of all insect species so far examined (Fig. 11) and has even been reported for crustacean species (Duman-Scheel et al. 2002). Although the spatial expression patterns of *dpp* are quite diversified in the developing eyes of different species (Fig. 11), the eye development-promoting role of *dpp* can likewise be presumed to be conserved but awaits functional test. The same applies to the retinal differentiation-promoting role of *hh*.

Breakdown of Genetic Redundancy of ey *and* toy *During Dipteran Evolution*

Some of the *dac*-, *ey*-, and *toy*-related data in *Tribolium* suggest substantial rewiring of the regulatory interactions among these conserved players in eye development. The prime example is the redundant interaction of *ey* and *toy* during adult eye development in *Tribolium*, in conjunction with *dac* (Yang et al. 2009a). These relationships contrast with the upstream roles of *ey* and *toy* in the *Drosophila* retinal gene network (Gehring 2002). The *Tribolium* findings are not surprising given that functional redundancy is one of the proximate and ultimate causes for the conservation of duplicated genes (Force et al. 1999). The fact that the level of redundancy is lower in the developing *Drosophila* system may be tied to the more dramatic reorganization of genetic interactions during the evolution of the eye–antennal disc-patterning mechanisms. This may have led to a stronger degree of functional differentiation between *ey* and *toy* due to novel subfunctionalization opportunities. Along these lines, Lynch and Wagner (2011) have initiated a debate regarding the ancestral regulatory status of *ey* in comparison to *toy* in *Drosophila*.

At this point, the lack of data on how *ey* and *toy* act in direct-developing species like the grasshopper and cricket represents one of the most glaring gaps in the comparative study of insect eye development. There is little doubt that these highly awaited data will yield further important insights regarding the developmental organization of the early embryonic head as well as the gene regulatory organization of cells in the postembryonic growth zone of the eye.

Divergence of Eye Primordium Growth Activation

The comparative analysis of *eyg* in eye development also points toward profound differences between *Drosophila* and more ancestrally organized insects. At the surface, the opposite effects of downregulating *eyg* in *Drosophila* and *Tribolium* could be considered to reflect changes in the architecture of the eye specification gene network. However, there are arguments to conclude that these differences are more likely to reflect fundamental differences specifically in primordium growth activation. In *Drosophila*, *eyg* is part of the N-signaling-induced growth-promoting genet network that is pivotal for triggering the rapid tissue growth in the developing eye disc (for review, see Dominguez and Casares 2005). The discrepancy of *eyg* function in *Tribolium* and *Drosophila* may thus be explained by the smaller size of the eye in *Tribolium*, requiring less tissue proliferation. A second possibility is that the N-signaling-mediated organizer originated more recently in conjunction with the evolution of the *Drosophila* eye disc during dipteran evolution (Melzer and Paulus 1989). Consistent with this, an evolutionarily derived status of the N-initiated growth activation mechanism would explain the noncompartmentalized expression patterns of *fng* and *Dl* in the grasshopper (Dong and Friedrich 2005). A new data point in

support of this model has come from the silk moth. Similar to *Manduca*, this lepidopteran develops its 3,000-ommatidia large compound eye from a late-forming eye disc (Yu et al. 2012). The silk moth mutant *flügellos* has been found to represent a null allele of Bombyx *fng* (Sato et al. 2008). Importantly, while *fng* mutant animals are characterized by wing defects, the development of the compound eye is not affected in dramatic ways. This suggests that the dramatic growth of the lepidopteran eye does not depend on *fng* as in *Drosophila*. In conclusion, these data demonstrate that the N- and *eyg*-involving activation of growth in the *Drosophila* eye disc is not a conserved component of eye disc development in holometabolous insects. This compelling evidence notwithstanding, additional genes will need to be examined in the lepidopteran models before definitive conclusions can be drawn regarding the derived state of N-initiated growth activation module in the *Drosophila* eye disc.

Embryonic Versus Postembryonic Adult Eye Primordium Determination

Another fundamental question waiting to be addressed concerns the specification of the adult retina primordium in ancestrally organized holometabolous species like *Tribolium* and *Manduca*. To get a taste of the foundational nature of this issue, one has to remember that the late postembryonic specification of the adult eye primordium in *Drosophila*, based on molecular genetic analysis, came as a surprise to the *Drosophila* field (Baker 2001; Kumar and Moses 2001). The preceding consensus was that this step takes place in the embryo, during the subdivision of the embryonic visual anlage into its major constituents (Postlethwait and Schneiderman 1971; Wieschaus and Gehring 1976). Assuming that the late specification of the eye primordium is the consequence of the evolution of the highly derived integrated eye–antennal imaginal disc of *Drosophila* (Fig. 13), it is reasonable to hypothesize that the specification of the adult eye primordium in the lateral larval head capsule takes place during embryogenesis in species with late eye discs like *Manduca* or no disc formation like *Tribolium* (Fig. 9). Otherwise, one has to postulate a postembryonic patterning mechanism, which drives the specification and activation of the adult eye primordium in the static head epithelium of the last instar larva.

