

Leeches of the genus *Helobdella* as model organisms for Evo-Devo studies

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Abstract Model organisms are important tools in modern biology and have been used elucidate mechanism underlying processes, such as development, heredity, neuronal signaling, and phototropism, to name but a few. In this context, the use of model organisms is predicated on uncovering evolutionarily conserved features of biological processes in the expectation that the findings will be applicable to organisms that are either inaccessible or intractable for direct experimentation. For the most part, particular species have been adapted as model organisms because they can be easily reared and manipulated in the laboratory. In contrast, a major goal in the field of evolutionary developmental biology (Evo-Devo) is to identify and elucidate the differences in developmental processes among species associated with the dramatic range of body plans among organisms, and how these differences have emerged over time in various branches of phylogeny. At first glance then, it would appear that the concept of model organisms for Evo-Devo is oxymoronic. In fact, however, laboratory-compatible, experimentally tractable species are of great use for Evo-Devo, subject to the condition that the ensemble of models investigated should reflect the range of taxonomic diversity, and for this purpose glossiphoniid leeches are useful. Four decades ago (1975), leeches of the species-rich genus *Helobdella* (Lophotrochozoa; Annelida; Clitellata; Hirudinida; Glossiphoniidae) were collected in Stow Lake, Golden Gate Park, San Francisco, CA (USA).

These and other *Helobdella* species may be taken as Evo-Devo models of leeches, clitellate annelids, and the superphylum Lophotrochozoa. Here we depict/discuss the biology/taxonomy of these Evo-Devo systems, and the challenges of identifying species within *Helobdella*. In addition, we document that *H. austinensis* has been established as a new model organism that can easily be cultivated in the laboratory. Finally, we provide an updated scheme illustrating the unique germ line/soma-differentiation during early development and speculate on the mechanisms of sympatric speciation in this group of aquatic annelids.

Keywords Evo-Devo · *Helobdella* · Leeches · Model organisms · Germ line

Introduction

The British naturalist Charles Darwin (1809–1882) is well known for his zoocentric book *On the Origin of Species*, one of the most highly cited monographs in the history of biology (Darwin 1859). Less well known, however, is the fact that Darwin devoted much of the second half of his scientific career to the study of plants (Kutschera and Briggs 2009). As part of this work, Darwin introduced the grass seedling as a model organism for experimental plant research in his book *The Power of Movements in Plants* (Darwin 1881). His description of the phototropic bending response in dark-grown grass coleoptiles led to the discovery of the plant hormone auxin, and “Darwin’s model organisms” are still in use today (Kutschera and Niklas 2009; Kutschera and Wang 2015).

In 1904, the American geneticist Thomas Hunt Morgan (1866–1945) established his “Fly-laboratory” at Columbia

Dedicated to the memory of Ernst Mayr (1904–2005).

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University, using a common, small insect species, the fruit fly *Drosophila melanogaster*, as an experimental organism to study genetic variation. 6 years later, Morgan became one of the founders of the chromosomal theory of heredity, which was integrated into the Neo-Darwinian theory of that time (Mayr 1942). In the history of biology Morgan was a prominent “anti-selectionist” (Morgan 1916; Mayr 1982). Importantly, however, he was also the mentor of Theodosius Dobzhansky (1900–1975), who began to study the genetic basis of speciation in flies of the genus *Drosophila*, in 1928, in Morgan’s Fly-lab. Later, Dobzhansky, together with Ernst Mayr (1904–2005), founded the Synthetic Theory of Biological Evolution (Reif et al. 2000; Kutschera 2009, 2013; Kutschera and Niklas 2004).

The establishment of the first model organisms, grass coleoptiles and fruit flies (Darwin 1881; Morgan 1916) was followed by later introductions of diverse taxa, including viruses and bacteria, as well as many eukaryotes (some of which are shown in Fig. 1). Several models were chosen because particular advantageous features outweighed the difficulties in procuring and maintaining them; for example the giant axon and giant synapse of the squid *Loligo* enabled fundamental discoveries in neurophysiology, despite the fact that physiologists and biophysicists working with *Loligo* generally must travel to where their subject can be obtained. For the most part, however, species that emerged as models (e.g., the axolotl *Ambystoma mexicanum*, the nematode *Caenorhabditis elegans*, the frog *Xenopus laevis*, the zebrafish *Danio rerio*, the house mouse *Mus musculus*, and the mustard plant *Arabidopsis thaliana*) are, like *Drosophila*, easy to maintain, breed and manipulate in the laboratory (or green house). These models have therefore been extensively studied and far more is known about their taxonomy, development, reproduction and genetics compared to other taxa (Brenner 1974; Hossfeld 1996; Love 2009; Rader 2004; Koornneef and Meinke 2010; Ankeny and Leonelli 2011; Kolb et al. 2013; Reiss et al. 2015). The large communities of researchers dedicated to intensively studied model organisms also provide “economy of scale” so that, as new experimental resources emerge (whole genome sequencing, gene expression databases, knock-out libraries, etc.), they are first brought to bear on these models.

Notwithstanding the power of the model organism approach, however, the focus on just a few intensely studied taxa poses a limitation for understanding how the remarkable diversity of (animal and plant) body plans has evolved through modification of developmental mechanisms, a field now called evolutionary developmental biology, or Evo-Devo (Akam 1998; Hall 1999; Jenner and Wills 2007; Mueller 2007; Olsson et al. 2010). By definition, Evo-Devo requires developmental comparisons among taxa representing diverse branches of the

phylogenetic tree, and is therefore concerned at least as much with differences among taxa as with their similarities. It has also been pointed out that the factors that lead scientists to adopt certain species as “model systems” (e.g., small body size, rapid development, and short life cycle) may have correlates (canalized development, condensed genomes) that renders them less representative of evolutionary developmental diversity than might be predicted from their phylogenetic distribution alone (Hall 1999; Bolker 1995, 2014).

