Fossils, genes and the evolution of animal limbs

Neil Shubin, Cliff Tabin & Sean Carroll

The morphological and functional evolution of appendages has played a crucial role in the adaptive radiation of tetrapods, arthropods and winged insects. The origin and diversification of fins, wings and other structures, long a focus of palaeontology, can now be approached through developmental genetics. Modifications of appendage number and architecture in each phylum are correlated with regulatory changes in specific patterning genes. Although their respective evolutionary histories are unique, vertebrate, insect and other animal appendages are organized by a similar genetic regulatory system that may have been established in a common ancestor.

The origin of evolutionary novelties raises some of the most fundamental questions of biology. How do new structures arise? Can they evolve *de novo* or are they generally derived from pre-existing structures? And what is the developmental and genetic basis for their origin and modification[1]?

The adaptive evolution of vertebrates and arthropods to aquatic, terrestrial and aerial environments was accomplished by the invention of many novel features, especially new types of appendages. Enormous progress has been made in the past few years in understanding appendage development in both phyla. These genetic discoveries can be integrated with palaeontological data to address some of the principal events in the history of animal designs.

We will first examine the origin and evolution of vertebrate limbs and digits and of arthropod legs and insect wings. In both phyla we are confronted with a similar issue, namely the origin and adaptive modification of serially homologous organs. We will integrate palaeontological and developmental evidence that suggests that major innovations are largely derived from pre-existing developmental systems and will illustrate the potential genetic regulatory changes that enabled appendage evolution. Then we will explore the significance of newly discovered genetic similarities between arthropod and vertebrate appendages—similarities that have been retained despite more than 500 million years (Myr) of independent evolution. We will develop the hypothesis that the evolution of successively derived limb types, from lobopods to insect wings, and from agnathan fins to tetrapod limbs appears to be due, in part, to the successive cooption and redeployment of signals established in primitive metazoans. These examples illustrate how comparative developmental genetics can provide a mechanistic explanation of the origin and evolution of structures when palaeontological data are robust and important new hypotheses about evolutionary history when the fossil record is silent.

Origin and diversification of tetrapod limbs

Vertebrate limb diversity was produced by changes in the number, position and shape of structures that can be traced to Ordovician[2,3] (463–439 Myr) through Late Devonian[2–4] (409–362 Myr) fossils. The demands of feeding and locomotion in Ordovician and Silurian seas led to a surprising variability of the earliest known appendages: some forms possessed a continuous anterior fin that ran the length of the body, others had paired fins that projected immediately behind a head shield, and still other primitive vertebrates had no paired fins at all (Fig. 1). A body plan with two sets of paired appendages, pectoral and pelvic, is a derived feature that first appears in later jawed vertebrates (gnathostomes)[2–4]. The number of paired appendages has been highly conserved ever since their origin: the evolution of new gnathostome body plans primarily involved a modification of existing paired appendages rather than the invention of whole new sets (acanthodians are the only exception to this generalization). Therefore, the origin of more recent novelties, such as digits, involved the modification of genetic systems first established in more primitive vertebrates.

Serial homology and adaptive diversification

Primitive genetic systems must have provided a framework for the evolutionary integration of pectoral and pelvic appendages. Digits, for example, arose at the same time in the hand and foot: there is no Devonian tetrapod that has fingers and no toes[2–5]. Even in the post-Devonian world many unique designs appeared simultaneously in forelimbs and hindlimbs, as witnessed by chameleons, ungulates and ichthyosaurs. Obviously, serially homologous appendages can also evolve independently, an extreme case being the modification of pectoral appendages into wings in bats, birds and pterosaurs. Even in these extremely modified forelimbs, however, numerous similarities are retained between wing and leg.

The linkage between forelimbs and hindlimbs appears to be an ancient feature that resulted from patterns of gene cooption during the evolution of Palaeozoic fish. Serially homologous paired appendages are seen in Palaeozoic placoderms, acanthodians, chondrichthyians and osteichthyans[2–4]. In addition, pectoral and pelvic fins have evolved in parallel in almost all major gnathostome clades. There are numerous genetic parallels in pectoral and pelvic development that could account for these patterns of concerted evolution[6,7]. *Hox* genes, in particular, are likely to have been involved in the evolution of serial homology[8]. The earliest vertebrate appendages (unpaired fins) presumably did not utilize *Hox* genes; *HoxA* and *HoxD* genes are not expressed during the outgrowth of zebrafish unpaired fins[9]. Although these *Hox* genes were probably not involved in the origin of outgrowths in basal vertebrates, their later recruitment in the development of paired appendages was a key step in establishing serially homologous designs. The *HoxD* genes that came to play a role in appendage development are a subset of those involved in specifying regional identities along the caudal body axis (caudal neural tube, gut, somitic and lateral plate mesoderm)[6–10]. This suggests one of two situations: either nested patterns of *Hox* expression were originally present in a caudal set of paired outgrowths, and were later recruited in the development of a cranial set of outgrowths[8], or similar *Hox* genes were recruited in pectoral and pelvic outgrowths at the same time in the evolutionary history of vertebrates (Fig. 1). In either case, pectoral and pelvic appendages were genetically linked early in their history and could have evolved together, presumably because the development of these appendages had already been brought under similar regulatory controls.

Superimposed on these ancient genetic parallels are secondary differences in gene expression and interaction that may have served as the basis for the independent evolution of pectoral and pelvic appendages. *Hox* gene expression in extant tetrapod limbs is dynamic and encompasses at least three distinct phases initiated

successively in the primordia of the stylopod, zeugopod and autopod[7] (Fig. 2). The presence of three distinct phases of *Hox* expression in limbs may reflect the observation that all tetrapods maintain a standard pattern of organization (Fig. 2), whereas specific differences in expression (or gene interaction) in each phase could result in the independent modification of pectoral and pelvic appendages[7]. Phase II *Hox* expression is practically identical in the fore- and hindlimb buds of mice, which possess generally similar skeletal patterns. In contrast, the wings and legs of chicks have very different skeletal patterns and different patterns of phase II *Hox* expression as well[7,11]. Surprisingly, phase III expression is very similar in chick wing and leg buds, indicating that aspects of the derived structure of chick wings are established by some other genetic means. Candidates include *Hox* genes of other clusters that are differentially expressed in the wing and leg buds[12] and the *T-box* genes, another family of putative transcription factors differentially expressed in the forelimb and hindlimb buds[13]. Combinatorial action between genes may explain different functional requirements for the *HoxA* and *HoxD* genes in the forelimb and hindlimb. For example, homozygous deletion of both *HoxA-11* and *HoxD-11* results in almost complete loss of the zeugopod in the forelimb but not in the hindlimb[14], despite the fact that these genes have equivalent patterns of phase II expression in both limbs. A possible explanation for the observed differences between the appendages may be that there is expression of the paralogous gene *HoxC-11* during phase II in the hindlimb but not the forelimb, where it may act redundantly with *HoxD*-11.

Learning to crawl: the fin-to-limb transition. Some regions of vertebrate appendages are more variable than others[4,15,16]. The invention of flippers, wings and other specialized limbs often involved significant changes in the pattern of distal structures rather than proximal ones[15]. Two broad notions of the homology of distal structures have emerged over the past 130 years: one that sees digits as being unique to tetrapods[17,18] and another that sees antecedents of digital structure in the fins of sarcopterygian fish[19,20]. Both genetic and fossil data support the hypothesis that digits are evolutionary novelties[21,22] (Fig. 3).

The origin of digits is associated with the evolution of new temporal and spatial patterns of gene expression and regulation[7,9,21]. In extant tetrapods, the development of digits correlates with a reversal in the anteroposterior order of expression of *Hox* genes in phase II and phase III[7,23] (Figs 2, 3). Recent studies of teleosts

