Evolving Form and Function: Fossils and Development

Proceedings of a symposium honoring Adolf Seilacher for his contributions to paleontology, in celebration of his 80th birthday

Derek E. G. Briggs, Editor

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Conquering Land, Air and Water: The Evolution and Development of Arthropod Appendages

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Abstract
Arthropods are one of the most successful and diverse animal groups on the planet. With an ability to adapt to a vast spectrum of ecological niches, arthropods have come to dominate the oceans, land and air, largely through changes in developmental strategies and through the modification and specialization of body parts. Clearly, to anyone who has looked at butterfly wings, lobster claws or spider jaws, the appendages of arthropods have evolved striking innovation and diversification of form. Classical work has shed much light on the structural similarities of these appendages and the significance of their differences. Molecular and genetic studies on the arthropod model system *Drosophila melanogaster* have given researchers a good understanding of the molecular basis for appendage development. Yet very little is known about the processes that underlie the diversity of limb form. In recent years there has been considerable progress towards understanding the developmental basis for this diversification. Comparisons of the expression patterns of known limb patterning genes in diverse arthropod groups reveal a striking conservation in some of the pathways, as well as differences that may help explain morphological diversity. Here we explore what is known about appendage development in arthropods, focusing on how homologs of the patterning genes involved in *Drosophila* leg and wing development are deployed during the development of appendages of other arthropods. Additionally, we highlight how recent advances in gene-knockdown technology have allowed researchers to begin testing the function of these genes in systems outside *Drosophila.* Finally, we discuss what this information can tell us about the general principles guiding appendage development in arthropods and what insights it provides into appendage diversity and evolution.

Keywords
Leg development, insects, crustaceans, chelicerates, Hox genes, proximodistal patterning.

Introduction

There are four main groups of living arthropods: (1) crustaceans; (2) hexapods, which include the insects and their close relatives; (3) myriapods, largely made up of centipedes and millipedes; and (4) chelicerates, featuring scorpions, spiders and horseshoe crabs (Table 1). Their appendages differ greatly in number, size and shape. The generalized arthropod appendage is made up of three elements: a protopodite, a telopodite and an exopodite (Figure 1). Historically these elements have been given many names, but we use the nomenclature favored by Boxshall (2004). Data from fossil

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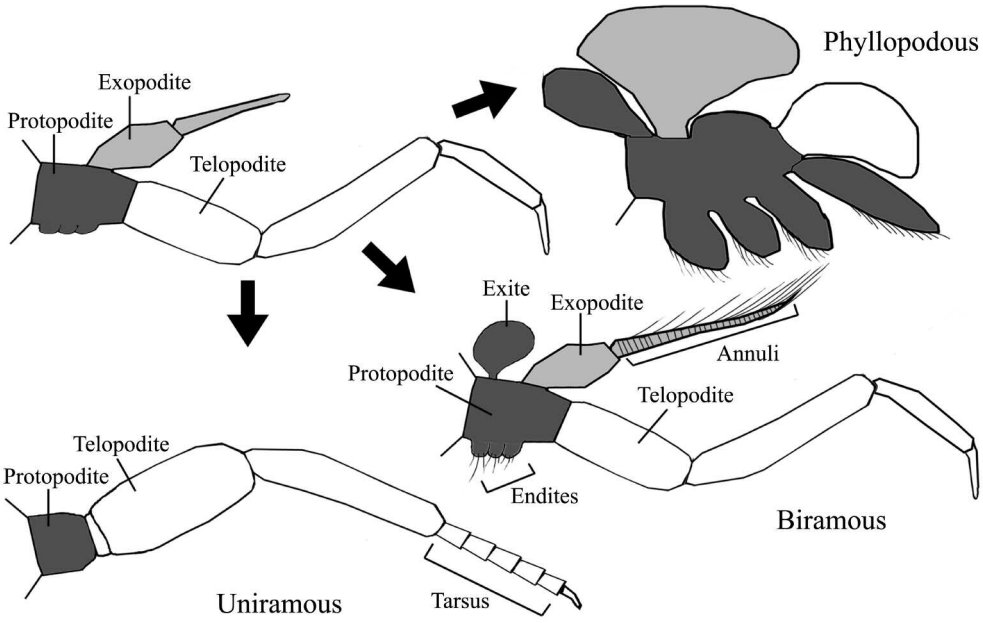

FIGURE 1. A hypothetical ancestral arthropod appendage showing the protopodite (dark grey), exopodite (light grey) and telopodite (white). The three examples of extant limb forms are thought to be derived from the ancestral form.

and extant examples provide insight into what an ancestral arthropod appendage may have looked like.

The protopodite is the basal or proximal element of the limb. The number of its segments varies among different species, but is thought to have consisted of a single segment ancestrally. In crustaceans the protopodite can be subdivided into as many as three segments, named the basis, coxa and precoxa (Boxshall 2004). The insect protopodite is similarly divided, but usually is referred to simply as the coxa. The protopodite can produce a variable number of unsegmented branches; dorsal branches are exites (also called epipods), while ventral branches are endites. Exites and endites, very common in crustaceans, are less so in hexapods, myriapods and chelicerates, although they do occur. Some theories have placed insect wings and spider opisthosomal appendages, such as the spinnerets (opisthosomal segments in spiders are posterior to the leg-bearing segments), as homologs of ancestral exites (Wigglesworth 1973; Averof and Cohen 1997; Damen and others 2002). Alternate views consider insect wings to be novel outgrowths of the body wall (Crampton 1916; Snodgrass 1935). We address this issue in greater detail below. Exites have not been found in early crustacean fossils, and have been posited to have arisen independently multiple times (Boxshall 2004). For this reason exites are not thought to be part of the ancestral arthropod appendage.

The telopodite is a segmented distal element that extends from the protopodite, and is most often the prominent element of the leg of insects, myriapods and chelicerates, and the locomotor or feeding appendage of crustaceans. The telopodite is made up of a variable number of "true" segments and any number of "false" segments. Each "true" segment is innervated by distinct muscle attachments, whereas "false" segments, or annuli, are not (Snodgrass 1935). In general terms, the telopodite has up to five or six primary segments, which, in insects, are the trochanter, femur, tibia, tarsus and pretarsus (or claw). Annulation (the making of "false" segments) of either the primary segments or the terminus of the telopodite can greatly increase the apparent number

Table 1. The major extant arthropod groups, highlighting the groups and species discussed in the text.

Group	Order	Species	Common Name
Chelicerates		*Cupiennius salei*	Spider
Myriapods		*Lithobius atkinsoni*	Centipede
		Glomeris marginata	Millipede
Hexapods	Diptera	*Drosophila melanogaster*	Fruit fly
	Lepidoptera	*Bombyx mori*	Silk moth
	Coleoptera	*Tribolium castaneum*	Flour beetle
	Hemiptera	*Oncopeltus fasciatus*	Milkweed bug
	Orthoptera	*Schistocerca americana*	Grasshopper
Crustaceans	Anostraca	*Artemia franciscana*	Brine shrimp
	Notostraca	*Triops longicaudatus*	Tadpole shrimp
	Isopoda	*Porcellio scaber*	Sow bug

of segments in an appendage. Annulation of primary segments is common in the tarsus of insects, and also occurs in the femur of some chelicerates (Snodgrass 1935; Boxshall 2004). Terminal annulation is most commonly found in the antenna, which bears a flagellum that can have as many as 300 annuli.

The exopodite is less well understood. Most prominent in crustaceans, the exopodite often takes the form of a second, segmented distal arm located dorsal to the telopodite. The exopodite rarely has more than a couple of true segments, but can have many annulations. An exopodite is frequently present in fossil crustaceans and trilobites, highly variable in extant crustaceans, and virtually nonexistent in other arthropods such as hexapods, myriapods and many chelicerates. Regardless, it is considered to be an ancestral feature of the arthropod limb because of its abundance in the fossil record (Manton 1977; Boxshall 2004). The loss of the exopodite correlates with the emergence of terrestrial life, presumably because the exopodite no longer had a function in a dry environment, or it hindered adaptation to the physical demands placed on an appendage used for walking on land. Appendages lacking an exopodite have just a single distal element and are referred to as uniramous, whereas those with both an exopodite and telopodite are called biramous.

The protopodite, telopodite and exopodite make up a generic arthropod appendage representing the ancestral form (see Figure 1). The great diversity of arthropod appendages arose through modification of this form. Reductions in the exopodite or telopodite, addition of exites and endites to the protopodite, and subdivision of segments by annulation are common modifications. Some appendages have proved difficult to derive from the generalized model. The phyllopodous limb of branchiopod crustaceans is a flattened appendage with many branches, none of which are segmented (Schram 1986). In this limb the exopodite and telopodite have presumably been reduced to single lobes, preventing an easy comparison to the generalized form (Manton 1977).