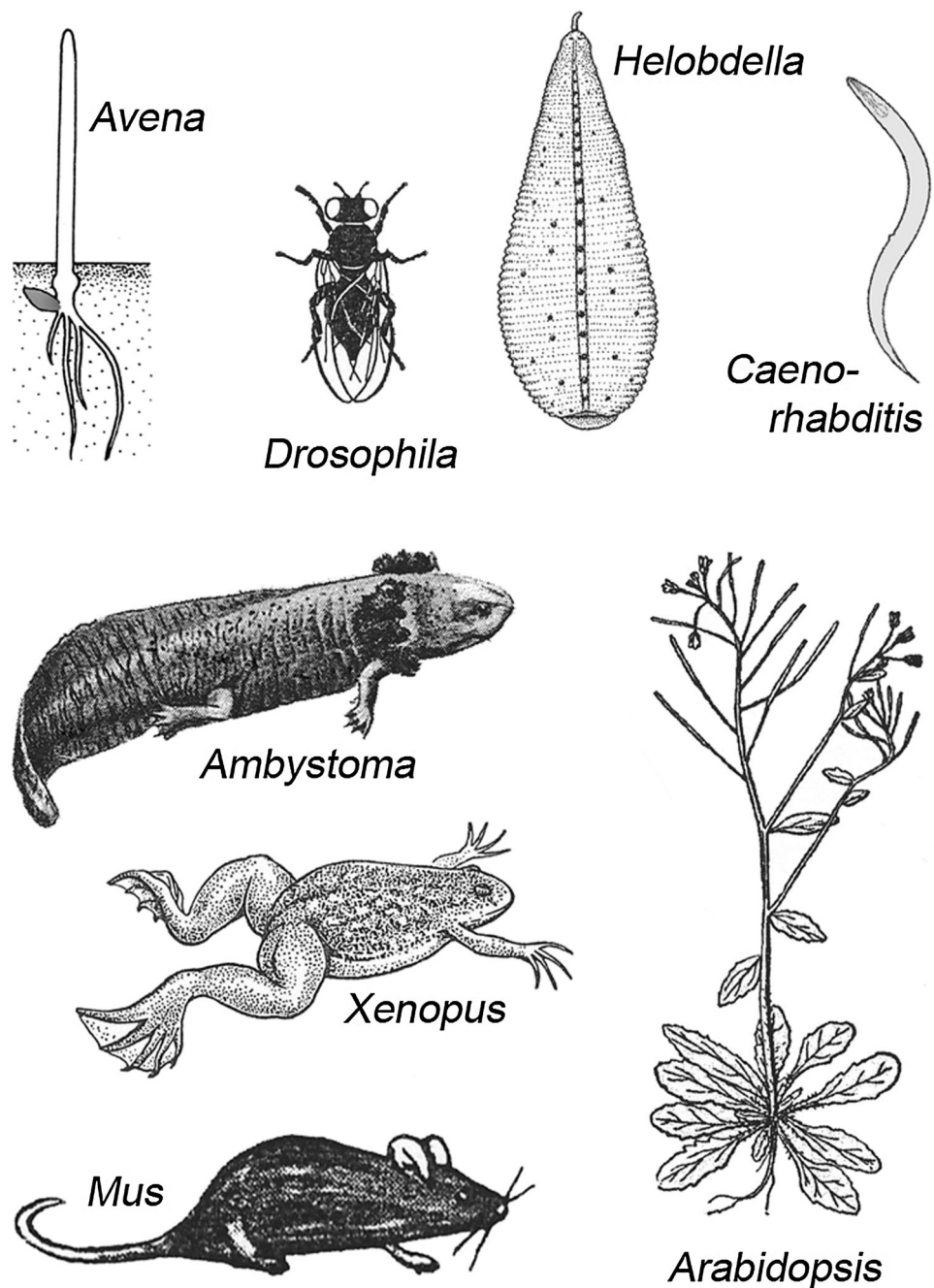
Thus, while some degree of experimental tractability is essential to elucidate developmental mechanisms for comparative purposes, the field of Evo-Devo requires us to identify and study additional models, in particular those representing previously under-studied branches of the phylogenetic tree, and to relax our demands for ease of culture and experimental simplicity. A potential side benefit of investigating new models is the element of bio-prospecting involved, i.e., the possibility that studying a new system may reveal either “new” cell-biological, or developmental processes, or provide particularly clear examples (or good experimental access) to phenomena that have previously been difficult to examine.

In this article, we describe the distribution, collection, laboratory maintenance, feeding and systematics of several “lab-friendly” freshwater glossiphoniid leech species in the genus *Helobdella* (Blanchard 1896), small clitellate annelids (invertebrate, segmented worms) that have evolved a high degree of parental care within the superphylum Lophotrochozoa (molluscs, annelids, flatworms and others). Pioneering studies on the development of glossiphoniid leeches were carried out in 19th century (reviewed in Sawyer 1986). Modern cellular and molecular techniques were brought to bear on studies of leech development beginning decades later. Since the mid 1970s, multiple studies have outlined processes of cell lineage and cell fate decisions, segmentation, spiral cleavage, and growth control in several different *Helobdella* species (see Weisblat and Kuo 2009, 2014). Unexpectedly, the genome of at least one sequenced *Helobdella* species is now known to have more extensive genome rearrangements—more than almost any other bilaterian animal yet examined (Simakov et al. 2013). We discuss a recent model of germline-soma-differentiation that may account for this highly dynamic genome.