Also the comparative framework of the transient arrest model of holometabolous visual system development predicts that both larval eye and adult eye precursor cell populations are committed in the embryonic visual anlage (Fig. 2). In the embryo, differentiation is initiated in the larval eye precursor but suppressed in the adult eye precursor cells. The latter, embedded in the lateral head epidermis, are maintained as a quiescent primordium until activation at the beginning of metamorphosis. This scenario is consistent with the positioning of the adult eye primordium in front of the larval eye in *Manduca* (Allee et al. 2006).

Of note, this anteroposterior alignment of larval and adult eye primordium seems not conserved in *Tribolium*. This may be due to the more extreme modification of the

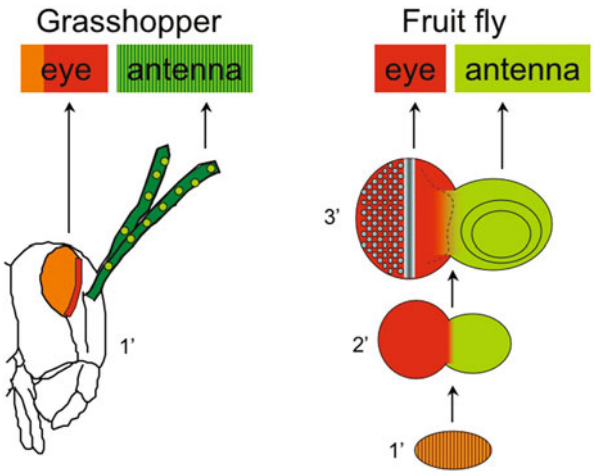

Fig. 15 Somatic stem cell reservoirs versus imaginal discs in insect eye development. In direct-developing insects like the grasshopper, the adult antenna and compound eye derive from organ-specific stem cell reservoirs (eye: *red*; antenna: *light green*) and differentiated cells of the nymph (eye: *orange*; antenna: *dark green*), which have been generated during embryogenesis. This mode of organ precursor tissue organization contrasts with the development of adult antenna and compound eye from the joint eye–antennal imaginal disc of *Drosophila*, which undergoes dramatic morphogenetic change through all three larval instars (1'–3')

Tribolium larval eyes in terms of accessory cell reduction and anatomical positioning in the larval head (Liu and Friedrich 2004). In *Manduca*, the larval eyes still form ommatidia-like subunits with lenses and pigment cells (Fig. 9; Allee et al. 2006).

Important work remains to be done to probe the previously discussed model by elucidating whether and how the precursor cells of the adult eye are set aside in more ancestrally organized systems like *Tribolium* and *Manduca* (Fig. 9). While interesting in its own right, answers to these questions will yield insights of broader significance. For one, they will add to our understanding of the molecular developmental evolution of holometabolous development, which after all was co-responsible for the unparalleled radiation of holometabolous insects (Kristensen 1999). Furthermore, the comparative evidence implies that the *Drosophila* eye–antennal imaginal disc is a derivative of the retinal growth zone in direct-developing insects, which most likely represents a tissue-specific stem cell population (Dong and Friedrich 2010; Fig. 15). If confirmed, the evolutionary transformation of the retinal growth zone in directly developing species to the *Drosophila* eye–antennal imaginal disc would be an example of how evolution reprogrammed stem cell populations to invent novel ways of body plan development.

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References

Allee JP, Pelletier CL, Fergusson EK, Champlin DT (2006) Early events in adult eye development of the moth, *Manduca sexta*. J Insect Physiol 52:450–460

Anderson H (1978) Postembryonic development of the visual system of the locust, *Schistocerca gregaria*. I. Pattern of growth and developmental interactions in the retina and optic lobe. J Embryol Exp Morphol 45:55–83

Baker NE (2001) Master regulatory genes; telling them what to do. Bioessays 23:763–766

Baonza A, Freeman M (2002) Control of *Drosophila* eye specification by Wingless signalling. Development 129:5313–5322

Bennet RR, Tunstall J, Horridge GA (1967) Spectral sensitivity of single retinula cells in the locust. Z Vgl Physiol 55:195–206

