

Comparative Developmental Genetics and the Evolution of Arthropod Body Plans

David R. Angelini¹ and Thomas C. Kaufman²

¹Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut 06269-3043; email: david.angelinuconn.edu

²Department of Biology, Indiana University, Bloomington, Indiana 47405-3700; email: kaufman@bio.indiana.edu

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Abstract

The arthropods display a wide range of morphological diversity, varying tagmosis, as well as other aspects of the body plan, such as appendage and cuticular morphology. Here we review the roles of developmental regulatory genes in the evolution of arthropod morphology, with an emphasis on what is known from morphologically diverse species. Examination of tagmatic evolution reveals that these changes have been accompanied by changes in the expression patterns of Hox genes. In contrast, review of the modifications to wing morphology seen in insects shows that these body plan changes have generally favored alterations in downstream target genes. These and other examples are used to discuss the evolutionary implications of comparative developmental genetic data.

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INTRODUCTION

The diversity of animal life is one of our world’s most mysterious and intriguing qualities. Individual species have multiplied to fill ecological niches through a dizzying array of physiological and morphological modifications. Modern evolutionary biology has begun to understand the mechanisms involved in speciation (28, 98). However, one of the principal and persistent mysteries remains the origins and evolution of the novel morphologies and body plans that arise among diverse species (71). In recent decades, investigators of comparative developmental genetics have applied tools and ideas from molecular and developmental biology to some of the questions of morphological evolution with heartening success. This new field has also been known as phylontogenetics, or more commonly “evo-devo”—the truncated catchall

named for two of its most influential parent disciplines, evolutionary and developmental biology.

One of the animal groups that has been a major beneficiary of comparative developmental genetics is the Arthropoda. [The others are vertebrates, basal chordates, and deuterostomes (e.g., 43, 66, 79).] Since their appearance in the Cambrian, approximately 530 mya, arthropods have dominated the animal world. They have evolved myriad variations on an anatomical theme. In practical, experimental terms, arthropod evo-devo has flourished as it has drawn on the well-established fields of entomology, carcinology (the study of crustaceans), and genetics. The first two have described a wealth of diverse morphology and body plans, while the latter has provided tools and new developmental hypotheses for their investigation.

Comparative Developmental Genetics and the Hox Genes

The homeotic complex (Hox) genes have emerged from genetic studies of the fruitfly, *Drosophila melanogaster*, as crucial early regulators of segment identity, and thus body plan organization, in arthropods. Hox genes are homeodomain transcription factors, remarkably well conserved in sequence and expression across the arthropods and other animals. Therefore, they provide a reliable and accessible experimental inroad to the study of diverse body plans. In general, Hox genes are expressed alone or in overlapping domains of adjacent body segments. They exhibit the intriguing feature of “colinearity,” appearing in the gene complex in the order in which they are expressed along the anterior-posterior (AP) body axis. Ten Hox genes are expressed along the body of most arthropods, where they are usually named for their orthologues in *Drosophila*. From anterior to posterior, these are *labial (lab)*, *proboscipedia (pb)*, *Hox3/zen*, *Deformed (Dfd)*, *Sex combs reduced (Scr)*, *Hox6/ftz*, *Antennapedia (Antp)*, *Ultrabithorax (Ubx)*, *abdominal-A (abd-A)*, and *Abdominal-B (Abd-B)*. In the insects, *Hox3/zen* and *Hox6/ftz* have been modified and do not function as typical Hox genes in this group (48).

Hox genes specify the identity of body segments and structures where they are expressed, and mutations result in a homeotic transformation to some other fate, often to a more posterior identity (reviewed in 47). In 1978, characterization of the homeotic *bithorax* mutations of *Drosophila* led Ed Lewis to presage the growth of comparative Hox work that would come decades later:

Flies almost certainly evolved from insects with four wings instead of two and insects are believed to have come from arthropod forms with many legs instead of six. During the evolution of the fly, two major groups of genes must have evolved: “leg-suppressing” genes which removed legs from abdomi-

nal segments of millipede-like ancestors followed by “halter-promoting” genes which suppressed the second pair of wings of four-winged ancestors. If evolution indeed proceeded in this way, then mutations in the latter group of genes should produce four-winged flies and mutations in the former group, flies with extra legs. (57)

This evolutionary scenario described by Lewis has not been borne out quite as he envisioned it—rather than the evolution of new genes, the evolution of regulatory interactions appears to have been key to body plan changes. As we discuss below, the details of Hox expression domains and timing, as well as the target genes controlled by specific Hox genes, have been associated with greater and lesser aspects of body plan evolution in a range of arthropod groups.

Several excellent reviews covering different aspects of Hox genes and their connections to arthropod evolution have been published in recent years (8, 47, 69). These articles have emphasized the commonalities and themes seen across the arthropods and other animals. Here, we have attempted to organize our discussion in terms of several of the major novelties in arthropod body plan evolution. In the course of this, we revisit some of the same topics and update their consideration with recent data. Principally, we hope to illustrate the diversity of arthropod morphology and review developmental genetic data relevant to its evolutionary plasticity. With this in mind, we do not limit ourselves to discussions of tagmosis or to the Hox genes. These have been fruitful lines of research, but they are necessarily just the beginning.

What is the Meaning of the “Body Plan” Concept?

A body plan is a basic pattern of anatomical organization shared by a group of animals (71). However, there is sometimes disagreement over what constitutes a body plan. Part of this confusion may be historical, but much

AP: anterior-posterior, as in anterior-posterior body axis

Hox: homeotic complex. A cluster of homeodomain transcription factors required to specify the identity of body segments along the AP body axis of arthropods and other animals

Homeosis: the transformation of the normal identity of an anatomical structure or body segment to another’s identity. Homeosis is usually considered in the context of mutations in developmental regulatory genes. Homeotic, regulatory mutations in these genes have also been proposed as a factor in some instances of body plan evolution

Tagmosis: the specialization and/or anatomical unification of adjacent body segments into “tagma” to facilitate certain behaviors, often through similar modification of appendages. Such behaviors may include gathering sensory information, feeding, locomotion of some sort, gas exchange, or brooding

of it doubtlessly stems from ambiguity inherent in the term. The first such conceptual grouping based on anatomy was the archetype defined by Richard Owen, who also established the more enduring idea of homology. An archetype was envisioned as all the possible variations upon an anatomical theme (64). However, in rejecting Darwin’s theory of evolution by natural selection, Owen’s archetype never addressed the relationships between distinct morphologies. In 1945, Joseph Henry Woodger first proposed the *bauplan* (literally a “structural” or “building” plan) as the collection of homologous anatomical features seen across the natural history of a group (70). This recast Owen’s idea in an evolutionary context, and became translated as “body plan.” The term is still sometimes erroneously used to denote an anatomical grade of organization. However, body plans remain a useful concept because they summarize a collection of ancestral and synapomorphic characters within a group, while accepting their various derivations, and asserting an implied hypothesis that these similarities appear due to the monophyly of the group.

Given this definition, where is it appropriate to apply the concept? Does it only apply at greater levels of classification, such as phyla? Can we speak of the arthropod or chordate body plans, but not apply the term to the anatomy of insects or tetrapods? We suggest that a useful concept should not be artificially limited, and see no problem in speaking of the body plan of any presumptively monophyletic group sharing a characteristic anatomy. It should be possible to consider “greater” or “lesser” levels of body plans. Indeed, this seems appropriate, given that significant morphological innovation has appeared within many phyla since their appearance in the Cambrian, and these may be no less significant to their natural history. Fitch & Sudhaus have made a similar argument based on changes in the body axes of nematode groups (35). Other examples of such innovations include the evolution of jointed limbs in sarcopterigid vertebrates, the appearance

of wings in pterygote insects, and the loss of segmentation in higher mollusks. Therefore, body plans may be related by degrees to synapomorphies seen within any clade.

What Defines the Arthropod Body Plan?