Materials and methods

The genus *Helobdella* comprises a group of small, flattened, proboscis-bearing freshwater leeches with one pair of dorsal anterior eye-spots (order Rhynchobdellidae). The genus is thought to have evolved in what is now South

Fig. 1 Model organisms that have been extensively studied by generations of biologists. Charles and Francis Darwin introduced in 1881 the etiolated grass seedling (*Avena sativa*, *Phalaris* sp.) as an experimental system for the analysis of plant growth. Other important model organisms are the Axolotl *Ambystoma* (1890), the house mouse *Mus* (1910), the fruit fly *Drosophila* (1916), the mouse ear cress *Arabidopsis* (1943), the frog *Xenopus* (1955), the round worm *Caenorhabditis* (1965), and the flat leech *Helobdella* (1976)



America, but now occurs in suitable habitats almost throughout the world (Siddall et al. 2005; Sket and Trontelj 2008). In the San Francisco (SF) Bay Area of California (CA), USA, we have collected *Helobdella* from the underside of stones, leaves or pieces of bark in different still and running freshwater ecosystems. Due to the vagaries of nature and human intervention, these are relatively changeable habitats. Between 1985 and 1988, numerous leeches were collected in the Stow Lake in the Golden Gate Park (SF) and in the running waters of the adjacent Botanical Garden. Another source of *Helobdella* was a

small creek running perpendicular to Palm Drive on the Campus of Stanford University (1985–1988). Between 2008 and 2015, Matadero Creek in Palo Alto contained a stable population of *Helobdella* that was analyzed twice per year (in February and September, respectively). Leeches of the genus *Helobdella* were also collected in the American River in Sacramento, California, and in several man-made ponds near Galt, California.

To grow *Helobdella* in laboratory cultures, glass jars (size ca. 20 cm in diameter, high ca. 8 cm) were filled with artificial pond water (1 % Instant Ocean spring water). The

depth of the water was ca. 4 cm and the jars were covered with non-transparent lids, so that the leeches (up to 500 per jar) were maintained in the shade (18–25 °C). Subject in part to the preferences of individual species, *Helobdella* were either fed on water snails (e.g., *Physa* sp., lab-reared or wild-caught), bloodworms (i.e., insect larvae of the genus *Chironomus*, available commercially in frozen form as pet food), or aquatic oligochaetes (e.g., *Tubifex* sp.).

All observations and experiments were carried out at least three times using different leeches collected in the same aquatic ecosystem. For documentation of the results, representative individuals are depicted.

Results

Species identification

To the unaided eye, *Helobdella* are somewhat drab, grayish-brown in appearance. Inspection of individual *Helobdella* under a dissecting microscope, however, reveals distinct patterns of pigmentation and other morphological features, which historically have provided the basis for identifying and distinguishing different species (Fig. 2). This approach leads to confusion, because there may be significant variation within at least some species, as well as strong similarities between what turn out to be different taxa. The problems are confounded by the fact that fixatives used to preserve museum specimens usually destroy the pigmentation. To circumvent these problems, rapidly

evolving sequences of the mitochondrial gene cytochrome-c-oxidase subunit 1 (CO1) are used to distinguish between species (Siddall and Borda 2003; Siddall and Budinoff, 2005; Bely and Weisblat 2006; Kutschera et al. 2013), complemented by photographing representative live individuals to document morphological features. Through such efforts, it now appears that *Helobdella* is a relatively speciose genus compared to other leech genera, and species designations are subject to revision (Borda and Siddall 2004a, b; Siddall et al. 2005, and see below). In the following discussion we use quotation marks to designate species whose status is unclear.

The San Francisco Bay area leech “*Helobdella triserialis*” (SF)

Four decades ago (1975), Roy T. Sawyer, then in the laboratory of Gunther S. Stent (1924–2008), collected glossiphoniid leeches of the genus *Helobdella* in Stow Lake, Golden Gate Park, San Francisco, California (USA). These small (ca. 10–20 mm long) leeches, characterized in part by a regular orthogonal array of white pigment spots on the dorsum, were maintained in the laboratory (Fig. 2) and fed on water snails (genus *Physa*). In the earliest research papers published using these lab populations (Weisblat et al. 1978, 1980), the Stow Lake leeches were assigned to the South American taxon *H. triserialis* (Blanchard 1849), according to the taxonomy in Sawyer (1972, 1986). But, it was subsequently realized that the CO1 sequence of this isolate differs significantly from that of *H. triserialis* as described from Bolivia (Bely and Weisblat 2006). This Stow Lake population persisted through 1988 (individuals were collected by U. K. between 1985 and 1988), but two decades later (between 2007 and 2014), no individuals of this species could be found in Stow Lake, nor elsewhere in Golden Gate Park, where populations of *H. californica* exist (Kutschera 1988, 1989, 2011). Similarly, populations apparently corresponding to “*H. triserialis*” (SF) were discovered and analyzed between 1985 and 1988 in a creek on the campus of Stanford University (Kutschera 1992), but in 2007, this population had disappeared.

In Fig. 3, the extensive parental care that is characteristic of all glossiphoniid leech species is illustrated, based on observations of captured “*H. triserialis*” (SF) individuals maintained in the laboratory. Fertilization is internal, and clutches range from a few to more than 100 zygotes. Each zygote is enclosed by a fertilization envelope, and is deposited from the female gonopore into one of several cocoons formed by secretions from specialized cells in the body wall of the clitellar segments (Sayers et al. 2009). The cocoons are then attached to the ventral side of the body (Fig. 3a). The yolky embryos can be isolated easily from the cocoons and cultured in a simple salt solution. In

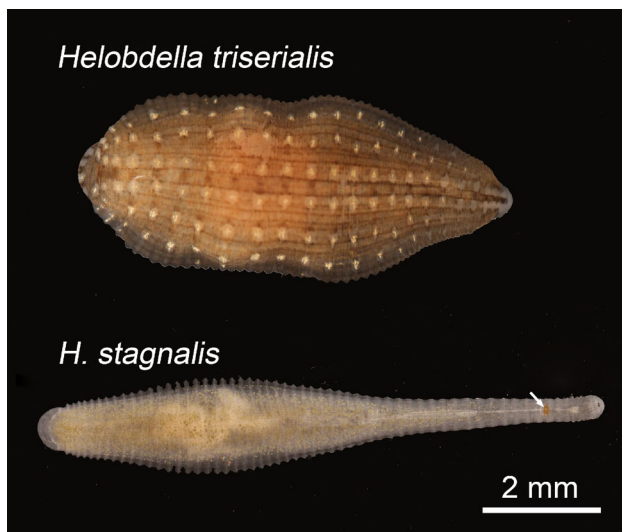


Fig. 2 Photograph of the co-occurring leech species “*Helobdella triserialis*” and “*H. stagnalis*”, dorsal view. The white arrow indicates the dorsal scute, which is characteristic of “*H. stagnalis*”. Both individuals were collected in Matadero Creek, Palo Alto, California, in September 2014

