

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".⁽⁸⁾ 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

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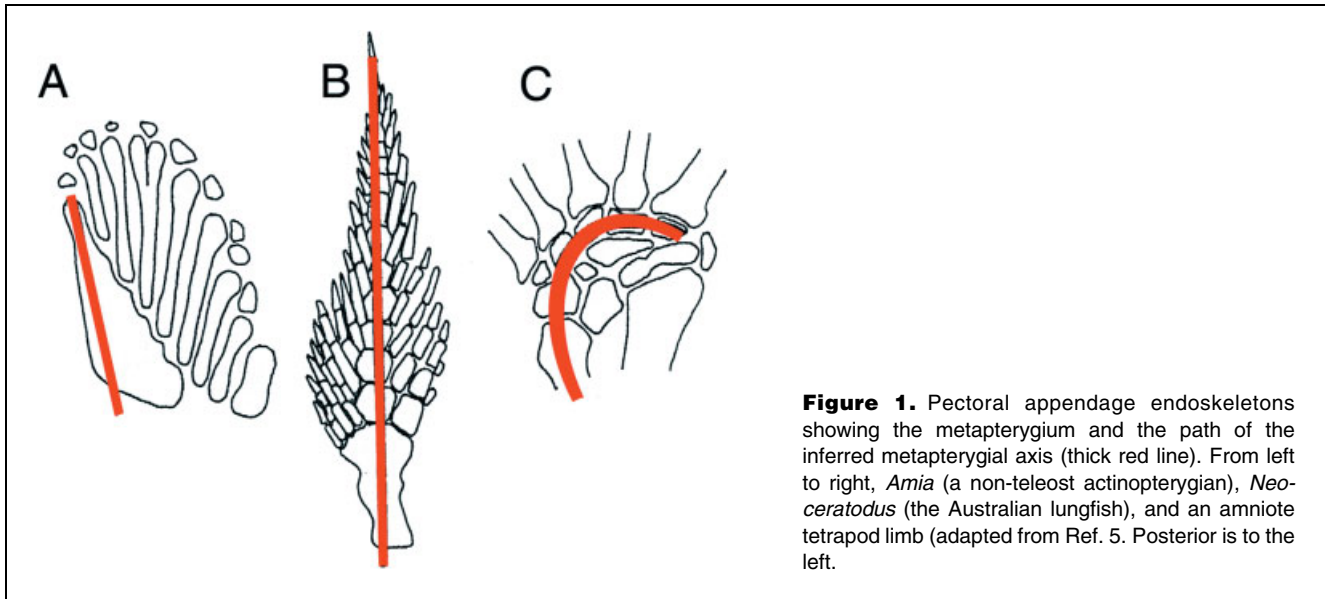
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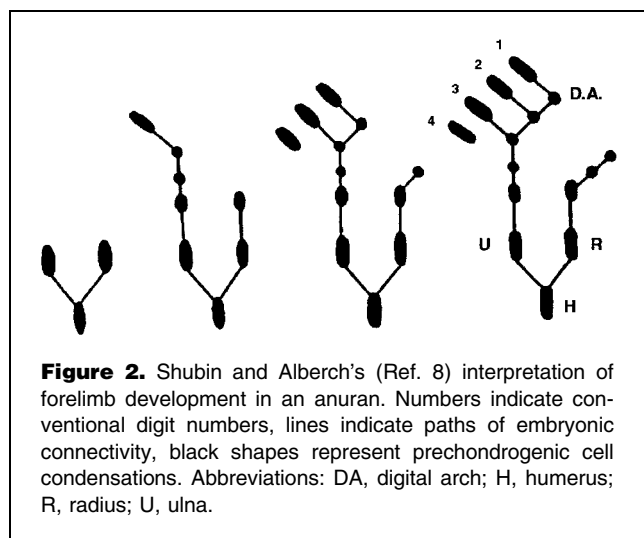
described as “one of the major morphological questions of the last century”. In lungfish, this axis develops from proximal to distal and so what was primitively distal (in sarcopterygian fins) is now, according to the model, anterior in anuran and amniote limbs. Thus, there has been a turning towards 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 precartilaginous connections 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⁾



'Embryonic connectivity' between precartilaginous 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.^(8,11) However, when Wolpert and Hornbruch⁽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 bilateral 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