Diversity of appendage form occurs not only between species but also within species. While we consider all appendages to have arisen from a common form, there are distinct differences in appendages depending on their position along the anteroposterior axis. The appendages of the first segment, the antennae, are largely uniramous in both extant and fossil arthropods, with the exception of malacostracan and remipede crustaceans. Trilobites had a series of nearly identical

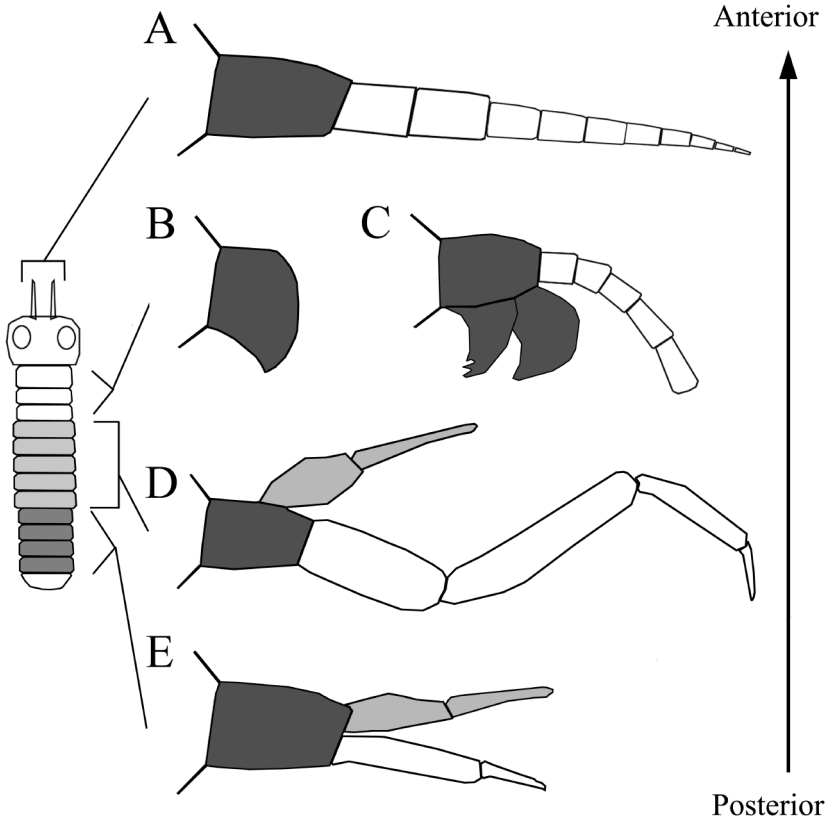

FIGURE 2. Appendage diversity along the AP axis of a generalized arthropod, with the protopodite (dark grey), exopodite (light grey) and telopodite (white) identified. **A,** Antenna. **B,** Gnathobasic mandible. **C,** Branched maxilla. **D,** Biramous leg. **E,** Biramous pleopod.

biramous appendages posterior to the antennae, which could be the ancestral arthropod state (Manton 1977). In contrast, the appendages of extant arthropod groups are regionally specialized (Figure 2). In insects, crustaceans and myriapods at least three segments posterior to the mouth bear gnathal appendages used for feeding. With some notable exceptions, these feeding appendages are more similar across species than they are to other appendages, such as the thoracic legs, within the same species. Likewise, arthropod thoracic legs are generally more similar across species than they are to the mouthparts or antennae. Thus, it is likely that the appendages of the ancestral arthropod had acquired some level of regional identity along the anteroposterior axis before the divergence of the major arthropod classes. Chelicerates are somewhat unusual, however, for while their segments display regional specializations, the different body segments do not directly compare with those from other arthropod taxa.

Drosophila—The Model Arthropod Leg

Drosophila melanogaster has served as the "model" arthropod because of the tremendous success of its use as an experimental organism in genetics. The adult *Drosophila* leg, representative of a typical insect leg, is made up of six segments: the coxa, trochanter, femur, tibia, tarsus and pretarsus (or claw) (Snodgrass 1935). It is generally assumed that the coxa represents the protopodite while the remainder of the leg represents the telopodite. In most arthropods appendages develop out from the embryonic body wall and are functional on hatching (Figure 3A–C). Various diver-

gent strategies have evolved, however, particularly in species with indirect development where animals pass through a larval stage before reaching adulthood. In some crustaceans, for example, the animal hatches with only the anteriormost appendages formed, and development of the other appendages takes place postembryonically. In many insect groups, the embryo hatches with underdeveloped larval limbs (see Figure 3C). During the last larval and pupal stages those legs continue to grow and differentiate to create the adult form. Importantly, the larval legs become the adult legs. In Diptera, such as *Drosophila*, however, an extreme version of this mode of development is quite divergent and not characteristic of other arthropods (see Figure 3A, D). During embryogenesis, ectodermal cell populations are set aside and internalized before hatching. These cells form the imaginal discs, larval structures that will become virtually all the adult structures, including the legs (Cohen 1993). Despite the divergent morphogenesis of *Drosophila* structures, most of what we understand of the genetic and molecular basis of arthropod appendage patterning is derived from research on the *Drosophila* leg and wing imaginal discs.

From a molecular standpoint, *Drosophila* leg development can be broken down into three stages: (1) the embryonic specification of the limb primordium; (2) the patterning of the proximal–distal (PD) axis; and (3) the elaboration and growth of the late instar or pupal disc (Figure 4).

Specification

The future leg imaginal disc begins as a small cluster of 20 to 30 cells in the embryo. The first molecular markers of these cells are the zinc-finger transcription factor *buttonhead* (*btd*) and the homeobox gene *Distalless* (*Dll*), which are activated during Stage 10 when the germ band undergoes elongation (see Figure 4). The transcription factor *btd* requires input from the Wingless (Wg) signaling pathway and is expressed in the leg domain at early Stage 10 (Estella and others 2003); *btd* marks the entire imaginal primordia as well as additional cells that presumably will form the peripodial membrane, an overlying layer of cells of the imaginal disc that does not produce the adult epidermis. Shortly afterwards, *Dll* is expressed within the *btd* domain. *Dll* activation requires Wg signaling, as well as *btd* and the closely related gene *Sp1* (Estella and others 2003). *Dll* seems to mark just the disc primordia, but it is not required for the specification of imaginal discs. *Dll* mutant embryos die before hatching due to a second role for *Dll* in neurogenesis, but if the mutant embryos are cultured inside an adult fly they can form imaginal discs (Cohen and others 1993). These discs become proximal leg structures, such as the coxa and trochanter, but lack distal structures, indicating the role of *Dll* in specifying distal identity.

By the end of Stage 10, *wg* resolves from uniform segmental stripes into disconnected ventral and dorsal stripes (Baker 1988); *btd* and *Dll* expression are now centered on the dorsal edges of the ventral *wg* stripe, restricted by the activity of the Decapentaplegic (Dpp) signaling pathway dorsally, and by activity from the epidermal growth factor receptor (EGFR) ventrally (see Figure 4; Goto and Hayashi 1997). Mutations in the Dpp pathway result in the expansion of the *btd* or *Dll* domain dorsally, and mutations in the EGFR pathway result in the expansion of the *btd* or *Dll* domain ventrally (Goto and Hayashi 1997; Estella and others 2003). The ability of Dpp to repress *Dll* is temporary; by Stage 12, the beginning of germ band retraction, *dpp* is expressed in spots near the dorsal edge of each ventral *wg* stripe, overlapping the dorsal *Dll* domain (see Figure 4; Cohen and others 1993). EGFR repression is also transient. During Stage 11, when the germ band is fully extended, EGFR actively signals within the *Dll*-expressing limb primordia (Kubota and others 2000). These apparent conflicts indicate that the patterning elements of the early limb field are under dynamic regulation.

Initial PD axis

During dorsal closure, at Stage 14, the bulk of the *dpp*-expressing cells move dorsally away from the *wg* stripe and limb primordium, except for a small strip of *dpp*-expressing cells that extend into the primordium (see Figure 4; Kubota and others 2003). At this stage the limb primordia, as indicated by the field of *btd*-expressing cells, has *Dll* expression in a group of cells at the center, while the genes *escargot* (*esg)* and *homothorax* (*hth*) are coexpressed in an outer ring encom-

