Emerging developmental genetic model systems in holometabolous insects

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Abstract

The number of insect species that are amenable to functional genetic studies is growing rapidly and provides many new research opportunities in developmental and evolutionary biology. The holometabolous insects represent a disproportionate percentage of animal diversity and are thus well positioned to provide model species for a wide variety of developmental processes. Here we discuss emerging holometabolous models, and review some recent breakthroughs. For example, flies and midges were found to use structurally unrelated long-range pattern organizers, butterflies and moths revealed extensive pattern formation during oogenesis, new imaging possibilities in the flour beetle *Tribolium castaneum* showed how embryos break free of their extraembryonic membranes, and the complex genetics governing interspecies difference in head shape were revealed in *Nasonia* wasps.

Introduction

The breakthrough in developmental genetics in the late 1970's and 1980's was to a large extent driven by research on a holometabolous insect, the "fruit fly" (vinegar fly) *Drosophila melanogaster* [1]. With the discovery of the homeobox, research in this species also inaugurated the molecular phase of evolutionary developmental biology [2,3], which remains a thriving field in the 21st century [4]. In the 1990's, results obtained in *Drosophila* became the starting point for systematically exploring the developmental gene network in other insects and beyond [5-9].

The pace with which new experimental insect systems and research questions have been added to the rapidly growing field of comparative developmental genetics has been accelerating in the last 20 years, owing to important technological advances, such as RNA interference (RNAi) [10,11], next-generation sequencing (NGS) [12], and genome editing technology [13,14]. But, owing to other challenges such as culture techniques, access to embryos, sample transparency, eggshell removal, or delivery of nucleic acids or proteins to the tissues of interest, the number of insect species in which developmental gene networks has been examined with functional approaches is still rather small. Here, we review progress with experimental systems that belong to the extremely large group of holometabolous insects, which make up roughly half of all species described and nearly 85% of the insects [15]. The Holometabola form a monophyletic group [16] whose defining developmental feature is the specialized larva, which is probably homologous to the less-specialized nymph of hemimetabolous insects [17-20]. Comparative developmental genetic studies have been carried out on all life stages but mostly on embryos. As embryologists, we focus here on species that

are suitable for functional genetic analysis in embryos. The examples that we choose belong to the four dominant holometabolous insect orders Diptera (midges, flies), Lepidoptera (moths, butterflies), Coleoptera (beetles), and Hymenoptera (sawflies, wasps, bees, ants).

Diptera

The Diptera include approximately 152,000 named species of midges and flies that evolved through several episodes of accelerated radiation since their late Permian origin around 250 million years ago (Figure 1, [21]). With the “fruit fly” *Drosophila melanogaster*, the dipterans also include one of the most studied genetic model organisms of animal development (<http://flybase.org/>). They are therefore exceptionally well suited for studying the evolution of developmental mechanisms at the molecular level. This has long been recognized in the *Drosophila* field where, by exploiting genetic hybridization approaches and germ line transformation technology, multiple *Drosophila* species have played an important role in understanding the genetic basis of evolutionary parallelisms and novelties [e.g. 22,23-29].

Additional dipteran species have been developed as developmental genetic models because they promise insights into the evolutionary origin of *Drosophila* traits, are interesting in their own right, or have translational potential in applied entomology. Here, we focus on distant relatives of *Drosophila* that have been shown to be amenable to developmental genetic studies at the functional level and review some of the reasons for which they have caught the eye of evolutionary developmental biologists.

a) Nematocera

The midges (“Nematocera”) represent multiple basal lineages of dipterans that originated early in the Triassic (247-208 MYA). Best known among them are the mosquitoes, which belong to the monophyletic Culicomorpha, one of the four major branches of lower dipterans. Work on the developmental gene network in mosquito embryos has high translational potential. Work on the developmental gene network in mosquito embryos has high translational potential [e.g. 30,31,32], but is difficult because the pigmented eggshell of the most important disease vectors in this group is difficult to remove and poses a significant obstacle to embryological work.

Nevertheless, the developmental gene network of a malaria vector, *Anopheles gambiae*, has been examined in a series of gene expression studies [33-37]. More recently, *Aedes aegypti*, a vector of Dengue, Yellow fever and Zika, has emerged as one of the first mosquito models in which the embryonic gene network has been probed, not only at the level of gene

expression, but also at the level of gene function, using gene knockdown through RNA interference [38-42]. The potential of *A. aegypti* as a developmental genetic model organism was recently demonstrated in an impressive study that combined expression profiling, germ line transformation, genome editing, and genetic approaches to identify a dominant male-determining factor in this species [43].

