

Why we have (only) five fingers per hand: Hox genes and the evolution of paired limbs

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Summary

Limb development has long been a model system for studying vertebrate pattern formation. The advent of molecular biology has allowed the identification of some of the key genes that regulate limb morphogenesis. One important class of such genes are the homeobox-containing, or Hox genes. Understanding of the roles these genes play in development additionally provides insights into the evolution of limb pattern. Hox gene expression patterns divide the embryonic limb bud into five sectors along the anterior/posterior axis. The expression of specific Hox genes in each domain specifies the devel-

opmental fate of that region. Because there are only five distinct Hox-encoded domains across the limb bud there is a developmental constraint prohibiting the evolution of more than five different types of digits. The expression patterns of Hox genes in modern embryonic limb buds also gives clues to the shape of the ancestral fin field from which the limb evolved, hence elucidating the evolution of the tetrapod limb.

Key words: Hox gene, limb evolution, digits, pattern formation, homeobox.

Introduction

In building a coherent story of the evolution of life, biologists have drawn upon information gleaned from many disciplines including paleontology, embryology and comparative anatomy. Most recently, molecular genetics has started to contribute to building this evolutionary synthesis. The first impact of molecular biology has been to help clarify phylogenetic relationships between species by examining relatedness reflected in genomic sequence homologies. As the relatively young molecular discipline matures, it will continue to be a source of new information which, when incorporated, will help to provide a fuller picture of evolutionary processes. For example, current research efforts have begun to unravel the molecular events underlying embryological morphogenesis. The study of these regulatory events provides the potential for insight into the mechanisms of evolutionary changes in morphology. This paper will explore the significance of recent advances in the molecular biology of limb development as it relates to developmental constraints on the morphology of modern limbs and the evolutionary origin of the tetrapod limb.

Five fingers, five toes

All modern tetrapods (four legged creatures), as well as all but a few fossil tetrapods, have limbs characterized by five or fewer digits. This has been viewed as an evolutionary enigma. Individuals of many species, including mice, chickens, dogs, cats and humans carry mutations which give rise

to extra digits (Fig. 1). Yet a pattern of digits greater than five has never been adopted as the norm in a lineage leading to a modern species. This is surprising in light of the apparent evolutionary advantage of having more digits in certain instances. There are examples ranging from frogs to panda bears where an additional 'finger' has evolved (Fig. 2). The new 'finger' is never a true digit, however, rather in each case it is a modification of bones of the wrist (Gould, 1980). Such a pseudo-digit evolves under circumstances when selection and/or developmental constraints act to maintain the morphology of the five true digits (Gould, 1980).

This evolutionary paradox can be explained in terms of developmental constraints if the developmental mechanism by which the number of digits on a limb is determined is distinct from the mechanism that specifies the different morphology of individual digits. Each of these mechanisms has inherent developmental constraints. It has been argued that the ability to select for an additional digit is constrained by the average size of the embryonic limb bud in a given population (Alberch, 1985). It will be argued here that there are genetic constraints on digit morphology which make it impossible to select for more than five unique digits. Polydactyly can arise, but at least two of the digits will have the same genetically determined 'identity'; leading to, for example, a second digit V rather than a novel digit VI. Therefore, tetrapod species rarely maintain a polydactylous sixth digit because simply having a duplicated structure might be of limited evolutionary use if it cannot subsequently be molded by selection for a distinct function. (In this paper two digits will be defined as having the same

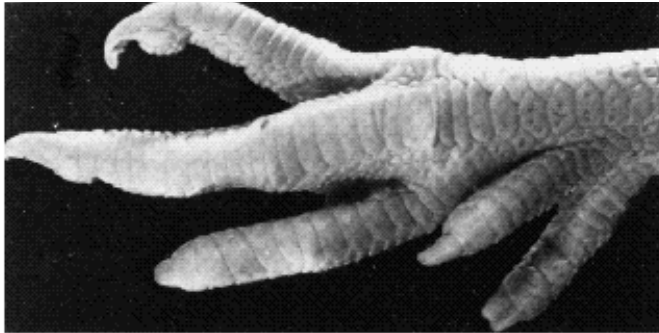


Fig. 1. Examples of polydactylous limbs. Human preaxial polydactyly of the hand (left) and chicken mutant *diplopodia-3* foot (right). Both exhibit an additional digit which morphologically closely resembles the neighboring digit 1 (an extra thumb).

'identity' if they have the same numbers of phalanges and are of a similar size and morphology. Empirically, a digit is considered to have the same identity as a second digit 'x' if, when examined in isolation by a morphologist, the first digit would be labeled as being a digit 'x'.)

