Branching, segmentation and the metapterygial axis: pattern versus process in the vertebrate limb

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Summary
Explanations of the patterns of vertebrate fin and limb evolution are improving as specific hypotheses based on molecular and developmental data are proposed and tested. Comparative analyses of gene expression patterns and functions in developing limbs, and morphological patterns in embryonic, adult and fossil limbs point to digit specification as a key developmental innovation associated with the origin of tetrapods. Digit development during the fin-to-limb transition involved sustained proximodistal outgrowth and a new phase of Hox gene expression in the distal fin bud. These patterning changes in the distal limb have been explained by the linked concepts of the metapterygial axis and the digital arch. These have been proposed to account for the generation of limb pattern by sequential branching and segmentation of precartilagenous elements along the proximodistal axis of the limb. While these ideas have been very fruitful, they have become increasingly difficult to reconcile with experimental and comparative studies of fin and limb development. Here we argue that limb development does not involve a branching mechanism, and reassess the concept of a metapterygial axis in limb development and evolution. BioEssays 24:460–465, 2002. © 2002 Wiley Periodicals, Inc.

Introduction
There has been considerable progress in understanding the molecular mechanisms involved in vertebrate limb development (reviewed in Ref. 1). These advances, together with new data from the fossil record, have renewed interest in the evolution of limbs. (2–7) In relating the phylogenetic evolution of limbs to their development, an important issue is the so-called metapterygial axis (Fig. 1). This axis refers to the main stem of branched patterns formed by fin metapterygia and associated radials. These patterns have long been interpreted as products of developmental branching during skeletal outgrowth, and there have been numerous attempts to trace, on a bone-by-bone basis, the fate of this ancestral fin axis in tetrapod limbs (reviewed in Ref. 8). Importantly, Shubin and Alberch’s influential model of limb development and evolution (8) represented a radical departure from such previous theories of 1:1 correspondence between the skeletal parts of fins, limbs and the metapterygial axis (hereafter called simply “the axis”). Instead, the axis (like limb patterns) was redefined as the product of sustained developmental branching and segmentation. Molecular, cellular and experimental embryological data, however, suggest that, while segmentation plays a key role in defining joints, limb development does not involve a branching mechanism. Although previous work has suggested that the limb skeleton develops by branching, we believe this to be a conflation of anatomical pattern and developmental process. Here we reassess the concepts of a metapterygial axis, digital arch, branching and segmentation in light of recent advances in our understanding of vertebrate limb development.

Branching, segmentation and the metapterygial axis
Shubin and Alberch, like Schmalhausen before them, proposed that, during tetrapod limb development, the metacarpals/metatarsals and the distal row of carpals/tarsals arise from a “digital arch.” (9) According to the model, the distal row of carpals and tarsals “bifurcate” to form the metacarpals and metatarsals, respectively. This influential and pervasive concept was first proposed in an attempt to define homologous regions of tetrapod limbs and the paired fins of sarcopterygians and actinopterygians. The essence of this hypothesis was that evolution of the digital arch itself resulted from a “bending” of the metapterygial axis of a sarcopterygian fin (Fig. 1), thereby providing a novel solution to what has been
described as "one of the major morphological questions of the last century". In lungfish, this axis develops from proximal to distal and so what was primitively distal (in sarcopterygian fins) is now, according to the model, anterior in anuran and amniote limbs. Thus, there has been a turning towards the anterior of the proximodistal axis during limb evolution, and anterior digits therefore correspond to the most distal region of limbs.

The digital arch model resulted from a study of patterns of chondrogenesis during amphibian limb development. The limb skeleton undergoes chondrification in a proximal-to-distal sequence, with the number of skeletal elements increasing with more distal segments of the limb. In our own forelimbs, for example, the number of skeletal elements increases proximodistally from one (humerus) to two (ulna and radius) to four (2 rows of four carpals) to five (metacarpals and phalanges). In their seminal study, Shubin and Alberch proposed that this pattern was generated by a mechanism that involved progressive branching and segmentation of cartilage condensations(8) (Fig. 2). The concept of a metapterygial axis in the tetrapod limb was interpreted to imply a posterior dominance during vertebrate limb development.(9) Thus, the apparent "branching" of the cartilaginous elements of the radius and ulna from the more proximal humerus is proposed to be asymmetric: the anterior (radial/tibial) side does not, in general, branch; by contrast the posterior (ulnar/fibular) side may branch to produce the ulnare/fibulare and intermedium. The majority of carpal and tarsal elements are hypothesized to arise from prechondrogenic condensations that can be traced to posterior regions of the limb (Fig. 2). Most of the distal branching events are believed to occur within a broad arch of branching mesenchyme, the digital arch, and it is this arch that, in amniotes and anurans, is thought to arise from condensations that originally branch from the posterior proximal carpals/tarsals. The digital arch model proposes that metacarpals/metatarsals arise by bifurcation from the carpals/tarsals, whereas the phalanges arise by segmentation from more proximal digital elements.(8–11)