The non-biting chironomids are distant relatives of mosquitoes on the Culicomorpha branch that are more closely related to the disease-transmitting black flies (*Simulium*). Chironomids have a long history in developmental biology. We owe to this group the first detailed description of insect embryogenesis, and the first wax models for teaching this subject [44]. Chironomid embryology in the 19th century and some 20th century sequels have been reviewed by the late insect embryologist Klaus Sander [45]. In the 20th century, *Chironomus* served as a model for establishing the concept of differential gene activity that was first proven using polytene chromosomes in the salivary glands chironomid larvae [46,47].

Chironomid embryos also served as a model for revealing the importance of localized RNA in insect eggs to establish head-to-tail polarity in the developing embryo. In 1964, Hideo Yajima had reported that the centrifugation, or partial ultra-violet light irradiation of *Chironomus* eggs suppresses anterior structures and induces the formation of a mirror image tail end [48]. Such “double abdomens” were also obtained with another chironomid species [49].

Subsequent work by Klaus Kalthoff and his collaborators, including biochemical approaches [50,51], provided evidence for localized maternal RNA in the anterior pole of the deposited egg as the cue for anterior body patterning [52]. This hypothesis was proven a decade later in *Drosophila* with the discovery of the homeobox gene *bicoid* [53]. Sander’s review of chironomid embryology ends with Yajima’s and Kalthoff’s experiments to which he refers as the first link between “classical” and “molecular” epochs of experimental embryology.

After the discovery of *bicoid*, chironomid embryology has been lingering in obscurity but Yajima’s and Kalthoff’s work became the starting point for its recent revival. Growing confidence that most flies lack a *bicoid* gene prompted the question of which localized RNA establishes head-to-tail polarity in chironomids [54-56]. This question was addressed by expression profiling of bisected *Chironomus riparius* eggs, RNA interference and genetic rescue experiments, and led to the discovery of a cysteine-clamp gene (*panish*), whose maternal transcripts are localized at the anterior pole and prevent double abdomen formation [57].

Since most dipterans lack homologs of both *bicoid* and *panish*, such developmental regulators may have evolved frequently *de novo* and it will be interesting to understand the structure of the developmental mechanisms that allow this unexpected evolutionary pattern. It

is also worth noting that with this study and the recent publication of a successful germline transformation experiment [58], *Chironomus riparius* has become a promising model for the functional analysis of embryonic gene networks in lower dipterans, which are currently short of suitable experimental systems.

Moth flies (Psychodidae) belong to an isolated branch of midges (Psychodomorpha) that probably originated very early during the dipteran radiation [15,21,59]. Within this group, *Clogmia albipunctata* (Psychodinae) is an attractive model for comparative molecular embryology in dipterans because of its isolated phylogenetic position, the ease with which it can be reared in the laboratory, and the possibility of controlled egg activation.

The eggs of this species develop synchronously and are fertilized in the ovary but start embryonic development only after being exposed to an osmotic shock, as reported for a related species [60]. Normally, this osmotic shock occurs at the time of oviposition into a moist environment. In the laboratory, embryonic development also starts when the eggs of dissected ovaries are exposed to water.

The early embryos lack morphologically distinct pole plasm and pole cells (precociously formed germ cells) as well as a cephalic furrow [61], a transient ectodermal fold that serves as a landmark in gastrulating embryos of higher flies (Cyclorrhapha) [58]. The first instar cuticle of *C. albipunctata* is rich in segment-specific morphological markers [62]. This is a considerable advantage of this experimental system because it facilitates the initial characterization of genetically induced perturbations of the embryonic gene network [63].

C. albipunctata has been used as a model in descriptive molecular embryology since the mid 1990's [56,64-71]. Embryonic transcriptome data and a staging scheme for embryos are also available [59,61]. Recently, protocols for RNA interference in embryos have been established (Eva Jiménez- Guri and Johannes Jaeger, personal communication; Yoseop Yoon and Urs Schmidt-Ott, unpublished data).

b) Brachycera

The flies (Brachycera) are monophyletic and include stout-bodied and fast-moving flies with short antennae. The brachyceran species that we review below all belong to the equally monophyletic higher Brachycera (Cyclorrhapha), which develop as maggots and pupate in the hardened skin of the last larval instar (puparium). Among them, the scuttle flies (Phoridae) and hover flies (Syrphidae) represent independent basal lineages and are therefore important for reconstructing the evolution of development in cyclorrhaphans.