The arthropods have traditionally been defined as segmented, appendage-bearing protostomes, protected by a cuticle that is periodically shed with growth (22). They are further distinguished from related groups, such as the onychophorans and tardigrades, by the fact that the appendages consist of podomeres with separate musculature and innervation (82). The specialization and/or anatomical unification of adjacent body segments, or tagmosis, helps to facilitate certain behaviors and varies greatly among arthropod groups. Tagmosis may also have followed convergent patterns along separate arthropod lineages, and several recent studies, based on molecular sequences and cladistic treatments of morphology, have questioned traditional arthropod groups, such as the Uniramia (54), Mandibulata (49), and Hexapoda (62). It has also fueled much debate over the phylogenetic relationships and monophyly of the extant arthropod classes (20, 21, 49, 73, 103). We frame our discussions in what we favor as the least controversial and most consensual of these phylogenies (20, 37, 72), summarized in **Figure 1**. Our favored phylogenetic hypothesis of arthropod relationships groups the insects, crustaceans, and myriapods into the Mandibulata. This group is unified by mouthpart homologies, and excludes the chelicerates.

DEVELOPMENTAL GENETICS OF ARTHROPOD TAGMOSIS

Evolution of the Arthropod Head

The union of anterior body segments into a well-developed head characterizes many of the extant arthropod groups. This presumably

provides the advantages of gathering the sensory and feeding appendages at the forward end of these active animals. Cephalization seems to have been associated with the recruitment of posterior segments into the head, coupled with a reduction of anterior segments. A recent morphological study by Budd (23) examined the head anatomy of several fossil arthropods in an attempt to address the homology of the large frontal appendages that characterize fossil crustacean-like species, such as *Yoboia* and *Fortiforceps*, as well as lobopods, such as *Aysbeaia*. This phylogenetic study included specimens that could be confidently assigned as basal members of the Chelicerata and Mandibulata, thereby including representatives of the extant crown groups without the interference of too many modern synapomorphies. In Budd's analysis, the crown groups (Mandibulata and Chelicerata) and trilobites formed a well-supported clade that includes fossil species lacking the frontal appendages, such as *Emeraldella* and *Cambrorhynchocope*. Based on this and other anatomical data, he suggests that the labrum seen in Mandibulata and Chelicerata may be evolutionarily derived from the frontal appendage. This is consistent with the expression of appendage patterning genes in the labrum (41) and with their functional requirement for proper labral development in insects and chelicerates (9, 80).

Hox Genes and Tagmosis

From genetic studies in *Drosophila*, Hox genes have been well characterized as high-level regulatory transcription factors, which act to impart specific identities upon the body segments and other structures in which they are expressed (reviewed in 47). In certain instances, the overlap of two or more Hox genes can produce fates distinct from those specified by either gene alone. For example, in the *Drosophila* labial imaginal disc, *pb* and *Scr* interact to direct proboscis development, where alone these genes specify only maxillary palp or leg (2). Therefore, the 10 ancestral arthro-

TAGMOSIS IN MAJOR ARTHROPOD GROUPS

Insects are the most tagmatically consistent arthropod class. They bear a head of fused segments, a thorax of three segments, followed by an abdomen of 10 or 11 segments.

The myriapods possess a well-organized head, similar to that of insects, followed by a varying number of homonymous trunk segments. Chilopoda (centipedes) bear one pair of legs on each trunk segment, while Diplopoda (millipedes) bear two pairs of legs on most segments. Pauropoda represent a curious intermediate state, where a segment as seen from the dorsal side spans what are apparently two segments ventrally.

Presumably, basal crustaceans consist of head and trunk tagmata. Among Malacostraca, the body plan consists of three tagmata: cephalon, pereon, and pleon. The appendages of the pereon and pleon are usually divided functionally to tasks such as walking, swimming, respiration, or brooding eggs, but these tasks do not always fall to the same tagma in different groups.

The basal chelicerates possess a body plan organized into three tagmata: prosoma, mesosoma, and metasoma. The prosoma bears the mouthparts and legs, whereas the mesosoma bears respiratory appendages. Arachnids have apparently eliminated the metasoma (16), and consist of two tagmata: prosoma and opisthosoma.

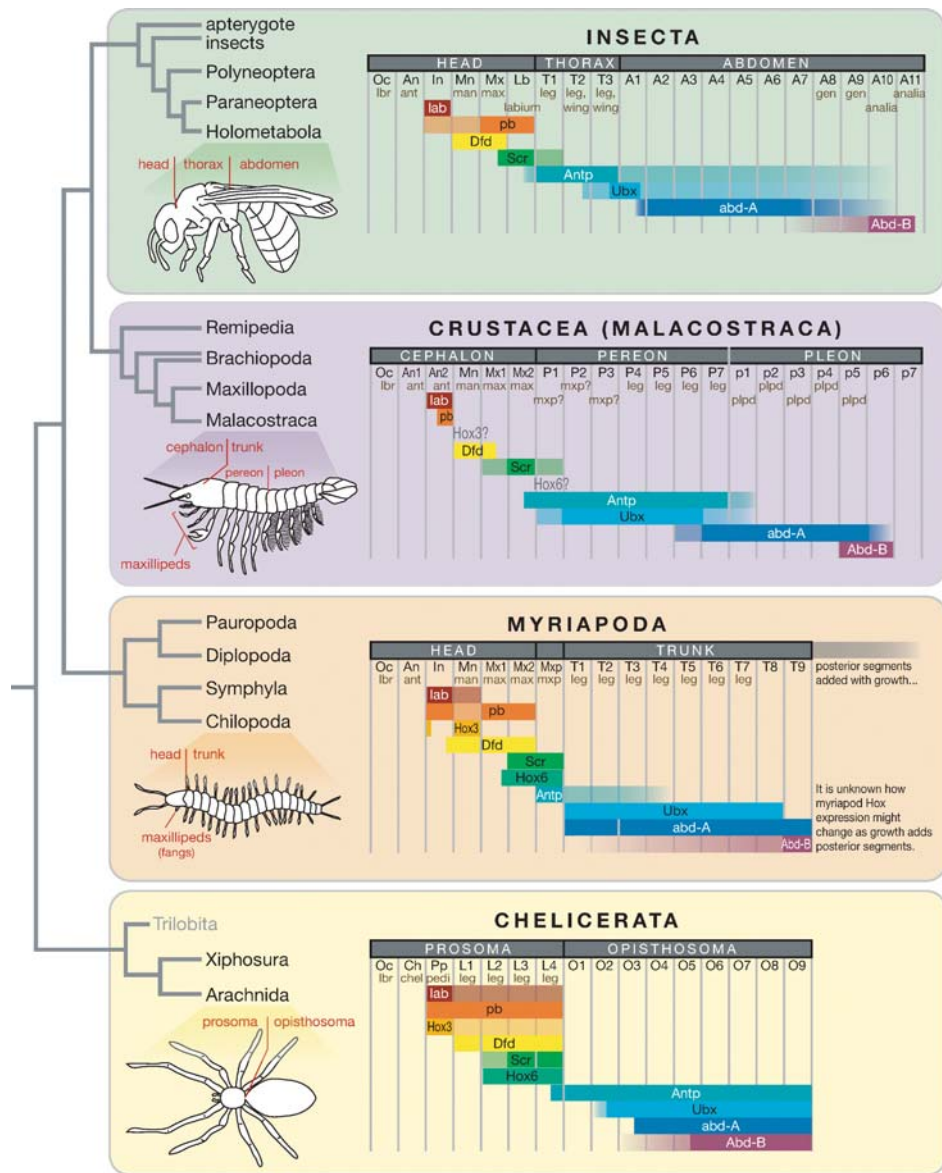
pod Hox genes are theoretically capable of specifying at least 20 unique body regions. (Assuming colinearity, if each Hox gene has an area where it is uniquely expressed and another in overlap with its neighbors, 2n-1 regions can be demarcated. An anterior Hox-free region adds one additional possible identity.) It is possible that such extensive differentiation exists within the central nervous system, but this has not been carefully examined. However, in the embryonic ectoderm of most arthropods, several Hox genes typically overlap in a given segment, such that far fewer than the theoretical maximum number of body regions is initially distinguished.