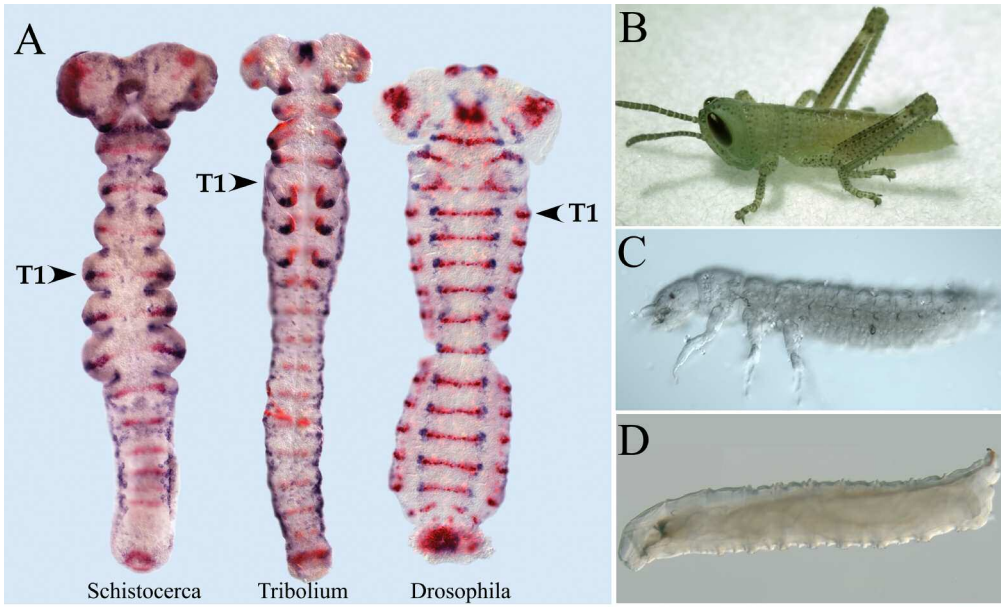

FIGURE 3. Modes of insect development. **A,** Grasshopper, beetle and fly embryos double stained for *wg* (red) and *dpp* (blue) mRNA; notice the absence of developing limb buds in *Drosophila*; *wg* and *dpp* patterns have a conserved spatial relationship; arrowheads mark the first thoracic segment (T1) in each embryo. **B–D,** Hatchlings: **B,** *Schistocerca* hatchling, which closely resembles the adult form. **C,** *Tribolium* larvae; notice the legs and head which will be modified slightly during the transition to adult structures. **D,** *Drosophila* larvae; notice the absence of legs and an external head.

passing the remainder of the primordia (Kubota and others 2003). This subdivides the primordium into approximate "distal" and "proximal" domains, forming the initial PD axis of the leg. During these later embryonic stages, the expression of both *esg* and *Dll* is dependent on Wg, EGFR and Dpp signaling (Kubota and others 2000, 2003). This marks a departure from the regulatory network used for the specification of the limb primordium, where *Dll* is repressed by both Dpp and EGFR activity. Thus, during embryogenesis there is a molecular transition from a stage of appendage specification to the onset of appendage pattern formation. During this transition, repressors of limb fate become necessary components of proper limb formation and the specification of the PD axis.

Imaginal disc development

At the end of embryogenesis the embryonic leg primordium is internalized and will become the larval imaginal disc. The imaginal disc initially is near the larval epidermis, but by the second instar it has separated from the epidermis and is connected only by a stalk. Molecularly, this transitional stage between the end of embryogenesis and the second instar is poorly understood. The spatial relationship of *wg, dpp, Dll* and *hth* during embryogenesis is retained in the larval imaginal disc. The imaginal disc maintains the dorsal–ventral relationship of *dpp* and *wg,* the proximal–distal relationship of *hth* and *Dll,* and the anterior–posterior relationship of the segment polarity genes from embryogenesis (see Figure 4; Basler and Struhl 1994; Abu-Shaar and Mann 1998; Wu and Cohen 1999). Expression of *wg* is in the anterior ventral quadrant, while *dpp* is expressed in a stripe along the anteroposterior axis, with higher expression dorsally (see Figure 4; Basler and Struhl 1994). Both Wg and Dpp are secreted proteins and the diffusion of Wg from ventral cells and Dpp from dorsal cells together form a gradient of a combined signal that patterns the PD axis of the leg (Lecuit and Cohen 1997). At the center of the disc the high level of both signals activates *Dll* transcription, thus defining the "distal" leg, and also represses *hth* from the

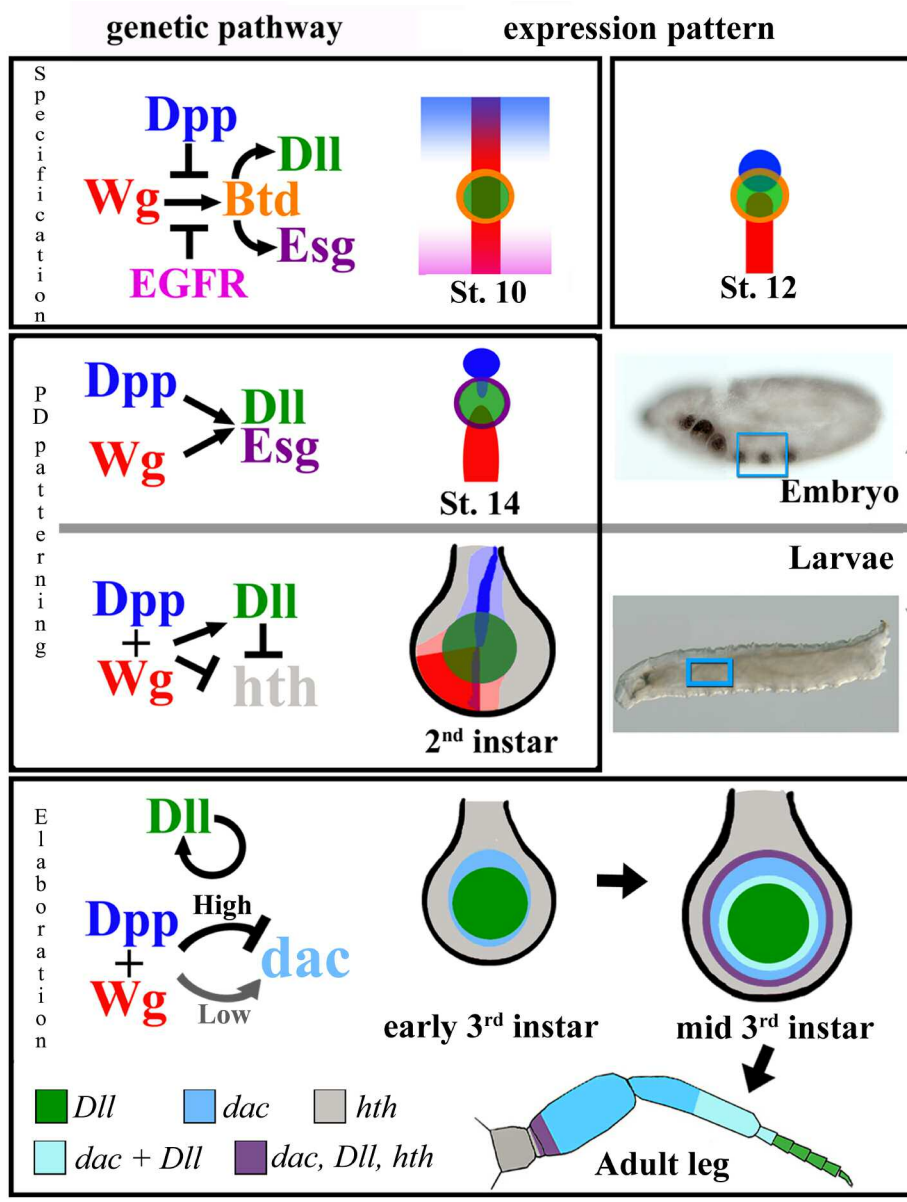

FIGURE 4. Genetic pathways and gene expression patterns in Drosophila limb development. At left is the genetic circuitry at each stage; at right are schematics of the corresponding expression patterns (dorsal is up, ventral down, and anterior is to the left). The top diagrams show embryonic development. A blue box shows the location of the limb domain on the embryo (labeled with a *Dll* antibody). The graded blue and pink in the Stage (St.) 10 diagram reflects the gradients of *Dpp* and EGFR signaling from the dorsal and ventral half of the embryo, respectively. After specification *dpp* is expressed in a spot adjacent to the *wg* domain. This likely marks the end of the specification step, but this domain of *dpp* has not been shown to play a role in proximodistal patterning. Note that the *Btd* domain encompasses the entire *Dll* domain as well as what is shown in orange. The bottom diagrams show imaginal disc patterning during larval development. The light red and blue in the imaginal disc schematics reflect the range of *Wg* and *Dpp* signaling, respectively; *dac* expression is shown in light blue. Under the current patterning model, the area of cells that sees high levels of both signals remains at a relatively fixed size at the center of the disc. During the third instar the disc grows and *Dll*-expressing cells at the perimeter end up outside the area of high signal and activate *dac* expression. *Dll*-expressing cells maintain *Dll* expression independent of Wg and Dpp signaling, creating an overlap of *dac* and *Dll* domains (aqua). Overlap of *hth, dac* and *Dll* is shown in purple. The adult leg shows the leg segments that correspond to the expression patterns in the third instar.

center of the disc. Initially the *Dll* and *hth* domains are complimentary and mutually exclusive, but by the end of the second instar these domains separate and a third gene, *dachshund* (*dac*), is activated at an intermediate position between *Dll*- and *hth*-expressing cells (see Figure 4; Lecuit and Cohen 1997). High levels of Wg and Dpp signaling repress *dac* from the center of the discs, but is activated by low levels of Wg and Dpp signal at that intermediate position (Lecuit and Cohen 1997). The regulation of *Dll* by the combined activity of Wg and Dpp in the imaginal disc differs from the regulation in the embryonic limb primordia where Dpp and Wg are separately necessary for *Dll* and *esg* expression on the dorsal and ventral side, respectively (Kubota and others 2003). After the first two larval instars the regulation network changes yet again.