Two species of phorids, *Megaselia abdita* and *Megaselia scalaris*, both well-known to forensic biologists [72,73], have been used to study the evolution of sex determination [74,75] and mechanisms of embryo development, including axis specification and patterning [76-82], extraembryonic membranes [83-86], and heterochronic shifts [87].

Laboratory cultures of these species are inexpensive and easy to maintain [88,89] and embryonic transcriptome data for *M. scalaris* and *M. abdita* are publically available [59,90]. PiggyBac-mediated germline transformation has been achieved in *M. abdita* [58]. Among the syrphids, *Episyrphus balteatus* has been used as a model for functional and comparative embryological studies [83,91,92]. A specific advantage of *E. balteatus* as a model in comparative dipteran embryology is the ease with which the eggshell can be removed. Limited embryonic transcriptome data are also available [93]. A disadvantage of this experimental system is that the maintenance of a laboratory culture is rather laborious, as it requires a supply of aphids and their host plant for larval feeding and egg collection.

One lineage of the Cyclorrhapha underwent a burst of rapid radiation in the early Tertiary (65-40 MYA), giving rise to roughly nine tenths of the cyclorrhaphan family diversity, including *Drosophila*, the house flies (Muscidae) and blow flies (Calliphoridae). As distant schizophoran relatives of *Drosophila*, the house flies and blow flies are suitable for generalizing findings in *Drosophila* for the Schizophora. The use of the house fly as a model in comparative molecular embryology of insects was initiated in the early 1990's [5]. The eggs of this species are much larger than those of *D. melanogaster* and also provide a good model for addressing questions of scaling during embryonic pattern formation, along with blow flies that also develop large eggs (e.g. *Calliphora erythrocephala*, *Lucilia sericata*) [94-98]. Sex determination in *Musca domestica* is polygenic and varies between populations, making this species an attractive model for the evolution of this process [99]. The genome of *Musca domestica* has recently been sequenced [100] and RNA interference as well as germ line transformation has been successful [101,102]. Blowflies (*Calliphora vicina*) also serve as a model for studying the evolution of spatial patterning [103,104].

Lepidoptera

The Lepidoptera (moths and butterflies) appear in the fossil record in the early Jurassic (190 MYA) and underwent a spectacular radiation in the Cretaceous and Tertiary (Figure 1, [15,16]). The monophyletic butterflies evolved in the early Tertiary within a group of higher moths, which includes about 60% of the described lepidopteran species.

By far the most important genetic model organism in the Lepidoptera is the silkworm *Bombyx mori* (Bombycoidea) [105], which is only known as a cultivated species [15]. *B. mori* belongs to one of the basal macrolepidopteran lineages. Advanced technologies for genetically manipulating this species have recently been reviewed [106], along with many other aspects of its biology (230 Pubmed-listed review papers). The wing patterns of various butterfly species have been studied in the context of pattern formation, evolutionary novelties, mimicry and sexual dimorphism, mostly using genetic and association mapping, transcriptome and genome sequencing, and gene expression analyses. This work, too, has recently been reviewed elsewhere [107,108].

The embryology of lepidopterans has been studied in a wide range of species, including representatives of the basal lineages [109]. This work has revealed aspects of lepidopteran embryology not found in other Holometabola, including specialized blastokinetic movements and extraembryonic membrane development, and the unique mode of blastoderm formation reported for some macrolepidopteran species and close relatives (Pyraloidea). In these species, the blastoderm forms without the syncytial blastoderm phase. Instead, individual cleavage nuclei cellularize and divide after reaching the periplasm of the egg. The evolution of this mode of blastoderm formation may correlate with extensive pattern formation during oogenesis (!), which has been reported for *B. mori* and the butterfly *Pararge aegeria* [110-114].

This unexpected finding prompts questions about differences in oogenesis between these species and other insects. A detailed analysis of the ovarian transcriptome of *P. aegeria* is already available but the effectiveness of RNA interference and genome editing in this species remains to be demonstrated [115]. The analysis of embryonic patterning in butterflies will require an experimental system with easy access to large numbers of ideally transparent eggs, in which the eggshell can be removed effectively, and in which both parental and embryonic RNA interference are effective. However, the efficiency of RNA interference varies considerably between lepidopteran species [116]. While a range of lepidopterans have now been found to be amenable to functional genetic studies in one way or the other [117,118], it remains to be demonstrated that any of these species is suitable for an efficient functional dissection of the embryonic gene network.

