

Pentadactyl Ground State of the Manus of *Alligator mississippiensis* and Insights into the Evolution of Digital Reduction in Archosauria



HANS C.E. LARSSON^{1*}, AUDREY C. HEPPLESTON¹,
AND RUTH M. ELSEY²

¹Redpath Museum, McGill University, Montreal, Quebec, Canada

²Louisiana Department of Wildlife and Fisheries, Rockefeller Wildlife Refuge, Grand Chenier, Louisiana

ABSTRACT

The three-fingered state of the avian manus poses intriguing questions about the evolution of digit reduction. Although digit reduction in most tetrapods appears to be the product of straightforward digit loss, avian digit reduction may have occurred with a dissociation of digit position from digit identity. The three digits of birds have the ancestral identities of I, II, and III but develop from an early pentadactyl ground state from digital anlage 2, 3, and 4. A series of hypotheses have been proposed in an attempt to explain this disparity, including a recent suggestion that the anteriormost condensation visible in the avian limb bud is in fact a vestigial structure from a hexadactyl ancestral ground state. We investigated this proposal by presenting sets of compatible evolutionary developmental trajectories starting from a hexadactyl state to test hypotheses of digit reduction. The development of skeletogenic mesenchymal condensations in a crocodylian, the closest extant relative to birds, is used to identify any extra precartilaginous digital vestiges. A developmental series of *Alligator mississippiensis* forelimb buds reveal only five digital anlagen, supports a pentadactyl ground state for the archosaurian manus, and rejects portions of the evolutionary developmental trajectories proposed. This condition lends further support to the contribution of a homeotic transformation during digit reduction in avian ancestry to account for the dissociation between digital identity and developmental position. *J. Exp. Zool. (Mol. Dev. Evol.)* 314B:571–579, 2010. © 2010 Wiley-Liss, Inc.

J. Exp. Zool. (Mol. Dev. Evol.)
314B:571–579,
2010

How to cite this article: Larsson HCE, Heppleston AC, Elsey RM. 2010. Pentadactyl ground state of the manus of *Alligator mississippiensis* and insights into the evolution of digital reduction in Archosauria. *J. Exp. Zool. (Mol. Dev. Evol.)* 314B:571–579.

The avian wing has figured prominently in discussions of digit reduction and homology. The three-fingered avian manus is of great interest because of the conflict between the evolutionary digital reduction and digital identities. The morphologies of the fully developed avian fingers can be traced to digits I, II, and III and followed from a stepwise loss of digits V and then IV in their theropod ancestry (Padian and Chiappe, '98; Sereno, '99; Dececchi and Larsson, 2009) (Fig. 1). However, modern bird fingers develop from digital positions 2, 3, and 4 from a normally developing pentadactylous groundstate (Larsson and Wagner, 2002). This conflict of position and identity spurred over a century of discussion (reviewed in Larsson and Wagner, 2002; Wagner,