What is usually observed is a correlation between the tagmatic boundaries of an arthropod's body plan and the overlap of Hox genes in that region (**Figure 1**). For example, in arachnids (7, 31, 86), *lab*, *pb*, *Hox3*, *Dfd*,

Scr, and *Hox6* are expressed in a nested pattern within the prosoma, where they overlap broadly. Similarly, in the opisthosoma expression of *Antp*, *Ubx*, *abd-A*, and *Abd-B* orthologues also overlap in a nested manner. In the Mandibulata, a distinct head usually gathers several pairs of appendages, which perform separate gnathal functions. This reaches an extreme in the decapod crustaceans, which may have as many as seven pairs of appendages

that function in feeding. In these arthropods, the Hox genes overlap very little in the head (5, 46, 47). It seems likely that this facilitates the specification of a greater number of distinct identities, although this has only been tested functionally in a handful of insect species.

Nevertheless, it is relatively uncommon for Hox genes to cross a tagmatic boundary. This is most clearly seen in the arachnids,



where segments of the prosoma and opisthosoma express separate sets of Hox genes. Only the *Antp* orthologue shows a small degree of crossover into the L4 prosomal segment, while it is predominantly expressed in the opisthosoma. In myriapods, the trunk is characterized by the expression of *Ubx* and *abd-A* (46). Again, *Antp* is the only Hox gene to cross this tagmatic boundary, although unlike its pattern in chelicerates, in the centipede it is predominantly expressed in the maxillipedial segment and tapers off posteriorly into the trunk. In the insects, *Antp* and *abd-A* chiefly specify the thorax and abdomen, respectively.

Among different crustacean body plans, the Hox genes of the trunk correlate in expression with tagmosis. Brachiopod crustaceans have a homonymous trunk, and in the brachiopod *Artemia franciscana*, *Antp*, *Ubx*, and *abd-A* overlap extensively in their expression (11). However, in the decapod *Procambarus clarkii* (5) and the isopod *Porcellio scaber* (4), expression of *Antp* and *Ubx* is restricted to the pereon, while *abd-A* expression appears in the pleon. Therefore, the boundaries of these tagmata are respected by the Hox genes.

So, does the overlap of Hox genes provide a molecular definition of tagmata? Not reliably, it appears. There are many instances where Hox expression crosses tagmatic boundaries, often at later stages of development to modify the fate of individual segments within a tagma. For example, in later stages of *Drosophila* embryogenesis, *Scr* and *Ubx* expression expand from neighboring tagmata into T1 and T3, respectively, to modify their identities. As is discussed below, these domains of expression likely evolved in connection with the placement and specialization of wings on the insect body plan.

T1: first thoracic segment or prothorax

The Recruitment of Maxillipeds in Crustacea

Hox genes are known to specify segment identity in *Drosophila*, alone or in combination. However, viewed from an evolutionary perspective, do Hox genes act passively to specify the identity of segments, or can they play an instructive role? In other words, how easily can modules of effector genes come under the regulation of different Hox genes or combinations of Hox genes? If Hox genes were to

Figure 1

The four most commonly recognized arthropod classes differ in body plan and Hox gene expression. A consensus tree of major arthropod groups is shown here. This tree is not meant to be exhaustive, and numerous taxa have been omitted. For each class, a representative of the most well-examined body plan is shown. The consensus patterns of Hox gene expression are also shown for these representative groups. Body segments and the appendages they typically bear are abbreviated. For segments: Oc, ocular; Ch, chelicerate; Pd, pedipalpal; L1, etc., first leg-bearing, etc.; O1, etc., first opisthosomal, etc.; An1, etc., first antennal, etc.; In, intercalary; Mn, mandibular; Mx1, etc., first maxillary, etc.; Mxp, maxillipedial; T1, etc., first thoracic, etc.; P1, etc., first pereonic, etc.; p1, etc., first pleonic, etc.; A1, etc., first abdominal, etc. Appendage abbreviations: lbr, labrum; chel, chelicerae; pedi, pedipalps; ant, antennae; man, mandibles; max, maxillae; mxp, maxillipeds; plpd, pleopods; gen, genitalia. The Holometabola include insect orders with true metamorphosis. Hemimetabolous pterygote insects comprise the Paraneoptera, which includes Hemiptera and allied orders, and the Polyneoptera, which includes Orthoptera, Phasmatodea, and others. Apterlygote insects are a paraphyletic assemblage that includes the firebrat *Thermobia* (*Zygentoma*). Malacostracan crustaceans include the isopods, decapods, and the “true shrimp.” Maxillopoda and Brachiopoda are diverse and possibly paraphyletic groups. Remipedia includes the barnacles and related crustaceans. Myriapoda includes Chilopoda (true centipedes), Symphyla (garden centipedes), Diplopoda (millipedes), and Pauropoda. The chelicerates are considered to be basal among the arthropods. Arachnida includes most extant chelicerates, including the Araneae (spiders), Acari (mites), as well as scorpions and others. The Xiphosura include extinct chelicerates as well as extant horseshoe crabs of the genus *Limulus*. The extinct trilobites are likely a basal lineage within Chelicerata.

passively identify segments, then evolutionary changes in tagmatic boundaries should be possible without shifts in Hox expression along the AP axis. In this case, network association between Hox genes and their target effectors must be flexible. However, if these network associations are not plastic, and individual Hox genes maintain stable regulatory connections to target genes, then tagmatic shifts would require changes in Hox expression. Apparently, body plan evolution has proceeded by both routes, as we will show. The following example suggests that evolutionary changes in Hox expression may be instructive.

Among the crustaceans, numerous lineages, including the Maxillipoda, Isopoda, and Decapoda, have modified thoracic appendages to roles in feeding. The body segments bearing these maxillipeds may also be incorporated into the cephalic carapace, as in eucarid decapods. In effect, this is a modification of body plan tagmosis at the level of these crustacean groups. Therefore, investigators have examined how the Hox genes are expressed in crustaceans with and without maxillipeds. Segments of the crustacean trunk express *Ubx* and *abd-A* (4, 5, 11). An antibody to the Ubx and Abd-A proteins with broad phylogenetic cross-reactivity allowed Averof & Patel to survey seven diverse crustacean species (13). This study included species with maxillipeds independently derived among the Maxillipoda and Malacostraca, and illustrated that the anterior boundary of Ubx/Abd-A correlates with the most anterior segment bearing walking legs. That is, segments with appendages recruited to function as maxillipeds during their evolutionary history no longer appear to express *Ubx*. Instead, maxilliped-bearing segments express the more anterior Hox genes, *Scr* and/or *Antp* (5). The shift in tagmatic boundary has been accompanied by a shift in Hox expression. Furthermore, different lineages have evidently employed similar modifications in the evolution of these convergent structures.

A possible reason for this may be that Hox genes anterior of *Ubx*, such as *Scr* or *Hox6*,

may have already been in control of target genes important to the function of gnathal appendages. If so, mutations changing Hox expression might have been more likely than those bringing these targets under the regulation of a new, more posterior Hox gene. In this way, the evolution of maxillipeds may be canalized by network architecture.

A related story provides further evidence for the instructive activity of Hox genes. The isopod crustacean *Porcellio scaber* has evolved a single pair of maxillipeds along an independent lineage from the decapods. In this species, *Ubx* expression also makes its anterior boundary at the maxilliped-bearing segment. However, Abzhanov & Kaufman have shown that during early embryogenesis, the appendage appears to develop as a leg and only later transforms into a distinct maxilliped (3). *Scr* is expressed in the maxilliped segment, but the protein is not detectable by antibody in the appendages until stages after the morphological transformation. Presumably, this delay in maxilliped identity results from the suppression of *Scr* mRNA translation. Although the purpose of the delay remains unknown, it provides a developmental example of how Hox genes may specify segment identity in a non-model organism.