Once such an experimental system has been identified, the molecular embryology of lepidopterans should quickly mature and become an exciting area of evolutionary developmental biology.

Coleoptera

The beetles (Order: Coleoptera) are the most speciose order of insects, with around 400,000 species, and make up a quarter of described animal diversity [119], and are an outgroup to Lepidoptera+Diptera (Figure 1, {Misof, 2014 #16}). The developmental genetics of this prodigious clade has been represented almost exclusively by the red flour beetle *Tribolium castaneum* in the molecular era. Reasons for this include its ease of use in the lab [120], applicability of RNA interference (RNAi) [121,122], germline transformation [123] and associated tools [124,125], and a fully sequenced genome [126]. *Tribolium* has largely been a model for understanding the short germ mode of embryonic segmentation, which involves progressive growth and patterning at the posterior (Figure 2, [127]), and usually an embryonic rudiment that is small relative to the size of the entire egg. This developmental mode bears superficial similarity to vertebrate segmentation and contrasts with the long-germ mode found in *Drosophila* and in some other derived holometabolous lineages, where patterning of the body plan is largely simultaneous and does not rely on significant extension of the embryo [127].

Recently, these resources have been augmented by the application of CRISPR/Cas9 genome engineering, which allows targeted gene disruption and insertion of exogenous sequences using homologous recombination [128]. In addition, *Tribolium* has shown itself to be amenable to high resolution 3-dimensional time lapse imaging using light sheet technology [129]. Finally, an innovative genome wide screen took advantage of the highly effective systemic RNAi in *Tribolium* [130]. This screen identified numerous genes that are involved in specific developmental processes including embryonic and appendage patterning and morphogenesis, stink gland function and ontogeny, and oogenesis [130]. The bioinformatics required to support this massive undertaking also resulted in the production of a robust and very useful web portal for *Tribolium* genomics and phenomics [131].

The powerful tools available in *Tribolium* have facilitated recent advances in developmental genetics. For example, deeper understanding of the wave-like modes of gene expression that pattern the late embryo as it grows and segments was achieved using novel live imaging and computer modeling techniques alongside well established histological approaches [132-135]. These results provide valuable insights into how the mechanisms employed in *Tribolium* are similar to and different from segmentation mechanisms in vertebrates.

Significant progress was also made in understanding the cellular basis of *Tribolium* embryogenesis. One particularly interesting study discovered a novel function of Innexin in the process of cellularization that does not exist in *Drosophila* [136]. Another uncovered a unique interaction between extraembryonic epithelia, as the embryo undergoes the last stages of

development before hatching [137]. The extraembryonic membranes are of additional interest, as they are highly reduced in size and function in *Drosophila*, while most insects have full and functional membranes [138]. Aside from their morphogenetic function, extraembryonic membranes also serve important roles in immunity and desiccation prevention [139,140]. A transcriptomic approach identified genes expressed specifically in the serosa [141], which can now be functionally examined in *Tribolium*.

Tribolium is also an important model for understanding the evolution of appendage development among the insects, and analyses of antenna, wing, and imaginal disc development [142-144] have yielded novel insights that will be built upon in the future.

While *Tribolium* has been the main model for beetle developmental genetics, several other species are emerging as models to address a broad palette of topics. Of particular note are the horned scarab beetles. These species are models for morphological polyphenism in response to ecological conditions [145,146]. Functional approaches for uncovering the molecular basis of the dramatically divergent developmental outputs that can be encoded in a single genome are available in the dung beetle *Onthophagus*. RNAi works [147], and a genome is currently in progress (<https://www.hgsc.bcm.edu/arthropods/bull-headed-dung-beetle-genome-project>). Another beetle, the firefly [148] is emerging as a model for the origin of evolutionary novelties. Finally, a carrion beetle that has adapted to a cave environment is emerging as an insect model of regressive evolution [149].

Tribolium has long been the main model for understanding short germ embryogenesis. However, the *Tribolium* mode is not necessarily typical for the beetles. Previous studies have shown that beetles like *Callosobruchus maculatus* have long germ embryogenesis, *Dermestes maculatus* has an intermediate type [150], and species such as *Atrachya menetriesi* [151] and *Diabrotica* [152] have extremely short germ rudiments. Transcriptomic and functional resources focused on embryonic patterning have been developed for *Callosobruchus* and *Atrachya* [153]. Similarly *Dermestes* is being developed into a tractable intermediate germ models system [154]. *Diabrotica* is a major agricultural pest, leading to the development of many techniques, such as RNAi, and genomic resources that could be applied to developmental questions related to patterning an extremely small germ rudiment [155].