Bentley D, Keshishian H, Shankland M, Toroian-Raymond A (1979) Quantitative staging of embryonic development of the grasshopper, *Schistocerca nitens*. J Embryol Exp Morphol 54:47–74

Bessa J, Gebelein B, Pichaud F, Casares F, Mann RS (2002) Combinatorial control of *Drosophila* eye development by *eyeless, homothorax,* and *teashirt*. Genes Dev 16:2415–2427

Beutel RG, Friedrich F, Hörnschemeyer T, Pohl H, Hünefeld F, Beckmann F, Meier R, Misof B, Whiting MF, Vilhelmsen L (2011) Morphological and molecular evidence converge upon a robust phylogeny of the megadiverse Holometabola. Cladistics 27:341–355

Blackburn DC, Conley KW, Plachetzki DC, Kempler K, Battelle BA, Brown NL (2008) Isolation and expression of *Pax6* and *atonal* homologues in the American horseshoe crab, *Limulus polyphemus*. Dev Dyn 237:2209–2219

Blum M, Labhart T (2000) Photoreceptor visual fields, ommatidial array, and receptor axon projections in the polarisation-sensitive dorsal rim area of the cricket compound eye. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 186:119–128

Bodenstein D (1953) Postembryonic development. In: Roeder KD (ed) Insect Physiology. Wiley, New York, pp 275–367

Brown SJ, Shippy TD, Miller S, Bolognesi R, Beeman RW, Lorenzen MD, Bucher G, Wimmer EA, Klingler M (2009) The red flour beetle, *Tribolium castaneum* (Coleoptera): a model for studies of development and pest biology. Cold Spring Harb Protoc pdb.emo126

Buschbeck E, Friedrich M (2008) Evolution of insect eyes: tales of ancient heritage, deconstruction, reconstruction, remodeling and recycling. Evolution Education and Outreach 1:448–462

Buschbeck EK (2005) The compound lens eye of Strepsiptera: morphological development of larvae and pupae. Arthropod Struct Dev 34:315–326

Buschbeck EK, Roosevelt JL, Hoy RR (2001) Eye stalks or no eye stalks: a structural comparison of pupal development in the stalk-eyed fly *Cyrtodiopsis* and in *Drosophila*. J Comp Neurol 433:486–498

Cavodeassi F, Diez Del Corral R, Campuzano S, Dominguez M (1999) Compartments and organising boundaries in the *Drosophila* eye: the role of the homeodomain Iroquois proteins. Development 126:4933–4942

Cavodeassi F, Modolell J, Campuzano S (2000) The Iroquois homeobox genes function as dorsal selectors in the *Drosophila* head. Development 127:1921–1929

Champlin DT, Truman JW (1998) Ecdysteroids govern two phases of eye development during metamorphosis of the moth, *Manduca sexta*. Development 125:2009–2018

Chang T, Mazotta J, Dumstrei K, Dumitrescu A, Hartenstein V (2001) Dpp and Hh signaling in the *Drosophila* embryonic eye field. Development 128:4691–4704

Cho KO, Choi KW (1998) Fringe is essential for mirror symmetry and morphogenesis in the *Drosophila* eye. Nature 396:272–276

Dearden P, Akam M (2000) A role for *Fringe* in segment morphogenesis but not segment formation in the grasshopper, *Schistocerca gregaria*. Development Genes Evolution 210:329–336

Dominguez M, Casares F (2005) Organ specification-growth control connection: new in-sights from the *Drosophila* eye-antennal disc. Dev Dyn 232:673–684