By the third instar the PD axis has been established. During the remaining stages this axis is further elaborated on and morphogenesis of the leg begins, including elongation and segmentation. During the third instar the disc undergoes extensive cell proliferation and the flat field of cells becomes convoluted, eventually undergoing eversion during the pupal stage. In the third instar, *Dll* no longer requires input from Wg or Dpp for its expression (Lecuit and Cohen 1997; Galindo and others 2002); *dac,* on the other hand, does. Consequently, as the disc grows *dac* repression from *wg* and *dpp* is lifted in the outer *Dll*-expressing cells, as they move farther from the center (see Figure 4; Lecuit and Cohen 1997). A proximal ring of *Dll* expression, independent of Wg and Dpp, also appears at this stage (Diaz-Benjumea and others 1994). The proximal ring overlaps both *hth* and *dac* and corresponds to the future trochanter (see Figure 4). The leg is now made up of overlapping and exclusive domains of *Dll, dac* and *hth.*

Other Arthropod Legs

Development of the *Drosophila* leg is extremely derived and not representative for many insects, much less arthropods in general. In most insects and all other arthropod groups limbs are direct outgrowths of the embryonic body wall (see Figure 3A). Nonetheless, the adult legs of flies and other insects are considered to be homologous structures, and as homologous structures it would seem likely that the developmental patterning that underlies leg development could be conserved. Indeed, despite the major differences between development in the appendages of flies and in those of other arthropods, there are some striking similarities in the expression of the leg patterning genes described above (Figure 5). Most of these similarities have been identified in the components of the proximodistal patterning network of the imaginal disc during larval development, without much focus on the other stages of *Drosophila* limb development. Given the multiple stages of limb development in *Drosophila,* it is essential that these comparisons are made appropriately. For example, does the specification phase in *Drosophila* correspond to the specification of limb fields in other insects, or is it a novel process evolved to create the imaginal primordia? Another challenge is to understand how the different stages of *Drosophila* limb development compare with limb patterning in other arthropods.

Elements of the proximodistal patterning system are well conserved, particularly within insects (see Figure 5). *Dll* protein, as recognized by a crossreactive antibody, is expressed in the distal region of the developing legs of every insect, spider and crustacean that has been examined (Panganiban and others 1997; Schoppmeier and Damen 2001; Prpic and Tautz 2003). In addition to the distal domain of *Dll* expression, which corresponds to distal tibia and tarsus, several insects, including flies, have a proximal ring of *Dll* that corresponds to the trochanter–femoral border (Abzhanov and Kaufman 2000b; Beermann and others 2001; Rogers and others 2002; Angelini and Kaufman 2004). Cloned in several insects, *dac* orthologs are expressed at an intermediate position between and partially overlapping the ring and tip domains of *Dll,* corresponding to the tibia and femur, much like *Drosophila* (Abzhanov and Kaufman 2000; Prpic and others 2001; Angelini and Kaufman 2004). As in flies, *hth* expression is limited to the proximal leg segments in the milkweed bug, *Oncopeltus fasciatus,* and the flour beetle, *Tribolium castaneum,* whereas its cofactor Extradenticle (Exd) is uniformly expressed (Prpic and others 2003; Angelini and Kaufman 2004). Nuclear localization of Exd (n-Exd) requires Hth, thus restricting the action of Exd to

proximal cells (Rieckhof and others 1997). In *Cupiennius, exd* expression is spatially restricted, while *hth* is expressed broadly (Prpic and others 2003). As it is the coexpression of these genes that is important to function and proximodistal patterning, their coexpression in the proximal leg indicates a conservation, at the protein level, between spiders and insects. Conservation of the protein expression likely extends to crustaceans, as the use of a crossreactive antibody to Exd showed that it is likewise restricted to the proximal legs of crustaceans (Abzhanov and Kaufman 2000). The dorsoventral relationship of *dpp* and *wg,* the upstream activators of proximodistal patterning genes in the *Drosophila* imaginal disc, is also conserved in the legs of other arthropods. In the insects, spiders and millipedes that have been examined, *wg* is expressed ventrally in segmental stripes and *dpp* is initially localized in a dorsal patch at each limb tip; this is consistent with a role for the two genes in creating a gradient of activity centered at the tip of the leg (Jockusch and others 2000; Niwa and others 2000; Schoppmeier and Damen 2001; Prpic 2004).

While the general organization of the proximodistal patterning genes in arthropods is relatively well conserved, the details of the patterns show some divergence. Although all insects express *Dll* in the distal tip and in a proximal ring of the leg, they differ in how that patterns takes form. In *Drosophila* the proximal ring domain appears de novo after the distal domain through an unknown mechanism (Diaz-Benjumea and others 1994). In *Tribolium* and *Schistocerca* two domains form through the down regulation of *Dll* in an intermediate domain (Beermann and others 2001; Prpic and others 2001; Giorgianni and Patel, unpublished observation). It remains unclear how this down regulation of *Dll* corresponds with the onset of *dac* expression in this intermediate domain. The *Dll* pattern in spiders and the isopod crustacean *Porcellio scaber* differs from that in insects in that *Dll* persists in a single continuous domain throughout development, as opposed to separate domains at the distal tip and the proximal ring (Abzhanov and Kaufman 2000b). This results in a change in the relative spatial relationship of *dac* and *Dll* (see Figure 5). *Dll* and *dac* expression domains are initially mutually exclusive in the *Drosophila* leg disc and only overlap partially at later stages. The exclusive spatial relationship of *dac* and *Dll* in *Drosophila* is due to the repression of *dac* and activation of *Dll* at high levels of the combined Wg and Dpp signal. The partial overlap is then caused by the initiation of the proximal *Dll* ring and the relief of *dac* repression as the leg grows. In spiders and crustaceans there is extensive overlap of *dac* and *Dll* expression, indicating a divergence in the regulation of these genes outside insects. It remains unclear what impact an altered relationship of these genes has on morphology, but some hints are provided by comparisons of the leg and antenna of flies (see below).

While most studies have compared the expression patterns in legs of other arthropods to those in the *Drosophila* imaginal disc, there is some evidence from *Tribolium* and the grasshopper *Schistocerca americana* of phases comparable to the early patterning events in the *Drosophila* embryo. A hallmark of the specification stage in *Drosophila* is the activation of *Dll* by Wg signaling, and the repression of *Dll* by Dpp. Before the emergence of limb buds in *Tribolium* or *Schistocerca, Dll* expression starts in a small group of cells at the edge of the segmental wg stripes (Nagy and Carroll 1994). Further, *Dll* turns on in advance of *dpp* in *Tribolium,* while *dpp* and *Dll* are activated at approximately the same time in *Schistocerca* (Giorgianni 2004). When *dpp* begins in the limb field it is expressed in a domain that partially overlaps the *wg* stripe, within the *Dll* domain (Jockusch and others 2000; Giorgianni and Patel 2004). If *Dll* is indeed independent of Dpp signaling during this initial phase, then it hints that embryonic limb patterning in *Drosophila* is not just for creating imaginal discs, but is potentially part of a conserved molecular ground plan for limb development.

There is also evidence that the third phase of *Drosophila* limb patterning has a counterpart in other arthropods. As the leg of *Tribolium* or *Schistocerca* grows, *dpp* expression is repressed from the tip of the leg and becomes expressed in a ring. In direct developing *Schistocerca,* more rings appear over the course of development in and between many of the leg segments (Jockusch and others 2000; Giorgianni and Patel 2004). In the spider, *dpp* forms a ring in each of the leg segments as well (Schoppmeier and Damen 2001; Prpic and others 2003). In each case, when the *dpp* rings appear the leg has grown substantially and a system where Dpp and Wg are still both re-