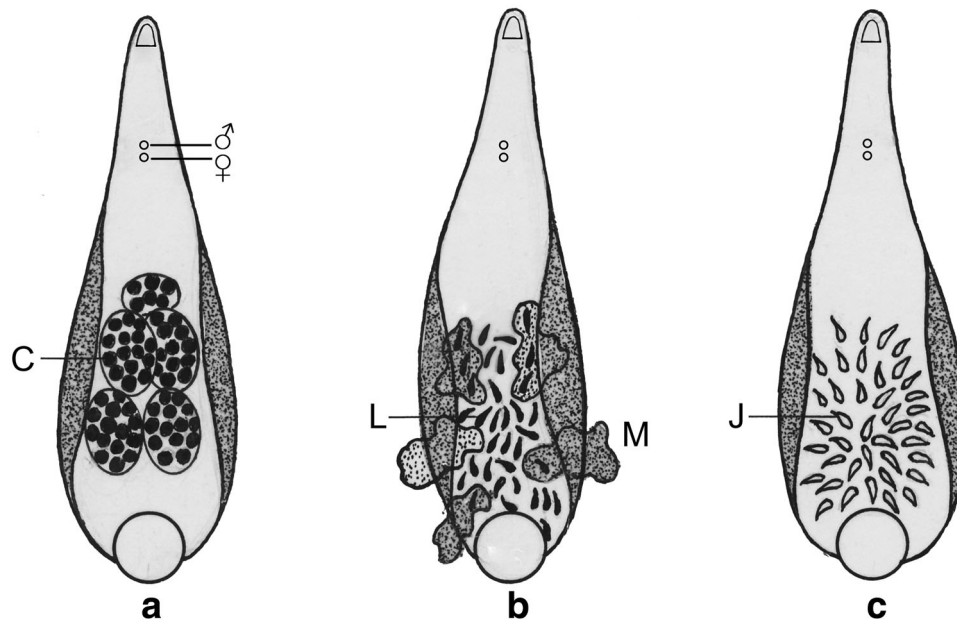


Fig. 3 Scheme illustrating cocoon production and the development of juvenile leeches in “*Helobdella triserialis*”, based on observations in lab cultures. In this representative example, 5 cocoons (C) are attached to the ventral aspect of the parent (a). Male and female gonopores are indicated. As the cocoon membranes (M) disintegrate and the partially developed embryos (usually designated as “larvae”,

L) hatch from the fertilization envelope, they adhere to the ventral body wall of the parent via anterior ventral adhesive organs (b). A few days later, the juveniles (J) have developed functional suckers and use them to attach to the ventral side of the parent (c). Length of the leech: ca. 12 mm

normal development, the cocoons disintegrate as the developing leech embryos emerge from the fertilization envelope, before their suckers are formed. At this time, they dangle passively from the parental venter via adhesive secretions from ventral anterior glands (Fig. 3b). Then, as their posterior suckers become functional, the developing leeches hang by those suckers, still on the parental venter, digesting their residual yolk. After both suckers are functional and the yolk is fully digested, the juvenile leeches ride to their first few meals still attached to the underside of the parent (Fig. 3c). Following the completion of their reproductive functions, adults of “*H. triserialis*” and other *Helobdella* species eventually initiate a process of senescence, culminating in the death of the organism. Senescence is characterized by gradual reduction in motility and feeding, accompanied by pronounced swelling of the body and loss of pigmentation in some species, or by shriveling and increased pigmentation in others.

Figure 2 shows a representative specimen of “*H. triserialis*” SF collected in Matadero Creek in Palo Alto (CA), ca. 1 km away from the campus creek, where large populations of these leeches existed earlier (1985–1988). Schmerer et al. (2013) have shown that the CO1 sequence of “*H. triserialis*” (Palo Alto; GenBank no. KC771417) is 99 % identical to that of specimens collected in San Francisco, so that these populations represent one biospecies. We have confirmed this finding using specimens of

“*H. triserialis*” (Palo Alto) collected in Matadero Creek in 2013. “*H. triserialis*” co-occurs in its habitat (Matadero Creek) together with individuals of the species “*H. stagnalis*” (Fig. 2; see below).

In a recent report, we assigned “*H. triserialis*” Palo Alto to the taxon *H. papillata*, as recommended by Siddall and Borda (2003) and Klemm et al. (2012). However, a phylogenetic analysis based on CO1 sequences from GenBank revealed that “*H. triserialis*” (SF) Palo Alto is not identical with *H. papillata* (Schmerer et al. 2013). Thus, the taxonomic status of this leech is currently under investigation. Wedeen et al. (1990) have documented that this species (i.e., specimens from SF) reproduces via self-fertilization. We have corroborated this finding, using individuals collected in the Matadero Creek (Palo Alto).

The Sacramento leech “*Helobdella robusta*”: variability and biology

Problems with maintaining “*H. triserialis*” (SF) in permanent laboratory culture (gradual declines in the health and productivity of the colonies), combined with its disappearance from the original habitat (Stow Lake, GG Park, SF), led to the adoption of another *Helobdella* species as a lab model. This species was originally discovered as a by-catch with snails collected commercially from a creek flowing into the American River in Sacramento, CA (USA)

in the 1980s. This leech was later described as a new species, *H. robusta* (Shankland et al. 1992) served as model organism (Seaver and Shankland 2000), and was eventually included as one of three species for whole genome sequencing in a lophotrochozoan genome initiative (Simakov et al. 2013). Curiously, the later analysis of CO1 sequences revealed the existence of two distinct species of “*H. robusta*” at the site where it was originally collected (Bely and Weisblat 2006). The reproductive biology of these leeches has not yet been detailed (Rogers et al. in preparation) but it is also self-fertile (D.A.W. personal observation). Figure 4 shows four representative individuals of “*H. robusta*” taken from a large lab population, illustrating the range of variation in the number of papillae and longitudinal stripes within this species.