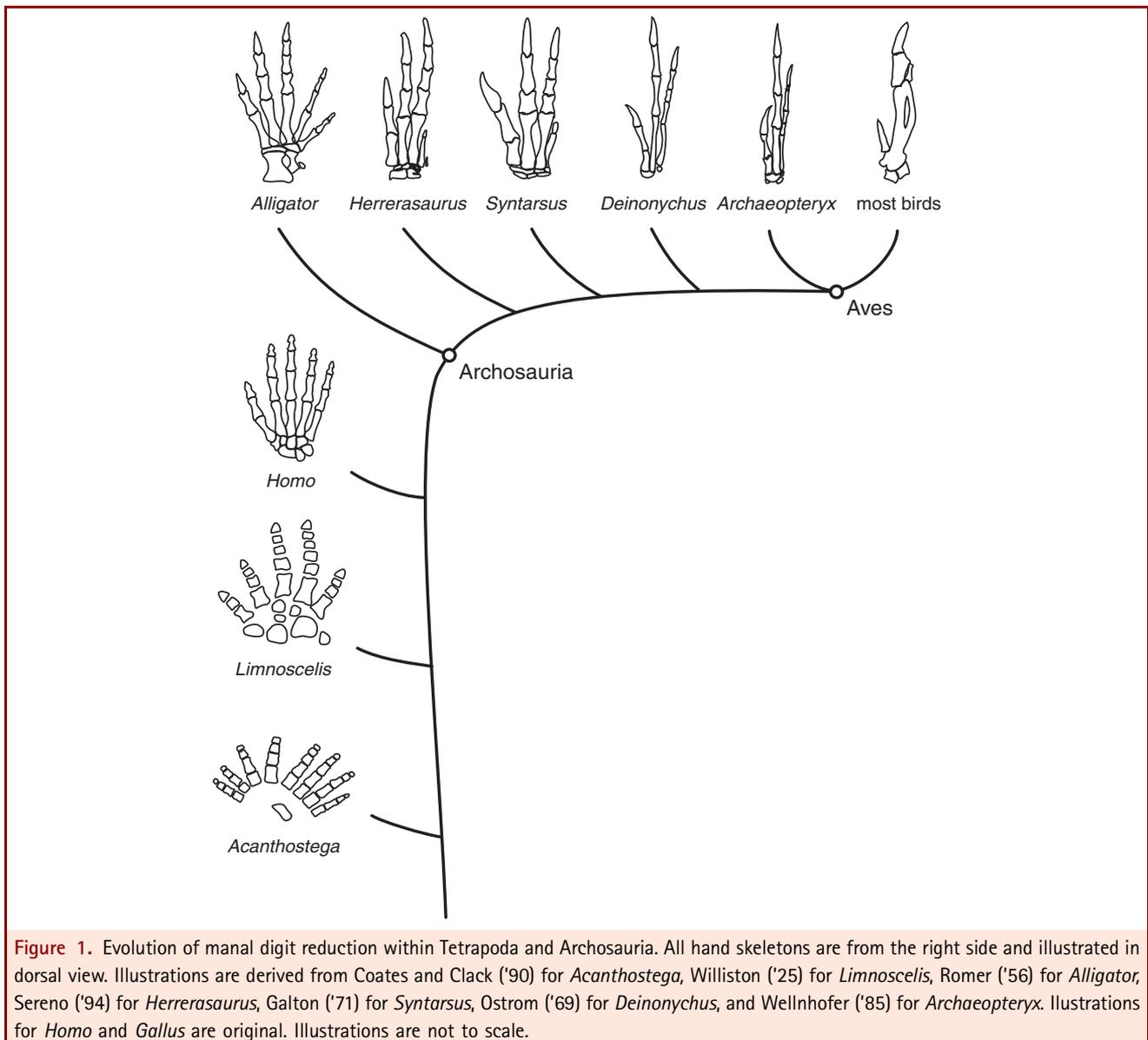
2005; Vargas and Fallon, 2005b) with numerous hypotheses suggested to explain this discrepancy.

Grant Sponsors: NSERC; CRC; FQRNT.

*Correspondence to: Hans C.E. Larsson, Redpath Museum, McGill University, 859 Sherbrooke St. W., Montreal, Que., Canada H3A 2K6. E-mail: hans.ce.larsson@mcgill.ca

Hans C.E. Larsson and Audrey C. Heppleston contributed equally to this manuscript.

Received 19 October 2009; Revised 8 May 2010; Accepted 11 May 2010
Published online 17 June 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jez.b.21362



Solutions to the discordance between avian digit position and identity span a range of competing hypotheses. These include alternative evolutionary origins of birds from an unknown non-dinosaurian ancestor (Burke and Feduccia, '97; Feduccia and Nowicki, 2002), convergent evolution of the anatomy of digits I–III in the avian digits II–IV (Kundrát et al., 2002; Galis et al., 2003), an avian-specific modification of the early digit development such that the first digit to chondrify was instead in digital position III (Chatterjee, '98), referred to as the Axis Shift Hypothesis (Larsson and Wagner, 2002), and a homeotic transformation with digit identities I–III developing from digital positions 2–4 (Wagner and Gauthier, '99). Homeotic transformations

are shifts in morphological identities from one position to another and were first identified in segmental identity changes in *Drosophila* (reviewed by Gehring, '94). Many of these homeotic transformations are associated with shifts in the expression boundaries of one or few homeotic genes such as Mads-box genes in flowers (Irish, 2003), Hox genes in arthropod body segments (Hughes and Kaufman, 2002) and vertebrate axial identities (Richardson et al., '98) and BMP signalling in chick wing fingers (Dahn and Fallon, 2000).