Dominguez M, de Celis JF (1998) A dorsal/ventral boundary established by Notch controls growth and polarity in the *Drosophila* eye. Nature 396:276–278
Dominguez M, Ferres-Marco D, Gutierrez-Avino FJ, Speicher SA, Beneyto M (2004) Growth and specification of the eye are controlled independently by Eyegone and Eyeless in *Drosophila melanogaster*. Nat Genet 36:31–39
Dong Y, Friedrich M (2005) Comparative analysis of Wg patterning in the embryonic grasshopper eye. Dev Genes Evol 215:177–197
Dong Y, Friedrich M (2010) Enforcing biphasic eye development in a directly developing insect by transient knockdown of single eye selector genes. J Exp Zool B Mol Dev Evol 314B:104–114
Dong Y, Dinan L, Friedrich M (2003) The effect of manipulating ecdysteroid signaling on embryonic eye development in the locust *Schistocerca americana*. Dev Genes Evol 213:587–600
Donner AL, Maas RL (2004) Conservation and non-conservation of genetic pathways in eye specification. Int J Dev Biol 48:743–753
Duman-Scheel M, Pirkl N, Patel NH (2002) Analysis of the expression pattern of *Mysidium columbiae wingless* provides evidence for conserved mesodermal and retinal patterning processes among insects and crustaceans. Dev Genes Evol 212:114–123
Egelhaaf A (1988) Evidence for the priming role of the central retinula cell in ommatidium differentiation of *Ephestia kuehniella*. Rouxs Arch Dev Biol 197:184–189
Erezyilmaz DF, Riddiford LM, Truman JW (2006) The pupal specifier *broad* directs progressive morphogenesis in a direct-developing insect. Proc Natl Acad Sci 103:6925–6930
Fahrenbach WH (1969) The morphology of the eyes of *Limulus*. II. Ommatidia of the compound eye. Z Zellforsch Mikrosk Anat 93:451–483
Force A, Lynch M, Pickett FB, Amores A, Yan YL, Postlethwait J (1999) Preservation of duplicate genes by complementary, degenerative mutations. Genetics 151:1531–1545
Friedrich M (2006) Continuity versus split and reconstitution: exploring the molecular developmental corollaries of insect eye primordium evolution. Dev Biol 299:310–329
Friedrich M (2008) Opsins and cell fate in the *Drosophila* Bolwig organ: tricky lessons in homology inference. Bioessays 30:980–993
Friedrich M, Benzer S (2000) Divergent *decapentaplegic* expression patterns in compound eye development and the evolution of insect metamorphosis. J Exp Zool B Mol Dev E 288:39–55
Friedrich M, Caravas J (2011) New insights from hemichordate genomes: prebilaterian origin and parallel modifications in the paired domain of the Pax gene *eyegone*. J Exp Zool B Mol Dev E 316:387–392
Friedrich M, Rambold I, Melzer RR (1996) The early stages of ommatidial development in the flour beetle *Tribolium castaneum* (Coleoptera, Tenebrionidae). Dev Genes Evol 206:136–146
Friedrich M, Dong Y, Jackowska M (2006) Insect interordinal relationships: insights from the visual system. Arthropod Syst Phylogeny 64:133–148
Gehring WJ (2002) The genetic control of eye development and its implications for the evolution of the various eye-types. Int. J. Dev Biol 46:65–73
Greenwood S, Struhl G (1999) Progression of the morphogenetic furrow in the *Drosophila* eye: the roles of Hedgehog, Decapentaplegic and the Raf pathway. Development 126:5795–5808
Hafner GS, Tokarski TR (1998) Morphogenesis and pattern formation in the retina of the crayfish *Procambarus clarkii*. Cell Tissue Res 293:535–550
Hafner GS, Tokarski TR (2001) Retinal development in the lobster *Homarus americanus*. Comparison with compound eyes of insects and other crustaceans. Cell Tissue Res 305:147–158
Harzsch S, Walossek D (2001) Neurogenesis in the developing visual system of the branchiopod crustacean Triops longicaudatus (LeConte, 1846): corresponding patterns of compound-eye formation in Crustacea and Insecta? Dev Genes Evol 211:37–43
Heberlein U, Wolff T, Rubin GM (1993) The TGF beta homolog *dpp* and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. Cell 75:913–926

Henze MJ, Dannenhauer K, Kohler M, Labhart T, Gesemann M (2012) Opsin evolution and expression in arthropod compound eyes and ocelli: Insights from the cricket *Gryllus bimaculatus*. BMC Evol Biol 12:163

Homberg U, Paech A (2002) Ultrastructure and orientation of ommatidia in the dorsal rim area of the locust compound eye. Arthropod Struct Dev 30:271–280

Inoue Y, Miyawaki K, Terasawa T, Matsushima K, Shinmyo Y, Niwa N, Mito T, Ohuchi H, Noji S (2004) Expression patterns of *dachshund* during head development of *Gryllus bimaculatus* (cricket). Gene Expr Patterns 4:725–731

Jackowska M, Bao R, Liu Z, McDonald EC, Cook TA, Friedrich M (2007) Genomic and gene regulatory signatures of cryptozoic adaptation: loss of blue sensitive photoreceptors through expansion of long wavelength-opsin expression in the red flour beetle *Tribolium castaneum*. Front Zool 4:24

Jun S, Wallen RV, Goriely A, Kalionis B, Desplan C (1998) Lune/eye gone, a Pax-like protein, uses a partial paired domain and a homeodomain for DNA recognition. Proc Natl Acad Sci U S A 95:13720–13725