Distinguishing pattern from process
These concepts of limb development are linked to a morphogenetic mechanism for cartilage formation that stemmed from a mechanical model in which cartilage condensation is seen as a mechanically inductive event in cartilage formation.(11) Moreover, the model suggests that limb development is sequential; proximal development is initiated by a single focal condensation and subsequent "distal development must proceed from this focus by branching and segmentation".(11)
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‘Embryonic connectivity’ between precartilage condensations is therefore interpreted as a snapshot of continuous, ontogenetic, sequences of branching and segmentation events\(^{(8)}\) (Fig. 2). In effect, the branching and segmentation model specifies an evolutionary developmental constraint: most elements of the limb must arise from elements that have already appeared.

These ideas have been very influential: they have been used as an elegant heuristic device for interpreting developmental analyses of zebrafish fins,\(^{(3,4)}\) and the relation of primitive tetrapod limb patterns to sarcopterygian fins.\(^{(12)}\) They have also contributed to a heated debate over the phylogenetic position of birds.\(^{(13)}\) However, they are very hard to reconcile with a number of recent and classical observations of limb development. Experimental evidence shows that specification of skeletal elements in the limb is not based on a mechanical process and that proximal elements are not necessary for distal element development. Specifically, the physical–mechanical model of branching and segmentation proposes that the condensation process—an early feature of cartilage development—is itself the basis for patterning.\(^{(6,11)}\) However, when Wolpert and Hombrich\(^{(14)}\) constructed double anterior limb buds by pinning together the anterior halves of two limbs prior to the initiation of cartilage condensation, a limb developed with two humeri, demonstrating that cartilage patterns are specified before condensation. Indeed, a wide variety of different types of evidence suggests that branching is not a mechanism of limb development, and that proximal limb elements are not required for digit development. Grafts of distal tips of limb buds to a neutral site, long before cartilage develops, show normal development of distal structures, which are therefore clearly not dependent on the presence of proximal cartilaginous condensations\(^{(15)}\) (Fig. 3). The presence of digits in patients with unilateral ulnar agenesis\(^{(16)}\) also argues against the hypothesis that digits branch from the distal ulna. Moreover, increasing doses of \(X\)-irradiation administered to limb-bud mesenchyme cells prior to the onset of chondrogenesis result in progressive loss of proximal structures, yet the digits remain relatively unaffected.\(^{(17)}\) Experimental manipulations of the limb bud (e.g., disaggregation and reaggregation of mesenchyme) can result in digits with a “branched” pattern, indicating that, in the absence of all positional information (as in the reaggregate system), cartilage condensations can be forced to split or bifurcate,\(^{(18)}\) although there is no evidence that normal limb development involves such a mechanism. If branching is the mechanism of digit formation, then an impermeable barrier placed distal to the ulna should either block digit development or result in digit formation proximal to the barrier. We tested this prediction by implanting a foil barrier into a chick wing bud at stage 20, when only the humerus and proximal ulna and radius have been specified.\(^{(19)}\) As seen in Fig. 4 (and as expected from previous experiments), digits developed normally, and they lie distal to the foil barrier. It is noteworthy that the ulna and radius also developed normally. Thus, there is abundant evidence indicating that development of distal structures does not require proximal branching and segmentation. In fact, all limb structures, not just those of the skeleton, appear by sequential specification and differentiation in proximal-to-distal sequence.\(^{(15,19–22)}\)

While there is no evidence for branching in the limb, it is equally clear that segmentation does play a key role in joint specification. Skeletal elements in the limb begin as continuous condensations of prechondrogenic mesenchyme that later undergo segmentation at the joints. Joint development begins with the repression of chondrogenesis at specific sites along the prechondrogenic condensation. The boundary between skeletal elements is sharpened by cell death.\(^{(23)}\) In long bones, joint formation is initiated by localized production of Wnt14, a secreted signaling molecule that is expressed at the sites of joint formation prior to segmentation.\(^{(24)}\) Wnt14 blocks chondrocyte maturation and may initiate the process of joint cavity development. Wnt14 induces Growth and Differentiation Factor 5 (GDF5),\(^{(24)}\) and loss-of-function mutations in the GDF5 gene result in loss of joints and, consequently, altered digit length.\(^{(25)}\) Thus, in contrast to branching, segmentation of prechondrogenic condensations is fundamental to normal limb development.