Reduction of Tagmata in Some Lineages

While most arthropods have successfully exploited an elaborate tagmosis, many others have evolved a secondarily simplified body plan. These include many parasites, species with reduced mobility, as well as those modified for microscopic habitats. Rhizocephala (Crustacea, Remipedia) are parasites of other crustaceans, and lack most typical crustacean structures, including most body segmentation (22). The “trunk” or abdomen of the body is extremely reduced in larval stages, and absent in adults. In the rhizocephalan *Sacculina carcini*, the larval abdomen expresses *Abd-B* before it degenerates at the end of larval development (19). Strangely, *abd-A* is apparently

not expressed. This is intriguing, since if selection favored a reduced abdomen in the lineage leading to *Sacculina*, it could have been possible for Hox genes in this tagma to activate apoptotic pathways to eliminate these segments. Apoptotic targets are regulated by *Dfd* and *Abd-B* in the *Drosophila* embryo to produce intersegmental furrows (59). It is possible that in an ancestor of *Sacculina*, *Abd-B* had similar regulatory connections to the cell death pathway, which were co-opted to the elimination of the abdomen.

Similarly, acarid mites of the suborder Parasitiformes lack obvious segmentation, and the opisthosoma is fused and dramatically reduced (22). Such extreme reductions are also seen in Hexapoda. Collembolans are a basal hexapod lineage, and the presumed sister group of the insects. The family Sminthuridae are noted for an abdomen that is reduced and fused to the thorax, giving the animals a globular appearance (29). Reduction of this kind even appears among the pterygote insects. The Coccoidea (scale bugs) are sexually dimorphic, and females are often sessile. The

female abdomen is reduced and lacks obvious segmentation (29). Numerous other examples exist, and these groups have certainly reduced their body plans independently. Therefore, it is tempting to speculate whether these reductions of the abdomen have also been accompanied by modifications in the roles of *abd-A* and *Abd-B* reminiscent of the Rhizocephala. Does *Sacculina* represent an extreme case, or might the elimination of Hox expression and/or the activation of apoptotic pathways provide a common evolutionary route to tagmatic reduction?

EVOLUTION OF LESSER BODY PLAN FEATURES IN INSECTS

Insect tagmosis is remarkably consistent. However, these arthropods have evolved amazing variations from this pattern. The developmental genetic mechanisms responsible for these varied morphologies are just now being explored. Much of this work has focused on modifications to wing morphology, and a guide to some of the taxa discussed below is given in **Figure 2**.

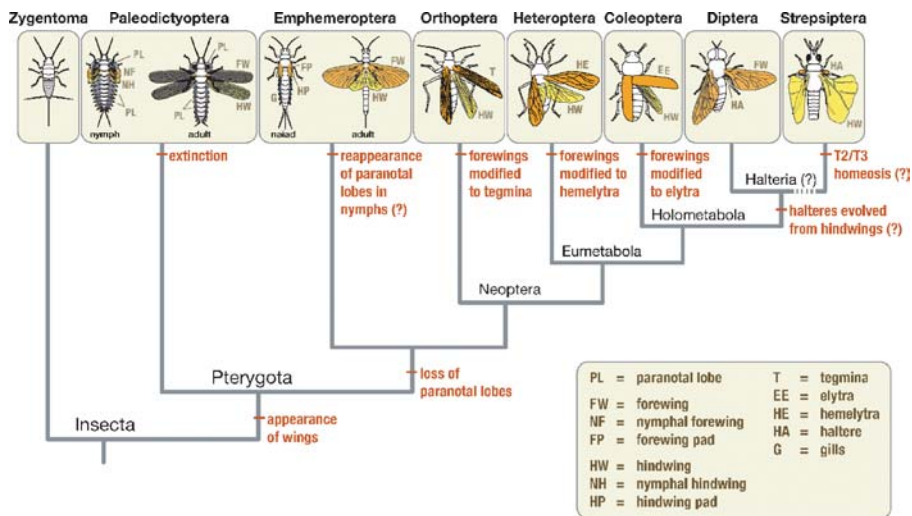


Figure 2

Some of the wing modifications seen among insects. The paranotal lobes seen in primitive insects are shown in blue. Forewings and their derivatives are shown in orange, while hindwings and derived structures are shown in yellow. One evolutionary scenario for the origins of these wing modifications is mapped onto a tree of these groups, based on the phylogeny of Wheeler and coworkers (97).

The Origin of Wings

One of the most distinctive features of the insects is their ability to fly. Debate over the evolutionary origins of insect wings has been a subject of much consideration in the past century. Two main hypotheses have been considered: The first asserts that wings evolved from outgrowths of the dorsolateral cuticle (36), first as an adaptation to parachuting or gliding (81), before articulation and muscle connections allowed powered flight. The second idea postulates that wings were modified from ancestral exites (dorsal projections) on the proximal legs and abdominal appendages of early insects (53). In turn, these exites are thought to derive from gill-like epipods of primitive crustacean-grade ancestors (12), and possibly homologous to the gills seen in aquatic larvae of some extant Ephemeroptera (mayflies).

Genetic studies of *Drosophila* have cataloged a number of ways in which wing development resembles development of the other appendages (reviewed in 61). In this species, the wing and leg imaginal primordia are derived from a shared pool of precursor cells. Wings utilize some of the same signaling mechanisms to establish axial polarity. These data seem to support the hypothesis that wings evolved from an appendage derivative.

However, it is possible that in evolution from a body wall outgrowth, appendicular developmental networks were co-opted for use in protowings. Thus, Averof & Cohen reasoned that it would be very unlikely to find genes principally involved in insect wing development in the epipods of crustaceans. To test this hypothesis, they determined the protein accumulation of two “wing gene” products in two crustaceans: the brachiopod *Artemia franciscana* and the malacostracan crayfish *Pacifastacus leniusculus* (12). The genes chosen were *pdm*, which is required early to specify the fate of the *Drosophila* wing disc but has only limited expression in the legs, and *apterous* (*ap*), which functions in determination of dorsal-ventral (DV) polarity in the wing. In *Artemia*, Pdm and Ap proteins were

found throughout the more distal of the two epipods, whereas in *Pacifastacus*, Pdm was detected in the single epipod of appendages in this species. These data provide molecular evidence for an evolutionary connection between epipods and wings, and support anatomical claims of their homology (53).

These researchers later expanded their investigation and found that *pdm* and *ap* are expressed in chelicerate structures, which have also been suggested as homologues of crustacean epipods (30). The Xiphosura are unique among modern chelicerates in that the opisthosomal segments bear appendages. In species such as the horseshoe crab *Limulus polyphemus*, the opisthosomal appendages are modified into gas exchange structures called book gills. These structures have been internalized as book lungs in the evolution of arachnids, such as the spider *Cupiennius salei*. Both book gills and book lungs express *pdm* and *ap* during embryonic development. In *Cupiennius*, these genes are expressed in the spinnerets of the opisthosoma (30), which are also thought to be an appendicular derivative (6, 22, 80). These studies suggest that derived structures such as insect wings, crustacean epipods, xiphosuran book gills, and the arachnid book lungs and spinnerets may all share a common ancestry. If so, this provides a remarkable example of the evolutionary flexibility of the arthropods.

These studies are useful, but should be interpreted cautiously. They represent only the expression data of two genes in four divergent species. One paradox of the wings-from-epipods hypothesis is that it requires each of the four independent lineages of primitively wingless hexapods to have lost wings or their precursors in parallel. Jockusch & Ober (50) have recently presented a critical review of the hypothesis of appendicular wing origin. They also used molecular markers and histology to show that, unlike *Drosophila*, the wing and leg primordia are not descended from a common pool of cells in *Tribolium* and the grasshopper, *Schistocerca*. Instead these authors propose that the appearance of *pdm* and *ap* in

wings and epipods may represent convergent co-option.

Suppression of Prothoracic and Abdominal Wings in Modern Insects

Early wings are thought to have been present on all thoracic and abdominal segments. The oldest fossil species identifiable as pterygote insects possess primitive wings on T2 and T3, and lateral winglets or “paranotal lobes” on T1 and abdominal segments A1 to A9 (53) (depicted in **Figure 2**). Unlike most modern insects, the wings and paranotal lobes of Paleozoic species were also present in the juvenile instars (101). Wing venation was very extensive in fossil specimens (55). Therefore, in several ways, modern insects have reduced the prominence of wings in their body plan (29). Venation has been reduced, while wing number has been restricted to two and limited to adults in most extant species.