The idea that the number of digits might be developmentally uncoupled from the mechanisms that assign each digit a unique morphology is suggested by the fact that many polydactylous mutants have extra digits with a morphology that closely resembles that of an adjacent digit rather than a unique structure (Fig. 1). A similar conclusion is implied by experiments where limb mesenchyme is disassociated and then repacked into a limb bud ectodermal hull. The disassociation destroys the anterior/posterior axis information which is encoded in the mesenchyme. The scrambled, reassociated cells will still form a limb-like structure including digits. (Finch and Zwillig, 1971; Patou, 1973). These digits lack distinctive identities along the anterior/posterior axis. This result argues strongly that some digit-organizing mechanism exists independent of positional information in the limb (Wolpert, 1989).

Molecular regulation of digit morphology

The best current candidates for genes specifying positional information are the Homeobox-containing genes. These appear to have been present in the last common ancestor

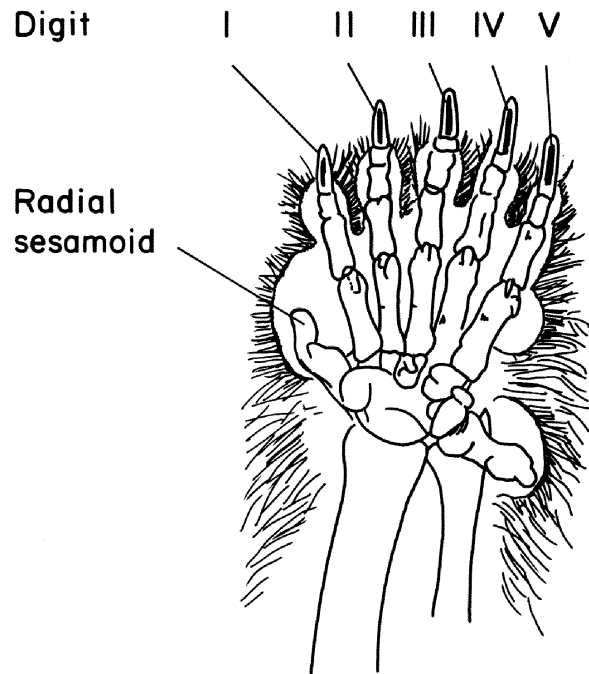


Fig. 2. The structure of the Panda's hand. A wrist bone, the radial sesamoid, is enlarged and due to a rearrangement of muscle attachments is able to function as an opposable digit. Objects such as bamboo, can be grasped between the radial sesamoid and true digit 1. Drawing adapted from Gould, 1980.

of vertebrates and insects. In *Drosophila* these genes do not break the animal into developmental units (such as segments) but rather define the identity of each unit. A different set of genes is responsible for setting up the number of repeating segments in the developing insect (gap genes, pair-rule genes and segment-polarity genes).

In vertebrates, the ancestral homeobox gene cluster was duplicated to give four homologous clusters. These are called Hox-1, Hox-2, Hox-3 and Hox-4 (Fig. 3). The members of all four clusters are expressed in anterior-to-posterior domains in both the embryonic central nervous system and body mesenchyme. (For a recent review see McGinnis and Krumlauf, 1992).

In the developing limb, the expression of the Hox genes of the various clusters divide the limb bud into regions along different axes. For example, the Hox-1 genes are expressed in differential domains along the proximal/distal axis (Yokouchi et al., 1991).

The Hox genes whose expression pattern seems of most relevance to subdividing the limb bud along the anterior/posterior axis (and hence to potentially specifying unique fates to each digit) are members of the Hox-4 cluster; in particular the five genes from the extreme 5' end of the cluster: *Hox-4.4*, *Hox-4.5*, *Hox-4.6*, *Hox-4.7* and *Hox-4.8*. These genes are found to be expressed in the posterior-most regions of the vertebrate embryo, overlapping the region from which the hindlimb bud forms as an outgrowth of the flank mesenchyme. Surprisingly, these same genes are also expressed in the forelimb bud. Based on the forelimb's more anterior position, one might have expected that different, more 3', Hox-4 genes would have been found in

the forelimb region. It has recently been shown (Dolle et al., 1989, 1991) that within each limb bud (hind- and fore-, although only the forelimb is published) these Hox-4 genes are expressed in a 3 → 5 /anterior→posterior order. These are actually not sequential domains of expression stripes, but rather are akin to a nested set, like russian dolls, with all of the genes being expressed at the same posterior border and each gene's expression extending to a successively more anterior position (Fig. 3). They are also expressed in a temporal order such that *Hox-4.4* is the first to be expressed. Subsequently *Hox-4.5* is activated, and so on. Each is initially expressed just at the posterior margin and then spreads anteriorly.

This pattern of expression is set up early in the limb bud. Subsequently the expression patterns shift, changing in both extent and exact orientation relative to the anterior/posterior axis. Nonetheless, early in limb development, when they first spread across the entire limb bud, the five Hox-4 genes subdivide the limb field into five zones, from posterior to anterior: (4.8 + 4.7 + 4.6 + 4.5 + 4.4) all expressed, (4.7 + 4.6 + 4.5 + 4.4) expressed; (4.6 + 4.5 + 4.4) expressed; (4.5 + 4.4) expressed and (4.4) expressed. Each of the five zones of the limb field can thus be considered to have a unique Hox code, or 'address'. (Izpisua-Belmonte et al., 1991). Fate mapping experiments at this stage of limb development demonstrate that regional domains of Hox expression correlate with the anlage of individual digits (Morgan and Tabin, unpublished observation).