Adult “*H. robusta*” preferentially feed on water snails and usually kills its host (i.e., prey) organism (Fig. 5). Other potential prey organisms, such as insect larvae (*Chironomus* sp.) or oligochaetes (*Tubifex* sp.), are ignored by hungry adults. Figure 6 illustrates how juvenile leeches that have been carried to the host by the brooding parent crawl into the shell of the snail and co-feed with the adult. Moreover, to enable feeding by young “*H. robusta*” that have been reared in isolation from the parent, we find it necessary to wound/kill the host snail with forceps or pins. We suggest that adult “*H. robusta*” provide for their young by immobilizing and damaging the soft body of the host snail thereby enabling the young to feed even on comparatively large snails that would otherwise elude them.

In addition to the normal process of senescence seen in other species, adult “*H. robusta*” are also subject to a lethal degenerative process called “head rot”, an apparently fungal infection which starts in the head region of the animal and progresses rearward (Fig. 7). In the case documented here, juveniles were still attached to the belly of the dying parent. In our experience, lab-reared populations

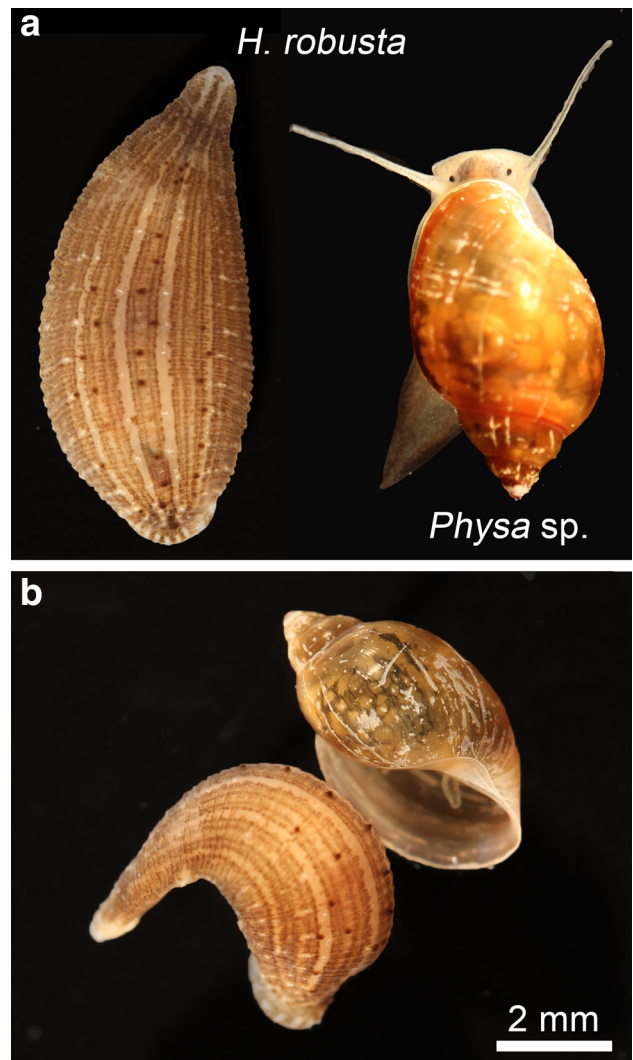


Fig. 5 Feeding on water snails in the leech species “*H. robusta*”. The adult hirudinean is attracted by a rapidly moving water snail (*Physa* sp.) (a). Within 1 h, the leech has sucked up the soft contents of the body of the snail, so that the empty shell is left (b)

Fig. 4 Variability in the dorsal patterns of stripes and papillae in the Sacramento leech “*H. robusta*”. The four individuals depicted (a–d) were from the same lab population (UC Berkeley, February 2013)

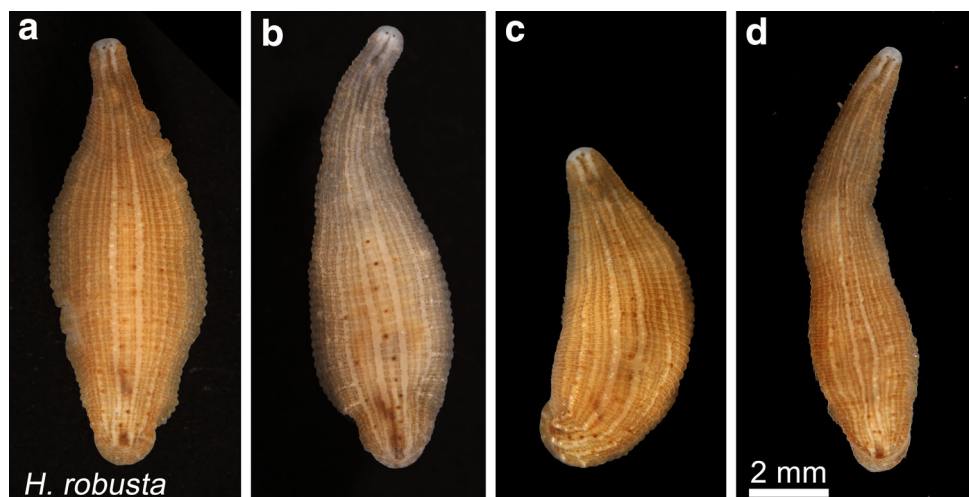
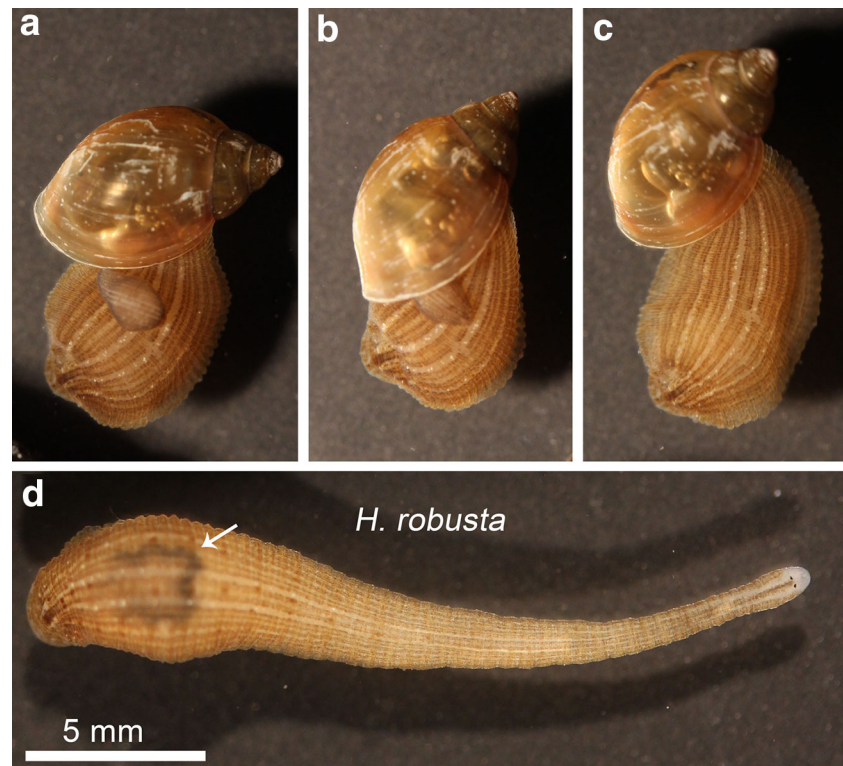