In *Drosophila*, input from thoracic Hox genes, such as *Antp*, is not required for the activation of wing development (25). Instead, it appears that several Hox genes act to repress wing development elsewhere along the body. In the prothorax (T1) of the *Drosophila* embryo, *Scr* is expressed in the dorsal ectoderm where it is required to suppress the development of wing primordia (25, 67). Dorsal T1 expression domains of *Scr* have been noted in other insects (77, 93). Similar expression also appears in the primitively wingless insect *Thermobia* (77) and the isopod crustacean *Porcellio scaber* (D.R.A. & T.C.K., unpublished data). Expression of *Scr* in these primitively wingless groups has led to the idea that the function of *Scr* in T1 wing suppression is an exaptation of pterygote insects. However, without functional data, we can only speculate about the potential function of this dorsal *Scr* expression domain in primitively wingless species.

In the insect abdomen, *Ubx* and *abd-A* apparently suppress wing development. Genes such as *snail*, which mark early wing discs in *Drosophila*, become ectopically expressed in

the abdomen of embryos lacking *abd-A* (25). In the milkweed bug *Oncopeltus*, depletion of *abd-A* causes ectopic abdominal leg development (10). Abdominal segments of *abd-A*-depleted individuals also acquire dorsal pigmentation similar to T3. This pigmentation marks the location of the juvenile hindwing pads in *Oncopeltus*, suggesting that *abd-A* depletion in this species may also relieve suppression of abdominal wing development.

Differentiation of Forewings and Hindwings

Most winged insects bear two pairs of wings: forewings on the mesothorax (T2) and hindwings on the metathorax (T3). However, the forewings and hindwings have distinct morphologies, which may allow the wings to cooperate aerodynamically to enhance flight proficiency (29). Alternatively, one pair of wings may be modified for the purposes of protection or display, as with the coleopteran elytra, or for balance, as with the halteres of Diptera and Strepsiptera. These more derived examples are discussed below, but first let us consider the basic issue of more subtle distinctions between forewing and hindwing.

For several decades, it has been known that the Hox gene *Ubx* was required to distinguish the identity of the T3 body segment from T2 in *Drosophila* (57). In *Drosophila* and other Diptera, the hindwings are modified into small balancing organs called halteres, and *Ubx* acts to repress wings on T3. So what is the situation of other insects in which wings are present on T3? Would *Ubx* expression be absent from the developing hindwings? Warren and colleagues (94) investigated this possibility in the butterfly *Precis coenia*, where they found that Ubx protein accumulated at high levels in the imaginal hindwing (T3) discs, but was absent from forewing (T2) discs. Weatherbee et al. showed that several wing patterning genes suppressed by Ubx in the *Drosophila* haltere disc are expressed in patterns similar to the *Drosophila* wing disc in both the forewing and hindwing discs of

A1, etc.: first abdominal segment, etc.

T2: second thoracic segment or mesothorax

T3: third thoracic segment or metathorax

Precis (96). Furthermore, a spontaneous mutation was identified in *Precis* in which *Ubx* fails to be expressed in patches of the hindwing (96). In the absence of *Ubx* protein, these patches adopt pigmentation patterns characteristic of the forewing. Conversely, hindwing pigmentation could be induced in the forewings of *Precis* by constitutive expression of *Ubx* from a viral vector (56). Therefore, rather than acting to repress wing development, it appears that *Ubx* acts to distinguish hindwing from forewing.

The Evolution of Dipteran Halteres from Hindwings

The differentiation of hindwings from forewings is taken to an extreme in the Diptera. In this group the hindwings are modified into a pair of balancing organs called halteres. In *Drosophila* the Hox gene *Ubx* is required to specify the halteres, and certain *Ubx* mutant alleles produce flies without halteres, but rather with two pairs of wings (57). *Ubx* intervenes at several levels to direct haltere development, by inhibiting genes involved in dorsal-ventral specification of the disc (60), organ size and shape, and bristle formation (95). *Ubx* is expressed in regions of the T3 body segment in a wide range of insects with varied modifications of fore- and hindwings (10, 17, 25, 102), and this may be an ancestral trait shared by pterygote insects. Therefore, it is likely that the evolution of halteres proceeded by a slow process as *Ubx* regulation was acquired within the *cis*-regulatory elements of genes functioning at each of these levels.

The Evolution of Elytra from Forewings in Coleoptera

The Coleoptera are perhaps the most successful lineage of animals, with over 350,000 described species (29). They are characterized by the modification of the forewings into elytra, a protective covering over the abdomen. Therefore, in Coleoptera, such as the red flour beetle, *Tribolium castaneum*, flight is depen-

dent on the hindwings (T3), which are similar to the single pair of wings (T2) in Diptera.

What is the role, if any, of *Ubx* in these modified forewings, the elytra? It is possible that *Ubx* acts to modify wing development to produce elytra from the T2 segment in *Tribolium*, just as it does in the T3 disc of *Drosophila* to produce halteres. Alternatively, *Ubx* may simply identify T3 structures and regulate a separate set of target genes to allow hindwing development in the beetle. Recently, these hypotheses were tested by Tomoyasu and coworkers, who used RNA interference to suppress *Ubx* function in *Tribolium* larvae (87). They found that with the reduction of *Ubx* activity the T2 and T3 dorsal imaginal discs develop as elytra. Similarly, mutations in the *Tribolium Scr* orthologue, which is expressed in the T1 segment, produce ectopic elytra-like structures on T1, rather than wing-like structures (14, 15). The *Antp* orthologue in *Tribolium* appears to have no influence on the identity of elytra or hindwings, since its suppression causes no defects in these structures (87). Therefore, in the absence of input from Hox genes, such as *Ubx*, the dorsal imaginal discs develop as elytra. This contrasts the situation in *Drosophila*, where in the lack of *Ubx* the imaginal discs develop as two pairs of wings (57). Furthermore, Tomoyasu and coworkers have shown that in *Tribolium*, *Ubx* does not repress target genes as it does in the haltere disc of *Drosophila*. Instead, these genes are expressed in unique patterns in the elytra that are independent of *Ubx* regulation. It seems likely that the ancestral role of *Ubx* has been to identify T3. During evolution, as T2 and T3 structures were modified differently in separate lineages, different target genes came under *Ubx* regulation in order to produce distinct morphologies unique to T3.

In this example, the unregulated (or at least Hox-free) pathway has diverged to produce the elytra developmental program. However, for the T2 discs to develop as elytra and to retain a functional pair of hindwings, *Ubx* would have to acquire regulatory control over wing

development in order to preserve this developmental mechanism. This is very different from the evolutionary scenario imagined for the Diptera, in which the evolution of a novel structure (halteres) was accompanied by the appearance of novel regulatory relationships to produce that structure.

Forewing modifications appear in other insect groups as well. The forewings of Orthoptera are modified into a leathery protective form called tegmina. Many species of Heteroptera feed on plants, from which they acquire toxic substances used to deter predators. The forewings or hemelytra are often thickened proximally and brightly colored to advertise the insects' toxicity. It is interesting to speculate whether modifications in wing patterning seen in the evolution of coleopteran elytra might also have been paralleled in the evolution of these other modified forewings, and whether the development of the more typical hindwings is dependent on suppression of the modified developmental mechanisms by *Ubx*.

Phylogenetic Homeosis Among the Strepsiptera?