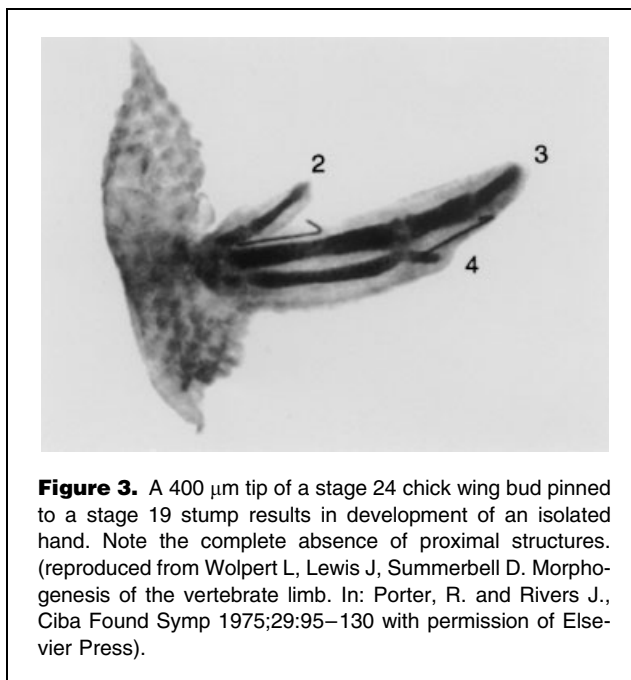


Figure 3. A 400 μm tip of a stage 24 chick wing bud pinned to a stage 19 stump results in development of an isolated hand. Note the complete absence of proximal structures. (reproduced from Wolpert L, Lewis J, Summerbell D. Morphogenesis of the vertebrate limb. In: Porter, R. and Rivers J., Ciba Found Symp 1975;29:95–130 with permission of Elsevier Press).

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

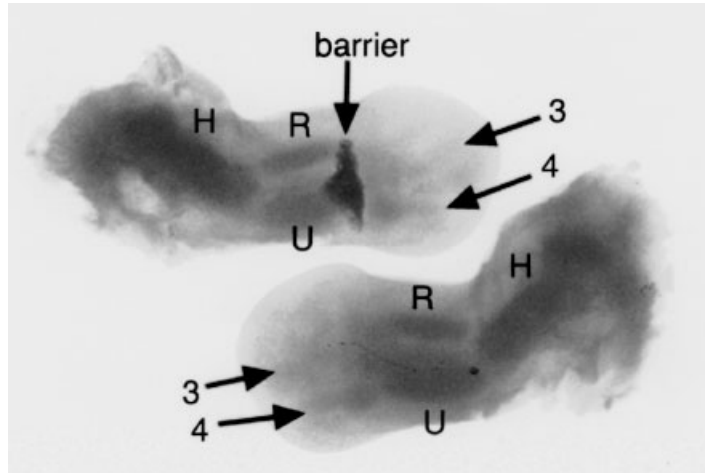


Figure 4. Top shows skeletal preparation of a chick wing 2 days after insertion of a foil barrier into the undifferentiated wing bud. Although the barrier was positioned at the level of the prospective radius and ulna, distal structures, including digits (arrows) developed normally. Compare with normal contralateral wing (bottom).

observed in leg buds.⁽²⁶⁾ In the wing bud, there is no evidence to support the idea that the digits arise by bifurcation from a digital arch.⁽²⁶⁾ 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,^(27,28) 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**).⁽²⁹⁾ 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⁽⁸⁾ is plausible when applied to lungfish and other sarcopterygian fins. However, if their definition is extended to characterize axes in fins beyond this clade,⁽³⁾ then inconsistencies arise. For example, the posterior radial of the pectoral fin in *Polypterus* (a non-teleost actinopterygian) has been identified as a metapterygium.⁽¹⁰⁾ However, polypterid pectoral fin development^(30,31) 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.^(4,32) Other examples are known in actinopterygians such as *Amia* (Bowfin), *Anguilla* (Eel), *Clupea* (Herring), *Gobius* (Goby),⁽³³⁾ *Betta* (Betta),⁽³⁴⁾ and the chondrichthyans *Pristiurus*⁽³⁵⁾ 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⁽⁸⁾ 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

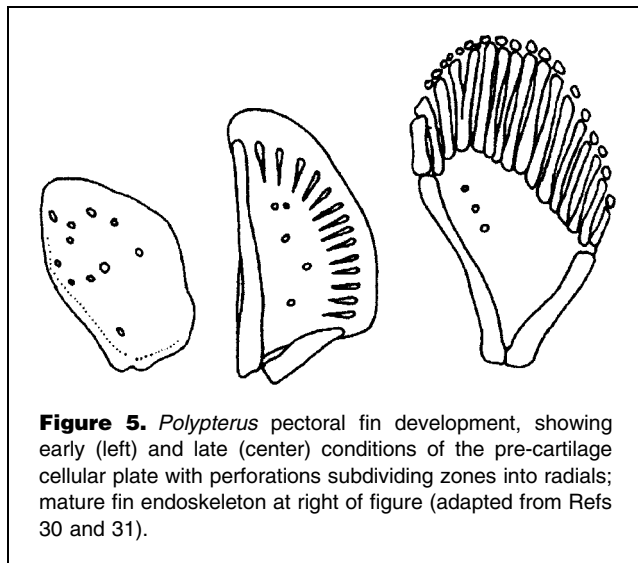


Figure 5. *Polypterus* pectoral fin development, showing early (left) and late (center) conditions of the pre-cartilage cellular plate with perforations subdividing zones into radials; mature fin endoskeleton at right of figure (adapted from Refs 30 and 31).