Kenyon KL, Ranade SS, Curtiss J, Mlodzik M, Pignoni F (2003) Coordinating proliferation and tissue specification to promote regional identity in the *Drosophila* head. Dev Cell 5:403–414

Klingler M (2004) *Tribolium*. Curr Biol 14:R639–R640

Konopova B, Jindra M (2008) Broad-Complex acts downstream of Met in juvenile hormone signaling to coordinate primitive holometabolan metamorphosis. Development 135:559–568

Koyama T, Syropyatova MO, Riddiford LM (2008) Insulin/IGF signaling regulates the change in commitment in imaginal discs and primordia by overriding the effect of juvenile hormone. Dev Biol 324:258–265

Kozmik Z (2008) The role of Pax genes in eye evolution. Brain Res Bull 75:335–339

Kristensen N (1999) Phylogeny of edopterygote insects, the most successful lineage of living organisms. Eur J Entomol 96:237–253

Kronhamn J, Frei E, Daube M, Jiao R, Shi Y, Noll M, Rasmuson-Lestander A (2002) Headless flies produced by mutations in the paralogous Pax6 genes *eyeless* and *twin of eyeless*. Development 129:1015–1026

Kumar JP (2009) The molecular circuitry governing retinal determination. Biochim Biophys Acta 1789:306–314

Kumar JP, Moses K (2001) The EGF receptor and Notch signaling pathways control the initiation of the morphogenetic furrow during *Drosophila* eye development. Development 128:2689–2697

Labhart T, Keller K (1992) Fine structure and growth of the polarization-sensitive dorsal rim area in the compound eye of larval crickets. Naturwissenschaften 79:527–529

Labhart T, Meyer EP (1999) Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. Microsc Res Tech 47:368–379

Lamb T (2011) Evolution's witness: how eyes evolved. Oxford University Press, USA

Lee JD, Treisman JE (2001) The role of Wingless signaling in establishing the anteroposterior and dorsoventral axes of the eye disc. Development 128:1519–1529

Lee MSY, Jago JB, Garcia-Bellido DC, Edgecombe GD, Gehling JG, Paterson JR (2011) Modern optics in exceptionally preserved eyes of Early Cambrian arthropods from Australia. Nature 474:631–634

Liu Z, Friedrich M (2004) The *Tribolium* homologue of *glass* and the evolution of insect larval eyes. Dev Biol 269:36–54

Liu Z, Yang X, Dong Y, Friedrich M (2006) Tracking down the "head blob": comparative analysis of *wingless* expression in the embryonic insect procephalon reveals progressive reduction of ocular segment patterning in higher insects. Arthropod Struct Dev 35:341–356

Lomer CJ, Bateman RP, Johnson DL, Langewald J, Thomas M (2001) Biological control of locusts and grasshoppers. Annu Rev Entomol 46:667–702

Lorenz MW (2007) Oogenesis-flight syndrome in crickets: age-dependent egg production, flight performance, and biochemical composition of the flight muscles in adult female *Gryllus bimaculatus*. J Insect Physiol 53:819–832

Lynch V, Wagner G (2011) Revisiting a classic example of transcription factor functional equivalence: are Eyeless and Pax6 functionally equivalent or divergent? J Exp Zool B Mol Dev Evol 316B:93–98

Ma CY, Zhou Y, Beachy PA, Moses K (1993) The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. Cell 75:927–938

MacWhinnie SGB, Allee JP, Nelson CA, Riddiford LM, Truman JW, Champlin DT (2005) The role of nutrition in creation of the eye imaginal disc and initiation of metamorphosis in *Manduca sexta*. Dev Biol 285:285–297

Maderspacher F, Bucher G, Klingler M (1998) Pair-rule and gap gene mutants in the flour beetle *Tribolium castaneum*. Dev Genes Evol 208:558–568

Mahfooz NS, Li H, Popadić A (2004) Differential expression patterns of the hox gene are associated with differential growth of insect hind legs. Proc Natl Acad Sci U S A 101:4877–4882

Maurel-Zaffran C, Treisman JE (2000) *pannier* acts upstream of *wingless* to direct dorsal eye disc development in *Drosophila*. Development 127:1007–1016

Melzer RR, Paulus HF (1989) Evolutionswege zum Larvalauge der Insekten -Die Stemmata der höheren Dipteren und ihre Abwandlung zum Bolwig-Organ. Z Zool Syst Evolutionsforsch 27:200–245