In comparing limbs of disparate species, the existence of genetically marked zones of the limb bud allows one to objectively correlate the digits arising in each. It has been previously argued that it is inappropriate to draw a one-to-one correspondence (to 'homologize') between digits of different phylogenetic groups on the grounds that the limb is formed from a global field. According to this argument, such a field, with internal properties, ultimately generates

similar structures in different organisms, but digits themselves are not independently regulated parts and should not be considered as such (Goodwin, 1984; Webster, 1984). Drawing homologies between analogous embryonic structures is only considered useful when there is a biological basis for believing that the parts are developmental units (Wagner, 1989). If the different Hox addresses control the morphological fate of digits in addition to marking the primordia of each, then they would provide a basis for considering digits to be developmental units. This view does not countermand the concept of global processes. Rather, the unique Hox codes provide a differential context over which global processes can then act, ultimately resulting in morphologically distinct digits.

The hypothesis that these early developmental Hox addresses specify ultimate digit identity has now been directly confirmed (Morgan et al., 1992). The chick hind limb has four digits. *Hox-4.6* is normally expressed in the presumptive anlage of the three posterior digits (designated digits 4, 3, and 2), but not in the anlage of the anterior-most digit 1. Using a viral vector, *Hox-4.6* was ectopically expressed throughout a developing chick leg bud. This does not alter the Hox-4 code (sum of all Hox genes expressed) of the three most posterior zones where *Hox-4.6* is already active. However, it changes the fourth expression pattern (4.4 + 4.5) to match that of the third pattern (4.4 + 4.5 + 4.6). The morphological consequence of this is to transform digit 1 into a morphology indistinguishable from digit 2 (Fig. 4). This effect is only seen when the infection is done early, at the time the endogenous expression of Hox-4 genes divides the limb bud into five distinct regions. Thus the early expression of Hox-4 genes can determine the identity of digits without affecting the number of digits. When two digits are formed from primordia expressing the same Hox code they develop identical morphologies.

The existence of polydactylous individuals and the results of experimental manipulations indicate that it may be relatively easy for an embryo to develop limbs with an extra digit. Indeed, the most frequently observed congenital malformation of the limb is polydactyly (Mellin, 1963; Bunnell, 1964; Tentamy and McKusick, 1978). However, the existing Hox-4 genes expressed in the limb bud only provide five distinct addresses, thus allowing for the specification of up to five distinct types of digits. In principle, an additional duplication of one of the Hox-4 genes could provide an expanded capacity for encoding position along the anterior-posterior axis. However, the Hox-4 genes are also coordinately expressed in the CNS and elsewhere in the body mesenchyme. Thus to alter their expression would affect more than just the limb. In theory, the effects of a newly derived Hox-4 gene could be limited to the limbs by creating a limb-specific promoter. That, however, would likely require first duplicating a Hox-4 gene and then fine-tuning its regulation. The initial step of this transformation could be lethal. Hence, polydactyly is a common condition (humans, chickens, etc.) but perhaps nothing useful evolutionarily can be done with it; or at very least it may be evolutionarily easier to modify the morphology of an already uniquely specified structure such as a wrist bone. Changing the morphology of a digit or wrist bone is likely to be distinct from specifying its identity, involving altering the

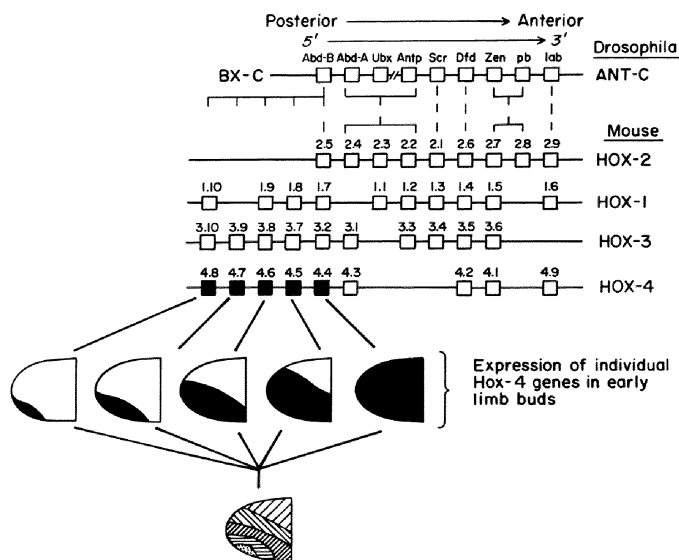


Fig. 3. The four vertebrate Hox gene clusters. Five members of the Hox-4 cluster (black boxes) are expressed in an overlapping nested pattern in the developing limb bud.