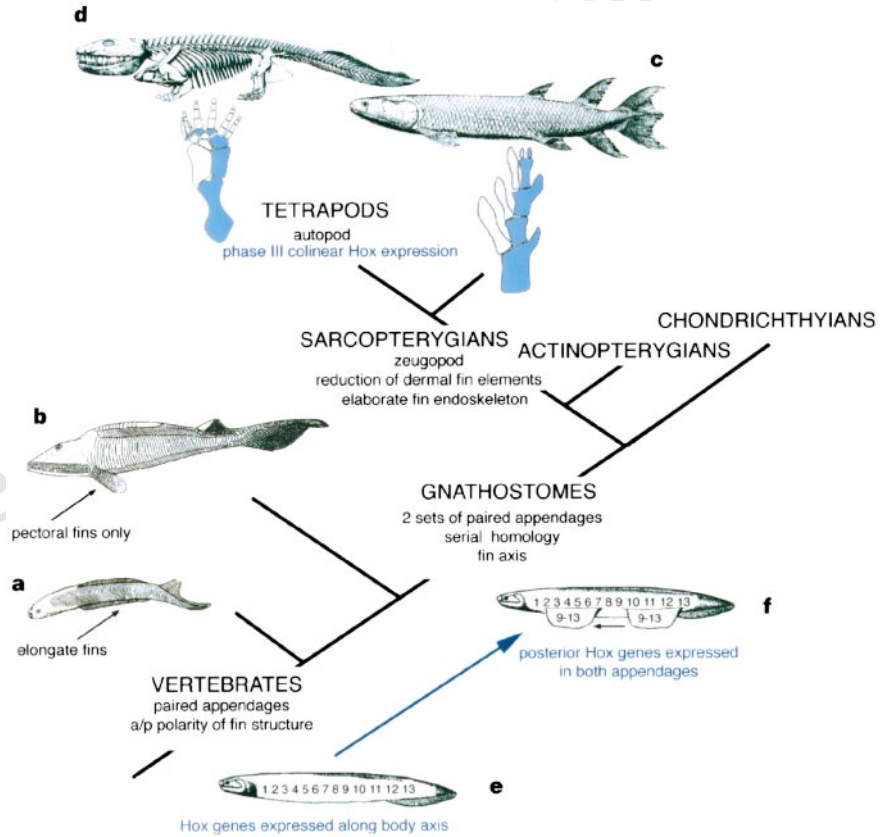

Figure 1 Major innovations of vertebrate paired appendages. Basal chordates, such as *Amphioxus* (not shown), do not possess appendages homologous to those of vertebrates. Unpaired, median fins are the earliest known vertebrate appendages[2]. Paired appendages are first encountered in Ordovician and Silurian jawless fish as elongate fins that extend laterally along the body wall (for example, *Jamoytius*; **a**) or as paired pectoral fins (osteostracans; **b**). Other basal vertebrates (not shown) do not possess any paired appendages. Multiple sets of paired appendages are a derived characteristic of jawed fish (gnathostomes). In many gnathostomes, pectoral and pelvic fins have often evolved in parallel. This pattern of concerted evolution suggests that pectoral and pelvic appendages shared similar regulatory genes in early stages of gnathostome evolution. A fin axis (blue) is seen in the fins of many gnathostomes, and it is a primitive characteristic for sarcopterygian fish (for example, *Eusthenopteron*; **c**) and tetrapods (for example, *Ichthyostega*; **d**). Sarcopterygian fins are derived in having a zeugopod and an elaborate endoskeletal fin skeleton. Digits develop within the distal portion of this extensive endoskeleton. The establishment of serially homologous appendages is proposed to result from gene cooption during the evolution of Paleozoic vertebrates. *HoxD* genes were probably not involved in the origins of body wall outgrowths in basal vertebrates because unpaired fins do not express these genes[9]. These *Hox* genes were initially involved in specifying regional identities along the primary body axis, particularly in caudal segments (**e**). One key step in the origin of jawed fish was the cooption of similar nested patterns of expression of *HoxD* genes in the development of both sets of paired appendages (**f**). This cooption may have happened in both appendages simultaneously, or *Hox* expression could have been initially present in a pelvic appendage and been coopted in the development of an existing pectoral outgrowth[8]. The reconstructions in **a** and **b** are modified from those in ref. 106, that in **c** from ref. 107, and that in **d** from ref. 108. The hind limb of *Ichthyostega* (**d**) is modified from ref. 5.

(zebrafish) have revealed patterns of *Hox* expression that are similar to patterns seen in proximal regions of tetrapod limbs[9,21]. Phase III *Hox* expression is not seen in the zebrafish and appears to be unique to the digital region of tetrapod limbs[7,9] (the expression of other sarcopterygians or more basal actinopterygians is not known). In addition, the different phases of *Hox* expression are not only discrete in tetrapod limbs, but they are regulated by separate *cis*-regulatory enhancer elements in each phase[24–26]. During phase II *Hox* expression in tetrapods, a complex set of enhancer elements is used within the regulatory region of each *Hox* gene of the cluster (as in the regulation of the *Hox* genes along the main body axis)[25,26]. However, regulation of all the *HoxD* genes in phase III depends upon a single enhancer upstream of the entire cluster[24,26]. The utilization of this distinct enhancer is consistent with the hypothesis that digits are evolutionary novelties because the development of the autopod is regulated differently from that of the rest of the limb.

The presence of phase III *Hox* expression in tetrapod limbs, and its absence in teleost fins, suggests that this pattern may be an apomorphy for tetrapods or a more inclusive group. In addition, the presence of a uniquely tetrapod enhancer for phase III *Hox* expression implies that this regulatory element is also more derived relative to conserved phase I and II enhancers. The shift from phase II to phase III collinear expression involves multiple genes expressed at different times and in different regions of the limb. If these changes were genetically independent then they would have required the joint evolution of numerous regulatory elements. If only a single enhancer was involved, then this shift could have produced a change in the expression of multiple genes in a small number of evolutionary steps. Furthermore, the utilization of the same enhancer in forelimbs and hindlimbs provides a developmental explanation of the observation that fingers and toes arose simultaneously in the fossil record.

We propose that the temporal and spatial shift in the expression of *Hox* genes during limb development correlates with transformations inferred from the fossil record. Devonian fossils provide morphological links between structures in fins and limbs. Sarcopterygian fins are dominated by an axis of segmented endoskeletal elements that extends from proximal to distal[2–4,27] (Figs 1, 3). This axis is most similar to tetrapod limbs proximally, where the humerus, radius and ulna (femur, tibia and fibula) can readily be compared between taxa[2–5,15,22]. Embryological and palaeontological data suggest that the axis of fins was developmentally bent during

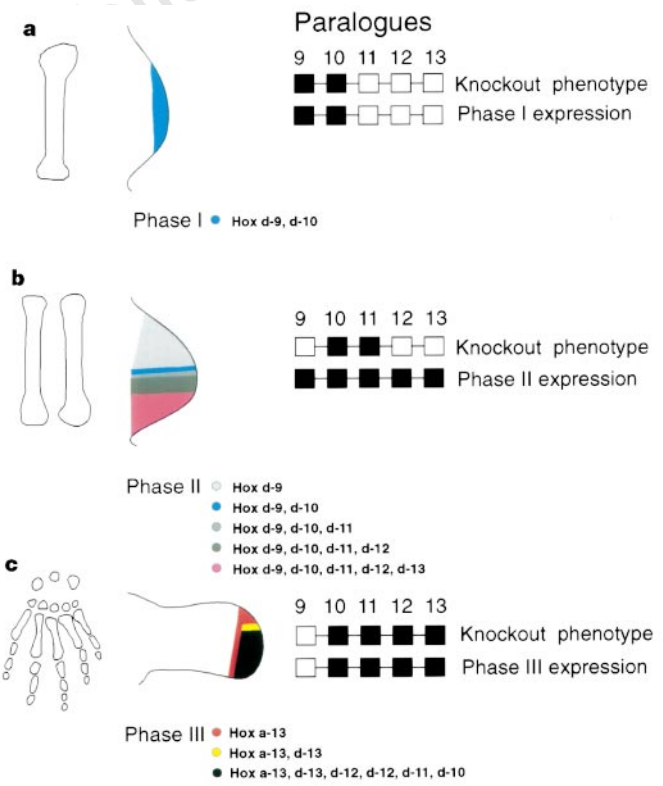

Figure 2 Stylopod, zeugopod, and autopod: patterning the limb. The tetrapod limb consists of three distinct compartments: **a**, the stylopod (upper arm and thigh); **b**, zeugopod (lower arm and calf); and **c**, autopod (hand and foot). This subdivision of the limb is supported by phylogenetic comparison[4], analysis of gene expression[7], and experimental manipulation[14,36–41,109–111]. There is a broad correlation between the position of a compartment and its evolutionary history[4]. The sylopod (**a**) is the most ancient (possibly of Late Silurian origins) whereas the zeugopod and autopod are the most recent (being first encountered in Devonian sarcopterygians). This same order of appearance of the three limb segments is recapitulated during development. Early removal of the apical ectodermal ridge (AER) results in a limb with only a stylopod; the zeugopod and autopod are produced after successively later surgeries[112,113]. The *Abd-B*-related genes of the *HoxD* cluster are expressed in a complex, dynamic pattern encompassing at least three distinct[7], independently regulated phases[24,26]. In the first phase (**a**; phase I), two of these genes (*HoxD*-9 and *HoxD*-10) are expressed across the entire limb bud[7]. This expression correlates with the time that the stylopod is specified[7,112]. Subsequently, a second phase of expression (**b**; phase II) is initiated in response to the secreted factor, *Sonic hedgehog*. Here, *Hox* genes are expressed in a nested set centred around the *Sonic*-expressing cells, with *HoxD*-13 being expressed in the most restricted domain, and *HoxD*-12 and *HoxD*-11 each encompassing a broader domain[7]. This pattern of expression coincides with the time of specification of the zeugopod and takes place in cells fated to form this segment. Finally, a third phase of expression (**c**; phase III) is initiated later during limb development, when these *Hox* genes are all expressed across the majority of the distal portion of the limb bud[7]. During this phase, the expression of *Hox* genes still appears to be a consequence of the *Sonic hedgehog* signal, but the relative responsiveness of the different genes has changed so that *HoxD*-13 now has the broadest expression domain and *HoxD*-12 and *HoxD*-11 are nested within it[7]. The phase III expression (**c**) patterns occur in the presumptive autopod at the time that segment is specified. The combination of the change in relative size of *Hox* expression domains with a phenomenon known as 'posterior prevalence' (the general rule that more 5′ genes in the *Hox* cluster are phenotypically dominant) results in different *Hox* genes playing pre-eminent roles in different limb segments: for example, *HoxD*-9 during phase I in the stylopod (**a**), *HoxD*-11 during phase II in the zeugopod (**b**), and *HoxD*-13 during phase III in the autopod (**c**). The expression of the dominant *Hox* genes in each phase is essential for the formation of the bones in each segment, as seen in their knockout phenotypes. The knockout phenotype of a gene consists of alterations in the pattern and shape of skeletal elements. The location of these modifications depends on the position of the gene within the cluster. For example, mice engineered to be deficient in both *HoxD*-11 and in *HoxA*-11 (the paralogous gene of the *HoxA* cluster) form limbs that are essentially missing the zeugopod[14] (**b**). Phenotypes of *Hox D-9*-deficient mice, in contrast, are specific to the stylopod[109] (**a**), whereas *Hox D-13* deficient mice primarily have defects in the autopod[39] (as do mice engineered to be deficient of *Hox A-13* paralogues[110,111]) (**c**). Knockout data are derived from refs 14, 36–41 and 109–111.

the origin of tetrapod limbs[27]. This scheme holds that there is a dramatic difference between the autopod and the zeugopod because the branching of the axis shifts from the anterior (preaxial) to the posterior (postaxial) compartment of the limb[7,27]. Proximal elements, such as the radius, project anteriorly from the axis, whereas distal elements, such as the digits, project from the posterior side of the axis. We propose that the reversal of morphological polarities in the appendages of Devonian vertebrates correlates with the reversal of *Hox* gene expression seen in phase III (Fig. 3f, g). As *Hox* expression in phase III is driven by a novel enhancer element, the axis was not bent *per se*; rather, a novel extension (with reversed morphological polarities) is considered to be added to it in the Late Devonian. This hypothesis is supported by a comparison of panderichthyid fins and tetrapod limbs. The fins of this sister-group of tetrapods (Fig. 3d, f) are highly reduced in comparison to other sarcopterygians (Figs 1e, 3b, c, c) and this reduction is most prominent distally. No potential homologues of digits, wrist or ankle bones are preserved in these fish[22].