Melzer RR, Michalke C, Smola U (2000) Walking on insect paths? Early ommatidial development in the compound eye of the ancestral crustacean, *Triops cancriformis*. Naturwissenschaften 87:308–311

Mito T, Noji S (2008) The two-spotted cricket *Gryllus bimaculatus*: an emerging model for developmental and regeneration studies. Cold Spring Harb Protoc: pdb.emo110

Monsma SA, Booker R (1996) Genesis of the adult retina and outer optic lobes of the moth, *Manduca sexta*. I. patterns of proliferation and cell death. J Comp Neurol 367:10–20

Moreaux L, Laurent G (2007) Estimating firing rates from calcium signals in locust projection neurons in vivo. Front Neural Circuits 1:2

Moses K, Rodrigues AB (2004) Growth and specification: fly Pax6 homologs *eyegone* and *eyeless* have distinct functions. Bioessays 26:600–603

Müller C, Rosenberg J, Richter S, Meyer-Rochow V (2003) The compound eye of *Scutigera coleoptrata* (Linnaeus, 1758)(Chilopoda: Notostigmophora): an ultrastructural reinvestigation that adds support to the Mandibulata concept. Zoomorphology 122:191–209

Nilsson D-E (1996) Eye ancestry: old genes for new eyes. Curr Biol 6:39–42

Niwa N, Inoue Y, Nozawa A, Saito M, Misumi Y, Ohuchi H, Yoshioka H, Noji S (2000) Correlation of diversity of leg morphology in *Gryllus bimaculatus* (cricket) with divergence in *dpp* expression pattern during leg development. Development 127:4373–4381

Papayannopoulos V, Tomlinson A, Panin VM, Rauskolb C, Irvine KD (1998) Dorsal-ventral signaling in the *Drosophila* eye. Science 281:2031–2034

Park T (1934) Observations on the general biology of the flour beetle, *Tribolium confusum*. Quart Rev Biol 9:36–64

Parthasarathy R, Tan A, Bai H, Palli SR (2008) Transcription factor *broad* suppresses precocious development of adult structures during larval–pupal metamorphosis in the red flour beetle, *Tribolium castaneum*. Mech Dev 125:299–313

Paterson JR, Garcia-Bellido DC, Lee MSY, Brock GA, Jago JB, Edgecombe GD (2011) Acute vision in the giant Cambrian predator *Anomalocaris* and the origin of compound eyes. Nature 480:237–240

Perez-Mendoza J, Campbell JF, Throne JE (2011) Effects of rearing density, age, sex, and food deprivation on flight initiation of the red flour beetle (Coleoptera: Tenebrionidae). J Econ Entomol 104:443–451

Perron M, Kanekar S, Vetter ML, Harris WA (1998) The genetic sequence of retinal development in the ciliary margin of the *Xenopus* eye. Dev Biol 199:185–200

Pichaud F, Casares F (2000) *homothorax* and *iroquois-C* genes are required for the establishment of territories within the developing eye disc. Mech Dev 96:15–25

Pichaud F, Desplan C (2002) Pax genes and eye organogenesis. Current Opionion in Genetics and. Development 12:430–434

Posnien N, Schinko JB, Kittelmann S, Bucher G (2010) Genetics, development and composition of the insect head–a beetle's view. Arthropod Struct Dev 39:399–410

Postlethwait JH, Schneiderman HA (1971) A clonal analysis of development in *Drosophila melanogaster*: morphogenesis, determination and and growth in the wild type antenna. Dev Biol 24:477–519

Prpic NM, Wigand B, Damen WG, Klingler M (2001) Expression of *dachshund* in wild-type and *Distal-less* mutant *Tribolium* corroborates serial homologies in insect appendages. Dev Genes Evol 211:467–477

Raymond PA, Barthel LK, Bernardos RL, Perkowski JJ (2006) Molecular characterization of retinal stem cells and their niches in adult zebrafish. BMC Dev Biol 6:36

Reifegerste R, Moses K (1999) Genetics of epithelial polarity and pattern in the *Drosophila* retina. Bioessays 21:275–285