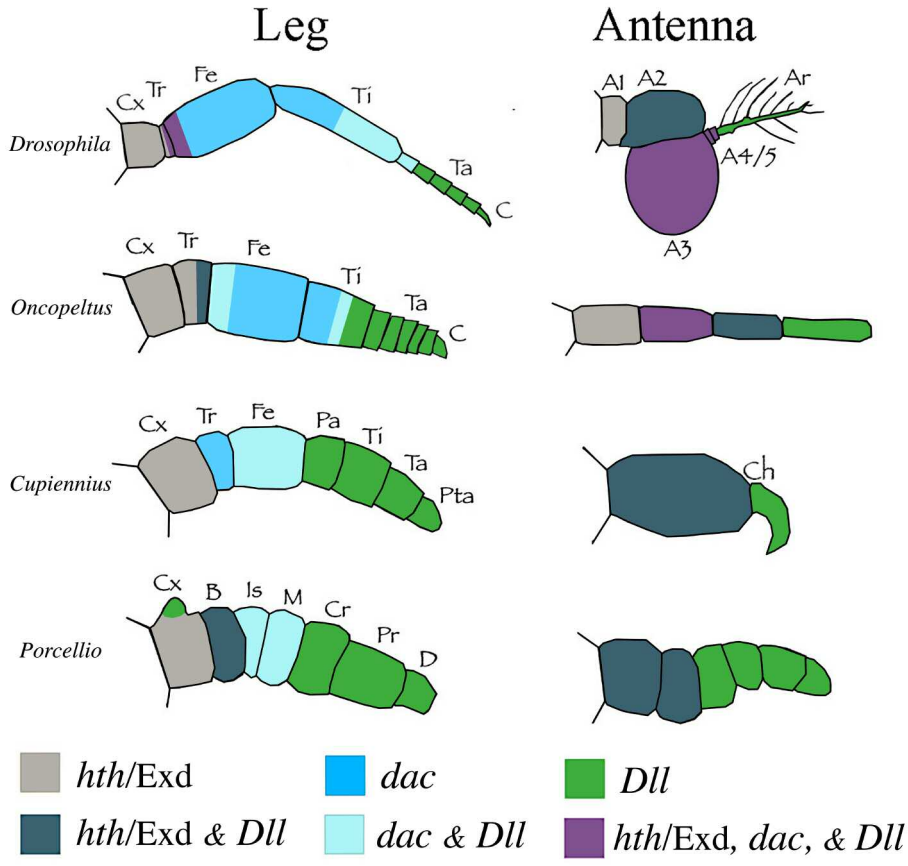

FIGURE 5. Legs and antennae from each of the major arthropod groups, with a map of gene expression. *Hth/Exd* coexpression (grey), *dac* (blue), *Dll* (green), *hth* and *Dll* (dark teal), *dac* and *Dll* (aqua), all three genes (purple). Damen and others (1998) have proposed that the *Cupiennius* antennal appendage gives rise to the chelicerae. *Abbreviations:* **B,** basis; **C,** claw; **Ch,** chelicerae; **Cr,** carpus; **Cx,** coax; **D,** dactyl; **Fe,** femur; **Is,** ischium; **M,** merus; **Pa,** patella; **Pr,** prodopus; **Pta,** pretarsus; **Ta,** tarsus; **Ti,** tibia; **Tr,** trochanter. Note that use of the same name for the various segments does not necessarily indicate that they are homologous structures.

quired to maintain *Dll* expression seems unlikely, in part because the domain of *Dll* is considerably larger than the *dpp* ring. This may signal a change in regulation analogous to the transition during the third instar when *Dll* becomes independent of Wg and Dpp signaling. These rings of *dpp* expression may play a role in the segmentation of the leg. Although they do not seem to have a direct counterpart in the *Drosophila* leg disc, rings of *dpp–lacZ* do appear in the tarsi during pupal life, indicating a potential role in the formation of the tarsal segments (Giorgianni and Patel 2004). In *Drosophila* the formation of the proximal ring of *Dll* occurs as the third limb patterning stage begins. Although the formation of these two domains differs in other insects, it can nevertheless signal a similar stage transition.

Mutants in *Tribolium* hint that there may be different phases of *Dll* regulation in beetles. A particular mutant *Dll* allele, which maps to the noncoding region, begins *Dll* transcription normally but loses expression in later stages (Beermann and others 2001). *Dll* reduction occurs at roughly the same time as the transition of *dpp* expression to a ringed pattern, or what may represent the third limb patterning stage. The loss of *Dll* transcription in this allele could occur because expression during the third stage of limb patterning requires a distinct enhancer, which is non-

functional in the mutant. Support of this interpretation comes from work on the *Drosophila Dll* enhancer, which showed that there are separate regions that control the regulation of *Dll* at the different stages of limb development. For example, a reporter gene that has been attached to a specific enhancer element is able to recapitulate faithfully the early onset of *Dll* in the embryo, but nothing later. Use of a second element turns on reporter expression at a later stage, corresponding to the proximodistal patterning phase, while a third is expressed in the imaginal disc (Vachon and others 1992).

These observations suggest that, like *Drosophila,* other arthropods have several distinct phases of appendage patterning and that these phases may have a conserved molecular basis. In insects, myriapods and chelicerates, *wg* and *dpp* are positioned in a way consistent with the creation of a gradient of signal centered at the distal tip of the leg. The readout of that gradient in flies involves *Dll, dac* and *hth*/Exd in domains from distal to proximal. Accordingly, homologs of *Dll, dac* and *hth*/Exd maintain a similar organization in other insects, spiders and crustaceans. There are some differences in the details of the spatial relationship of these genes, but overall they are expressed in conserved domains. There is also evidence in other insects of counterparts to the specification stage and to the elaboration and growth stages of the *Drosophila* model. This indicates that, despite its diverged morphogenesis, *Drosophila* may have a conserved patterning process and that the molecular models derived from *Drosophila* may be useful for understanding patterning events in other arthropods. If the stages are truly comparable, then there has been a remarkable heterochronic shift in *Drosophila* where the three patterning stages are spread across embryonic and larval development while the equivalent stages in the other insects all take place during embryogenesis. This highlights the importance of taking care to compare the expression data from other arthropods with the appropriate stage in *Drosophila* before drawing conclusions on the convergence or divergence of any pattern relative to the model. Understanding how these patterning genes function at the various stages will help us to draw the correct comparisons.

Functional Tests in Nonmodel Systems

Expression data from nonmodel arthropods indicate that the patterning pathways may be conserved at a molecular level. However, to support this claim more rigorously it is important to test gene function. The ability to generate mutant lines in *Tribolium,* and the development of gene knockdown technologies such as RNAi (which allows for the targeted degradation of specific mRNAs) for less genetically tractable systems, have allowed researchers to test whether or not the function of these genes is also conserved outside *Drosophila.* A functional role for *Dll* in the development of distal structures has been shown in several systems. In *Tribolium,* researchers have isolated several mutant alleles of the *Dll* homolog, in which legs are truncated at the coxa and do not form any distal structures (Beermann and others 2001). RNAi knockdown of *Dll* transcript in Oncopeltus resulted in the truncation of the leg beyond the femur (Angelini and Kaufman 2004). Similarly, RNAi of *Dll* in the spider *Cupiennius* severely truncated the appendages (Schoppmeier and Damen 2001). Knockdowns of *dac* and *hth* in *Oncopeltus* resulted in the loss of the tibia and the fusion of proximal leg structures, respectively (Angelini and Kaufman 2004). These results are consistent with mutational analysis of these genes in *Drosophila* (Mardon and others 1994; Casares and Mann 2001). Our knowledge of gene function in nonmodel systems is still very limited, but the coming years should provide a wealth of information as these knockdown techniques are applied to more genes in more systems.

Other Appendages

Our knowledge of the genes that pattern the leg can also be helpful in understanding the myriad of other appendage types in arthropods. The structure of the leg is relatively well conserved. Conversely, the other appendages—the antennae, mouthparts and genitals—have undergone drastic morphological evolution. These appendages express the same suite of genes for patterning the PD

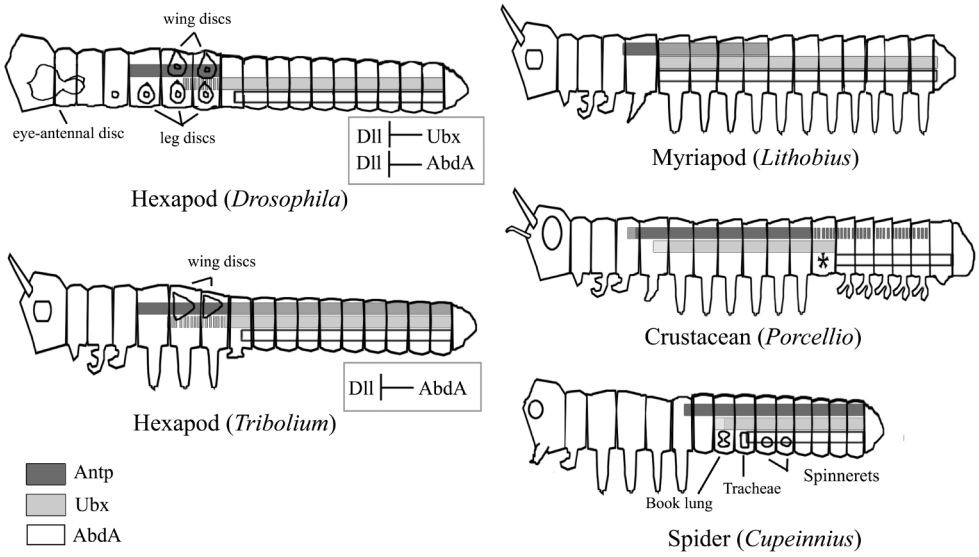

FIGURE 6. *Hox* gene expression in the major arthropod groups. *Antp* (hatched pattern of black and gray), *Ubx* (solid shades of light gray), *AbdA* (dark gray). Lightened shades within the same bar reflect lower levels of expression. Vertical lines reflect dynamic expression domains that shift over the course of development. The asterisk indicates that pleopods will form on this segment later in development. The regulation of *Dll* transcription by *Hox* genes in *Drosophila* and *Tribolium* is indicated. The mechanism of *Dll* regulation is not known in the other species. The book lung, trachae and spinnerets are in the *Cupiennius* opisthosomal appendages, and the spinnerets express *Dll* (Schoppmeier and Damen 2001). In some other spider species, all opisthosomal appendages express *Dll* (Popadic and others 1998).

axis, but the exact expression pattern of these genes varies. Interestingly, variations in the expression patterns are correlated with morphological variations.