Available evidence can begin to address many of the solutions outlined above (Wagner, 2005). The non-dinosaurian ancestry of birds cannot be justified in the face of overwhelming evidence for

the sister-taxon relationship between birds and deinonychosaurian theropod dinosaurs (Padian and Chiappe, '98; Prum, 2002). Over 100 osteological characters are shared between early birds and theropod dinosaurs (Sereno, '99) and all serious phylogenetic analyses nest Aves within Theropoda (e.g. from Gauthier, '86 to Livezey and Zusi, 2007). Furthermore, analysis of evolutionary rates indicates that node Aves does not undergo any significant evolutionary change of forelimb skeletal characters and that all Aves-specific forelimb anatomy evolved at high rates before the origin of birds within maniraptoran theropods (Dececchi and Larsson, 2009). The evolution of birds from theropod dinosaurs is further strengthened from musculoligamentous (Baier et al., 2007), integumentary (Ji et al., '98; Prum, '99; Prum and Brush, 2002), and endocranial (Larsson et al., 2000; Larsson, 2001) morphologies. The hypothesized convergence of avian digital identity with that of earlier pentadactyl theropod dinosaurs is also refuted by the stepwise progression of the loss of digits five and then four in basal theropods (Padian and Chiappe, '98; Sereno, '99; Larsson and Wagner, 2003) and the evolution of the avian forelimb skeletal morphology within maniraptoran theropods (Dececchi and Larsson, 2009). There is no evidence for a dramatic evolutionary convergence of posterior digits attaining anterior characters with the available fossil record.

Within these phylogenetic constraints, the Axis Shift and Frame Shift hypotheses are still possible. The presence of a normal pentadactyl state with a primary axis through digit IV at early digital mesenchymal condensations has rejected an avian-specific modification to the early limb patterning (Larsson and Wagner, 2002). The avian manus develops a normal pentadactyl condensation state from which the anteriormost digital anlagen (digital position I) fails to chondrify, whereas digital positions two, three, and four continue to develop the anatomies of digits I, II, and III, respectively. Digital position five does chondrify but develops into a short cartilaginous rod-like metacarpal that fails to ossify. Similar pentadactyl patterns were described using vascular patterning (Kundrát et al., 2002), *Sox9* expression (Welten et al., 2005), and a brief chondrified pentadactyl state appears in the ostrich (*Struthio*) (Feduccia and Nowicki, 2002). A schematic of the developmental pathways starting from a normal pentadactyl state to the avian condition are illustrated in Figure 2A (adapted from Larsson and Wagner, 2002; Fig. 2). The top route maps the homeotic transformation required under the Frame Shift Hypothesis and the bottom route maps the Axis Shift Hypothesis. This scheme illustrates how the presence of a digital anlage at position 1 refutes the Axis Shift Hypothesis with a posterior digit reduction while providing support for a posteriorward homeotic transformation of digital identity with an anterior digit reduction.

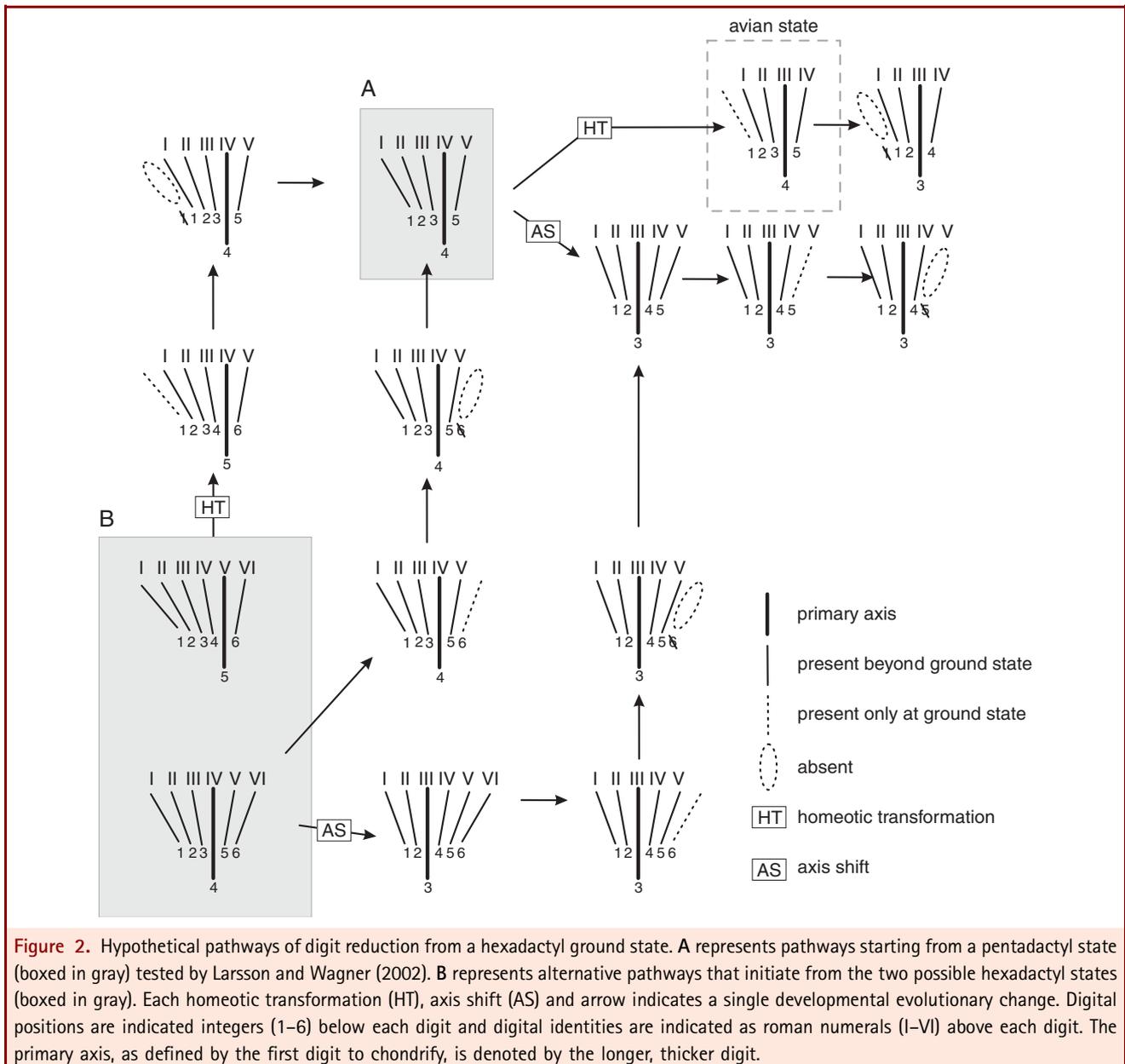
Although a homeotic transformation of digital identities remains plausible, opponents have argued that digital patterning is governed by developmental mechanisms too pleiotropic to be plausibly adaptive (Galis et al., 2003; Galis et al., 2005). These