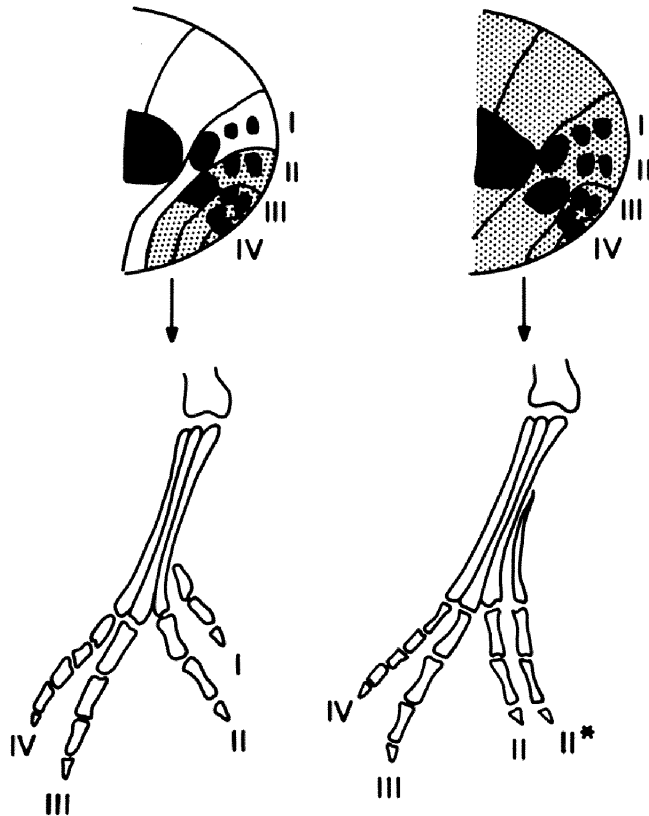


Fig. 4. Consequences of misexpression of *Hox-4.6* in the developing chick leg bud. The normal expression patterns of the *Hox-4* genes are shown (upper right) superimposed on a hypothetical fate map of the digits. Boundaries of each expression domain are drawn as lines and the region expressing *Hox-4.6* is shaded (see Fig. 2). Infection of the limb bud with a viral vector transducing *Hox-4.6* does not affect the Hox code of the regions destined to become digits 4, 3 and 2, however it gives the primordia of digit 1 a code identical to the adjacent digit 2 (upper left). The phenotypic result of such an experiment in the chick hind limb bud is to transform the wild-type digit 1 (lower left) into a phenocopy of digit 2 (lower right) in the resultant foot. Drawing based on Morgan et al., 1992.

properties of downstream genes on which the Hox genes act. That the Hox genes themselves do not directly specify the digit morphologies is evident from the fact that the same Hox genes are expressed in hind limb buds and forelimb buds, and in homologous limbs of different species such as the human arm and chicken wing.

Evolution of the tetrapod body plan

Given the developmental dangers of tampering with the Hox gene clusters, how might limbs have evolved in the first place? The limbs of tetrapods evolved from the paired pectoral and pelvic fins of their fish ancestors. While there is scant fossil evidence of the earliest stages of a paired fin evolution, the origin of those structures has long been a subject of comparative anatomical and embryological research. The evidence, (reviewed by Zangerl, 1981)

suggests that the paired fins evolved from a pair of ventrolateral skin folds extending along the length of the body axis (Fig. 5). Migration of mesenchymal cells from the body wall into these folds restricted the continuous fold into two separate domains to form the paired appendages. Consistent with the limbs evolving from lateral plate mesenchyme, the Hox genes, which form a nested set in the limb bud, are also expressed in an overlapping pattern in the paraxial mesoderm along the flank where, as in the limb, they are thought to provide positional information during development (Fig. 5). As in the limb, spatial domains of Hox genes along the main body axis have immediate effects on skeletal elements. Experimental alteration of the Hox code in the developing trunk, for example, causes homeotic transformations of ribs and vertebrae (Le Mouellic et al., 1992; Kessel and Gruss, 1991).

The fact that the expression domains of the Hox genes pre-existed in the lateral plate and somitic mesoderm along the body axis, allows the original limb field to be traced. The region of the flank encompassing the domains of expression of the *Hox-4.4 - Hox-4.8* genes is larger than the current extent of the embryonic hind limb bud (Fig. 6). This implies that the original region of the flank that contributed mesenchyme to the early ancestral fin was larger than that from which the tetrapod hind limb derives. Fossil evidence is consistent with this idea; fins of primitive fish often were broadly based (Romer, 1966; Halstead, 1968) (Fig. 7). As fins evolved, the fin field contracted so that the posterior Hox genes are no longer expressed in a strictly contiguous pattern in the hind limb and the flank.