processes that are unique to chondrogenic mesenchyme and are not an element in other arenas of connective tissue development. This now seems unlikely (see above). Instead, the fate of any mesenchymal cell is determined initially by positional information, and positional values will differ only slightly in cells whose progeny result in significantly different end structures. The limb musculature also emerges by apparent segmentation (subdivision of muscle blocks), but it is quite clear from exhaustive experimental evidence that such segregation of muscle masses is under the control of positional cues resident in limb-bud mesenchyme.^(36–38) There is no basis to presume a unique or special mechanism for acquisition of positional identity by chondrogenic cells in the limb bud. If fin and limb evolution occurs primarily by changes in such positional fields, then these changes affect all fibroblasts and their derivatives simultaneously. These changes in turn result in coordinated transformations of both soft and hard tissues of adults. Alternatively, soft tissue structures would have to be modified separately in order to accommodate changes in skeletal morphologies. Experimental embryology and molecular genetics of the limb rejects this view.

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.^(39–41) 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.

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References

1. Tickle C, Munsterberg A. Vertebrate limb development—the early stages in chick and mouse. *Curr Opin Genet Dev* 2001;11:476–481.
2. Tabin CJ. Why we have (only) five fingers per hand: hox genes and the evolution of paired limbs. *Development* 1992;116:289–296.
3. Coates MI. Fish fins or tetrapod limbs—a simple twist of fate? *Curr Biol* 1995;5:844–848.
4. Sordino P, van der Hoeven F, Duboule D. Hox gene expression in teleost fins and the origin of vertebrate digits. *Nature* 1995;375:678–681.
5. Shubin N, Tabin C, Carroll S. Fossils, genes and the evolution of animal limbs. *Nature* 1997;388:639–648.
6. Coates MI, Cohn MJ. Fins, limbs, and tails: outgrowths and axial patterning in vertebrate evolution. *Bioessays* 1998;20:371–381.
7. Ruvinsky I, Gibson-Brown JJ. Genetic and developmental bases of serial homology in vertebrate limb evolution. *Development* 2000;127:5233–5244.
8. Shubin NH, Alberch P. A morphogenetic approach to the origin and basic organization of the tetrapod limb. *Evol Biol* 1986;1:319–387.
9. Shubin NH. The implications of “The Bauplan” for Development and Evolution of the Tetrapod Limb. In: Hinchliffe JR, Hurlle JM, Summerbell D, editors. *Developmental patterning of the vertebrate limb*. New York: Plenum; 1990. pp. 411–422.
10. Shubin NH. The evolution of paired fins and the origin of tetrapod limbs. Phylogenetic and transformational approaches. *Evol Biol* 1995;28:39–86.
11. Oster GF, Shubin NH, Murray JD, Alberch P. Morphogenetic rules and evolution. *Evolution* 1988;42:862–884.
12. Coates MI, Clack JA. Polydactyly in the earliest known tetrapod limbs. *Nature* 1990;347:66–69.
13. Burke AC, Feduccia A. Developmental patterns and the identification of homologies in the avian hand. *Science* 1997;278:666–668.