Fig. 6 Feeding behavior of the leech “*H. robusta*”, adult individual with one juvenile. The leeches creep into the shell of the water snail (*Physa* sp.) (a, b, c) and suck up the soft parts of the host organism. After the shell is completely emptied, the gut of the adult leech is filled with the fluidized body contents of the water snail (white arrow) (d)



of “*H. robusta*” are also subject to irreversible decline and die-off after variable periods of time. As a result, yet another *Helobdella* was adopted for developmental studies, as described below.

The Texas leech *Helobdella austinensis*: a new model organism

In 1997, a colony of leeches superficially resembling “*H. robusta*” was established in the laboratory of M. Shankland at the University of Texas, Austin, with specimens collected at the mouth of Shoal Creek in Austin, Texas (Seaver and Shankland 2000). As Fig. 8 shows, this species is morphologically similar to “*H. robusta*”, but clearly distinguishable from “*H. triserialis*” Palo Alto (Fig. 2). A second colony, based on descendants from this first population, was established at UC Berkeley. These colonies have been stable for many years (though the Texas colony was eventually closed out intentionally), and other colonies have been founded from them; thus, this Texas leech has emerged as the main species for lab studies of *Helobdella* development. It became apparent over time that this leech differs significantly from “*H. robusta*” both developmentally (e.g., Kuo and Shankland 2004) and as judged by CO1 sequence divergence (Bely and Weisblat, 2006). 2 years ago, therefore, this species from Austin, TX, was described as a new species, *H. austinensis* (Kutschera et al. 2013). Further support for the depiction of *H. austinensis* as a

distinct species comes from the observation that it is incapable of self-fertilization, in contrast with both “*H. triserialis*” and “*H. robusta*” (M. Levine, V. Rogers, D.A.W., unpublished observations).

In the laboratory, *H. austinensis* feeds on water snails (*Physa* sp., *Planorbis* sp.), *Chironomus* larvae, and wounded crustaceans (*Asellus* sp., *Gammarus* sp.), but *Tubifex* worms are ignored. The stability of this species in the lab, coupled with the ability to maintain breeding populations on commercially available, frozen *Chironomus* larvae, has led to its current position as the most tractable representative of the genus *Helobdella*, and thus the most suitable leech species for studies of early annelid development.

The Two-eyed flat leech “*Helobdella stagnalis*”: type species of the genus

In 1758, the Swedish naturalist Carolus Linnaeus (Linnaeus 1758) described the first *Helobdella* species: the small, dark-gray, ca. 10 mm long European “two-eyed flat leech”, *H. stagnalis*, which therefore represents the type species of the genus *Helobdella* (Blanchard 1896; Castle 1900). “*H. stagnalis*” is a largely unpigmented leech characterized by the presence of a dorsal chitinous scute (Fig. 2). As defined primarily by this unambiguous feature, “*H. stagnalis*” appears to be a cosmopolitan species, found in freshwater ecosystems around the world, with the exception of Australia (Sawyer 1986). It co-occurs with the



Fig. 7 Degeneration and death in an adult individual of “*H. robusta*” by a fungal infection that originates in the head region of the leech (a, b). On the ventral side of the dead body of the mother leech, juveniles are still attached that leave the decaying adult (c, d)



Fig. 8 Two representative specimens of the variable Texas leech (*H. austinensis*), dorsal view. This species, which can easily be maintained in lab populations, serves as model organism for Evo-Devo studies in annelids

other *Helobdella* species we have collected between September 2007 and February 2015 in the Bay Area, CA (USA). Unexpectedly, however, different geographical isolates of this “species” exhibit CO1 sequence divergence

as great as that between various described species (Oceguera-Figueroa et al., 2010; D. V. Rogers and C. J. Winchell, personal communication). Thus, as for some of the other *Helobdella* species, the taxonomic status of “*H. stagnalis*” is no longer clear, either.