According to the lateral fin fold hypothesis, the two serially homologous appendages are assumed to have arisen simultaneously, as two distinct 'concentrations' of the orig-

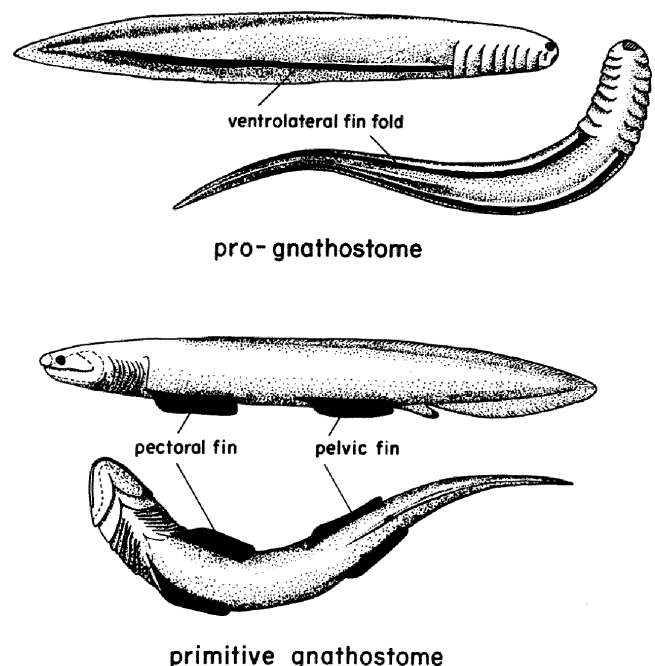


Fig. 5. Hypothetical representation of the ventrolateral fin fold in the prognathostome and the pectoral and pelvic fins derived from the fin fold in the primitive gnathostome (shown in lateral and ventral views). Drawing adapted from Jarvik, 1980.

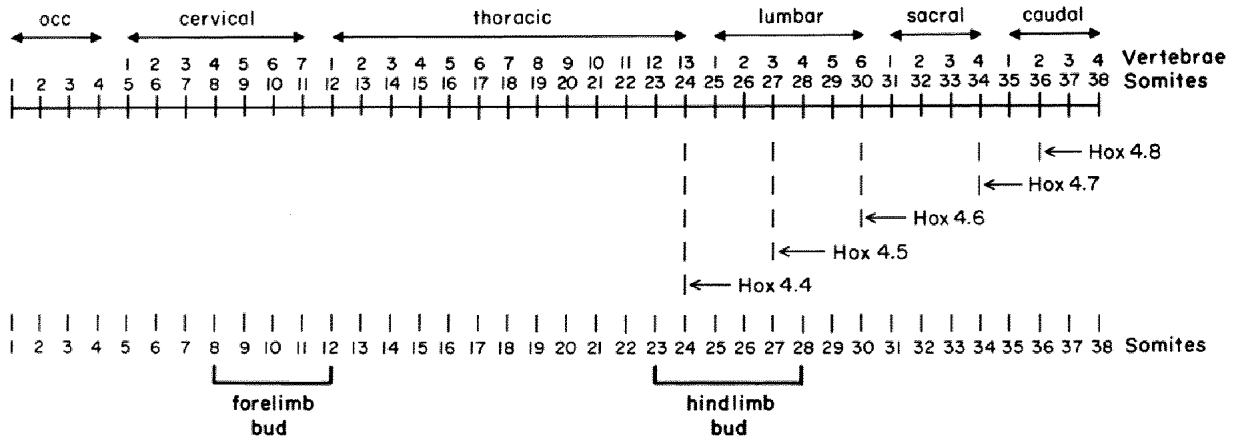


Fig. 6. Expression domains of the *Hox-4.4 - Hox-4.8* genes in the mesoderm along the flank of the mouse (based on Kessel and Gruss, 1991). The arrows delineate the anterior-most extent of each gene's expression.

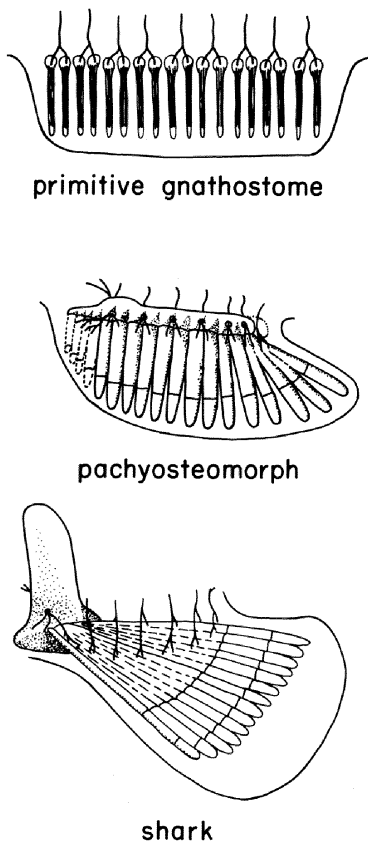


Fig. 7. Diagram of the early evolution of the pectoral fin in the primitive gnathostome, pachyosteomorph and shark showing the broad base of the primitive fin extending over a larger number of the metameric segments of the body wall than does the modern limb bud. Drawing adopted from Jarvik, 1980.