arguments do not necessarily preclude the occurrence of a frameshift. Homeotic transformations are relatively common within the segmental evolution of arthropods and axial skeleton of vertebrates and demonstrate that these developmental mechanisms can evolve without deleterious pleiotropic effects. Boundaries of discrete regions of the vertebral column shift readily throughout vertebrate evolution from the extremely long-necked elasmosaurs with over 50 cervical vertebrae, to frogs with only one (Carroll, '88). However, in some cases, vertebral identity appears to be constrained within large clades. Mammals, with the exception of sirenians and sloths (Buchholtz and Stepien, 2009), have only seven cervical vertebrae and this condition does appear to be stabilized by pleiotropic constraints (Galis, '99).

Some gene expression patterns reinforce the potential dissociation between digital position and number. The autopodial region that includes digit I in mouse expresses *Hoxd13* but not *Hoxd11* and *12* (Chiang et al., 2001) and has been used as an early marker for digit I. A similar expression pattern was found in chick wings (Ros et al., 2003) but shifted posteriorly to the second digital anlagen (Vargas and Fallon, 2005a). The only other pentadactylous amniote examined for these gene expression patterns in the developing autopodium is *Alligator mississippiensis*. Only *Hoxd11* was visualized in this taxon but nevertheless exhibited an expression pattern similar to mouse with expression in all digital regions except digit I (Vargas et al., 2008), confirming that the anteriormost digit of *Alligator* is indeed homologous to digit identity I in mouse and chick, at the level of Hox expression. Young et al. ('66) presented compelling evidence for another evolutionary dissociation between digit position and identity in the lizard *Chalcides chalcides*. A similar developmental position and anatomical conflict is present in this taxon. Digit I develops from digital position 2 yet also does not express *Hoxd11*.

More Than Five Fingers?

An alternative set of hypotheses of avian digit reduction must also be considered. Digital reduction has historically been discussed from a pentadactylous state (Holder, '83; Larsson and Wagner, 2002). Independent digit reductions in the theropod and numerous mammal and squamate lineages have all been traced back through the fossil record to pentadactylous ancestral states. However, the manus and pes of the earliest tetrapods were polydactylous. *Acanthostega*, for example, famously exhibits eight fingers (Coates and Clack, '90) (Fig. 1). Soon after their origin, tetrapods seem to have stabilized their autopodia to a pentadactylous pattern (Laurin, '98). Numerous lineages have since reduced digit numbers and many have lost them altogether (Caldwell, 2003) but few have ever evolved more than five digits. The only exceptions are some aquatic clades that evolved extra digits (polydactyly) to increase limb paddle surface areas. Some whales (Richardson and Oelschläger, '47), have extra digits and



some extinct ichthyosaurs and plesiosaurs had extra digits and extra phalanges (hyperphalangy) (Carroll, '88; Motani, 2005).