Are digits, or their functional equivalents, unique to tetrapods? Fins of rhizodontid fish have stunning similarities to tetrapod limbs[20] (Fig. 3). The fins of these Devonian fish contain up to eight endoskeletal radials that project distally; in *Sauripteris* (Fig. 3c), six of these rods terminate at the same proximodistal level. Either these radials are directly homologous to the six to eight digits of Devonian tetrapods (a hypothesis not supported by phylogenetic inference) or they are functional analogues. In either case, these rods reflect a site at which morphological polarities are reversed (for example, the radials of *Sauripteris* branch postaxially[20]), suggesting that phase III *Hox* expression may have arisen in this clade. Several different lineages of Devonian sarcopterygians appear to have evolved the same morphological solution to life in shallow freshwater environments. The tetrapod clade evolved true digits, whereas rhizodontids developed functional analogues. In both cases, the genetic shifts may well have been similar.

Adaptive diversification: how many fingers? Digit reduction is a dominant theme of tetrapod limb evolution; deviations from a pentadactyl pattern virtually always involve the loss of fingers or toes[5,28]. Polydactylous hands and feet have almost never been fixed in phylogeny, despite the presence of polydactylous variants within populations (individuals of many species including cats, dogs, mice, chickens and humans carry mutations that cause the formation of extra digits). This paradox can be explained in terms of evolutionary constraints by postulating a genetic limitation to digital evolution. One approach holds that the genetic mechanisms that determine the number of digits are distinct from those that regulate morphology, and that there are currently only five discrete genetic programs for

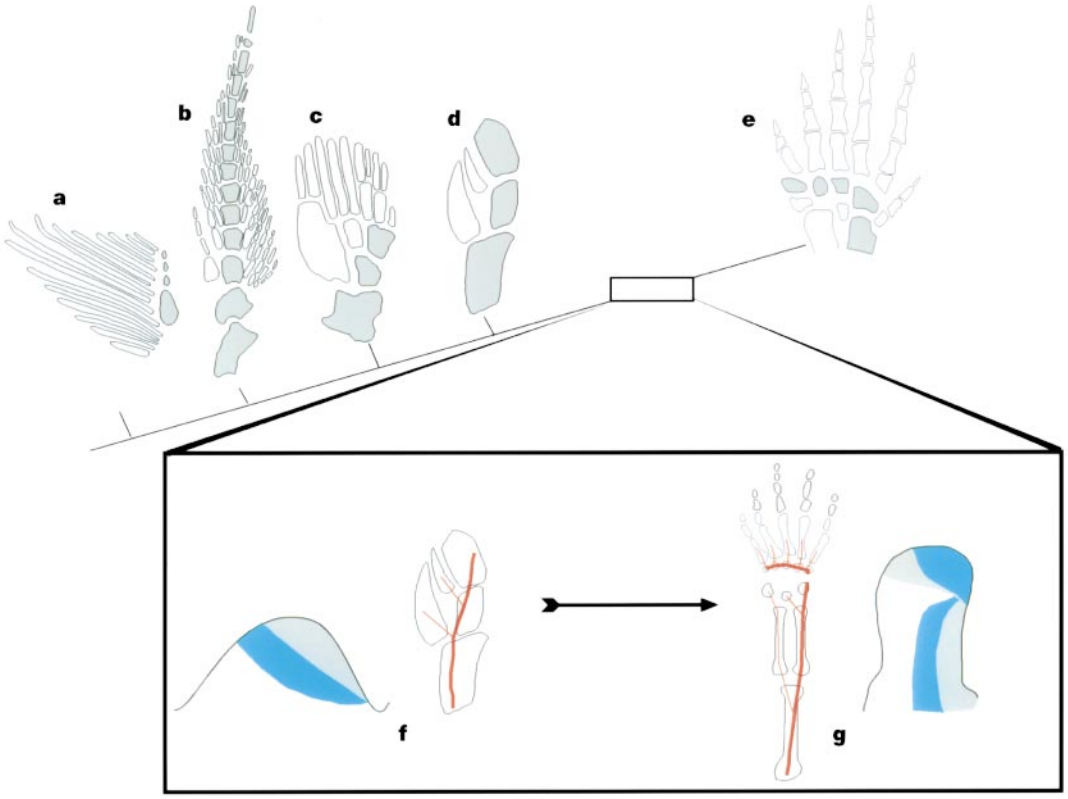

Figure 3 The origin of digits. A fin axis (grey) is present in the fins of chondrichthyians (**a**, *Cladoselache*), basal actinopterygians (not shown), and sarcopterygians (**b**–**e**). In sarcopterygians such as *Neoceratodus* (**b**), *Sauripteris* (**c**), *Panderichthyes* (**d**) and *Tulerpeton* (**e**), a single element (stylopod) articulates with the pectoral or pelvic girdle; all other proximal bones of the fin have been lost. The autopod is considered to be a synapomorphy of tetrapods because their nearest relatives do not have any apparent homologues of digits. Box: we propose that the origin of digits correlates with a novel pattern of *Hox* expression. **f**, The axis (red) of the fin of *Panderichthyes* is short and radials branch preaxially. Patterns of phase II *Hox* expression (**f**; *Hox D-11* in light blue, *Hox D-13* in darker blue) were most likely already in place in sarcopterygian fins. **g**, The proximal portion of the axis (red) of tetrapods compares with the entire axis of *Panderichthyes*. Phase III *Hox* expression reflects a reversal of the nested domains of expression of *HoxD-11* (**g**, light blue) and *HoxD-13* (**g**, dark blue) during the time when the autopod is specified. This reversal in the polarity of *Hox* expression is considered to be correlated with the origin of the autopod; digits differ from more proximal structures in that they lie on the postaxial side of the axis. Because this hypothesis relies on genetic comparisons between phylogenetically disparate taxa (for example, teleosts and tetrapods), the shift from phase II to phase III collinear expression may have evolved in more basal sarcopterygians. Analogues of digits are seen in the fins of other sarcopterygian fish (**c**, *Sauripteris*); the expanded endoskeletons of rhizodontids include as many as eight branched preaxial radials. Different lineages of sarcopterygians appear to be inventing similar solutions to life in shallow freshwater ecosystems. The reconstruction of *Tulerpreton* (**e**) is modified from ref. 114.

specifying unique digit morphology[28]. The primary genetic limitation is on the number of kinds of digits, not their absolute number. A specific prediction of this hypothesis is that polydactyly can arise, but at least two of the digits will have the same identity (that is, morphology). In this regard, it is easier to modify other carpal or tarsal bones to new functions than to create a new digit. Supporting this notion is the observation that the additional 'digits' in extant polydactylous taxa are typically modified carpal or tarsal bones (as in frogs and panda bears, for example). Unfortunately, we cannot as yet evaluate the genetic basis of this constraint because we do not understand the genetic mechanisms that regulate the differences between digits.

Many classical morphologists were interested in defining 'laws of form'—common trends that appear in widely different groups. Comparative analysis of diverse taxa now offers the promise of fundamental insights into these long dormant questions. The 360-Myr history of tetrapod limbs is witness to dramatic regularities in digital reduction[4,16,29]. One notion, 'Morse's law of digital reduction', contrasts the stability of the inside digits (III, IV) with the lability of outside ones (V, II, I)[30,31]. In virtually every known example of digital reduction, digits V, II and I are among the first to be lost and digits III and/or IV are typically retained in tetrapods that have the most extreme patterns of digital reduction. This pattern is widespread and has evolved independently in lizards[32,33], dinosaur and bird feet[34], and in mammals[35] (for example, ungulates). The major exception to this trend lies in the hands of theropod dinosaurs (that lose postaxial digits); theropod feet conform to Morse's law, as do the hands and feet of other dinosaurs[34].

Are regularities of digital evolution the product of developmental constraints upon variation? Knockouts of different *Hox* genes (*HoxD-11*, *HoxD-12*, *HoxD-13*, *HoxA-13*) lead to changes in the shape and number of bones in affected mice and these different genes often have overlapping effects[14,36–41]. One common result is the stability of the internal digits (IV, III) in knockouts of single genes or combinations of genes[41]. The parallels between the expectations of Morse's law and the results of experimental manipulation suggest that trends of digital evolution may have a developmental basis. The morphological effects of different gene knockouts may reflect the sequence of digital formation: the first digits to be affected are typically the last to form in development[37]. Although evolutionary patterns of digital reduction are unlikely to involve coding mutations of *Hox* genes, limb reduction may involve changes in the regulation of *Hox* genes or the genes that they control. Comparative analysis of gene expression and function in representative taxa could elucidate the mechanisms behind these general evolutionary trends. The lizard genus *Lerista*, for example, has species with five, four, three, two, and no toes—the range of states of digital reduction in this genus parallels that seen in virtually all other tetrapods[32].

Origin and diversification of arthropod limbs

> "Exites and endites of the proximal limb segment of arthropods, from trilobites to insects, have an extraordinary history. They have furnished most of the remarkable tools of the phylum: mandibles and other mouth parts; gills of trilobites and Crustacea; swimming and grasping appendages; gill-plates of Ephemeroptera … Their evolutionary potential is comparable with that of the vertebrate limb …" Wigglesworth (1972)[42].

The adaptive radiation of arthropods started much earlier than that of tetrapods. The Cambrian fossil record abounds with trilobites, arachnomorphs and crustacean-like forms, and contains many bizarre animals with spectacular appendages and body armour (Fig. 4). The 'arms race' of the Cambrian explosion may well have been a 'limbs race' among arthropods to evolve better sensory, locomotory, feeding, grasping and defensive appendages.

The most obvious feature of arthropod diversity is the number, morphology and function of their appendages (Figs 4, 5). Antennae, mouth parts, walking legs, grasping and swimming appendages are all modifications of a basic jointed limb structure that defines the phylum. It is generally though that jointed legs evolved from simple unjointed appendages such as the lobopodia found in the probable sister group of the arthropods, the Onychophora (Fig. 5a). The diversity of Cambrian arthropods and lobopodans (Fig. 4) indicates that the transition from lobopods to jointed appendages occurred before the Cambrian (570–510 Myr). Fossils from this period are scarce and the reconstruction of these transitions mostly relies on the comparative analyses of later, Cambrian fossils[43,44].