inally continuous fold. The molecular data, however, would suggest that the genetic program of the pelvic fin evolved first and was subsequently reactivated anteriorly and employed in the formation of the pectoral fin. If the two sets of fins had evolved independently from flank mesenchyme one would have expected that the Hox genes

expressed in the anterior flank would have been carried by laterally migrating mesenchyme into the pectoral fin and the Hox genes expressed in the posterior flank would have been maintained in the pelvic fin. Instead, (assuming that the *Hox-4* gene expression in fin buds is consistent with the expression patterns found in limb buds, a testable hypothesis), the genes expressed along the posterior flank are expressed in the primordia of both the fore and hind appendages. Not only are the same Hox genes expressed in both developing appendages but they are expressed in identical spatial and temporal patterns. The expression of posterior genes in the anterior appendage thus may indicate that the pelvic and pectoral fins evolved by both adopting molecular mechanisms present in a common ancestral posterior fin.

It should be noted that the fossil evidence is somewhat at odds with this conclusion. The earliest fins in the fossil record are pectoral fins seen in some fossil agnathans such as *Hemicyclospis* (Romer, 1966; Carroll, 1988). However, the hypothesis that pectoral appendages are derived from pelvic ones is so strongly supported by a strict genetic interpretation that it is worth entertaining the possibility that these early agnathans were themselves derived from a lineage that had already developed two set of paired appendages and that the posterior pair was secondarily lost.

According to a controversial view, unlike the pectoral fin, the shoulder girdle with which it articulates may have evolved from a modified branchial arch (Zangerl, 1981). Molecular evidence consistent with this separate origin of the shoulder comes from a different Hox gene, *Hox-3.3*. This gene is expressed in the anterior of the embryonic body wall at the base of the branchial arches. It is also specifically expressed in the extreme proximal, anterior region of the fore limb bud, but not the hind limb bud, (Oliver et al., 1988). Indirect experimental evidence implicates *Hox-3.3* in playing a role in the morphogenesis of the shoulder region (Oliver et al., 1990).

Once the expression of the Hox-4 genes, and other posteriorly expressed Hox genes from the Hox-1 cluster, were incorporated into the fin field they were presumably used to assign positional addresses across the anterior-posterior axis

of the developing bud. The current view of the hypothetical primitive fin condition is of a metamereric series of simple rays consisting of basal and radial elements. The original set of expressed genes from the Hox-1, and Hox-4 clusters would together have allowed a large number of unique ray identities to have been specified. Note that the total number of rays could exceed the total number of Hox genes. The Hox genes would only affect the number of distinct morphological types of rays that could be uniquely specified.

The evolutionary transition from fish fin to tetrapod limb includes (in part) a reorientation of the digits from a primitive anterior position to a distal position; a reorganization of the proximal region of the limb as well as a reduction in the number of fin rays to the five elements ultimately forming digits. Part of the way this was accomplished is reflected in the modern expression patterns of the Hox genes in the limb bud. The presumed primitive expression patterns of all the Hox clusters in the early fin bud would have paralleled the anterior-to-posterior expression domains seen in the posterior flank. This pattern is still observed in the earliest stages of limb bud growth (Fig. 8). However, as the development of the bud proceeds the Hox gene expression patterns shift so that they divide the limb bud along orthogonal axes: the Hox-4 genes into anterior/posterior domains and the Hox-1 genes into proximal/distal domains. (The expression pattern of the Hox-3 homologues has not yet been described in the limb bud.) This reorien-