Supernumerary digits have been hypothesized for a broad set of modern taxa. A prepollex has been identified in the early development of some anurans (Emery, 1890), a primate (Schmidt-Ehrenberg, '42), and a prehallux in some urodeles (Steiner, '21), respectively, and may be hypothesized as a vestige of the ancestral polydactylous condition. The latter author even claimed to have found six pedal digital anlage (reviewed by Galis et al., 2002). These supernumerary digit-like structures have never been observed to develop into chondrified or ossified skeletal elements

but may represent condensations of nonskeletal structures such as tendons and ligaments. However, an interesting theory was presented by (Holmgren, '33) to suggest the extra condensations are indeed digital anlage and represent vestiges of the ancestral tetrapod condition. At the time, the condition in *Acanthostega* was not known, but a hypothesis can be made that the earliest polydactylous condition may be present in some form, within the evolution of the ground state of tetrapod limbs. In theory, at least, the fact that most tetrapods exhibit a stable pentadactyl condition does not require a stable pentadactylous ground state during early embryogenesis.

A Hexadactyl Ground State?

Welten et al. (2005) explored the hypothesis of a hexadactyl ground state for avian ancestry, although acknowledge that it has the “disadvantage [of] that there is no direct evidence of its support” (Welten et al., 2005, p 20) and “no adult archosaur has six distinct digits” (Welten et al., 2005, p 26). They proposed a route of digital reduction that would not require shifts of digital identities from digital positions with positions 2–6 expressing digital identities I–V (Welten et al., 2005). However, we point out that this starting condition still requires an earlier identity shift so is not completely free of a hypothesis of digital homeotic transformation.

A hexadactyl state provides a suite of alternative pathways of digital reduction. Figure 2B illustrates each possibility. Each developmental route is derived by minimizing the number of developmental changes per step. Such a procedure is in line with characterizing evolutionary changes as minimal, independent units of change (Lewontin, '78; Wagner, 2001). For example, beginning at a hexadactyl ground state with a primary axis passing through digital position 5, a homeotic transformation, followed by a reduction in digital position 1 will produce a manus with six digital anlage and digital identities I–V on digital positions 2–6. The primary axis through digital position 5 will then be identified as digit IV. Further complete loss of digital anlage 1 will yield only five digitalanlagen, which would have to be renumbered 1–5 as no trace of an anterior anlage would be observed. Thus, the former digital position 5 would be renumbered as position 4 and identified as digit IV. This state would be equivalent to the normal pentadactyl state. Following this methodology, there are only two most plausible starting states for a hexadactyl ground state, each of which has two possible developmental evolutionary routes to arrive to a pentadactyl state. Although it is true that a hexadactyl ancestral state could have the primary axis passing through any of the six digits, we will only use the states that have the axis passing through digital positions four and five to not stray too far from known developmental pathways. All other hypothetical axis positions could be derived by additional backward steps to the pathways in Figure 2 following the minimal step approach outlined above. Each pathway will not be discussed at length in the text, but rather a general statement that multiple pathways are possible to arrive at similar end states. The utility of this approach is that if not all ancestral states of the pathway are present in modern embryos, the observation of intermediate states may help identify which pathways can be rejected and which can be supported. Additionally, this path network also provides a formal set of hypotheses to test using a descendent taxon's development (Larsson and Wagner, in review).

Although some early tetrapods and secondarily aquatic lineages do express more than five digits, the majority of tetrapods, extinct and extant, have a maximum of five. Archosauromorpha is no exception with all known taxa having

no more than five digits. The presence of a hexadactyl ground state for Archosauria can thus only be assessed within the embryology of extant taxa. Although outgroup comparisons will be important for state change polarization, the condition at Archosauria can only be assessed with modern archosaurian taxa. Although the ground state for Aves appears to be pentadactyl, the ground state for Crocodylia, the only other extant archosaur, is unknown. Crocodylia exhibit five ossified manal and pedal digits that develop directly from five chondrified digits (Müller and Alberch, '90). Modern crocodiles also express a similar posterior digit reduction in their manus. Digits I–III have the general amniote phalangeal formula of 2–3–4 and all bear unguis. Digits IV and V are reduced in crocodiles in that they bear no unguis and are reduced to only four and three phalanges, respectively (Kükenthal, 1893). The general amniote manual phalangeal formula is 2–3–4–5–3 with the majority amniotes and nonamniote reptiliomorphs sharing this condition. The anterior emphasis of digit function and retention with a trend to reducing digits IV and V in crocodiles and dinosaurs suggests postaxial manal digital reduction may be synapomorphic for at least Archosauria. We chose to examine the early mesenchymal skeletogenic patterning in the manus of *A. mississippiensis* because of this taxon's accessibility in North America and the direct manus to manus comparison. Although the crocodylian pes also develops only five digits, its ground state will not directly influence the manal ground state reconstruction for Archosauria.