Populations of “*H. stagnalis*” can be easily maintained in the laboratory, feeding avidly on *Chironomus* larvae and other aquatic invertebrates, especially oligochaete worms of the genus *Tubifex* (Kutschera and Wirtz 1986, 2001). Indeed, “*H. stagnalis*” can be a serious pest affecting the commercial production of aquatic oligochaetes such as *Lumbriculus* sp. Nonetheless, this “species” is rarely used for developmental studies. In our experience, this could be because anyone attempting to remove the cocoons or embryos from “*H. stagnalis*” might easily become discouraged. In sharp contrast to any of the other species described here, “*H. stagnalis*” is extremely agile, ceaselessly twisting and contorting itself so as to frustrate any, but the most adept scientist who seeks to remove its embryos without damaging them and/or the adult “mother” leech. This struggle is accompanied by secretion of copious amounts of mucous (by the leech), which further complicates handling the embryos, even if they can be removed. [We now realize that these problems can be circumvented by anesthetizing the cocoon-bearing adults by exposure to clove oil (10 μ l per 50 ml medium; C.J. Winchell, personal communication)]. It might be thought that this dramatic behavioral difference between “*H. stagnalis*” and the other *Helobdella* species reflects a further refinement of the extensive parental care exhibited by glossiphoniid leech species. We speculate, however, that the physical prowess of “*H. stagnalis*” serves primarily for feeding, and that their capacity for rapid movements have co-evolved with that of their oligochaete hosts, which also exhibit active escape responses (note that the other three *Helobdella* species discussed here, “*H. triserialis*”, “*H. robusta*”, and *H. austinensis*, do not feed on *Tubifex* worms). In senescence, the “*H. stagnalis*” we have studied do not exhibit the anterior-to-posterior progressing degeneration that is typical for “*H. robusta*” (Fig. 7). Instead, this species tends to lose pigmentation and swells substantially before dying.

Finally, it should be noted that the biology of the invasive species *H. europaea* (Kutschera 1987, 2004) has been studied in some detail. However, with respect to Evo-Devo, this taxon is of subordinate importance.

Discussion

A major goal for Evo-Devo biologists is to understand the ontogenetic changes that explain the phylogenetic development of the phenotype. As one strategy to achieve this

goal, scientists individually or collectively compare the developmental patterns of diverse species, mapping similarities and differences onto phylogenetic trees that depict the relationships of the species under investigation. Similarities suggest developmental features that might have been present in their last common ancestor, while differences reflect changes in developmental processes leading from that ancestor to either one or both of the descendants.

Given resource limits, there is an unavoidable tradeoff in the Evo-Devo enterprise between sampling many taxa and studying any available species in depth to understand the range and perhaps the limits of what evolution has explored in terms of changing developmental processes and body plan diversity. Although zoological Evo-Devo dominates this branch of the life sciences (Akam 1998; Hall 1999; Olsson et al. 2010; Minelli and Baedke 2014), it should be noted that plant biologists have also adapted this approach. Specifically, the reconstruction of the evolutionary “green lineage” of photoautotrophic organisms rests to a large extent on the principles of Evo-Devo research (Niklas and Kutschera 2009, 2010). This botanical aspect is not further discussed here.

For the most part, Evo-Devo is undertaken as an essentially retrospective process, aimed at reconstructing the history of developmental changes underlying body plan diversification and/or to deduce the genome, developmental processes and basic structure associated with ancient forms, such as the last common ancestor of a taxon of interest. For that purpose, there is an emphasis on identifying and studying organisms that are thought to be representative of basal groups, and a parallel tendency to dismiss so-called “derived” species as irrelevant to the field. Despite the predominance of the retrospective approach to Evo-Devo, it is of interest to complement it with what we may call a prospective approach, concerned with appreciating the wide range of possible variation in genome architecture and developmental processes that have been explored by evolution and how such variants may contribute to the emergence of new body plan features. For this aspect of Evo-Devo, derived or rapidly evolving species may be of particular interest.

As outlined briefly above, a variety of studies focusing on the biogeography and development of the genus *Helobdella* has revealed an unexpected diversity of species within this genus, compared to other genera of glossiphoniid leeches (Oceguera-Figueroa et al. 2010; Salas-Montiel et al. 2014). Correlated with this, whole genome sequencing revealed that the genome of “*H. robusta*” has undergone extensive rearrangements relative to the inferred bilaterian, protostome and lophotrochozoan ancestors that are unmatched even by the rapidly evolving genomes of the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. This is seen at the chromosomal

level, as judged by the extensive loss of macrosynteny relative to other genomes, and at the micro-scale by the near total disruption of the famous Hox gene cluster, which remains largely or completely intact in two other lophotrochozoans sequenced in parallel with *Helobdella* (Simakov et al. 2013).

Loss of macrosynteny requires that genes have changed their relative positions not just within their original chromosome (e.g., by inversion mutations), but also from one chromosome to another, for example by translocation mutations that shift sections of one chromosome to another. Heritable (i.e., germ line) translocations are normally lethal because they result in mismatched chromosomes and altered gene dosages when the gametes of the mutant individual unite with those of a wild-type conspecific through mating and sexual reproduction.

Many species “set aside” their germ line precursors early in development, which reduces the number of potentially damaging mitotic divisions to which the germ line is subjected in each generation (Extavour and Akam 2003; Niklas and Kutschera 2014, 2015); in the nematode *C. elegans*, for example, primordial germ cells (PGCs) are set aside from somatic lineages at the fourth zygotic mitosis (Kimble and Hirsh 1979). For *Helobdella*, however, the male and female PGCs share a common lineage with segmental mesoderm for 23 rounds of zygotic mitosis (Cho et al. 2014; Weisblat and Kuo 2009, 2014). A modified and updated scheme illustrating this novel finding is depicted in Fig. 9.