Morphological studies of Cambrian lobopodans have investigated the series of innovations that led to the basal arthropod design. These novelties include: the evolution of external segmentation; sclerotization; and, most important to our discussion, the origin of jointed, biramous appendages. Whereas the taxonomic relationships between Cambrian forms and extant arthropods are uncertain, Cambrian taxa with different types of 'arthropodization' and

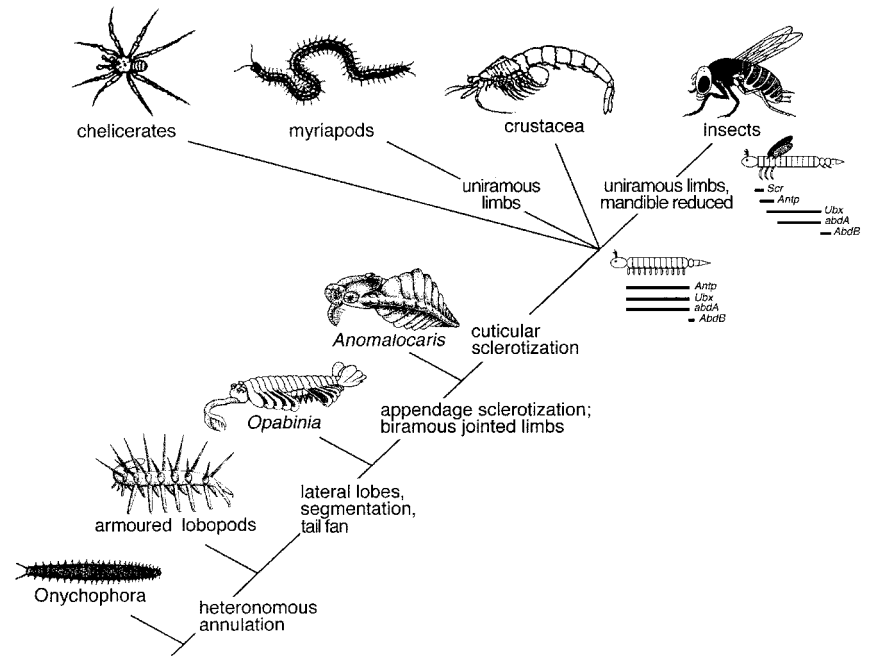

Figure 4 The lobopod–arthropod transition and the diversification of arthropod limb patterns. Several innovations occurred in the lobopod–arthropod transition[43]. Various lopopods that may represent different degrees of 'arthropodization' are depicted. *Opabinia* is shown in partial cutaway view[43] to reveal the lateral lobes and ventral lopopodia. The relationships among the major arthropod groups is an unresolved polychotomy. The most basal euarthropod was probably fully sclerotized with jointed, biramous limbs[115], and a homonomous trunk. Uniramous limbs evolved in the terrestrial arthropods. The subdivision of the trunk and differentiation of individual limbs in modern insects involved regulatory changes in *Hox* gene domains along the many body axis from an ancestor in which trunk appendages and *Hox* gene domains were mostly identical (*Hox* scheme is adapted from ref. 53).

limb morphology can be identified[43]. For example, the Cambrian *Aysheaia* and the Recent *Peripatus*, possess simple, unjointed uniramous limbs (Fig. 4), but lack the annulation and armour typical of more derived lobopodans such as *Hallucigenia*[43] (Fig. 4). Other lobopodans, such as *Opabinia*, are externally segmented and possess lateral lobes above the ventral lobopods[43] (Figs 4, 5b). Fusion between the gill-like lateral lobes and ventral lobopodia may have given rise to the biramous limb[43,45–47] (Fig. 4c). In forms such as *Anomalocaris*, this fusion was accompanied by limb segmentation and sclerotization (Figs 4, 5). Full cuticular sceloratization, then, arose in primitive arthropods.

Serial homology and adaptive diversification. The adaptive radiation of primitive arthropod limbs entailed considerable changes in their number, pattern and function. Two trends are evident. First, in arthropods in general and certain lineages in particular, there have been increases in the number of different limb types. For example, advanced lobopods had perhaps four types of appendages (frontal, jaw, trunk and tail fan) and only one type of trunk appendage, whereas insects possess up to ten distinguishable limb-derived appendages and four or five different trunk appendages (Fig. 4). A second trend has been the dramatic diversification of homologous appendages—not just in size or morphological detail. Major changes in limb organization have evolved, such as the evolution of unbranched walking legs in insects and changes in mandible architecture in myriapods, crustacea and insects (Fig. 4).

The developmental genetics of limb formation and identity in Recent arthropods may help to shed light on the diversification of arthropod limbs. *Hox* genes have played a different role in arthropod evolution from that seen in tetrapods. Studies of *Drosophila melanogaster* have revealed that each type of appendage is typically specified by a single or a pair of *Hox* genes acting in the individual body segment that gives rise to a particular appendage[48]. For example, the *Antennapedia* gene acts in all three pairs of walking legs but the distinct morphology of the first, second and third pair of walking legs is determined by the *Sex combs reduced*, *Antennapedia*, and *Ultrabithorax Hox* genes[49], respectively. In the antenna, no *Hox* gene is active. If *Hox* gene function is lost or ectopically activated in individual segments, the identity of the corresponding appendage is transformed. Thus, loss of *Antennapedia* transforms second leg to antennal structures[50] and expression of *Antennapedia* in the antenna transforms it to a leg[51]. Importantly, loss of all *Hox* gene functions in insects results in a dead embryo bearing antennae on all segments[52]. This demonstrates that the potential to form a limb exists in all segments, but the type of limb formed is determined by individual *Hox* genes.

The specification of different limb types by different *Hox* genes or combinations of *Hox* genes differs from the nested pattern of *Hox* genes expressed in the limbs of vertebrates and has important implications for the pattern of morphological evolution in the arthropods. Different, but serially homologous, arthropod limbs are distinguished by the action of different *Hox* genes that modify the interpretation of a common set of positional signals. Thus, the increase in appendage diversity between lobopods, primitive crustacea and insects must have involved the diversification of *Hox* gene regulation and function in the arthropod trunk. Comparative studies of *Hox* gene expression between crustacea (for example, branchiopods) and insects support this notion. In the branchiopod thorax, the expression of the *Antp*, *Ubx* and *abd-A Hox* genes is coincident and the morphology of thoracic limbs is uniform[53]. In insects, these same *Hox* genes differentiate the middle thorax, posterior thorax and anterior abdomen (Fig. 5). The adult hexapod abdomen is legless and this is due to the direct repression of limb formation by products of the *Ubx* and *abd-A Hox* genes[54]. These gene products do nor repress limb formation in branchiopods[55].

The diversity of the architecture of putatively homologous appendages has fuelled many debates about arthropod relationships. For example, walking legs and mandibles can differ so much between taxa that it has been suggested that the arthropods had multiple ancestors (that is, they are polyphyletic)[56], but phylogenetic[57,58] studies have refuted this. In addition, developmental studies suggest that different limb architectures arise through modifications of a common genetic program. For example, the *Distal-less* (*Dll*) gene controls the development of the distal portion of *Drosophila* limbs[59] and is expressed in the distal domains of limbs in all arthropods studied so far[50,60,61]. However, *Dll* is not expressed in insect or developing adult crustacean mandibles[55] but is expressed in myriapod mandibles[62]. These data agree with fossil evidence suggesting that crustacean and insect mandibles were reduced from the primitive whole-limb mandible by truncation of the mandibular proximodistal axis.

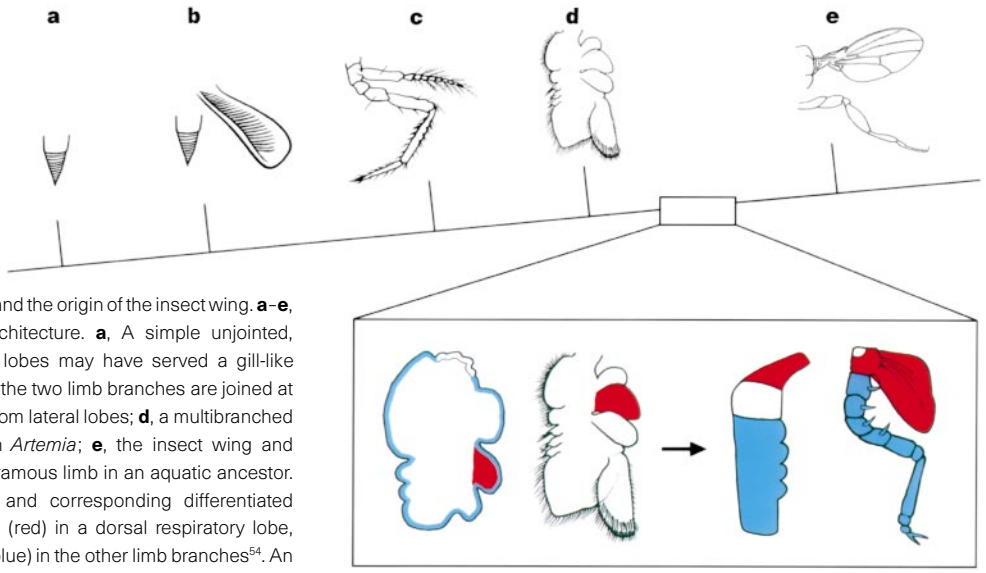

Figure 5 The evolution of the arthropod limb and the origin of the insect wing. **a**–**e**, Some of the major transitions in limb architecture. **a**, A simple unjointed, annulated lobopodium; **b**, separate lateral lobes may have served a gill-like function; **c**, a jointed biramous limb in which the two limb branches are joined at the base, an upper branch is often derived from lateral lobes; **d**, a multibranched limb found in the branchiopod crustacean *Artemia*; **e**, the insect wing and uniramous leg appear to derive from a polyramous limb in an aquatic ancestor. Box: left, a developing polyramous limb and corresponding differentiated structure. The *apterous* gene is expressed (red) in a dorsal respiratory lobe, whereas the *Distal-less* gene is expressed (blue) in the other limb branches[54]. An ancestor–descendent relationship between the limbs in the box is not implied. Right, separation of the dorsal respiratory lobe from the ventral limb primordium in a primitive pterygote such as a Paleodictoptera nymph. The proto-wing at this stage was probably a gill-like structure on all trunk segments and still attached to the base of the limb. The *apterous* and *Distal-less* genes play critical roles in wing and leg formation in *Drosophila*.