RESULTS

The series of developing *A. mississippiensis* forelimbs used in this study ranged from Ferguson ('85) stages 14–17 to capture the early skeletal condensations of the manus. Each stage was sampled at least three times to avoid selection of abnormal embryos. Selections from this series are presented in Figure 3. At stage 14, the ulna and radius have begun to chondrify and skeletogenic mesenchymal condensations have begun to spread into the handplate but have not established any discernible structure (Fig. 3A). By stage 15, the handplate has expanded anteroposteriorly (Fig. 3B). Skeletogenic condensations have spread to fill the posterior half of the handplate. This condensing mass has established the putative carpal mass with no discernible internal patterning to reflect later carpal morphology. The condensations have also spread distally to form faint traces for the rays of digitalanlagen 4 and 5. The anteriodistal portion of the carpal mass has a scalloped margin and may indicate the earliest signs of digitalanlagen outgrowth. At early stage 16, four digits are clearly developed (Fig. 3C). Digits 3 and 4 have begun to chondrify, whereas digits 2 and 5 are still in a condensed state.

The maximal number of skeletogenic mesenchymal condensations within the forelimb autopodium is present by late stage 16. Digitalanlagen are arranged in much the same pattern as that of birds, described earlier. There are only five digitalanlagen

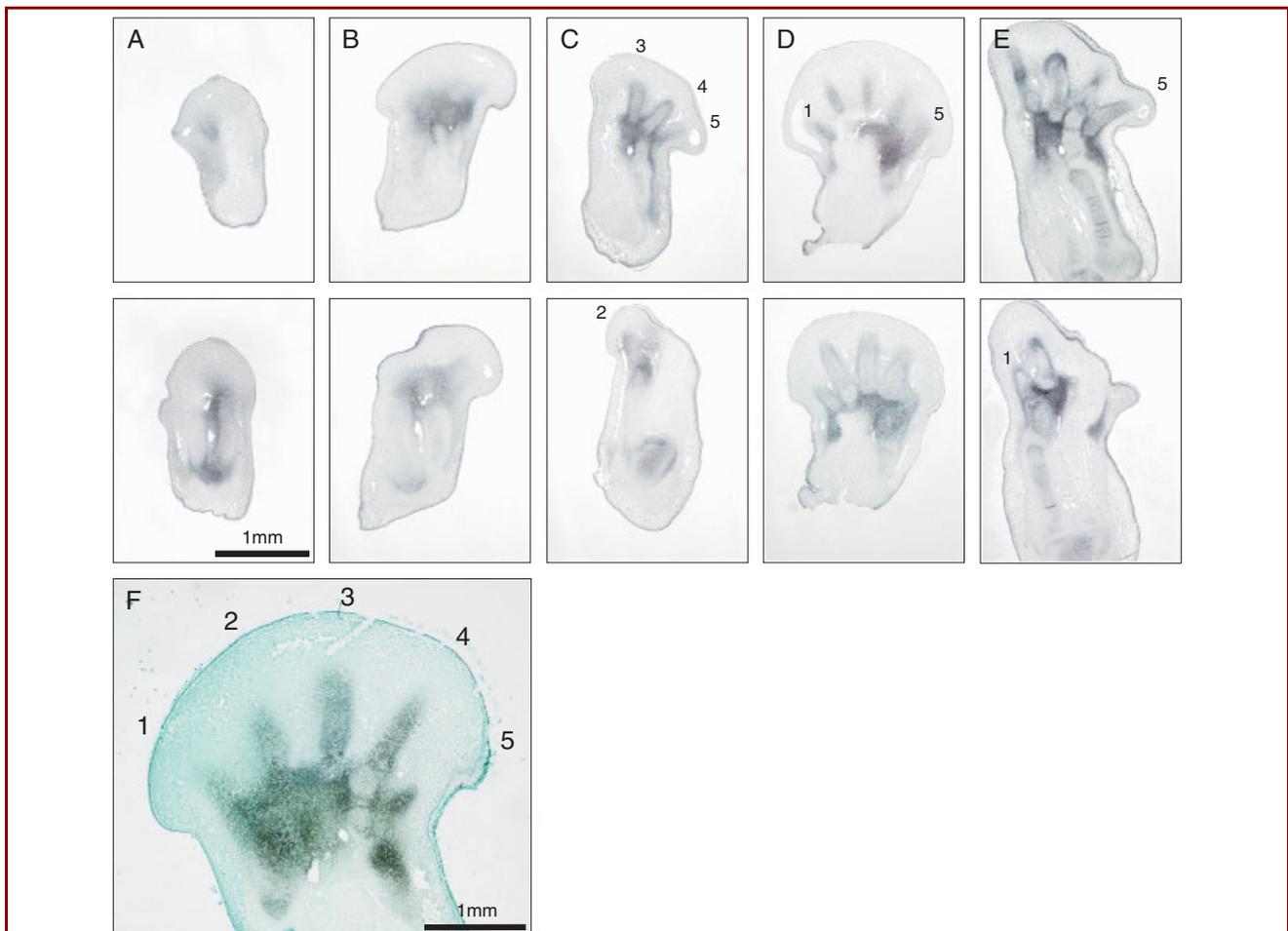


Figure 3. Skeletogenic mesenchymal condensations within the handplate of *Alligator mississippiensis* during the early development of digital anlage. All specimens are right limb buds in dorsal aspect. Sections through the handplate from Ferguson ('85) stages 14 (A), 15 (B), early 16 (C), late 16 (D), and 17 (E) are stained with PNA conjugated with horseradish peroxidase. Two sections from each specimen are shown for each stage to present all digits. An enlarged section from a specimen from stage late 16 illustrates the five digital anlagen (F). A–E are at equivalent scales. Numbers represent digital positions.

(Fig. 3D and F). The anterior and posteriormost digital anlagen are located near the extreme anterior and posterior edges of the handplate and no trace of peripheral digits were found, neither through vascular anatomy nor mesenchymal condensations. The digital anlagen at position 3 is the largest in length and width, followed by in decreasing order, digital positions 4, 2, and 1 and 5. All digital anlagen are unsegmented and joined to a relatively undifferentiated condensed carpal mass. Only the ulnare and distal carpal IV have begun to chondrify at this stage. At stage 17, all digits have begun to chondrify (Fig. 3E). No mesenchymal condensations are present at the anterior and posterior extremes of the handplate. Externally, the five manual digits are visible at this stage as discrete ridges on the handplate (Ferguson, '85).

DISCUSSION