**Digit development and the metapterygial axis**

Shubin and colleagues\(^{(5)}\) have rejected that idea that digits arise by a simplistic anterior bending of the axis, and fate maps of chick wing bud mesenchyme show only a modest anterior expansion distally, and even less expansion has been
observed in leg buds. Further problems arise when one considers the mechanism by which digits are specified. Numerous experiments have shown that a signal (now known to be mediated by Sonic hedgehog) from the polarizing region or ZPA can specify pattern along the anteroposterior axis, and that the patterning mechanism for this axis is separable from patterning along the (conventional) proximodistal axis. The ZPA thus ultimately patterns both “proximal” long bones (e.g., ulna and radius) and “distal” digits along this anteroposterior axis (Figs. 1 and 2). This is difficult to reconcile with the digital arch as an extended metapterygial axis, which would in turn imply that digits are in fact patterned along the proximodistal axis relative to the body wall, with digits 1 and 2 being the most distal. Again this idea is at variance with the observation that an attenuated ectopic signal from the polarizing region specifies an additional digit 2 in chicks (which have a normal anteroposterior digit pattern of 2-3-4). According to the developmental constraints of the digital arch model, such duplications of digit 2 without digits 3 and 4 should not be possible.

Taken together, these experimental data make the proposed mechanism for cartilage formation and bending of the primitive fin axes (i.e., the digital arch) in limb evolution implausible. And, just as the developmental significance of cellular connections between cartilage condensations is open to question, the significance of the digital arch in a comparative context also deserves similar re-evaluation.

The metapterygial axis in evolution

It is important to distinguish between the metapterygium as a skeletal unit and the metapterygial axis as an inferred feature of development. The skeletal metapterygium is a complex posterior radial present in paired fins. Larger than preceding anterior radials, it usually articulates with two or more secondary radials along the anterior and distal surfaces (Fig. 1). Metapterygia plus secondary radials therefore form asymmetrically patterned structures dominating the posterior region of adult fin skeletons. Primitively, pectoral fins of all gnathostomes include a metapterygium but, in living groups, this structure is most notably absent from teleosts.

If used merely as a description of skeletal pattern elaboration, Shubin and Alberch’s redefinition of the metapterygial axis is plausible when applied to lungfish and other sarcopterygian fins. However, if their definition is extended to characterize axes in fins beyond this clade, then inconsistencies arise. For example, the posterior radial of the pectoral fin in Polypterus (a non-teleost actinopterygian) has been identified as a metapterygium. However, polypterid pectoral fin development shows no metapterygial axis-like features. Instead, a flat precartilage cellular plate is first perforated and then split distally to form radials in a posterior-to-anterior sequence (Fig. 5). A similar perforated cellular plate has been described in zebrafish pectoral fin development. Other examples are known in actinopterygians such as Amia (Bowfin), Anguilla (eel), Clupea (Herring), Gobius (goby), Betta (Betta), and the chondrichthyans Pristiurus and Scyliorhinus (R. Freitas and M.J.C., unpublished data). Given that some of these fins are described as having a metapterygium whereas others are not, the utility of the developmental criterion for metapterygial axis identification appears limited (phylogenetically) to sarcopterygians.

A further issue surrounding the concept of an evolutionarily conserved fin axis is the presumption that it represents a primary morphological datum which can be used to judge homologies (synapomorphies) of subsidiary skeletal structures. However, this premise requires that the formation of limb skeletons during earliest embryogenesis takes place by
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In conclusion, the preskeletal patterns identified by Shubin and Alberch (8–11) (and others) are obviously important elements in our understanding of evolutionary dynamics. The details of the patterns of skeletal development during ontogeny can be useful phylogenetic data. (36–41) Examples include variations in the spatial positions and temporal orders of appearance of the limb skeleton of urodeles relative to amniotes. (39) In this way, the novelties of hand- and foot-plate patterns indicate the evolution of developmental specializations (apomorphies) unique to tetrapods and subgroups of tetrapods. However, the link between such patterns and underlying developmental processes is not as simple as formerly thought. There are mismatches. Adult metapterygia do not necessarily develop from segmenting and branching patterns of pre-chondrogenic cell condensations; the branched appearance of these prechondrogenic patterns is not in itself reflective of a branching morphogenetic process (as was formerly interpreted). Therefore, in this example (vertebrate paired appendages), we can no longer use these patterns as simple proxies for the phylogenetic distribution of shared (and thus homologous) developmental mechanisms.

Lastly, the overwhelming evidence that limbs do not develop by branching renders moot the question of which mesenchymal condensation “gives rise” to particular skeletal elements in the autopod. (42) We argue that process-based explanations of such changes lie within the details of developmental genetic regulation of patterning and morphogenesis, and not in physical–mechanical models (11) and their implied constraints on pattern diversity. Indeed, as our knowledge of the developmental genetic control of morphology continues to improve, it is likely that other mechanically based explanations of such events will also be supplanted by an improved understanding of the molecular mechanisms of pattern formation.

Acknowledgments

This paper is dedicated to the memory of Pere Alberch, who, given the opportunity, would surely have contested the significance of its contents. We thank Neil Vargesson for comments on the manuscript.

References