Richards S, Gibbs RA, Weinstock GM, Brown SJ, Denell R, Beeman RW, Gibbs R, Bucher G, Friedrich M, Grimmelikhuijzen CJ, Klingler M, Lorenzen M, Roth S, Schroder R, Tautz D, Zdobnov EM, Muzny D, Attaway T, Bell S, Buhay CJ, Chandrabose MN, Chavez D, Clerk-Blankenburg KP, Cree A, Dao M, Davis C, Chacko J, Dinh H, Dugan-Rocha S, Fowler G, Garner TT, Garnes J, Gnirke A, Hawes A, Hernandez J, Hines S, Holder M, Hume J, Jhangiani SN, Joshi V, Khan ZM, Jackson L, Kovar C, Kowis A, Lee S, Lewis LR, Margolis J, Morgan M, Nazareth LV, Nguyen N, Okwuonu G, Parker D, Ruiz SJ, Santibanez J, Savard J, Scherer SE, Schneider B, Sodergren E, Vattahil S, Villasana D, White CS, Wright R, Park Y, Lord J, Oppert B, Brown S, Wang L, Weinstock G, Liu Y, Worley K, Elsik CG, Reese JT, Elhaik E, Landan G, Graur D, Arensburger P, Atkinson P, Beidler J, Demuth JP, Drury DW, Du YZ, Fujiwara H, Maselli V, Osanai M, Robertson HM, Tu Z, Wang JJ, Wang S, Song H, Zhang L, Werner D, Stanke M, Morgenstern B, Solovyev V, Kosarev P, Brown G, Chen HC, Ermolaeva O, Hlavina W, Kapustin Y, Kiryutin B, Kitts P, Maglott D, Pruitt K, Sapojnikov V, Souvorov A, Mackey AJ, Waterhouse RM, Wyder S, Kriventseva EV, Kadowaki T, Bork P, Aranda M, Bao R, Beermann A, Berns N, Bolognesi R, Bonneton F, Bopp D, Butts T, Chaumot A, Denell RE, Ferrier DE, Gordon CM, Jindra M, Lan Q, Lattorff HM, Laudet V, von Levetsow C, Liu Z, Lutz R, Lynch JA, da Fonseca RN, Posnien N, Reuter R, Schinko JB, Schmitt C, Schoppmeier M, Shippy TD, Simonnet F, Marques-Souza H, Tomoyasu Y, Trauner J, Van der Zee M, Vervoort M, Wittkopp N, Wimmer EA, Yang X, Jones AK, Sattelle DB, Ebert PR, Nelson D, Scott JG, Muthukrishnan S, Kramer KJ, Arakane Y, Zhu Q, Hogenkamp D, Dixit R, Jiang H, Zou Z, Marshall J, Elpidina E, Vinokurov K, Oppert C, Evans J, Lu Z, Zhao P, Sumathipala N, Altincicek B, Vilcinskas A, Williams M, Hultmark D, Hetru C, Hauser F, Cazzamali G, Williamson M, Li B, Tanaka Y, Predel R, Neupert S, Schachtner J, Verleyen P, Raible F, Walden KK, Angeli S, Foret S, Schuetz S, Maleszka R, Miller SC, Grossmann D (2008) The genome of the model beetle and pest *Tribolium castaneum*. Nature 452:949–955

Ridley AW, Hereward JP, Daglish GJ, Raghu S, Collins PJ, Walter GH (2011) The spatiotemporal dynamics of *Tribolium castaneum* (Herbst): adult flight and gene flow. Mol Ecol 20:1635–1646

Rogers SM, Harston GW, Kilburn-Toppin F, Matheson T, Burrows M, Gabbiani F, Krapp HG (2010) Spatiotemporal receptive field properties of a looming-sensitive neuron in solitarious and gregarious phases of the desert locust. J Neurophysiol 103:779–792

Roonwal ML (1936) Studies on the embryology of the African migratory locust, *Locusta migratoria migratorioides* Reiche and Frm. (Orthoptera, Acrididae) II-Organogeny. Philos Trans R Soc Lond B 227:175–244

Salvini-Plawen L, Mayr E (1977) On the evolution of photoreceptors and eyes. Evol Biol 10:207–263

Sanchez D, Ganfornina MD, Bastiani M (1995) Contributions of an orthopteran to the understanding of neuronal pathfinding. Immunol Cell Biol 73:565–574

Sato K, Matsunaga TM, Futahashi R, Kojima T, Mita K, Banno Y, Fujiwara H (2008) Positional cloning of a *Bombyx wingless* locus *flugellos* (*fl*) reveals a crucial role for *fringe* that is specific for wing morphogenesis. Genetics 179:875–885

Savard J, Tautz D, Richards S, Weinstock GM, Gibbs RA, Werren JH, Tettelin H, Lercher MJ (2006) Phylogenomic analysis reveals bees and wasps (Hymenoptera) at the base of the radiation of holometabolous insects. Genome Res 16:1334–1338