Hymenoptera

The hymenoptera are the basally branching lineage of the Holometabola (Figure 1, [16,156]), meaning that an early bifurcation of the holometabolan clade gave rise to the lineage leading to modern hymenopterans, and another lineage that would produce all of the other

holometabolous orders. Thus, the Hymenoptera are a crucial sampling point for understanding the evolutionary history of traits occurring in this lineage. They are also highly diverse with over 125,000 described species, and likely many thousands more that remain to be described [15].

Two species have been the main models for hymenopteran developmental genetics of embryonic patterning: The wasp *Nasonia vitripennis*, and the honeybee *Apis mellifera*. *Nasonia* and *Apis* represent the oldest divergence within the Apocrita (all ants, bees, and wasps) [157], which represents the vast majority of hymenopteran diversity. *A. mellifera* has a well annotated and assembled genome [158], is amenable to embryonic RNAi [159], and germline transformation using transposons has recently been demonstrated [160]. *N. vitripennis* also has a sequenced and annotated genome [161], and is amenable to a variety of gene knockdown approaches, including embryonic RNAi, parental RNAi, and morpholino mediated knockdown [162-164]. Germline transformation has had limited success in *Nasonia*, while CRISPR/Cas9 based methods seem to work (JAL, Michael Perry, personal communication). *Nasonia* embryos are relatively easy to work with, and the content of the eggs facilitates fluorescent imaging, which paved the way for multiplex in situ hybridization, which in turn aids the robust interpretation of RNAi experiments [165]. A large web portal for hymenotperan genome sequences is now available (<http://HymenopteraGenome.org>)[166], This database is complemented by a *Nasonia*-specific database with additional annotations and functionality (www.waspatlas.com) [167]

Nasonia and *Apis* are used as models for an independent evolutionary derivation of long germ embryogenesis. While *Drosophila* is now held up as the exemplar of the long germ type, it should be noted that *Apis* was used to introduce this concept [127]. In both *Nasonia* and *Apis*, the expression domains of early zygotic segmentation genes (gap genes) are approximately comparable to those in *Drosophila* [159,168-171]. This indicates that long-germ patterning mechanisms evolved partly in parallel in wasps and flies. (Figure 2).

In *Drosophila*, the pair-rule segmentation genes function downstream of the gap genes. These genes are expressed in stripes of two-segment periodicity and the borders of these stripes are set directly by short range gradients of gap gene proteins [172,173]. Surprisingly, pair-rule gene expression dynamics differ between *Nasonia* and *Drosophila*. For example, some of the pair-rule genes of *Nasonia* are expressed in a wave-like pattern that is reminiscent of the progressive segmentation process in short germ embryos such as *Tribolium* (Figure 2, [163]).

Thus, in contrast to *Drosophila*, it appears that regionalization (reflected in gap gene expression domains), and the process of demarcating segments within the regions (reflected in

pair-rule expression) may be partially decoupled, especially at the posterior, in the *Nasonia* embryo. This may reflect the rule for long germ embryogenesis, and *Drosophila* the exception, as indications from other long germ embryos are that posterior pair-rule patterning is not complete at gastrulation, while all relevant gap domains are in place and in similar proportions to those seen in *Nasonia* and *Drosophila* (e.g., *Apis* [159,174], *Clogmia* [175], and *Callosobruchus*, ([150], JAL personal observation)).

While the implications of long germ embryogenesis are most obvious for the anterior-posterior axis, the dorsal-ventral (DV) axis is also patterned more completely before gastrulation in long germ embryos compared to short ([176-178], Figure 2). The *Nasonia* DV patterning system recently revealed divergent strategies among holometabolous insects to achieve robust DV patterning outputs. [165]. In *Drosophila*, a morphogen gradient of activated Toll signaling is the sole source of embryonic DV polarity [178]. A role for Toll in axial polarity is unique to insects, while most other animals use bone morphogenetic protein (BMP) signaling to establish embryonic DV polarity [179]. Surprisingly, it was found that BMP signaling is required for almost all embryonic polarity and patterning in *Nasonia* while Toll has a much reduced role in comparison to *Drosophila* [165]. These findings indicate that the most recent common ancestor of *Nasonia* and *Drosophila* used both Toll and BMP signaling to partition the DV axis. Thus, during the convergent evolution of long germ patterning in *Drosophila* and *Nasonia*, the problems of increased demand for patterning information were solved in divergent ways.