The branching of arthropod limbs has been very important to their functional evolution. Different limb branches can be specialized for respiration, locomotion and a variety of other functions. Chelicerates, trilobites and aquatic crustacea have biramous or polyramous limbs and first appear in the Cambrian, whereas the terrestrial myriapods, insects and crustacea have unbranched (uniramous) limbs and appear much later in the Silurian[63] and Devonian[64], respectively. It has been argued that the ancestral arthropod was biramous[43,45–47]. Comparisons of *Dll* expression and limb outgrowth in various types of crustaceans and insects reveal that all limb types arise from the same relative anteroposterior position within the body segment but differ in their dorsoventral branch points[55]. This suggests that uniramy, biramy and polyramy are the products of shifts in signals along the dorsoventral axis of the body wall (or appendage) and that additions or reductions in branch number may evolve readily. This flexibility was crucial to the later evolution of perhaps the most significant invention by any arthropod—wings.

Learning to fly: the leg-to-wing transition. Early in the Devonian, before tetrapods arose, one major animal group had already invaded land—the insects. The subsequent evolution of flight presaged the enormous radiation of insects: these taxa now comprise more than two-thirds of all known animal species. The evolution of insects from an as-yet uncertain arthropod ancestor and the emergence of winged (pterygote) forms involved major transitions in limb architecture and function.

Few evolutionary mysteries have inspired more theories than the origin of insect wings. It is not certain when wings first arose because the Early Devonian insect fossil record is scanty[64]. All of the major pterygote groups appear by the Carboniferous (362–290 Myr) and are assumed to have arisen earlier. Fundamentally, discussion on the origin of wings has focused on whether they are novelties or whether they are modified versions of ancestral structures. If wings were derived from existing structures, what were their anatomical origins and initial functions?

One of the longest-held models is the 'paranotal' theory, which holds that wings are novelties derived from hypothetical rigid extensions of the body wall of a terrestrial ancestor[65]. A second hypothesis, the 'limb-exite' model proposes that insect wings evolved in a series of transitions beginning with the polyramous exite-bearing legs of an aquatic pterygote ancestor[42,66]. According to this model, proximal limb elements were modified to flap-like structures and adapted to spiracular or movable gill covers to facilitate respiration. These sack-like pro-wings were found on all thoracic and abdominal segments and became stronger as ancestral pterygote nymphs (similar to Recent mayfly larvae) used them presumably for propulsion. As insect lifestyles became more amphibiotic, some insects might have used a rudimentary proto-wing for surface skimming, as seen in extant stoneflies[67]. Finally, wings acquired the mechanical strength and flexibility (with corrugation and veins) and the supporting musculature to support active flight.

Recent studies of the developmental regulatory mechanisms controlling wing formation and number in *Drosophila* suggest a close ontogenetic and evolutionary relationship between legs and wings. For example, in *Drosophila*[68] and other Diptera, the wing arises in close association with the leg. Cells that give rise to the wing field in *Drosophila* actually migrate out of the developing ventral limb field[68]. There are important regulatory differences that distinguish the development of the sheet-like wing from the tubular structure of the leg. One key difference is that adult and developing wings are divided into discrete dorsal and ventral compartments whereas legs are not. In *Drosophila*, the definition of dorsal versus ventral cell fates is orchestrated by the *apterous* gene which is expressed only in dorsal cells and is necessary for wing but not leg formation[69]. Clearly, *apterous*, which regulates several crucial downstream signalling components, and is involved in a conserved dorsal pattern of expression in insect wings[70], was coopted into a distinct role in dorsoventral patterning at some stage of wing evolution. *apterous* primitively could have specified a dorsal compartment (or branch) of an ancestral polyramous appendage or an evolving proto-wing. Remarkably, this is exactly what has been found in a recent study[71]. The dorsal branch of a branchiopod crustacean respiratory epipodite specifically expresses the *apterous* gene and one other developmental marker of the insect wing field (Fig. 5d). This suggests that Recent wings evolved from the respiratory lobe of an ancestral polyramous limb, probably first appearing in the immature aquatic stages as gill-like structures, such as those found on all trunk segments of extinct Paleodictyoptera or extant mayfly larvae (Fig. 5d). Wings subsequently emerged as adult appendages and acquired greater strength and flexibility for sustained flight (Fig. 5e).

Interestingly, if the respiratory epipodite origin of insect wings is correct, then wings may have an even deeper origin, not just in the aquatic ancestor of pterygotes, but in lobopodans. The origin of the biramous limb has been postulated to involve the fusion of the gill-like structure of lateral lobes (such as those in *Opabinia*) with the ventral lobopod[43,45–47] (Fig. 5c). If this is true, then the wing may indeed be derived from lateral lobes, not of a terrestrial insect as once thought, but of a much more distant lobopodan ancestor.

Deep homology and origin of appendages

It is clear from the fossil record that chordates and arthropods diverged at least by the Cambrian. The appendages of these two groups are not homologous because phylogenetically intermediate taxa (particularly basal chordates) do not possess comparable structures. The most surprising discovery of recent molecular studies, however, is that much of the genetic machinery that patterns the appendages of arthropods, vertebrates and other phyla is similar. These findings suggest that the common ancestor of many animal phyla could have had body-wall outgrowths that were organized by elements of the regulatory systems found in extant appendages. We now describe these similarities and use them to consider the origin of animal limbs.

In *Drosophila*, the anteroposterior (AP) axis of the leg or wing imaginal disc (the larval precursor to the adult appendages) is divided into two compartments (reviewed in ref. 49). The posterior half of the disc expresses the gene *hedgehog*[72,73], which encodes the key signal that initiates AP patterning (Fig. 6). In response to *hedgehog*, a thin layer of cells running along the border of the anterior and posterior compartments is induced to produce another secreted protein encoded by the gene *decapentaplegic* (*dpp*)[74]. *dpp*, in turn, is a long-range signal providing positional information, and hence differential AP fates, to cells in both compartments[75–79]. Misexpression of either *hedgehog* or *dpp* in the anterior of the disc results in AP mirror-image duplications of limb structures[74].

The AP axis of the vertebrate limb is set up in a very similar manner (Fig. 6). A key organizing signal is *Sonic hedgehog* (*Shh*), one of the three direct homologues of the *Drosophila* gene *hedgehog*[80–83]. Like *hedgehog*, *Shh* is localized posteriorly in the limb bud. Misexpression of *Shh* anteriorly causes AP mirror-image duplications analogous to those caused by *hedgehog* misexpression in the fly imaginal disc[74,83]. In addition, *Bmp-2*, one of the two vertebrate homologues of the arthropod *dpp* signalling protein, is expressed in the limb bud in response to *Shh*[23]. Unlike *Drosophila dpp*, *Bmp*-2 does not have the ability to cause full limb duplications. However, it clearly functions as a secondary signal in the *Shh* pathway, polarizing the overlying ectoderm[84].

Signals organizing proximodistal (PD) outgrowth in vertebrate and insect appendages also operate similarly. In the insect imaginal wing disc, PD outgrowth is organized by a specialized set of cells running the length of the dorsoventral (DV) border, the 'wing margin' (Fig. 6). The dorsal compartment of the wing is characterized by the expression of the transcription factor *apterous*. *apterous* specifies dorsal-specific cell fate[69] and controls the expression of a secreted protein called *fringe*[85]. The interface between cells expressing

and cells not expressing *fringe* becomes the wing edge or margin[85]. A key downstream effector of *fringe* activity is encoded by *Serrate*[86]. In response to *fringe*, *Serrate* is induced, leading to the activation of several downstream effector genes[87–90] and the production of a signal at the margin which organizes the growth of the wing blade[87–89].

Unexpectedly, outgrowth of vertebrate limbs appears to be established by a very similar genetic cascade. Outgrowth of the limb bud is driven by signals from a specialized ectodermal structure, the apical ectodermal ridge[91] (AER), which, like the wing margin, runs along the DV border of the limb. Remarkably, a vertebrate homologue of *fringe*, called *Radical-fringe*, is expressed in the dorsal half of the limb ectoderm prior to formation of the AER[92]. At the border between cells expressing *Radical-fringe* and cells not expressing *Radical-fringe*, a homologue of *Serrate*, *Ser-2*, is induced and the AER forms. A *Radical-fringe* boundary is required to form the AER and ectopic radical *fringe* can induce an additional AER on the ventral surface[93].

There are also parallels between the regulation of the DV axis in vertebrate and arthropod appendages (Fig. 6). Genes specifying DV polarity in both groups have been identified. In *Drosophila*, the early ventral expression of the gene *wingless*, a member of the *Wnt* family of secreted factors, is necessary for the proper DV patterning of the wing[94,95]. Subsequently, the expression of the transcription factor *apterous* defines the dorsal compartment and specifies dorsal cell fates[69]. In the vertebrate limb, the early expression of a different *Wnt* family member is also required for DV patterning. *Wnt7a* is specifically expressed throughout the dorsal ectoderm and is necessary and sufficient for many aspects of dorsal patterning[96–98]. *Wnt7a* acts by inducing mesodermal expression of *Lmx-1*[97,98]. Like *apterous*, *Lmx-1* is a related member of the LIM-homeodomain family of transcription factors. As with *apterous* (*Drosophila*), *Lmx-1* (vertebrates) expression defines a dorsal compartment, being expressed early throughout the dorsal half of the limb bud, and it is sufficient to convey dorsal cell fate[97,98].

The simplest phylogenetic implication to draw from these comparisons is that individual genes that are expressed in the three orthogonal axes are more ancient than either insect or vertebrate limbs (Fig 6). Indeed, several of the regulatory systems seen in arthropod and vertebrate limbs are also involved in the development of other organs in a variety of taxa. The phylogenetic distribution of regulatory circuits and morphological structures presents two major interpretations: either similar genetic circuits were convergently recruited to make the limbs of different taxa or a set of these signalling and regulatory systems are ancient and patterned a structure in the common ancestor of protostomes and deuterostomes[99].