A more complex instance of wing modification exists in a little-known group of insects: the Strepsiptera. Adult males of these endoparasitic insects have halteres similar to those of Diptera. Strangely, strepsipteran halteres are found on T2, whereas T3 bears functional wings. The phylogenetic position of the Strepsiptera among insect orders has been controversial, but molecular and morphological data exist supporting a sister-group relationship between Strepsiptera and Diptera, termed the Halteria (97, 100). If the Halteria are indeed a monophyletic group, then it is interesting to consider the evolution of halteres among this proposed clade. Did halteres evolve separately in each lineage? From hindwings in Diptera and forewings in Strepsiptera? And if so, were these insects somehow predisposed, genetically or otherwise, for such anatomical modifications? Alternatively,

Whiting & Wheeler have suggested that the haltere developmental mechanism was somehow co-opted from one thoracic segment to the other after the divergence of these lineages (100). They point out that mutations in *Ubx* in the early strepsipteran lineage, similar to the *bithorax* and *postbithorax* (57) regulatory alleles of *Drosophila Ubx*, could cause *Ubx* expression in T1 rather than in T3, leading to a phylogenetic homeosis of wings and halteres. Bennett and colleagues have examined *Ubx* expression in embryos of one strepsipteran species (18; R. Bennett, personal communication). Unexpectedly, *Ubx* expression was limited to the first abdominal segment, and did not appear in either T2 or T3. Although it is possible that later expression could appear in imaginal discs, these data suggest that an ancestral homeotic-type mutation in the Strepsiptera appears unlikely.

However, it remains possible that Strepsiptera diverged from Diptera after the evolution of halteres along a dipteran-style body plan. In such a scenario, the loss of *Ubx* expression in the T3 segment may have permitted the development of T3 wings. The existing haltere developmental mechanism may then have become activated by some other factor expressed in T2. *Antp* is one candidate for this role. More studies in these experimentally challenging but evolutionarily intriguing insects will be necessary to resolve these issues.

The Appearance of Winglessness Among Insects

Reduction and loss of structures is one of the more common modifications seen in various taxa. From the nearly ubiquitous paranotal lobes of Paleozoic insects to modern forms, insects have exploited the advantages of reduction in wings. Winglessness has also offered other niches, which have been invaded by members of the Pterygota.

In the social Hymenoptera, nonreproductive castes of ants are wingless, facilitating a subterranean lifestyle (44). Sufficient larval nutrition produces an increase in juvenile

JH: juvenile hormone. An insect hormone used to trigger developmental events, such as the survival of wing discs in juvenile ants

hormone (JH) levels, which is required for development of queens. Part of this developmental program is the maturation of T2 and T3 wings. Individuals receiving less food (and hence producing less JH) develop as sterile, wingless workers (63). The mechanisms of wing specification and patterning are considered to be well conserved, based on the similar expression of orthologues in *Drosophila* (Diptera) and the lepidopteran *Precis* (24, 96). Therefore, working with four species of ants, Abouheif & Wray investigated the expression of genes known for their roles in the wing development of other insects (1). Their study confirmed that these orthologues were conserved in their expression patterns in the wings of reproductive castes. Surprisingly, their data from nonproductive castes also revealed that wing development was disrupted at different points in these different species.

Ants of *Pheidole morrisi* appear in two nonreproductive wingless castes: soldiers and minor workers. The fate of the T2 wing discs in nonreproductive castes of *Pheidole* is determined by a second JH requirement. JH exposure at the second checkpoint specifies soldiers, in which the T2 wing discs evert at the prepupal stage, but proceed to die apoptotically, whereas the wing discs of workers disintegrate before the end of larval development (78). Abouheif & Wray also found that the wing development pathway is disrupted much earlier in *Pheidole* workers than in soldiers (1). It was unexpected to find that wing development could be interrupted in so many different ways in species for which wingless castes are a shared ancestral trait. This may have been due to the neutral drift of developmental networks (see below).

At least two possible routes for the evolution of these interruptions of wing development are possible. We can presume that since all extant and fossil ant species possess wingless nonreproductive and winged reproductive castes (44), selection has acted to maintain this social arrangement. Therefore, winglessness in workers must not come at the cost of

winglessness in all castes. This would imply a genomic conservation of the wing developmental network. It is possible that this network receives input from the reception of JH and wing patterning is actively blocked at that point in its absence. If so, the point of interface between the JH and wing development pathways must be fairly flexible because it apparently varies between species and castes. Alternatively, when JH is not supplied at a critical stage, pathways related to JH reception (about which very little is known) could directly activate cell death in the wing discs. In this scenario, the default developmental pathway would be to produce wings in the style of the reproductive castes. Variation in the timing of JH checkpoints, between species and castes, would also vary the timing of cell death relative to the stage of wing patterning. This second hypothesis seems more likely, since castes that are determined by later JH checkpoints, like *Pheidole* soldiers, generally have a later point at which wing development is interrupted.

Winglessness has also evolved in solitary insects. Unlike polyphenic social insects, which apparently maintain the wing developmental pathways, wingless solitary insects could in theory lose the wing developmental network due to accumulations of neutral mutations. The phasmids, or stick insects (Phasmatodea), exhibit elaborate camouflage resembling vegetation. Winglessness also appears in the majority of species, while others possess a range of reduction in wing morphology. Partial wing loss or reduction is sometimes sexually dimorphic, with males retaining wings and flight ability. A recent molecular phylogeny by Whiting (99) found that, whereas winged forms are basal among the Phasmatodea, the majority of wingless species are derived from a single lineage. However, remarkably, wings also appear to have reappeared within this largely wingless group.

Flight involves the coordination of nerves and muscles, as well as the aerodynamics of wing shape. Genetic studies of *Drosophila* wing development have shown that while key

genes involved in wing patterning may function in multiple developmental processes, the genetic network they comprise to facilitate wing development is not used elsewhere (26). Without selection to maintain these regulatory connections, it is expected that mutations eliminating them should be neutral and accumulate with relative speed. Therefore, once lost, the reappearance of a complex trait, such as winged flight, has been considered very unlikely. Nevertheless, Whiting concludes based on parsimony that the reappearance of wings may have occurred on as many as four separate occasions during phasmid evolution (99). Acceptance of these findings implies that our understanding of genetic network evolution may be somewhat incomplete.

The Evolution of Foreleg Combs

Insect diversity encompasses far more than wing modifications. For other traits, few genetic data may be available. However, one area of some research is the occurrence of foreleg combs. These are most familiar as the sex combs of *D. melanogaster*. Sex combs are a row of large anterior-ventral bristles at the distal end of the first tarsal segment (the basitarsus) of *Drosophila* males. Males use these structures in courtship, where they stroke the female's abdomen to stimulate ovulation (27). This fairly minor anatomical feature is nonetheless specified by a Hox gene and lends itself to the name *Sex combs reduced* (*Scr*). *Scr* is expressed in the T1 leg disc, including a domain corresponding to the location of the adult sex comb in males. Loss of *Scr* activity in the T1 leg discs of *Drosophila* results in legs lacking sex combs, while ectopic expression can produce sex combs on the T2 and T3 legs (67).

A similar structure appears in the milkweed bug *Oncopeltus fasciatus*. In this species, combs appear on the distal foretibia of both sexes and are used for grooming their long rostrum. *Oncopeltus Scr* is also expressed in an anterior-ventral patch of the T1 legs, which appears to correspond with the location of the foretibial

comb (77). Depletion of *Scr* activity by RNAi in *Oncopeltus* eliminates the foretibial combs (45).

The similarity of combs in these species is somewhat surprising. Foreleg combs appear relatively rarely and are found among the Heteroptera and Diptera, as well as some Coleoptera and Hymenoptera. Therefore, *Oncopeltus* and *Drosophila* have apparently evolved foreleg combs independently. *Scr* is expressed in a patch of the distal embryonic T1 legs of the cricket *Acheta domestica*, despite the lack of any comb-like structures in this species (77). Since this domain of *Scr* expression may be widely conserved, it has been suggested to act as an exaptation, facilitating the evolution of combs (77). However, an obvious question becomes, why combs?

A broader survey of insects reveals that the distal tibia or proximal tarsi of the T1 legs often bear unique specialized structures. Although some Coleoptera have foreleg combs, similar to *Oncopeltus*, others bear large spine-like cuticular outgrowths on the T1 basitarsi. The foretibia of Embiidina (web spinners) bear glands used to produce silk threads. Furthermore, some Orthoptera, including *Acheta*, possess tympanal hearing organs on the distal tibia of the forelegs. It seems likely that, rather than being uniquely associated with combs, *Scr* expression in the T1 legs may specify many if not all of these structures. However, confirmation of such a regulatory relationship must await functional analyses in these other species.