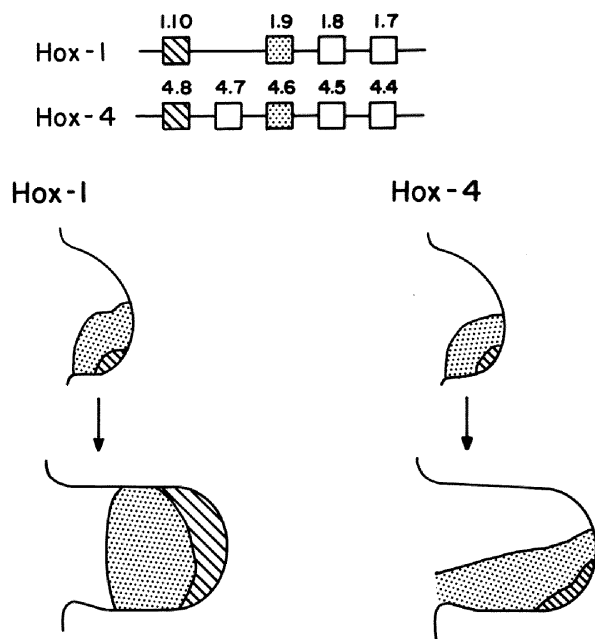


Fig. 8. Expression patterns of Hox-1 and Hox-4 genes in the limb bud. The chromosomal gene order of these clusters are diagrammed at top. For clarity, the expression pattern of only two genes are drawn for each cluster: *Hox-4.8* and its homologue *Hox-1.10* (striped) and *Hox-4.6* and its homologue *Hox-1.9* (stippled). For the full set of Hox-4 genes see Fig. 2. Initially, the Hox genes of both clusters are expressed in a nested set emanating from the distal posterior margin. Later in development Hox-4 genes divide the bud into anterior/posterior domains. The Hox-1 genes are expressed in an orthogonal pattern dividing the bud into proximal/distal domains.

tation has the effect of changing a linear code into a set of Cartesian coordinates. This allows the specification of the series of elements along the proximal/distal limb axis. The Hox-1 and Hox-4 genes together could have specified a total of nine anterior/posterior values in the original fin field. However, as a consequence of using the Hox-1 genes to form an orthogonal grid, only five different regions can be specified along the anterior/posterior axis by the Hox-4 genes alone.

This unified view of the mechanism of specification of the proximal/distal and anterior/posterior axes is in marked contrast to the traditional paradigm that these two axes are controlled in very different ways. Traditionally, the proximal/distal axis is thought to be determined during the growth of the limb bud. Under the apical ectodermal ridge (or AER), there is a region of rapidly dividing mesenchymal cells termed the 'progress zone' (Summerbell et al., 1973). As the bud grows out, cells left further from the AER begin to differentiate. As cells leave the progress zone, their proximal/distal value becomes fixed. The longer they remain in the progress zone, the more distal structures they will produce (Summerbell et al., 1973; Wolpert et al., 1975). In contrast, control of the anterior/posterior axis is controlled by a different region of the limb bud. If tissue from the posterior margin of a limb bud is transplanted to the anterior margin, limbs develop with mirror-image duplications along the anterior-posterior axis (Saunders and Gasseling, 1968). This region, termed the 'zone of polarizing activity' (or ZPA) is considered to be a source of a diffusible signal which provides positional information in a concentration-dependent manner (Wolpert, 1969). The molecular data also suggests that the proximal/distal and anterior/posterior axes are independently regulated by Hox gene expression (Dolle et al., 1989). The traditional concepts can be reconciled with the molecular model of Hox genes as encoding positional values along both axes by postulating that the Hox-1 and Hox-4 gene clusters respond differentially to the AER and ZPA signals. The evolution of two signaling centers, which both influence the Hox gene expression patterns, is thus a key event in the evolution of the tetrapod limb.

The origins of these signaling mechanisms, like the Hox genes they control, can be found in the development of the primary body axes. The Hox genes are expressed in an overlapping pattern along the anterior/posterior axis of the trunk. This primary expression pattern is initiated in the mesoderm during its induction as cells invaginate through the primitive streak. Hensen's node at the tip of the primitive streak has ZPA activity when transplanted to limb buds. (Saunders and Gasseling, 1983; Hornbruch and Wolpert, 1986). Moreover, the ZPA activity can be traced through embryogenesis moving from the node along the flank and into the posterior limb bud (Hornbruch and Wolpert, 1991). Thus, the limb bud ZPA activity was likely derived from the flank Hox-signaling system. The ZPA was, therefore, probably the signaling center, which originally influenced Hox gene expression in the ancestral posterior bud; and which was subsequently reactivated in the separate pectoral and pelvic buds. The AER initially may have simply regulated mesenchymal proliferation and outgrowth. The AER then secondarily evolved to also influence Hox gene

expression in the elaboration of the orthogonal limb axes. It will be interesting to compare the effect of ZPA and AER removal on Hox gene expression in limb and fin buds.

The basic limb pattern is the same in the tetrapod fore limb and hind limb; for example, humerus, ulna and radius in the forelimb; femur, tibia and fibula in the hindlimb. This pattern is also seen in an osteolepiform Devonian fish *Sauripterus* (Hall, 1843), because of which, the osteolepiforms are considered candidates for the tetrapod ancestral group. Molecular evidence has confirmed that tetrapods evolved a single time from finned ancestors (Hedges et al., 1990). However, the limb itself evolved independently twice: from the pectoral fin and from the pelvic fin. This is reflected in the fact that despite their general similarity there are significant differences between the fore and hind patterns such as the elbow joint flexing backwards while the knee joint flexes forwards. The antecedents for these differences can be seen in fish prior to the evolution of the limb (Rackoff, 1980).

The striking similarity in the overall fore limb and hind limb bone patterns may be a direct consequence of the fact that although the fore limb and hind limb buds evolved independently, each evolved by reorienting the expression of the same Hox-1 and Hox-4 genes along orthogonal axes. The common limb structure then results from the effect these genes have on downstream target genes.