The first model holds that genes and/or genetic circuits were convergently recruited for limb development during the evolution of vertebrates and arthropods. These genes would not be involved in appendage development in the common ancestor of vertebrates and arthropods; each gene or circuit was involved in other developmental events. This notion would require the parallel cooption of members of similar gene families, acting along different developmental axes to pattern an outgrowth of the body wall in at least two taxa. The evolution of limbs in each group would, then, have involved the convergent recruitment of numerous genes to define

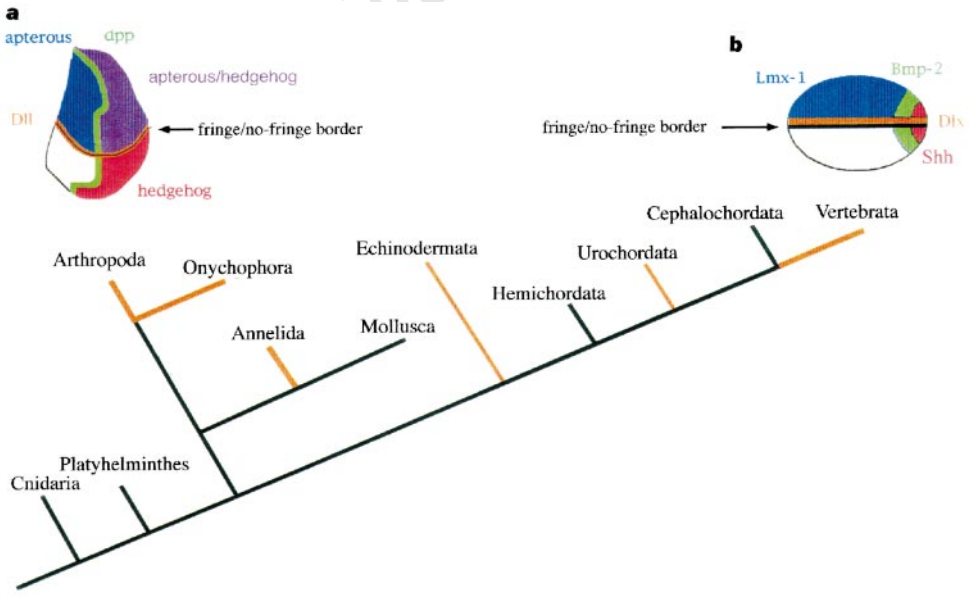

Figure 6 A cladogram of selected metazoans shows the distribution of major genes involved with appendage development. Homologous signals are deployed in similar locations in the limb primordia of arthropods (**a**, *Drosophila*) and vertebrates (**b**, chick). Equivalent orientations (dorsal up, anterior left) of a chick left wing bud and a *Drosophila* left wing imaginal disc are shown. *Sonic hedgehog* (red) in the chick, and its homologue *hedgehog* (red and purple, where coexpressed with *apterous*) in the fly are produced in a posterior domain. These factors induce the expression of secondary patterning signals in the appendages: overlapping expression of *Bmp-2* (green) in the chick and adjacent expression of *dpp* (green), the *Bmp-2*-homologue, in the fly. Dorsal cell fates are specified in both systems by *LIM* homeodomain transcription factors expressed throughout the dorsal half of the appendage primordia: *Lmx*-1 (blue) in the chick, and *apterous* (blue and purple, where coexpressed with *hedgehog*) in the fly. The outgrowth of both appendages is driven by a specialized group of cells (the AER in the chick and the wing margin in the fly) running along the anteroposterior axis at the junction of the dorsal and ventral compartments (yellow). These key groups of cells are specified in both the chick and the fly by the border between dorsal cells expressing the gene *fringe* and ventral cells not expressing *fringe*. For simplicity in viewing, conserved genes in signal transduction (such as the *hedgehog* receptor, *patched*) and other parallels between the two systems (such as the expression of *Wnt* genes) are not shown. *Distal-less* homologues (*Dll* in *Drosophila* and *Dlx* in the chick) are drawn in orange. *Distal-less* orthologues are expressed in a wide variety of animal appendages, including the lobopods of onycophorans, the tube feet of echinoderm, and the wings of birds and flies (orange). The limbs of these taxa are not homologous as appendages because phylogenetically intermediate groups do not possess comparable structures. This suggests at least two phylogenetic possibilities: either similar genetic circuits were convergently recruited to make the limbs of different taxa, or these signalling and regulatory systems are ancient and patterned a different structure (presumably another type of outgrowth) in the common ancestor of protostomes and deuterostomes[99].

similar developmental axes. If confirmed, this hypothesis would provide a stunning case of convergent evolution.

The second model is that some of these genes or circuits were components of an ancestral genetic regulatory system that was used to pattern a structure in the common ancestor of vertebrates and arthropods. This ancestral structure need not have been homologous to arthropod or vertebrate limbs; the regulatory system could have originally patterned any one of a number of outgrowths of the body wall in a primitive bilaterian for example. The genes themselves were initially involved in other developmental events; the key step in animal limb evolution was the establishment of an integrated genetic system to promote and pattern the development of certain outgrowths. Once established, this system provided the genetic and developmental foundation for the evolution of structures as diverse as wings, fins, antennae and lobopodia.

The evaluation of these two alternative models requires consideration of several factors that could affect the identity and deployment of appendate-patterning genes. First, given the independent histories spanning more than 500 Myr of the lineages being compared, one should expect regulatory differences among patterning systems. Even the insect wing and leg, which are both probably derived from an ancestral polyramous appendage, have acquired different patterning mechanisms. Because individual regulatory components appear to have been gained, lost and modified during insect wing and leg evolution there is no *a priori* reason to expect that either structure would be more genetically similar to vertebrate limbs. Second, one member of a gene family may substitute for another during normal development. Indeed, some of the genes that are deployed similarly in arthropods and vertebrates are not strictly orthologous. Such substitution could be the product of either convergent evolution or descent with modification (by substitution) among redundant genes. Third, it could be argued that the presumed inversion of the DV body axis during deuterostome evolution would imply a corresponding inversion of appendage DV axis patterning signals, an expectation that is not met by the observed expression of *apterous*- and *fringe*-related genes in vertebrate limb buds. A parallel in reversal of the DV axes of the body and the limbs would be expected if vertebrate and insect limbs were structurally homologous to a common ancestral appendage which predated the reversal. However, we know that the modern vertebrate limb evolved after the DV inversion of the body axis. Thus, a DV inversion in the patterning of the limbs would not necessarily be predicted, whether the axis patterning genes were independently coopted for appendage formation according to the first model, or whether they were coopted as a unit, but regulated independently of the body axis, according to the second model.

One can argue many ways from the comparison of only two taxa: the alternative phylogenetic hyopotheses need to be tested by additional comparative data. Evidence in support of an ancient common mechanism for the formation of outgrowths of the body wall comes from phylogenetic comparison of the expression of the transcription factor *Distal-less* (*Dll*)[100] (Fig. 6). *Dll* is expressed at the distal end of growing insect limbs[55,60,61], and is essential for appendage outgrowth[59]. *Dll* orthologues are expressed in the distal portion (AER) of the embryonic limb buds of vertebrates, the ampullae and siphons of tunicates, the tubefeet of echinoderms, the parapodia of annelids, as well as Onychophoran lobopodia[100]. The expression of *Dll*-related genes could represent convergent utilization of the gene. However, the fact that out of the hundreds of transcription factors that potentially could have been used, *Dll* is expressed in the distal portions of appendages in six coelomate phyla makes it more likely that *Dll* was already involved in regulating body wall outgrowth in a common ancestor of these taxa (Fig. 6). The additional parallels between vertebrate and arthropod limbs suggest that this ancestral outgrowth may have also been patterned along the three orthogonal axes.

If a conserved outgrowth patterning system was available for coption in the evolution of vertebrate limbs, then it must have been used in patterning non-limb outgrowths in basal taxa. Genetic studies provide an example of at least one secondary outgrowth patterned along these axes that predated the evolution of vertebrate limbs: the branchial arches. As the branchial arches grow out from the cranial region of the chick embryo, they express important components of the limb patterning system in similar developmental regions[101,102]. Like arthropod and vertebrate limbs, the branchial arches contain localized, posterior expression of a *hedgehog* gene, in this case *Shh*[101,102]. Furthermore, *Shh* is coexpressed with *Bmp* posteriorly[101]. Yet more similarities lie in the DV and AP axes: *fringe*-expressing cells are initially confined to the dorsal ectoderm and later are restricted to distal regions of the outgrowth where *Distal-less* orthologues are also expressed[93]. The ectopic deployment and modification of an existing patterning program, such as that of the branchial arches, may have given rise to the predecessors of vertebrate appendages.