The antennae

One of the most remarkable *Drosophila* appendage mutations ever isolated is the dominant gain-of-function mutation in the Hox gene *Antennapedia* (*Antp*) that transforms antennae to legs (Gehring 1966; Postlethwait and Schneiderman 1971). This dramatic transformation in appendage identity strengthened the notion that the antennae and legs both derive from an ancestral appendage. Subsequent analysis has revealed not only the extent to which the patterning of antennae and legs are similar, but also the importance of the differences. The *Drosophila* antennae form from half of the eye–antennal disc (Cohen 1993). The adult antenna is made of five segments (A1 through A5) and the arista. As in the leg, *wg* and *dpp* are expressed in opposing ventral and dorsal domains of the imaginal disc (Diaz-Benjumea and others 1994). Similarly, *Dll, dac,* and *hth* maintain distal, intermediate and proximal domains (Dong and others 2001). The regulation of these genes, however, shows some distinct differences to those in the leg. The *Dll* domain is continuous from the distal center of the disc to its proximal, or outer, extent (Dong and others 2001). As a result, the *dac* domain is contained within the *Dll* domain. The expression of *hth* is in a large domain that extends from the proximal edge of the disc and overlaps with the entire *dac* domain (see Figure 5). This difference in *hth* regulation between leg and antennae is of major importance to determining their divergent morphologies. In the leg, *Dll, dac* and *hth* mutually antagonize each other, maintaining largely exclusive domains (Abu-Shaar and Mann 1998). In the antenna, the three genes overlap extensively and do not have the same ability to regulate one another (Dong and others 2001). Further, the loss of *hth* results in the transformation of antennal tissue into leg tissue (Casares and Mann 1998). A major factor in this regulation is likely to be the

Antp gene, which is expressed in the leg but not the antenna. *Antp* functions to repress *hth* in most of the leg disc. Loss of *Antp* results in the derepression of *hth* (Casares and Mann 1998). Further, loss of *Antp* in the entire disc results in the transformation of the leg to an antenna (Struhl 1981). On the other hand, misexpression of *Antp* in the antennal disc, as in that remarkable gain-of-function allele, results in the repression of *hth* and transformation to a leg-like structure (Gibson and Gehring 1988; Casares and Mann 1998). It is not known whether the regulation of *hth* by *Antp* is direct or indirect, but it is a reasonable possibility that *Antp* allows the mutually antagonistic regulation of *Dll, dac* and *hth.* Thus, in these two distinct appendages, Wg and Dpp create a gradient for the PD axis, a gradient that is interpreted by *Dll, dac* and *hth.* However, their interpretation, or the subsequent regulation among them, is altered by the presence or absence of *Antp.* This is a striking example of morphological variation correlating with changes in the spatial relationship of a limited number of genes, in this case *Dll, dac* and *hth.*

While the *Drosophila* antenna is a highly derived structure, antennae in most other insects, as well as some crustaceans, are much more leg-like. Nonetheless, the expression of proximodistal patterning genes in the antennae of other insects shows a greater similarity to the antenna than to the leg of *Drosophila* (see Figure 5). In the antennae of *Oncopeltus, Dll, dac* and *hth* overlap considerably; the *dac* domain is positioned completely within both the *hth* and *Dll* domain (Angelini and Kaufman 2004). In other insects, such as *Tribolium, Schistocerca* and *Gryllus, Dll* and n-Exd (and presumably *hth* expression) maintain the exclusive domains seen in the legs, but *Dll* is in a continuous domain in the antennae (Jockusch and others 2000; Niwa and others 2000). And at least in *Tribolium, dac* expression in the antenna differs from that in the leg; it is weakly expressed through most of the antenna and overlaps with either *Dll* or *hth* (Prpic and others 2001). The absence of a domain that expresses *dac* but not *Dll* or *hth* is common to antennae in all these insects.

The gnathal appendages

The gnathal appendages of insects and their counterparts in other arthropods are specialized for manipulating and ingesting food. As a result, they have some of the most dramatic and divergent morphologies of any appendage (Snodgrass 1935). The gnathal appendages of *Drosophila* are particularly derived, while those of *Tribolium* and *Schistocerca* are thought to be more similar to those of ancestral insects. Despite the extensive research on the development of *Drosophila* leg, wing and antennae, the *Drosophila* gnathal appendages remain largely unstudied. This is partly because the *Drosophila* mandible is virtually nonexistent, the adult maxilla is reduced relative to other arthropods and derives from the eye–antennal disc, and the labium derives from two small labial discs to form a unique sponging apparatus used for feeding. It has been shown that the labial disc expresses *Dll* at its tip, with weak levels of n-Exd, in a nonoverlapping domain (Abzhanov and others 2001).

The insect mandible is considered to be gnathobasic; that is, it is made solely of the proximal limb segment (Manton 1977). The concept of a gnathobasic mandible is supported by the lack of Dll expression in this appendage (Popadic and others 1998). The mandible of myriapods and some crustaceans is also gnathobasic, and likewise the mandibular segment does not express Dll (Prpic and Tautz 2003). The mandibles of some crustaceans, however, have a distal telopodite and, not surprisingly, Dll is expressed where a mandibular telopodite is present (Browne and Patel 2000).

The maxillary and labial appendages of *Tribolium* and *Schistocerca* both bear a leg-like palp and ventral endites. The labial appendages fuse medially in insects to form the bottom lip of the feeding apparatus. In *Tribolium* and *Schistocerca,* the maxillary and labial appendages seem to be patterned similarly to the leg (Giorgianni and Patel 2004; Jockusch and others 2004). Both *wg* and *dpp* are present at the tip of the appendage, where *Dll* is expressed. Dll and n-Exd take up complimentary domains except for a ring of overlap in the palp (similar to that in the leg). In *Tribolium, dac* is present in a ring in the palp and in a larger, more proximal domain, a pattern that differs from that in the leg (Prpic and others 2001). The ventral branches of the maxilla and labium also express *Dll,* but do not seem to form through a redeployment of the PD axis pat-

terning mechanism. The branches of the gnathal appendages differ from the initial limb primordia in that *wg* and *dpp* are not positioned as if to specify a second PD axis, and Dll and n-Exd overlap throughout the branch (Giorgianni and Patel 2004; Jockusch and others 2004). These branches always originate in the coxa (protopodite) and do not contain more than a single segment. Thus, there seem to be distinct mechanisms that pattern the branches and the primary outgrowths. Studying the development of these branches in insects may help us understand the development of more complex crustacean appendages.

In the development of arthropod appendages from legs to mouthparts to antennae, the basic patterning elements are conserved. Dpp and Wg signaling form a signal gradient that specifies the PD axis by activating target genes, *Dll, dac* and *hth,* in an order from distal to proximal. However, the specific relationship of *Dll, dac* and *hth* can vary from appendage to appendage and from species to species. In the striking case of the *Drosophila* antenna and leg, it is the absence or presence of a Hox gene that dictates this relationship, and is predictive of the final morphology. It remains to be seen, however, whether this is a feature common to other arthropod appendages.

The Role of Hox Genes

The major role of Hox genes in the diversification of body segments has long been recognized. Hox mutations in *Drosophila, Tribolium* and *Bombyx* can result in the transformation of one type of appendage into a different one. As described above, *Drosophila* thoracic discs, mutant for *Antp,* give rise to antennae instead of legs. The loss of *Antp* results in the overlap in expression of the proximodistal genes *Dll, dac* and *hth,* which then activate a series of antenna-specific targets and presumably produce the distinct morphology (Casares and Mann 1998; Dong and others 2001). In *Bombyx,* mutations in the *Antp* homolog likewise transform the first thoracic legs to antennae (Nagata and others 1996). Mutants in the *Tribolium Antp* homolog, known as *prothoraxless,* also transform legs into antennae (Beeman and others 1993). The striking effect that Hox genes have on appendage morphology makes a strong case for their role as agents of evolutionary change.