Although a hexadactyl ground state may seem at first to be setting up a strawman hypothesis to test, the possibility of such a state before chondrogenesis did exist. The aim of this work was to establish a framework to test such hypotheses of digital developmental evolution. We emphasize the importance of attempting to distill developmental evolutionary hypotheses into discrete and putatively independent developmental mechanisms. Signature of a hexadactyl ground state may have been present in the skeletal developmental sequence according to our formulation of digital reduction from a hexadactyl ancestor. The earliest tetrapods were polydactylous and traces of these extra digits may have been present in the early limb patterning of modern tetrapods.

Furthermore, the observation of an anterior digital anlage to digit I in birds does lend to more complex evolutionary scenarios (Welten et al., 2005). Possible routes of the developmental evolution of digit reduction in Figure 2 represent a necessary step to formulating the hypothesis of a hexadactyl groundstate to test. Although the initial starting condition may be tested in a straightforward manner, testing the transitions along the entire hypothetical trajectories may not be so. Moreover, although modern developmental biology may shed light on ancient transitions, they need not always do so (Wagner and Larsson, 2003). If only portions of a transition can be gleaned from developmental data of modern taxa, then mapping out the entire hypothetical transition may prove useful to support at least a part of it.

In the present case, the pentadactyl ground state in the manus of birds and *Alligator* indicates such a state was most parsimoniously present at Archosauria. Although the pentadactyl state for *Alligator* could be parallel to that of birds, we maintain parsimony at this point to say this data reinforces a pentadactyl ground state for at least Archosauria. The pentadactyl ground state is also likely the plesiomorphic condition for all modern tetrapods, although we are currently surveying a broader set of taxa to confirm this. Although a hexadactyl state is plausible within tetrapods, we find no evidence for it at the level of Archosauria. The presence of a plesiomorphic pentadactyl ground state at Archosauria refutes the possibility of the peculiar avian manus developing directly from a hexadactyl state and all the hypotheses of digital reduction and identity that offers. Therefore, hypotheses of avian digit reduction within Archosauria must begin from a pentadactyl ground state outlined in Figure 2A and the distal portions of Figure 2B. The two possible hexadactyl ground states presented in Figure 2 both lead to a pentadactyl state that is indistinguishable from that present in early bird development and thus the data present for modern archosaurs does not suggest a polydactylous ground state for at least this clade. One other route from a hexadactyl state passes to a pentadactyl state with the primary axis passing through digital position 3. That route, however, toward the avian condition from this particular pentadactyl state has already been rejected by Larsson and Wagner (2002). If a hexadactyl ground state before Archosauria is to be tested, non-archosaurian taxa must be used.