Moreover, while most clitellate annelids are simultaneous hermaphrodites (Elliott and Kutschera 2011), the genus *Helobdella* is, to the best of our knowledge, unique for its class in that most of the species examined can reproduce by self-fertilization. We speculate that this combination of features in *Helobdella* creates a “perfect storm”, favoring genome scrambling and sympatric speciation in this group of aquatic annelids. This hypothesis rests on the following three observations: (1) the delayed segregation of germ line from soma during development (Fig. 9) provides increased opportunities for mutations, including chromosomal translocations in the lineages leading to PGCs; (2) the co-segregation of male and female germ line precursors makes it likely that any such translocations will be shared by eggs and sperm on that side of the animal; (3) the capacity for self-fertilization favors the probability of a union between eggs and sperm sharing the same translocation. This scenario leads to the rescue of otherwise lethal translocation mutations, thus accelerating the process of genome scrambling and providing for the efficient reproductive isolation of nascent species that share the same habits and ecological niches (Mayr 1942, 1982). We note that this same combination of features would tend to suppress the inheritance of recessive lethal mutations, so that, despite

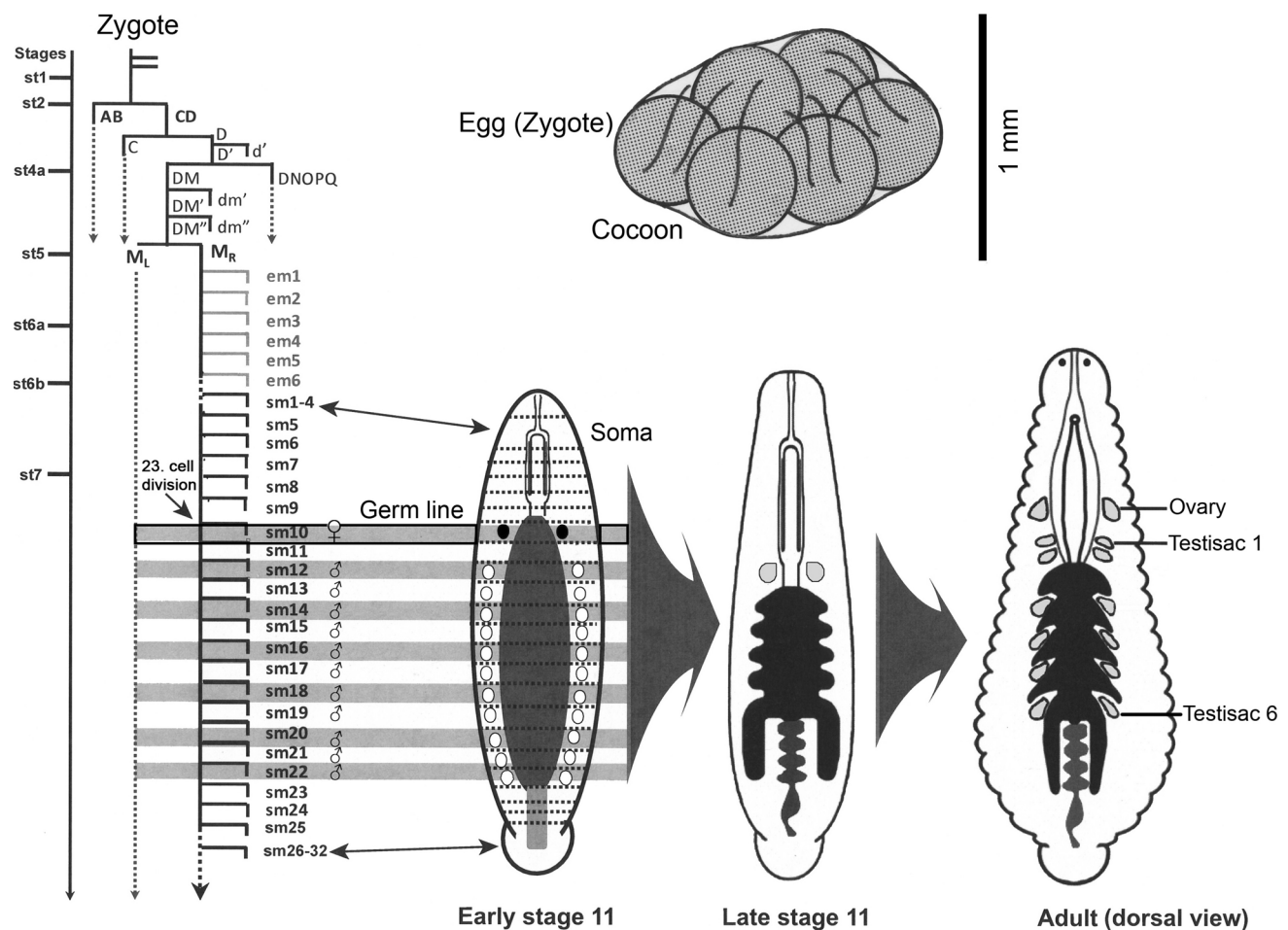


Fig. 9 Schematic rendering of a cocoon with fertilized eggs (zygotes), and cell lineage diagram in leeches of the genus *Helobdella*. The model highlights the divisions leading from the zygote to the segregation of male and female germ line from somatic

cells. Note that the male and female germ lines segregate from one another only after 23 rounds of zygotic mitoses (adapted from the corrected version of Cho et al. 2014)

the rapid evolution of the *Helobdella* genome in terms of rearrangements, the branch lengths of individual protein coding genes are normal (Simakov et al. 2013).

Our speculation regarding the mechanisms for sympatric speciation and genome rearrangements in *Helobdella* predicts that even closely related *Helobdella* species will differ by translocation mutations. Moreover, if self-fertilization among clitellate annelids is limited to the genus *Helobdella*, and self-fertilization is essential for genome scrambling to occur, then genomes of other clitellate species should be more similar to other annelids and the scrambling should be limited to *Helobdella*. These predictions may be tested by sequencing the genomes of other clitellates, and comparative genomic analyses.

In conclusion, if one is to be entirely rigorous, any species of animal can only truly be a “model” for itself, especially from the point of view of those interested in Evo-Devo. Nonetheless, glossiphoniid leeches of the genus

Helobdella provide experimentally tractable embryos that can be studied to provide clues as to the nature of developmental changes accompanying the evolution of leeches, clitellate annelids, spiralian, lophotrochozoans, bilaterians, and metazoans, according to one’s interests and the species chosen for comparison (Lai et al. 2009).

Moreover, the genus *Helobdella* is comprised of numerous morphologically derived species relative to other annelids, and with what appears to be a highly dynamic genome compared to most other bilaterian animals. Hence, these freshwater leeches may be particularly well-suited for the prospective Evo-Devo approach of exploring the permissible range of possible genome architectures and associated developmental processes.

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