14. Wolpert L, Hornbruch A. Double anterior chick limb buds and models for cartilage rudiment specification. *Development* 1990;109:961–966.
15. Wolpert L, Lewis J, Summerbell D. Morphogenesis of the vertebrate limb. *Ciba Found Symp* 1975;29:95–130.
16. Bozner P, Blackburn W, Cooley NR Jr. Bilateral ulnar agenesis: case report and review of the literature. *Pediatr Pathol Lab Med* 1995;15:895–913.
17. Wolpert L, Tickle C, Sampford M. The effect of cell killing by x-irradiation on pattern formation in the chick limb. *J Embryol Exp Morphol* 1979;50:175–193.
18. MacCabe AB, Saunders JW, Pickett M. The control of the anteroposterior and dorsoventral axes in embryonic chick limbs constructed of dissociated and reaggregated limb-bud mesoderm. *Dev Biol* 1973;31:323–335.
19. Summerbell DA. A quantitative analysis of the effect of excision of the AER from the chick limb-bud. *J Embryol Exp Morphol* 1974;32:651–660.
20. Summerbell D, Lewis JH. Time, place and positional value in the chick limb-bud. *J Embryol Exp Morphol* 1975;33:621–643.
21. Summerbell D, Lewis JH, Wolpert L. Positional information in chick limb morphogenesis. *Nature* 1973;244:492–496.
22. Watanabe A, Ohsugi K, Ide H. Formation of distal structures from stumps of chick wing buds at stages 24–25 following the grafting of quail tissue from X-irradiated distal limb buds. *J Exp Zool* 1993;267:447–453.
23. Nalin AM, Greenlee TK Jr, Sandell LJ. Collagen gene expression during development of avian synovial joints: transient expression of types II and XI collagen genes in the joint capsule. *Dev Dyn* 1995;203:352–362.
24. Hartmann C, Tabin CJ. Wnt-14 plays a pivotal role in inducing synovial joint formation in the developing appendicular skeleton. *Cell* 2001;104:341–351.
25. Storm EE, Huynh TV, Copeland NG, Jenkins NA, Kingsley DM, Lee SJ. Limb alterations in brachypodism mice due to mutations in a new member of the TGF beta-superfamily. *Nature* 1994;368:639–643.
26. Vargesson N, Clarke JD, Vincent K, Coles C, Wolpert L, Tickle C. Cell fate in the chick limb bud and relationship to gene expression. *Development* 1997;124:1909–1918.
27. Tickle C, Summerbell D, Wolpert L. Positional signalling and specification of digits in chick limb morphogenesis. *Nature* 1975;254:199–202.
28. Riddle RD, Johnson RL, Laufer E, Tabin C. Sonic hedgehog mediates the polarizing activity of the ZPA. *Cell* 1993;75:1401–1416.
29. Yang Y, Drossopoulou G, Chuang PT, Duprez D, Marti E, Bumcrot D, Vargesson N, Clarke J, Niswander L, McMahon A, Tickle C. Relationship between dose, distance and time in Sonic Hedgehog-mediated regulation of anteroposterior polarity in the chick limb. *Development* 1997;124:4393–4404.
30. Budgett JS. On the structure of the larval *Polypterus*. *Trans Zool Soc Lond* 1902;16:315–346.
31. Bartsch B, Gemballa S. On the anatomy and development of the vertebral column and pterygiophores in *Polypterus senegalus* Cuvier, 1829 (“Pisces,” Polypteriformes). *Zool Jb Anat* 1992;122:497–529.
32. Grandel H, Schulte-Merker S. The development of the paired fins in the zebrafish (*Danio rerio*). *Mech Dev* 1998;79:99–120.
33. Kälin JA. Die paarigen Extremitäten der Fische (Pterygia). In: Bolk L, Göppert E, Lubosch W, editors. *Handbuch der vergleichenden Anatomie der Wirbeltiere*. Vol 5. Berlin und Wien: Urban and Schwarzenberg; 1938. pp. 1–70.
34. Mabee PM, Trendler TA. Development of the Cranium and Paired Fins in *Betta splendens* (Teleostei: Percomorpha): Intraspecific Variation and Interspecific Comparisons. *J Morph* 1996;227:249–287.
35. Rauther M. *Echte Fische*, Teil 1. Bronn’s Klassen und Ordnungen des Tierreichs. 6. Band: Wirbeltiere, 1. Abteilung: Pisces. Leipzig: Akademische Verlagsgesellschaft mbH; 1940.
36. Schroeter S, Tosney KW. Spatial and temporal patterns of muscle cleavage in the chick thigh and their value as criteria for homology. *Am J Anat* 1991;191:325–350.
37. Kardon G. Muscle and tendon morphogenesis in the avian hind limb. *Development* 1998; Oct 125:4019–4032.
38. Yamamoto M, Gotoh Y, Tamura K, Tanaka M, Kawakami A, Ide H, Kuroiwa A. Coordinated expression of Hoxa-11 and Hoxa-13 during limb muscle patterning. *Development* 1998;125:1325–1335.
39. Blanco M, Alberch P. Caenogenesis, developmental variability and evolution of the carpus and tarsus of the marbled newt, *Triturus marmoratus*. *Evolution* 1992;46:667–687.
40. Caldwell M.W. Ontogeny and phylogeny of the mesopodial skeleton in mosasauroid reptiles. *Zoological Journal of the Linnean Society* 1996; 116:407–436.
41. Wake DB, Shubin N. Limb development in the Pacific giant salamanders, *Dicamptodon* (Amphibia, Caudata, Dicamptodontidae). *Can J Zool* 1998; 76:2058–2066.
42. Garner JP, Thomas ALR. Counting the fingers of birds and dinosaurs. *Science* 1998;280:355.