The origin and structure of the tetrapod limb has previously been interpreted in terms of a branching process of precartilaginous condensation (Shubin and Alberch, 1986). This suggests that the downstream targets of the Hox-1 and Hox-4 genes may be molecules that alter the cell adhesive properties that promote the bifurcation process (Yokouchi et al. 1991).

While branching may be a central process in limb development, there is an important difference between the bifurcation model as proposed by Shubin and Alberch and the Hox-based model of limb evolution discussed here. Shubin and Alberch view the tetrapod limb pattern as resulting from the bending of a single ancestral fin axis. The molecular evidence, in contrast, suggests that the limb owes its derivation to the reorientation of gene expression patterns such that two sets of genes which both controlled position along the primordial anterior/posterior axis, now each control position along one of two perpendicular axes. Mechanistically, it is not the bending of one axis but the splitting from one to two axes. Partially reoriented Hox expression patterns which are not fully orthogonal might have led to intermediate forms of fins.

Subsequently, when a proto-tetrapod fish began to alter its developing fin buds to create structures useful for more than just swimming, it became important to create differentiated digits to replace the fin rays. I have proposed that the five posterior Hox-4 genes, which divided the embryonic limb field, provided a means by which the development of each digit could be uniquely modified. The use of these genes, however, would only allow for the specification of five digits. In seeming contradiction with this, recent fossil evidence indicates that some of the earliest tetrapods had a greater number of digits. For example, the *Acanthostega* forelimb has eight digits (Coates and Clack, 1990). It is, of course, impossible to know the expression pattern

of genes from fossils. However, examination of some of these early polydactylous tetrapod limbs suggests that they were also limited to five independently specified digits.

When the developing proto-limb bud was first subdivided into five zones by Hox-4 expression domains, each region could have included the anlage for multiple digits. The use of the Hox-4 genes to specify digit identity does not preclude growing more than five digits, rather it prohibits developing more than five morphologically distinct types of digits. Examination of the forelimb of *Acanthostega* reveals that it indeed possessed only five morphological types of digits: two identical digits of type I, one digit of type II, two identical digit type IIIs, two identical digit type IVs, and one digit type V; eight digits in all, but a total of only five types (Fig. 9). This is a rare example of an evolutionary incipient structure seen in a species in transition from multiple fin rays to a uniquely specified hand. Even though the limb was polydactylous the pattern was firmly pentate.

As discussed above, this is also the case in many modern polydactylous mutants (eg. extra 'thumbs' on humans, extra digit 2 in *Diplopodia* mutant chickens). However, in modern tetrapods there is no selective advantage for maintaining these supernumerary digits. In contrast, for *Acanthostega* the multiple-digit pattern provided a broad hand which would have been useful in an aquatic environment. As tetrapods moved out of the water and on to land they

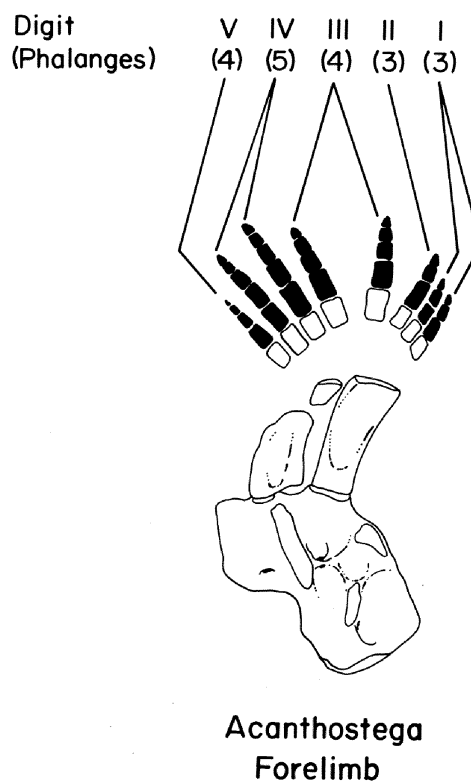


Fig. 9. Reconstruction of *Acanthostega* forelimb (after Coates and Clack, 1990, and M. Coates personal communication). The digits are numbered to indicate distinct 'identity'-types as defined in the paper, not intended as necessarily homologous to modern tetrapod digits 1-5.

would no longer need as many fanned out digits. Once that occurred, I would suggest the loss of the redundantly specified supernumerary digits and the resulting five-digit (or fewer) structure was inevitable. Had we descended from a species with a reduced number of digits, we might be counting in base eight, but it could never have been base twelve.

Hox genes clearly contribute to the regulation of limb pattern. Their exact place in the evolution of the limb is speculative, but their importance in modern limb development indicates that they played a central evolutionary role. Many of the hypotheses discussed here can be directly tested by comparative studies of Hox gene expression in different existing vertebrate lineages. Increasing knowledge about the functions of the Hox genes in development will give greater insight into their exact role in the evolution of the body plan.

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