Determination of whether two structures are homologous depends on the hierarchical level at which they are compared[103–105]. For example, bird wings and bat wings are analogous as wings, having evolved independently for flight in each lineage. However, at a deeper hierarchical level that includes all tetrapods, they are homologous as forelimbs, being derived from a corresponding appendage of a common ancestor. Similarly, we suggest that whereas vertebrate and insect wings are analogous as appendages, the genetic mechanisms that pattern them may be homologous at a level including most protostomes and deuterostomes. Furthermore, we propose that the regulatory systems that pattern extant arthropod and vertebrate appendages patterned an ancestral outgrowth and that these circuits were later modified during the evolution of different types of animal appendages. Animal limbs would be, in a sense, developmental 'paralogues' of one another; modification and redeployment of this ancient genetic system in different contexts produced the variety of appendages seen in Recent and fossil animals. □

Neil Shubin is in the Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; Cliff Tabin is in the Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA; and Sean Carroll is at the HHMI and Laboratory of Molecular Biology, University of Wisconsin, 1525 Linden Drive, Madison, Wisconsin 53706, USA

1. Müller, G. B. & Wagner, G. P. Novelty in evolution: Restructuring the concept. *Ann. Rev. Ecol. Syst.* **22,** 229–256 (1991).
2. Coates, M. I. The origin of vertebrate limbs. *Development* (suppl.) 169–180 (1994).
3. Coates, M. I. Fish fins or tetrapod limbs—a simple twist of fate? *Curr. Biol.* **5,** 844–848 (1995).
4. Shubin, N. The evolution of paired fins and the origin of tetrapod limbs. *Evol. Biol.* **28,** 39–85 (1995).
5. Coates, M. I. The Devonian tetrapod *Acanthostega gunnari* Jarvik: postcranial anatomy, basal tetrapod interrelationships and patterns of skeletal evolution. *Trans. R. Soc. Edinb.* **87,** 363–421 (1996).
6. Johnson, R. & Tabin, C. The long and short of *hedgehog* signaling. *Cell* **81,** 313–316 (1995).
7. Nelson, C. E. *et al.* Analysis of *Hox* gene expression in the chick limb bud. *Development* **122,** 1449–1466 (1996).
8. Tabin, C. J. & Laufer, E. *Hox* genes and serial homology. *Nature* **361,** 692–693 (1993).
9. Sordino, P., van der Hoeven, F. & Duboule, D. *Hox* gene expression in teleost fins and the origin of vertebrate digits. *Nature* **375,** 678–681 (1995).
10. Kessel, M. & Gruss, P. Murine developmental control genes. *Science* **249,** 374–379 (1990).
11. Mackem, S., Ranson, M. & Mahon, K. Limb-type differences in expression domains of certain chick *Hox*-4 genes and relationship to pattern modification for flight. *Prog. Clin. Biol. Res.* **383** *A*, 21–30 (1993).
12. Peterson, R. J., Papenbrock, T., Davada, M. M. & Awgulewitsch, A. The murine *Hoxc* cluster contains five neighboring *abdB*-related Hox genes that show unique spatially coordinated expression in posterior embryonic subregions. *Mech. Dev.* **47,** 253–260 (1994).
13. Gibson-Brown, J. J. *et al.* Evidence of a role for *T-box* genes in the evolution of limb morphogenesis and the specification of forelimb/hindlimb identity. *Mech. Dev.* **56,** 93–101 (1996).
14. Davis, A. P., Witte, D. P., Hsieh-Li, H. M., Potter, S. S. & Capecchi, M. R. Absence of radius and ulna in mice lacking *hoxa-11* and *hoxd-11*. *Nature* **375,** 791–795 (1995).
15. Vorobyeva, E. & Hinchliffe, J. R. From fins to limbs. *Evol. Biol.* **29,** 263–311 (1996).
16. Hinchliffe, J. R. & Johnson, D. R. *The Development of the Vertebrate Limb* (Clarendon, Oxford, 1980).
17. Holmgren, N. On the origin of the tetrapod limb. *Acta Zoologica* **14,** 185–295 (1933).
18. Holmgren, N. Contribution on the question of the origin of the tetrapod limb. *Acta Zoologica* **20,** 89–124 (1939).
19. Watson, D. M. S. On the primitive tetrapod limb. *Anat. Anzeiger* **44,** 24–27 (1913).
20. Gregory, W. K. & Raven, H. C. Studies on the origin and early evolution of paired fins and limbs. *Ann. N. Y. Acad. Sci.* **42,** 273–360 (1941).
21. Sordino, P. & Duboule, D. A molecular approach to the evolution of vertebrate paired appendages. *Trends Ecol. Evol.* **11,** 114–119 (1996).
22. Ahlberg, P. E. & Milner, A. R. The origin and early diversification of tetrapods. *Nature* **368,** 507–512 (1994).
23. Yokouchi, Y. *et al.* Homeobox gene expression correlated with the bifurcation process of limb cartilage development. *Nature* **353,** 443–445 (1991).