The expression of *Scr* in the T1 legs may be an evolutionary innovation of the pterygote insects. *Scr* expression is absent from the T1 limbs of the apterygote *Thermobia* (77), and this species lacks any distinctive morphology on the distal forelegs. However, as noted, all pterygotes in which *Scr* expression has been examined show a correlation of the T1 leg domain with specialized structures, such as combs or tympanal organs. Since *Scr* is a regulator of transcription, it is plausible that this conserved domain has been predisposed to acquiring regulation over some aspects of

RNAi: RNA interference. A method for depleting gene activity by the introduction of a double-stranded length of RNA transcript.

DSD:

developmental systems drift. The process by which neutral mutations may cause random changes in regulatory networks over evolutionary time

morphology in the context of the T1 leg. Many of these, such as the large bristles of foreleg combs, are likely to come at a fairly mild fitness cost to individuals. If they appear with relative frequency over evolutionary time, selection may favor them occasionally, especially when sexual pressures apply (65), as with *Drosophila*.

DISCUSSION

Implications of Developmental Systems Drift

All genetic material is subject to random mutation. Often mutations fail to alter DNA in any deleterious way. Kimura has described how these neutral mutations may accumulate, unaffected by selection since they do not influence phenotypic fitness (51). In time, as two lineages diverge from an ancestor, the similarity of orthologous sequences will drift apart from one another. True & Haag have proposed a similar form of drift acting at the level of ontogenetic networks (88), and arising from two factors. The first is Kimura's theory of neutral evolution in genes. The second comes from the insights of developmental genetics that most phenotypes are the product of complex networks of interacting regulatory genes (32). As a simple example, imagine that two lineages diverge from an ancestor in whom a phenotype is the product of a developmental network in which one regulatory gene product, A, activates an intermediate regulatory gene, B, that then activates an effector gene, C. If in one lineage, the *cis*-regulatory elements of gene C, the effector, change such that they may bind protein A, then gene B is functionally redundant. A subsequent mutation may then eliminate gene B or its binding site in the regulatory region of gene C without an effect on the phenotype. After the fact, examination of the network in each lineage would reveal that despite identical phenotypes, the network architecture in each is different. Although this is a simplistic example, such divergence in developmental net-

works may have occurred in the suppression of wing development in worker ants, as reviewed above. True & Haag have catalogued a number of other instances acting at various phylogenetic levels (88), and they have dubbed this phenomenon developmental systems drift (DSD).

The implications of DSD to comparative developmental genetics are quite important. DSD suggests that species may possess vastly more diverse genetic networks than can be predicted from their morphologies. One example from our own work involves the activation of the gene *spalt* in the antennae of insects. In *Drosophila* and the milkweed bug *Oncopeltus*, *spalt* is expressed in similar domains where it is required for formation of antennal joints (34, 92). *spalt* is activated cooperatively by *Distal-less* and *homothorax* in the antennae of *Drosophila* (34); however, these genes appear not to act in this way to specify *spalt* expression in *Oncopeltus* (9a). We suspect that in *Oncopeltus*, *spalt* is specified by a different unidentified factor. Since *spalt* performs similar developmental functions in each species, it seems unlikely that selection would directly alter the network architecture upstream of *spalt*. It is possible these network changes are related to other adaptive morphological changes, but random drift in the network architecture seems the simpler scenario.

Another important caveat of the DSD concept is that the divergence of networks can confound the candidate gene approach. As an example, let us reconsider our hypothetical 3-gene regulatory network from above. If the species that retains gene B as an intermediate were a model organism, then an investigator might choose to explore its orthologue's function in the other lineage. However, this study would be fruitless since gene B is no longer a component of the pathway in the second lineage. While this information is useful in understanding the regulatory evolution of these species, these sorts of negative data are rarely reported by themselves. Furthermore, if gene A is unknown in either species, then the

investigator has no more candidates for which to examine the regulation of gene C.

Fortunately, as genomic technology becomes more universally applicable, DSD may present fascinating opportunities for research. Theoretical modelers of developmental networks have been puzzled over the seeming robustness that networks exhibit in models, and have met with a frustrating lack of experimental data that describe the range of possible values for parameters such as gene expression rates (89). One problem may be that species are likely to exhibit a much narrower range of values than the total number of workable sets possible. However, DSD suggests that a diverse collection of species will provide a wide range of developmental parameter sets. Such experiments have been done with at best a few species and have been labor intensive (42, 74). However, a high-throughput method is needed for such analyses of large species numbers. If a large enough number of species could be assayed for the transcription levels of genes involved in one developmental process, such data would describe the range of transcription levels that are functionally possible to facilitate that process. Importantly, relationships between genes would also become obvious from such data. For example, perhaps genes A and B are consistently expressed strongly while gene C is at low levels, or vice versa, but never at high levels for all three. It is likely that such a relationship would be meaningful in the context of the network's architecture. Such large-scale genomic experiments would help explain, from principle, rather than post hoc explanations, how genetic networks specify morphology.

Integrating Comparative Developmental Conclusions into Evolutionary Biology

Most comparative developmental genetic data have been collected from a wide phylogenetic sampling. In contrast, few studies have examined morphological and developmental

genetic changes that might be relevant to speciation or at least resulting from relatively short-term isolation. This leads to an important question: Can comparative developmental genetics address the origins of novel morphologies?

This is a frequent criticism of macroevolutionary evo-devo. It is true that at such a phylogenetic scale, the origins of morphology may be only a matter of informed speculation. However, it would be wrong to then dismiss macroscale developmental studies as irrelevant to evolutionary biology. Like physiology and anatomy, the development of an organism is the end product of its evolution to date. Such studies describe the possible mechanisms by which organisms may be patterned, allowing us to begin to understand the limitations and language that genetic networks use in patterning organisms.

However, investigators of comparative developmental genetics have increasingly turned to smaller phylogenetic scales. Here, evo-devo can provide useful data on the origins of morphological novelty. Aside from exploring specific instances of morphological radiation, these studies also bear on the two major hypotheses of morphological evolution. The first is the traditional infinitesimal evolutionary model of Fisher, by which morphology changes by small quantitative degrees over generations due to selection and adaptive fitness advantages of some segregating alleles (34a). The second hypothesis arises from the observation that evolution of new morphology has occurred rapidly in the fossil record. This has led to speculation that spontaneous homeotic mutations or "hopeful monsters" might account for such changes (39, 40). This issue is still open, as it now seems that evidence exists to support morphological evolution by infinitesimal changes as well as mutations in developmental regulatory genes. However, these mechanisms appear in different contexts, which may mark an important distinction between them.

The concept of the hopeful monster was first suggested in 1940 by Goldschmidt (39),

and arose from his work on mutations in the *Drosophila* homeotic genes. He proposed that variably penetrant homeotic mutations might introduce the changes in morphology seen between species and higher taxa. The idea was derided, partly because Goldschmidt used it in support of more eccentric genetic theories (33). However, recent comparative studies of drosophilids have emerged that seem to suggest sudden mutation in developmental regulatory genes or networks can produce morphological differences between closely related species.

Evidence for Hopeful Monsters

Abdominal pigmentation varies widely within the *D. melanogaster* species group. It is sexually dimorphic in some species, in which it may play a role in mate choice. Kopp and colleagues have shown that the transcription factor *bric-a-brac* (*bab*) is required for specification of the melanic pattern in the abdomen (52). *bab* is an important developmental regulator, which also functions in the development of the limbs. In *D. melanogaster*, where black pigmentation is found over much of the male abdomen, *bab* is also activated by *Abd-B* and *doublesex* (*dsx*), the primary identifier of somatic sex. However, in other species, the expression of *bab* correlates with pigmentation rather than with sex (in sexually monomorphic species) or *Abd-B* expression (in species where pigmentation is restricted to more posterior segments). This suggests that since the radiation of these species, estimated to be within the last 5–10 million years (58), mutations in the regulatory regions of *bab* have altered inputs from *dsx* and the Hox genes. Therefore, mutations in this developmental regulatory gene (*bab*) have resulted in significant morphological divergence among these species.