Correlative evidence from diverse crustaceans supports the hypothesis that changes in Hox gene expression can be responsible for evolutionary changes in appendage morphology. Here, the expression of Hox genes in distinct body regions parallels shifts in appendage morphology (Figure 6). Below we highlight the effect of two Hox genes, Ubx and AbdA ([see Hughes and Kaufman 2002 for a detailed review of additional Hox genes). Averof and Patel (1997), using a crossreactive antibody that recognizes both Ultrabithorax (Ubx) and abdominal A (abdA), showed that the transformation of thoracic appendages to maxillipeds (gnathal-like appendages used for feeding), which occurs in crustacean evolution, correlates with shifts in the anterior expression boundary of Ubx/abdA. Branchiopods, such as *Artemia franciscana* and *Triops longicaudatus,* have nearly identical thoracic appendages and Ubx/abdA staining occurs throughout the thoracic segments. On the other hand, some of the anterior thoracic appendages of decapods, such as lobsters and shrimp, are modified to aid in feeding. These specialized limbs are more similar, in appearance, to the gnathal appendages than to the walking legs. In these crustaceans the anterior boundary of Ubx/abdA has shifted posteriorly to include only the segments that produce walking legs; the maxilliped-bearing segments do not stain for Ubx/abdA. Furthermore, weak levels of Ubx/abdA correspond to morphologies intermediate between leg and mouthpart.

In *Porcellio,* where *Ubx* and *abdA* have been cloned, they clearly occupy distinct domains (Abzhanov and Kaufman 2000a). *Ubx* is expressed throughout the thoracic segments, while *abdA* is restricted to abdominal segments. Further, n-Exd does not occur in the abdominal appendages, which is consistent with a similar pattern in the *Drosophila* embryo and supports a role for the Hox genes in the proximodistal patterning network. The molecular differences between the two body regions correlate with the distinct appendage types in each: the thoracic appendages are uniramous, elongate walking legs, while the abdominal appendages are short biramous pleopods.

Ubx and abdA also play an important role in insect appendage development. The specifica-

tion of limb fields in the early *Drosophila* embryo is limited to the three thoracic segments due to repression of *btd* and *Dll* by Ubx and abdA (Vachon and others 1992; Castelli-Gair and Akam 1995). Estella and co-workers (2003) showed that in a *Ubx* mutant, *btd* was expressed in the first abdominal segment, the only segment in the *Ubx* domain that does not also express *abdA*. The repression of *Dll* has been shown to be a result of direct repression by Ubx in combination with Hox cofactors Exd and Hth (Gebelein and others 2002). The ability of Hox proteins to repress Dll is transient, however, and does not function at later stages of embryogenesis nor during larval life (Castelli-Gair and Akam 1995).

In *Tribolium,* Ubx and abdA have distinct functions in limb development. The anterior boundaries of *Ubx* and *abdA* expression domains are offset. In the first abdominal segment (A1) *Ubx* is expressed throughout and *abdA* is restricted to the posterior third of the segment (Shippy and others 1998; Bennett and others 1999). *Tribolium* embryos form a pleuropod, a *Dll*-expressing appendage used for hatching, on A1. The *Dll*-expressing cells are restricted to the anterior two-thirds of the segment. Loss of *abdA* results in the formation of pleuropods along the entire abdomen. Loss of *Ubx* results in the transformation of the pleuropod to a leg. When both genes were knocked down through RNAi, legs formed along the entire abdomen (Lewis and others 2000). Thus in *Tribolium, Ubx* does not repress limb development, but plays a role in the modification of the limb, while *abdA* maintains the role of limb repressor.

In crustaceans, on the other hand, limbs are present in the segments that express Ubx and abdA, so neither plays the role of a limb repressor (limbs are present throughout the thorax and abdomen of many crustaceans) and, as described above, both seem to modify limb morphology. The difference between the ability of crustacean and insect Ubx to repress limb development is likely to be the result of changes to the Ubx protein (Galant and Carroll 2002; Ronshaugen and others 2002).

In insects, *Ubx* expression also eventually extends into a subset of the thoracic appendage primordia (at a time after it can no longer repress *Dll*) and functions in differentiating neighboring appendages within the thorax. The difference between the fly wing, which forms on the second thoracic segment, and the haltere, a stubby organ used for balance that forms on the third thoracic segment, is due to differential expression of *Ubx*; expression of *Ubx* in the haltere disc is responsible for repressing wing fate (Weatherbee and others 1998). Strikingly, removal of *Ubx* function in the developing third thoracic segment results in a four-winged fly (Lewis 1978). During pupal development, Ubx is even necessary for the formation of the unique trichome (hair) pattern on the second thoracic leg (Stern 2003).

Clearly Hox genes have a major influence on limb morphology. The boundaries and levels of Hox expression differ considerably from species to species and these shifts correlate with morphological changes. As shown by studies of Ubx, modifications of the protein itself can also change function. Finally, there is some evidence that one way in which Hox genes act is through modification of the proximodistal patterning network.

Wings, Spinnerets and Exites

Hox genes are likely to have a major role in the diversification, modification and changes in the number of appendages. Branches of the protopodite (exites and endites) have arisen and been lost many times in evolution and are highly diverse morphologically. The absence of exites from the oldest crustacean fossils suggests that they were not a feature of the ancestral arthropod appendage. Regardless, exites and endites are abundant in both fossil and extant crustaceans. Exites arise from a dorsal position on the protopodite and are never segmented. Exites commonly form a gill, a thin flat structure used for gas exchange in crustaceans. Although exites are rare on terrestrial arthropod legs, as in most insects, it has been argued that structures such as insect wings and chelicerate opisthosomal appendages are homologous with crustacean exites (Wigglesworth 1973; Averof and Cohen 1997; Damen and others 2002).

The evolutionary origin of insect wings has long been contentious. Snodgrass (1935) consid-

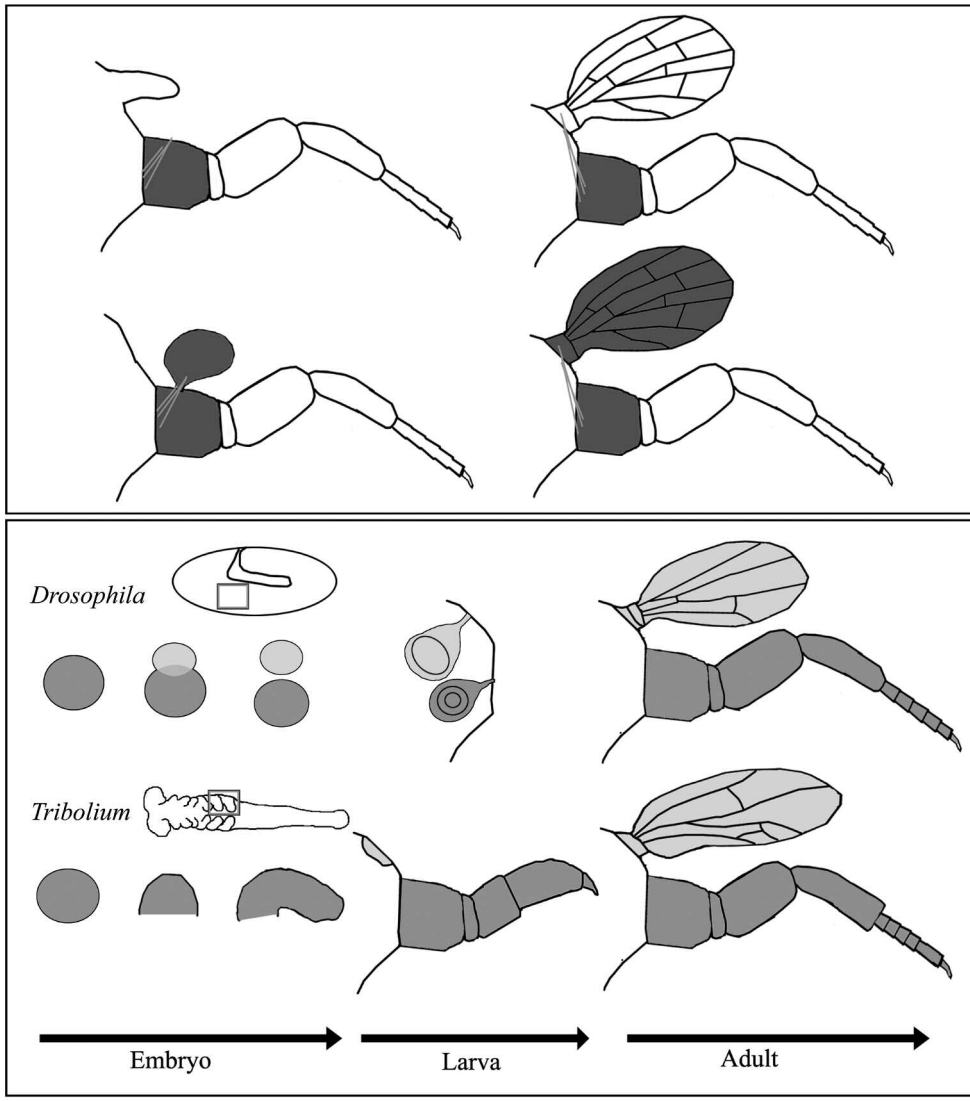

FIGURE 7. Scenarios for the origin of insect wings. **A,** Wings may have arisen as novel outgrowths of the body wall (top) or as displaced exites from a crustacean ancestor (bottom). In the bottom scheme the wing derives from the protopodite (dark grey). Light grey lines are wing muscles. **B,** Different origins of the wing in *Drosophila* and *Tribolium.* In *Drosophila,* the leg (light grey) and wing (medium grey) derive from the same population of cells in the embryo. The split primordia form separate imaginal discs, which evert during pupation. *Tribolium* appendages form directly from the body wall and there is continuous development of the leg through the embryonic, larval and adult stages. The wing primordium only becomes apparent late in larval life and appears on the dorsal body wall.

ered wings to be novel outgrowths of the body wall (Figure 7). Under this theory, wings arose through the modification of paranotal extensions and eventually became adapted for flight. The opposing view is that the insect wing is homologous with an exite from a crustacean appendage that was displaced to the dorsal body wall (Wigglesworth 1973). Wigglesworth's arguments for a gill-to-wing hypothesis centered on the similarity of wings to the wing-like abdominal gills of a mayfly, and the similarity of those gills to the abdominal styli in a basal nonwinged insect. These styli are also present on thoracic appendages and arise on the coxa, as exites. The implication is that (1) exites can occur independently of the rest of the leg and (2) abdominal styli are homolo-

gous to gills. The connection between abdominal gills and wings is generally well accepted. However, it is not entirely clear that crustacean exites, or the abdominal styli of basal hexapods, are truly homologous with mayfly gills.