24. Gerard, M., Duboule, D. & Zakany, J. C. Cooperation of regulatory elements involved in the activation of the *Hoxd-11* gene. *Compt. R. Acad. Sci. III* **316,** 985–994 (1993).
25. Beckers, J., Gerard, M. & Duboule, D. Transgenic analysis of a potential *Hoxd-11* limb regulatory element present in tetrapods and fish. *Dev. Biol.* **180,** 543–553 (1996).
26. van der Hoeven, F., Zakany, J. & Duboule, D. Gene transpositions in the *HoxD* complex reveal a hierarchy of regulatory controls. *Cell* **85,** 1025–1035 (1996).
27. Shubin, N. & Alberch, P. A morphogenetic approach to the origin and basic organization of the tetrapod limb. *Evol. Biol.* **20,** 318–390 (1986).
28. Tabin, C. J. Why we have (only) five fingers per hand: *hox* genes and the evolution of paired limbs. *Development* **116,** 289–296 (1992).
29. Holder, N. Developmental constraints and the evolution of vertebrate digit patterns. *J. Theor. Biol.* **104,** 451–471 (1983).
30. Morse, E. On the tarsus and carpus of birds. *Ann. Lyc. Nat. Hist.* **10,** 141–158 (1872).
31. Shubin, N., Crawford, A. & Wake, D. Morphological variation in the limbs of *Taricha granulosa* (Caudata: Salamandridae): Evolutionary and phylogenetic implications. *Evolution* **49,** 874–884 (1995).
32. Greer, A. Limb reduction in the Scincid lizard genus *Lerista*. 2. Variation in the bone complements of the front and rear limbs and the number of postsacral vertebrae. *J. Herpetol.* **24,** 142–150 (1980).
33. Lande, R. Evolutionary mechanisms of limb loss in tetrapods. *Evolution* **32,** 73–92 (1978).
34. Gauthier, J. Saurischian monophyly and the origin of birds. *Mem. Calif. Acad. Sci.* **8,** 1–55 (1986).
35. MacFadden, B. J. *Fossil Horses* (Cambridge Univ. Press, 1992).
36. Davis, A. P. & Capecchi, M. R. Axial homeosis and appendicular skeleton defects in mice with a targeted disruption of *hoxd-11*. *Development* **120,** 2187–2198 (1994).
37. Davis, A. P. & Capecchi, M. R. A mutational analysis of the 5′ *HoxD* genes: Dissection of genetic interactions during limb development in the mouse. *Development* **122,** 1175–1185 (1996).
38. Favier, B. *et al*. Functional cooperation between the non-paralogous genes *Hoxa-10* and *Hoxd0*-11 in the developing forelimb and axial skeleton. *Development* **122,** 449–460 (1996).
39. Dollé, P. *et al*. Disruption of the *Hoxd*-13 gene induces localized heterochrony leading to mice with neotenic limbs. *Cell* **75,** 431–441 (1993).
40. Favier, B., LeMeur, M., Chambon, P. & Dollé, P. Axial skeleton homeosis and forelimb malformations in *Hoxd*-11 mutant mice. *Proc. Natl Acad. Sci. USA* **92,** 310–314 (1995).
41. Capecchi, M. R. Function of homeobox genes in skeletal development. *Ann. N. Y. Acad. Sci.* **97,** 34–37 (1996).
42. Wigglesworth, V. B. Evolution of insect wings and flight. *Nature* **246,** 127–203 (1973).
43. Budd, G. The morphology of *Opabinia regalis* and the reconstruction of the arthropod stem-group. *Lethaia* **29,** 1–14 (1996).
44. Hou, X. G. & Bergström, J. Cambrian lobopodians—ancestors of extant onychophorans? *Zool. J. Linn. Soc. Lond.* **114,** 3–19 (1995).
45. Simonetta, A. M. & Delle Cave, L. in *The Early Evolution of Metazoa and the Significance of Problematic Taxa* (eds Simonetta, A. M. & Conway Morris, S.) 189–244 (Cambridge Univ. Press, 1991).
46. Budd, G. A Cambrian gilled lobopod from Greenland. *Nature* **364,** 709–711 (1993).
47. Chen, J. Y., Ramsköld, L. & Zhou, G. Q. Evidence for monophyly and arthropod affinity of Cambrian giant predators. *Science* **264,** 1304–1308 (1994).
48. Carroll, S. B. Homeotic genes and the evolution of arthropods and chordates *Nature* **376,** 479–485 (1995).
49. Struhl, G. Genes controlling segmental specification in the *Drosophila* thorax. *Proc. Natl Acad. Sci. USA* **79,** 7380–7384 (1982).
50. Struhl, G. A homoeotic mutation transforming leg to antenna in *Drosophila*. *Nature* **292,** 635–638 (1981).
51. Gibson, G. & Gehring, W. J. Head and thoracic transformations caused by ectopic expression of *Antennapedia* during *Drosophila* development. *Development* **102,** 657–675 (1988).
52. Stuart, J., Brown, S., Beeman, R. & Denell, R. A deficiency of the homeotic complex of the beetle *Tribolium*. *Nature* **350,** 72–47 (1991).
53. Averof, M. & Akam, M. *Hox* genes and the diversification of insect–crustacean body plans. *Nature* **376,** 420–423 (1995).
54. Vachon, G. *et al*. Homeotic genes of the Bithorax complex repress limb development in the abdomen of the *Drosophila* embryo through the target gene. *Cell* **71,** 437–450 (1992).
55. Panganiban, G. *et al*. The development of crustacean limbs and the evolution of arthropods. *Science* **270,** 1363–1366 (1995).
56. Manton, S. M. *Mandibular Mechanisms and the Evolution of Arthropods* Vol. 247 (British Museum and Queen Mary College, London, 1964).
57. Wheeler, W. C., Cartwright, P. & Hayashi, C. Y. Arthropod phylogeny: a combined approach. *Cladistics* **9,** 1–39 (1993).
58. Boore, J. L., Collins, T. M., Stanton, D., Daehler, L. L. & Brown, W. M. Deducing the pattern of arthropod phylogeny from mitochondrial DNA rearrangements. *Nature* **376,** 163–165 (1995).
59. Cohen, S. M. & Jürgens, G. Proximal–distal pattern formation in *Drosophila*: cell autonomous requirement for *Distal-less* gene activity in limb development. *EMBO J.* **8,** 2045–2055 (1989).
60. Cohen, S. *et al*. *Distal-less* encodes a homeodomain protein required for limb development in *Drosophila*. *Nature* **338,** 432–434 (1989).
61. Panganiban, G., Nagy, L. & Carroll, S. B. The development and evolution of insect limb types. *Curr. Biol.* **4,** 671–675 (1994).
62. Popadic, A., Rusch, D., Peterson, M., Rogers, B. T. & Kaufman, T. C. Origin of the arthropod mandible. *Nature* **380,** 395 (1996).
63. Jeram, A. J., Selden, P. A. & Edwards, D. Land animals in the Silurian: Arachinids and myriapods from Shropshire, England. *Science* **250,** 658–661 (1990).
64. Kukalová-Peck, J. *The Insects of Australia* 2nd edn (Cornell University Press, Ithaca, NY, 1991).
65. Snodgrass, R. *Principles of Insect Morphology* (McGraw-Hill, New York, 1935).
66. Kukalova-Peck, J. Origin and evolution of insect wings and their relation to metamorphosis, as documented from the fossil record. *J. Morphol.* **156,** 53–126 (1978).
67. Marden, J. H. & Kramer, M. G. Surface-skimming stoneflies: A possible intermediate stage in insect flight evolution. *Science* **266,** 427–430 (1994).
68. Cohen, B. *et al*. Allocation of the thoracic imaginal primordia in the Drosophila embryo. *Development* **117,** 597–608 (1993).
69. Diaz-Benjumea, F. & Cohen, S. M. Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell* **75,** 741–752 (1993).
70. Carroll, S. B. *et al*. Pattern formation and eyespot determination in butterfly wings. *Science* **265,** 109–114 (1994).
71. Averof, M. & Cohen, S. M. Evolutionary origin of insect wings from ancestral gills. *Nature* **385,** 627–630 (1997).
72. Lee, J. J. *et al*. Secretion and localized transcription suggest a role in positional signaling for products of the segmentation gene *hedgehog*. *Cell* **71,** 33–50 (1992).
73. Tabata, T. *et al*. The *Drosophila hedgehog* gene is expressed specifically in posterior compartment cells and is a target of *engrailed* regulation. *Genes Dev.* **6,** 2635–2645 (1992).
74. Basler, D. & Struhl, G. Compartment boundaries and the control of *Drosophila* limb pattern by *hedgehog* protein. *Nature* **368,** 208–214 (1994).
75. Posakony, L., Raftery, L. & Gelbart, W. Wing formation in *Drosophila melanogaster* requires *decapentaplegic* gene function along the anterior–posterior compartment boundary. *Mech. Dev.* **33,** 69–82 (1991).
76. Capdevila, J. & Guerrero, I. The *Drosophila* segment polarity gene *patched* interacts with *decapentaplegic* in wing development. *EMBO J.* **6,** 715–729 (1994).
77. Sanicola, M., Sekelsky, J., Elson, S. & Gelbart, W. M. Drawing a stripe in *Drosophila* imaginal discs: negative regulation of *decapentaplegic* and *patched* expression. *Genetics* **139,** 745–756 (1995).
78. Nellen, D., Burke, R., Struhl, G. & Basler, K. Direct and long-range actions of a *Dpp* morphogen gradient. *Cell* **85,** 357–368 (1996).
79. Lecuit, T. *et al*. Two distinct mechanisms for long-range patterning by *Decapentaplegic* in the *Drosophila* wing. *Nature* **381,** 387–393 (1996).
80. Echelard, Y. *et al*. *Sonic hedgehog*, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75,** 1417–1430 (1993).
81. Krauss, S., Concordet, J. P. & Ingham, P. W. A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. *Cell* **75,** 1431–1444 (1993).
82. Chang, D. T. *et al*. Products, genetic linkage and limb patterning activity of a murine *hedgehog* gene. *Development* **120,** 3339–3353 (1994).
83. Riddle, R. D. *et al*. *Sonic hedgehog* mediates the polarizing activity of the ZPA. *Cell* **75,** 1401–1416 (1995).
84. Tickle, C. Genetics and limb development. *Dev. Genet.* **19,** 1–8 (1996).
85. Irvine, K. & Weischaus, E. *fringe*, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development. *Cell* **79,** 595–606 (1994).
86. Spreicher, S., Thomas, U., Hinz, U. & Knust, E. The *Serrate* locus of *Drosophila* and its role in morphogenesis or imaginal discs: control of cell proliferation. *Development* **120,** 535–544 (1994).
87. Kim, J., Irvine, K. & Carroll, S. Cell recognition, signal induction, and symmetrical gene activation at the dorsal-ventral boundary of the developing *Drosophila* wing. *Cell* **82,** 795–802 (1995).
88. Couso, J. P., Knust, E. & Martinez Arias, A. *Serrate* and *wingless* cooperate to induce *vestigial* gene expression and wing formation in *Drosophila*. *Curr. Biol.* **5,** 1437–1448 (1995).
89. Diaz-Benjumea, F. J. & Cohen, S. *Serrate* signals through *Notch* to establish a *Wingless*-dependent organizer at the dorsal/ventral compartment boundary of the *Drosophila* wing. *Development* **121,** 4215–4225 (1995).
90. Kim, J. *et al*. Integration of positional signals and regulation of wing formation and identity by *Drosophila vestigial* gene. *Nature* **382,** 133–138 (1996).
91. Todt, W. L. & Fallon, J. F. Development of the apical ectodermal ridge in the chick wing bud. *J. Embryol. Exp. Morphol.* **80,** 21–41 (1984).
92. Rodriguez-Estaban, C. *et al*. *Radical fringe* positions the apical ectodermal ridge at the dorsoventral boundary of the vertebrate limb. *Nature* **386,** 360–361 (1997).
93. Laufer, E. *et al*. Expression of *Radical fringe* in limb-bud ectoderm regulates apical ectodermal ridge formation. *Nature* **386,** 366–373 (1997).
94. Williams, J. A., Paddock, S. W. & Carroll, S. B. Pattern formation in a secondary field: A hierarchy of regulatory genes subdivides the developing *Drosophila* wing disc into discrete sub-regions. *Development* **117,** 571–584 (1993).
95. Couso, J. P., Bate, M. & Martinez-Arias, A. A *wingless*-dependent polar coordinate system in *Drosophila* imaginal discs. *Science* **259,** 484–489 (1993).
96. Parr, B. A. & McMahon, A. P. Dorsalizing signal *Wnt-7a* required for normal polarity of D-V and A-P axes of mouse limb. *Nature* **374,** 350–353 (1995).
97. Riddle, R. D. *et al*. Induction of the LIM homeobox gene *Lmx-1* by *Wnt-7a* establishes dorsoventral pattern in the vertebrate limb. *Cell* **83,** 631–640 (1995).
98. Vogel, A. *et al*. Dorsal cell fate specified by chick *Lmx1* during vertebrate limb development. *Nature* **378,** 716–720 (1995).
99. Raff, R. *The Shape of Life* (Univ. Chicago Press, 1996).
100. Panganiban, G. *et al*. The origin and evolution of animal appendages. *Proc. Natl Acad. Sci. USA* **94,** 5162–5166 (1997).
101. Wall, N. A. & Hogan, B. L. M. Expression of *bone morphogenetic protein*-4 (*BMP*-4), *bone morphogenetic protein-7* (*BMP-7*), *fibroblast growth factor*-8 (*FGF*-8) and *Sonic hedgehog* (*SHH*) during branchial arch development in the chick. *Mech. Dev.* **53,** 383–392 (1995).
102. Marigo, V., Scott, M. P., Johnson, R. L., Goodrich, L. V. & Tabin, C. J. Conservation in *hedgehog* signaling: induction of a chicken *patched* homolog by *Sonic hedgehog* in the developing limb. *Development* **122,** 1225–1233 (1996).
103. Roth, V. L. Homology and hierarchies: Problems solved and unresolved. *J. Evol. Biol.* **4,** 167–194 (1991).
104. Wagner, G. P. The origin of morphological characters and the biological basis of homology. *Evolution* **43,** 1157–1171 (1989).
105. Bolker, J. A. & Raff, R. A. Developmental genetics and traditional homology. *BioEssays* **18,** 489–494 (1996).
106. Carroll, R. L. *Vertebrate Paleontology* (Freeman, San Francisco, 1988).
107. Jarvik, E. *The Structure and Evolution of the Vertebrates* Vol. 1 (Academic, New York, 1980).
108. Jarvik, E. The Devonian tetrapod *Ichthyostega*. *Fossils and Strata* **40,** 1–213 (1996).
109. Fromental-Ramain, C. *et al*. Specific and redundant functions of the paralogous *Hoxa*-9 and *Hoxd*-9 genes in forelimb and axial skeleton patterning. *Development* **122,** 461–472 (1996).
110. Mortlock, D. P., Post, L. C. & Innis, J. W. The molecular basis of hypodactyly (Hd): a deletion in *Hoxa13* leads to arrest of digital arch formation. *Nature Genet.* **13,** 284–289 (1996).
111. Mortlock, D. P. & Innis, J. W. Mutation of *HOXA13* in hand–foot–genital syndrome. *Nature Genet.* **15,** 179–181 (1997).
112. Saunders, J. The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. *J. Exp. Zool.* **108,** 363–403 (1948).
113. Summerbell, D., Lewis, J. H. & Wolpert, L. Postional information in chick limb morphogenesis. *Nature* **244,** 492–496 (1973).
114. Lebedev, O. A. & Coates, M. I. The postcranial skeleton of the Devonian tetrapod *Tulerpeton curtum* Lebedev. *Zool. J. Linn. Soc.* **113,** 302–348 (1995).
115. Hou, X. G., Bergström, J. & Ahlberg, P. *Anomalocaris* and other large animals in the Lower Cambrian Chenjiang fauna of southwest China. *Geol Forening. Forhandling.* **117,** 163–183 (1995).

Acknowledgements. We thank P. Ahlberg, G. Budd, A. C. Burke, M. Coates, A. Meyer, G. Panganiban, P. Sniegowski, D. Wake, R. S. Winters, L. Wolpert and members of our laboratories for their critiques of drafts of this manuscript. S.B.C. is an investigator of the HHMI. C.T. is supported by grants from the NIH and the American Cancer Society. N.S. is supported by grants from the NSF, from the National Geographic Society and from the Research Foundation of the University of Pennsylvania.

Correspondence and requests for materials should be addressed to N.S. (e-mail: nshubin@sas.upenn.edu).