Schinko J, Hillebrand K, Bucher G (2012) Heat shock-mediated misexpression of genes in the beetle *Tribolium castaneum*. Dev Genes Evol 222:287–298

Schroder R (2003) The genes *orthodenticle* and *hunchback* substitute for *bicoid* in the beetle *Tribolium*. Nature 422:621–625

Singh A, Kango-Singh M, Parthasarathy R, Gopinathan KP (2007) Larval legs of mulberry silkworm *Bombyx mori* are prototypes for the adult legs. Genesis 45:169–176

Smith WC, Price DA, Greenberg RM, Battelle BA (1993) Opsins from the lateral eyes and ocelli of the horseshoe-crab, *Limulus polyphemus*. Proc Natl Acad Sci U S A 90:6150–6154.

Sokoloff A (1972) The biology of *Tribolium*. Clarendon Press, Oxford

Stambolic V, Ruel L, Woodgett JR (1996) Lithium inhibits *glycogen synthase kinase*-3 activity and mimics *wingless* signalling in intact cells. Curr Biol 6:1664–1668

Suzuki Y, Truman JW, Riddiford LM (2008) The role of *Broad* in the development of *Tribolium castaneum*: implications for the evolution of the holometabolous insect pupa. Development 135:569–577

Suzuki Y, Squires DC, Riddiford LM (2009) Larval leg integrity is maintained by Distal-less and is required for proper timing of metamorphosis in the flour beetle, *Tribolium castaneum*. Dev Biol 326:60–67

Takagi A, Kurita K, Terasawa T, Nakamura T, Bando T, Moriyama Y, Mito T, Noji S, Ohuchi H (2012) Functional analysis of the role of *eyes absent* and *sine oculis* in the developing eye of the cricket *Gryllus bimaculatus*. Dev Growth Differ 54:227–240

Towner P, Harris P, Wolstenholme AJ, Hill C, Worm K, Gartner W (1997) Primary structure of locust opsins: a speculative model which may account for ultraviolet wavelength light detection. Vision Res 37:495–503

Treisman JE, Rubin GM (1995) *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. Development 121:3519–3527

Truman JW, Riddiford LM (2002) Endocrine insights into the evolution of metamorphosis in insects. Annu Rev Entomol 47:467–500

Truman JW, Hiruma K, Allee JP, MacWhinnie SGB, Champlin DT, Riddiford LM (2006) Juvenile hormone is required to couple imaginal disc formation with nutrition in insects. Science 312:1385–1388

Uhlirova M, Foy BD, Beaty BJ, Olson KE, Riddiford LM, Jindra M (2003) Use of Sindbis virus-mediated RNA interference to demonstrate a conserved role of Broad-Complex in insect metamorphosis. Proc Natl Acad Sci U S A 100:15607–15612

Vishnevskaya TM, Cherkasov AD, Shura-Bura TM (1985) Spectral sensitivity of photoreceptors in the compound eye of the locust. Neurophysiology 18:69–76

Wiegmann BM, Trautwein MD, Kim JW, Cassel BK, Bertone MA, Winterton SL, Yeates DK (2009) Single-copy nuclear genes resolve the phylogeny of the holometabolous insects. BMC Biol 7:34

Wieschaus E, Gehring W (1976) Clonal analysis of primordial disc cells in the early embryo of *Drosophila melanogaster*. Dev Biol 50:249–263

Wilson M, Garrard P, McGinness S (1978) The unit structure of the locust compound eye. Cell Tiss Res 195:205–226

Yang X, Weber M, ZarinKamar N, Wigand B, Posnien G, Friedrich R, Beutel R, Damen W, Bucher G, Klingler M, Friedrich M (2009a) Probing the *Drosophila* retinal determination gene network in *Tribolium* (II): the Pax6 genes *eyeless* and *twin of eyeless*. Dev Biol 333:215–227

Yang X, ZarinKamar N, Bao R, Friedrich M (2009b) Probing the *Drosophila* retinal determination gene network in *Tribolium* (I): the early retinal genes *dachshund*, *eyes absent* and *sine oculis*. Dev Biol 333:202–214

Yu L, Zhou Q, Zhang C, Pignoni F (2012) Identification of *Bombyx atonal* and functional comparison with the *Drosophila atonal* proneural factor in the developing fly eye. Genesis 50:393–403

ZarinKamar N, Yang X, Bao R, Friedrich F, Beutel R, Friedrich M, 2011. The Pax gene eyegone facilitates repression of eye development in *Tribolium*. Evodevo 2:8

Zuker CS (1994) On the evolution of eyes: would you like it simple or compound? Science 265:742–743