Another example comes from the larval cuticle morphology of drosophilids. The larvae of *Drosophila sechellia* have a naked cuticle, where *D. melanogaster* possess trichomes. Sucena & Stern have shown by quantitative genetic analysis that remarkably the *shaven-*

baby (*svb*) locus is the exclusive source of this morphological difference. The *D. sechellia* naked cuticle “phenotype” is not complemented in crosses to *D. melanogaster svb* mutants, which also have a naked cuticle phenotype (85), suggesting that *D. sechellia svb* bears a spontaneous mutation similar to the experimentally generated *D. melanogaster svb* mutant. Naked cuticle morphology has appeared independently several times among drosophilids, and using similar methods, the same group has shown that similar noncomplementing mutations at the *svb* locus have accompanied the morphology each time (84). Furthermore, among two populations of *D. borealis* the *svb* locus possess wild-type (that is, *D. melanogaster*-like) and mutant (*D. sechellia*-like) alleles in different interfertile populations (84). The *svb* locus encodes a zinc-finger transcription factor, which is also required for female fertility. Mutations in the coding sequence produce fertility defects. These *ovo* alleles are genetically separable and complement *svb* alleles, which are due to mutations in the regulatory regions of the gene. This suggests that evolution of naked cuticle may be channeled to proceed via *svb*-type regulatory mutations.

Genetic Evidence for Infinitesimal Morphological Evolution

It is easy to imagine evolutionary scenarios for pigmentation and cuticle morphology that include hopeful monsters. Presumably, mutations affecting these traits come at some initial fitness cost, but it is likely not so great that they could not segregate under some conditions, be tested by selection, and come to fixation in some lineages. Therefore, evolutionary experiments in lesser body plan features could be fairly common. However, can the same occur for greater body plan features? Because of the high mortality associated with homeotic transformation of body plan, mutations of the type studied by geneticists seem unlikely. It may also be an unnecessary possibility.

Polymorphisms exist among wild populations for at least one Hox gene, *Ubx*. Often these polymorphisms are only manifest phenotypically in extraordinary environmental circumstances. For example, Gibson & Hogness have shown that naturally occurring *Ubx* polymorphisms in *D. melanogaster* produce varying haltere defects when larvae develop in the presence of ether (38a). Stern has also shown that *Ubx* polymorphisms can lead to variation in the bristle patterns of the T3 leg in hybrids with *D. simulans* (83). Nor is this phenomenon unique to *Ubx* or *Drosophila*, as revealed by recent studies of polymorphism in human populations (76). Variation in human interleukin-4 appears to have phenotypic trade-offs for immune fitness, and changes at this locus relative to those of the great apes appear to indicate positive selection (75). Therefore, it appears that variation normally segregates in “genes of large effect.”

These examples deal with traits that are manifest only under extreme environmental circumstances. (We may consider hybridization an extreme cellular environment for a haploid genome. As for humans—modern life has often been called extremely taxing, but this may be especially true considering the historical influence of disease on survival.) However, it is possible for environmentally induced traits to become phenotypically fixed under strong selection. This concept of “genetic assimilation” was proposed by Waddington (91), based on experiments he conducted in *Drosophila*. Waddington heat-shocked a line of flies and observed that wing crossveins were absent in a moderate percentage of individuals. However, after 12 generations of heat shock followed by selection for a crossveinless phenotype, flies developed without crossveins even in the absence of the heat stimulus (90). Other examples of genetic assimilation have been reviewed by Pigliucci & Murren (68). It should be pointed out that genetic assimilation is not usually associated with infinitesimal evolution. From the standpoint of phenotype, it is easy to understand why—since morphol-

ogy changes rapidly under strong selection. However, it operates on the same infinitesimal molecular changes and the interactions of fitness and selection.

To summarize, polymorphisms exist in high-level regulatory genes, and even if they are not of immediately apparent fitness value, it may be possible for the environment to select and fix novel morphologies arising from this variation. Alternatively, for traits with a potentially low fitness cost, mutations affecting developmental regulatory genes may produce novel morphologies in the style of hopeful monsters. In general, mutations in *cis*-regulatory elements appear more commonly than those in coding sequences, and it follows then that evolution may tend to favor alterations in the regulatory networks of developmental genes. Taken together, these processes may contribute to the adaptive success of populations that ultimately result in isolation and speciation (28, 68).

Future Directions

The remaining task of comparative developmental genetics is to explain why differences in developmental networks produce differing morphologies. The on-going comparisons of network architecture in morphologically diverse and phylogenetically distant species will help to describe the range of possible mechanisms for conserved and derived structures. This will provide the first insights into how diverse structures may differ developmentally. However, this still will not explain why species differ morphologically. To pose a similar question as an example, does our current understanding of metathorax specification or wing patterning really tell us why the *D. melanogaster* wing adopts its particular adult morphology, as opposed to that of *D. virilis*? Not really. Although we hope to have demonstrated here that inroads are being made into this next level, illustrating how regulatory genes can influence the detailed aspects of morphology and change with their evolution. It will be necessary to

Genetic assimilation: the phenomenon in which environmentally induced traits become expressed in the absence of the original environmental stimulus. It is thought to require persistent strong selection for the trait.

Canalization: the theory formulated by Waddington (91) that developmental genetics may constrain evolution, such that certain phenotypes are more likely than others. He also suggested that during the course of parallel morphological evolution in separate lineages, similar genetic changes might be required or more likely in the evolution from one state to another.

identify downstream effector genes, characterize their functions in diverse species, and understand their connections to the regula-

tors that ultimately direct them in the production of cells comprising diverse morphological structures.

SUMMARY POINTS

1. Extensive morphological diversity exists among arthropods, in their tagmosis, appendage modifications, as well as lesser features, such as the placement of sensory structures, endocrine organs, and cuticular structures. All of these morphological traits may be considered part of the body plan at various taxonomic levels. Very little of this lesser morphological variation has been studied with developmental genetic methods.
2. Arthropod tagmosis correlates with the expression of Hox genes, and evolutionary changes in tagmatic boundaries have correlated with shifts in expression of some Hox genes.
3. In insects, the Hox genes *Scr*, *Ubx*, and *abd-A* have been central to the evolution of wing placement on the body. Modifications to wing morphology have involved changing downstream targets for *Ubx*.
4. Morphological evolution may proceed by two proposed mechanisms: infinitesimal mutation or homeotic (hopeful monster-type) mutation.
5. Available comparative genetic data suggest that the former mechanism may be prominent in the evolution of tagmatic body plan changes, while spontaneous homeotic mutations may contribute to the evolution of lesser body plan features.
6. Evolutionary changes in developmental genes seem preferential to regulatory sequences, which conserve the structure of the encoded protein, but may change the architecture of developmental networks.

FUTURE DIRECTIONS

The extensive exploration of expression patterns in diverse species must be followed up by functional analyses. This will help test earlier conclusions and provide insights into the mechanisms producing biological diversity. Unlike expression studies, functional experiments can also allow analysis of epistatic relationships.

Genetic studies have made *Drosophila* by far the most well understood arthropod. However, to investigate structures and processes that may be absent or highly modified in *Drosophila*, it will be necessary to take forward genetic approaches with other species. Such screens are currently under way with species such as *Tribolium*.

Genetic studies have identified high-level regulatory genes important in development, but these genes represent a small fraction of the genome. To understand how they truly specify morphology, it will be necessary to determine their connections to downstream effector genes.

Development of high-throughput methods will be needed for quantitative analysis of expression levels. This will provide rapid comparisons of genetic networks in separate genotypes or species.

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Together with *The Strategy of The Genes* (1957, London, Allen & Unwin), this text outlined many of Waddington's ideas, such as canalization and developmental constraint, which have been revisited again in recent decades.



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ERRATA

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