Nasonia is also emerging as a powerful model for understanding the molecular basis of the evolution of adult form. Morphologically distinct species can be mated in the lab to produce fertile hybrid females. These hybrid females will lay eggs as virgins, and all of the progeny will be haploid, hybrid males [164]. The haploidy of males greatly facilitates downstream genetic analysis, and has allowed the identification of causative loci underlying species differences in wing shape [180,181]. The powerful tools available in *Nasonia* were recently applied to understand the evolution of head shape, where multiple genes are involved in head shape differences between species, and complex epistatic interactions lead to novel phenotypes in hybrid males [182].

Parasitic wasps related to *Nasonia* in the superfamily Chalcidoidea are also being sequenced. One, *Copidosoma floridanum*, is polyembryonic, meaning that from one laid egg, many individual wasps will emerge (up to 1000)[183]. This mode of embryogenesis is highly divergent and must incorporate numerous novelties, the nature of which is still largely unknown. Another species, *Trichogramma pretiosum*, lays an extremely small egg, which develops into a larvae that shows no outward signs of segmentation until pupation [184]. Finally, a genome of

the fig-wasp *Ceratosolen solmsi* has been sequenced [185]. While its complex life history will inhibit its adoption as a developmental model organism, its genome will be an important resource in understanding the evolution of molecules involved in Chalcid development.

The genomes of many other hymenopteran species have been or are currently being sequenced which will be a boon for understanding the patterns of developmental evolution in this clade where an incredible amount of developmental diversity resides. This includes a social wasp [186], multiple bee [187,188], and several ant [189-192] species. While development is not the main focus of these communities, it is already clear that ants, bees, and wasps will be quite informative developmental models, especially in regard to developmentally plastic traits (e.g., [193-195]).

Also of note are the genomes of insects stemming from the basal hymenopteran lineages. *Orussus abientus* represents the sister lineage of the Apocrita, and could thus serve as an important model for understanding the evolutionary origin of unique features found in the higher wasps, ants, and bees, such as the “wasp waist” which is found in most Apocritan lineages, but is absent from *Orussus*, or the giant eggs produced in this genus, which can be longer than mother producing them [196]. However, so far little appears to be known about the development of these creatures. In contrast, the more basally branching sawfly *Athalia rosae* has a history as a laboratory model. It is amenable to a wide array of functional techniques including RNAi [197], germline transformation [198], and TALENs [199]. Some work on embryonic patterning has been performed [200], and recently patterning of the wings has become a topic of interest [201]. *Nasonia* and *Apis* share some unusual embryonic features, such as mode of gastrulation and behavior of the extraembryonic membranes [177,202]. The basally branching hymenopterans will be valuable in understanding the origin of these novel morphogenetic mechanisms.

In general the Hymenoptera should provide a rich resources for understanding the limits (or lack thereof) of gene regulatory networks in producing novel developmental strategies, whether in the face of endoparasitism of another insect's egg [203,204], or developing in social milieu [205,206].

Conclusion

Besides the traditional arthropod model *Drosophila*, many new arthropod models are now available for probing developmental genetic questions. In particular, the past few years has witnessed a revolution in both functional approaches and genomic resources that are available for “emerging” model organisms to probe developmental genetic questions. As we have shown,

holometabolous insects are an ideal group to capitalize on these new resources to drive our understanding of how developmental systems evolve. We envision that with even more species being introduced as functionally tractable models, this trend will continue for some time. In the future, knowledge about effectively rearing and handling species might become a key limitation. We therefore believe that providing a broad zoological background to the next generation of developmental geneticists is an important educational goal.

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Figure 1: Phylogeny of Holometabola. Branch points and divergence times are based [16]. Note that the divergence times are subject to ongoing debate and the fossil record of some groups (e.g. Hymenoptera) begins much later [15].

Figure 2. Comparison of long and short germ embryos. A. In long germ embryos, all segmental fates are specified in the embryo prior to gastrulation upper drawing, approximately proportional to their sizes in the emerging larva (lower drawing). In addition, several distinct fates are robustly patterned along the dorsal-ventral axis. **B.** In short germ embryos, only anterior segments are patterned prior to gastrulation (top image). Patterning of posterior segments proceeds as the embryo lengthens (lower two images). Fewer fates along the DV axis are specified prior to gastrulation, and much patterning necessarily takes place during the growth phases. Red= mesoderm, shades of green=different ectodermal fates, blue=extraembryonic. H=head segments, T=thoracic segments, A=abdominal segments, gz=growth zone.

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Figure 1

Figure 2

A

Long Germ

B

Short Germ