More recently, Averof and Cohen (1997) showed molecular support for a common origin of wings and exites. They cloned homologs of the *Drosophila* wing patterning genes *nubbin* (*pdm*) and *apterous* (*ap*) and showed that they were both specifically and strongly expressed in the exite of a crustacean (*Artemia*) appendage. They concluded, based on the conservation of the patterns of these genes, that the two structures are related. However, using patterning gene conservation to homologize two distinct structures requires caution, because it is difficult to distinguish between a shared history and the redeployment of that gene in a new or different structure.

Boxshall (2004) noted that basal hexapods lack exites or wings, and expressed doubt that a structure in the common ancestor evolved into wings in the insect lineage and into exites in crustaceans, but was lost in basal hexapods. It is worth considering two scenarios of homology: (1) wings and exites derive from a common structure or cell population (that is, an exite became a wing); or (2) they both derive from a common patterning cassette used by different cell populations (see Figure 7). Given that exites have such a varied history, the second scenario where wings, gills and exites are patterned by a common mechanism despite different structural origins seems more likely. A problem with the first scenario is that it does not explain how an exite could be physically separated from the leg and yet persist on the dorsal side of the body wall. In an argument for the first scenario, Wigglesworth (1973) pointed out that the major wing muscles originate in the leg coax, indicating a common origin (see Figure 7A). Snodgrass (1935) considered this a cooption of leg muscles by the novel wing structures.

Further arguments for the first scenario of a common origin of leg and wing derive from studies of the origin of the wing primordia in dipterans. Although wings and legs develop from separate imaginal discs they share a common origin in *Drosophila* (Cohen and others 1993; Goto and Hayashi 1997). The initial Dll-expressing primordia that produce the leg discs also produce the wing discs. Shortly after specification, the primordium split into a ventral population that will become the leg primordium and a second population of cells that moves dorsally and is the future wing disc (Cohen and others 1993). The ventral population retains Dll expression while the dorsal population shuts it down and begins expression of the transcription factor *vestigial* (*vg*). The loss of Dll is necessary for wing fate, as misexpression of Dll in the dorsal population results in the transformation of wing into leg (Gorfinkiel and others 1997). While Dll is a marker for ventral appendages, *vg* is a marker for the wing, and ectopic *vg* expression can cause the transformation of leg into wing (Kim and others 1996). Furthermore, during the time when the primordia are combined, *dpp* is expressed in a spot just dorsal to the *wg* stripe (Cohen and others 1993). The *dpp*-expressing cells move dorsally with the wing primordium, where *dpp* is necessary for maintaining wing fate (Kubota and others 2000).

The wing and leg also share a common origin in the basal dipteran *Dacus.* Anderson (1963) showed that the wing disc develops from the leg imaginal discs and that the two remain physically connected during larval stages. These striking findings were used to support the first scenario, in which the dorsal part of the limb field, which presumably would normally becomes the exite, is physically separated from the leg. However, while wing and leg primordia may derive from a single cluster of cells, this does not mean that the wing is an exite. The wing imaginal disc also produces the entire dorsal thorax, which makes the common primordium equally supportive of the scenario in which the wing is an outgrowth of the dorsal body wall. Without a more detailed fate map of those early primordial cells it is difficult to draw conclusions about the significance of a common primordium.

To better understand the origin of wings it will be necessary to determine the patterning elements that control exite specification, and to resolve the origin of wing primordia in other insects. Most insects do not form wing imaginal discs like those in Diptera (see Figure 7B). The wings do not become obvious until a late larval stage, when they are first recognizable as thickenings of the larval ectoderm (Quennedey and Quennedey 1990; Svacha 1992). These ectodermal

thickenings fold internally, producing a wing disc that emerges during pupation. *Drosophila* wing primordia, on the other hand, delaminate as a flat sheets of cells during embryogenesis, and remain largely detached from the larval ectoderm until pupation (Cohen 1993). Given the late stage at which the wings of nondipterans become evident, it is unclear whether there are wing "primordia" that are specified during embryogenesis. To conclude that wings derive from appendage exites requires that "wing" fate is specified in part of an original limb field. Barring late cell movement from the leg to the dorsal body wall (which has never been described), this means that wing specification should occur during embryogenesis, as in *Drosophila.* Unfortunately, isolation of orthologs of the earliest wing-specific markers, such as *vg,* in phylogenetically primitive insects has thus far eluded researchers. If a population of cells is specified to form wings, it is remarkable that they behave just like the rest of the larval ectodermal cells through most of development while retaining a capacity to develop wings.

Chelicerate opisthosomal appendages—the feathery book gills of horseshoe crabs, and book lungs and spinnerets in spiders—form posterior to the walking legs (see Figure 6). The nature of these appendages is still largely unclear; they could represent specialized remnants of legs, exites, or novel body wall structures. Using a crossreactive antibody, Damen and co-workers (2002) showed that, like wings and crustacean exites, the book gills of horseshoe crabs and book lungs and spinnerets of a spider all express Pdm in a strong and specific manner. Further, they cloned the *ap* homolog and showed that it too was expressed in the opisthosomal appendages. If the opisthosomal appendages are truly exites then this represents a remarkable example of the formation of an exite in the absence of any other leg structures. If legs are repressed by Hox genes in the spider opisthosoma in the same way as insects, then repression does not affect the whole limb primordium, as in flies, but is restricted to the protopodite and telopodite.

Conclusions

Arthropods show an amazing diversity of appendages. Underlying this diversity is a basic conserved mechanism for patterning the PD axis. From limited studies in insects, myriapods and chelicerates, it seems that *wg* and *dpp* may have a conserved role in setting up the initial PD axis. Information is notably lacking from crustaceans, for which *dpp* expression has yet to be described. Examination of the three proximodistal patterning genes *Dll, dac* and *hth* has revealed a conservation of order along the PD axis as well as some distinct differences in their patterns relative to each other. Hox genes seem to allow morphological diversity among serially homologous appendages. It seems likely that they impart some of this diversity by affecting the relationships among the proximodistal patterning genes.

Understanding the developmental basis for the diversity of appendage forms is a problem of great interest. Research on *Drosophila* has laid the groundwork and provided a model for the molecular basis of appendage development. However, *Drosophila* appendage development is highly derived. Thus translating our extensive knowledge of the *Drosophila* leg to most other arthropods is hindered by our uncertainty in how to properly connect the expression of patterning genes in other arthropods to the distinct functions that these genes have at the different stages of *Drosophila* leg development. In emerging insect model systems such as *Tribolium* and *Oncopeltus* leg morphogenesis is much more similar to that of other arthropods. These systems provide the best candidates for developing a detailed molecular view of leg development in a system outside *Drosophila.* A deeper understanding of leg development in a system such as *Tribolium* will make comparisons to legs in other arthropod groups and to serially homologous appendages both within and outside of the system easier. Also required is a greater understanding of the morphological processes that occur during early embryonic leg development in these arthropod systems, which will help us interpret changing expression patterns and could give us markers to make comparisons between species.

By continuing to examine known patterning genes, their temporal and spatial relationship to one another, and their function, we can compare basic patterns to the dynamic patterning mech-

anisms that create a leg, and so better understand how changes could have led to the divergent array of appendages found in nature. It will also be of great interest to expand the collection of studied genes beyond those most commonly pursued, as described in this review. For example, the EGFR pathway also plays a dynamic and critical role in the development of the leg at multiple stages of development (Campbell 2002), yet very little is known about this pathway in other arthropods. Likewise, the Notch signaling pathway is involved in segmentation of the *Drosophila* leg late in larval development (Rauskolb 2001). It will be interesting to see whether this pathway is conserved in other arthropod legs, and when and how it is deployed. Future studies detailing leg patterning pathways in more appendage forms tells us about the plasticity of the pathway and help us to understand how arthropods have been able to produce such an incredible diversity of appendages.

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