Developmental evolution of digital reduction in theropods now shifts back to an argument centered on the discrepancy between the three avian digits with identities I–III developing from digital anlagen II–IV within the framework of a pentadactyl ground state. Although a homeotic transformation has been proposed (Wagner and Gauthier, '99) and supported by skeletogenic condensation patterning (Larsson and Wagner, 2002) and Hox expression patterns (Vargas et al., 2008) more experimental work needs to be done to further this hypothesis and fully reject an argument of evolutionary convergence (see above). One key line of inquiry will be to assess the potential

flexibility of shifting digital identities across digital positions. Preliminary experiments suggest digital identity is not fixed to digit position (Dahn and Fallon, 2000; Drossopoulou et al., 2000; Towers et al., 2008) and may offer insight into developmental mechanisms involved in a potential homeotic transformation in the avian lineage. Recent work targeting the *Shh* pathway and its associated mechanisms of chick digital development have further revealed a potentially simple mechanism for digital reduction with an associated posteriorward homeotic transformation of digital identities (Vargas and Wagner, 2009). Future work will have to determine whether morphological correlates exist for experimentally induced reductions and homeotic transformations to further implicate the roles of these mechanisms in the evolution of non-avian theropods.

MATERIALS AND METHODS

A. mississippiensis embryos were collected at the Rockefeller Wildlife Refuge (Cameron Parish, LA) and fixed in Dent's fixative and shipped to Montreal. The collection was comprised of a series of embryos from multiple clutches collected over the course of several days. Embryos were then stored in absolute methanol at 4°C. After dissection from their eggs, each embryo was staged following Ferguson ('85), refixed in 10% neutral buffered formalin, and stored in 70% ethanol. One forelimb bud from at least three individuals per stage ranging from stages 14 to 17 were embedded in paraffin and sectioned at 5 µm intervals. HRP bound peanut agglutinin lectin staining (Sigma L7759) was used following Dunlop and Hall ('95). HRP was exposed using a metal-enhanced DAB kit (Sigma D-0426). This lectin has high affinity to β1,3GalβNAc disaccharide residues (Zschäbitz, '98). These glycoproteins are expressed on the cell surfaces of condensing cells and have been used as markers for early skeletogenic mesenchymal condensations. Fast green was used as a counter stain and photographs were taken using a Nikon DS-Fi1 digital camera mounted on a Nikon SMZ1000 stereomicroscope.

ACKNOWLEDGMENTS

This manuscript benefited greatly from critical reviews by Alexander Vargas (twice) and three anonymous reviewers. Funding for this work was supplied by NSERC and CRC to H.C.E.L. and FQRNT to A.H.

LITERATURE CITED

- Baier DB, Gatesy SM, Jenkins FA. 2007. A critical ligamentous mechanism in the evolution of avian flight. *Nature* 445:307–310.
- Buchholtz EA, Stepien CC. 2009. Anatomical transformation in mammals: developmental origin of aberrant cervical anatomy in tree sloths. *Evol Dev* 11:69–79.
- Burke AC, Feduccia A. 1997. Developmental patterns and the identification of homologies in the avian hand. *Science* 278:666–668.

- Caldwell MW. 2003. "Without a leg to stand on": on the evolution and development of axial elongation and limblessness in tetrapods. *Canadian J Earth Sci* 40:573–588.
- Carroll RL. 1988. *Vertebrate paleontology*. New York: Freeman and Co.
- Chatterjee S. 1998. Counting the fingers of birds and dinosaurs. *Science* 280:355a.
- Chiang C, Litingtung Y, Harris MP, Simandl BK, Li Y, Beachy PA, Fallon JF. 2001. Manifestation of the limb prepattern: limb development in the absence of sonic hedgehog function. *Dev Biol* 236:421–435.
- Coates MI, Clack JA. 1990. Polydactyly in the earliest known tetrapod limbs. *Nature* 347:66–69.
- Dahn RD, Fallon JF. 2000. Interdigital regulation of digit identity and homeotic transformation by modulated BMP signaling. *Science* 289:438–441.
- Dececchi TA, Larsson HCE. 2009. Patristic evolutionary rates suggest a punctuated pattern in forelimb evolution before and after the origin of birds. *Paleobiology* 35:1–12.
- Drossopoulou G, Lewis KE, Sanz-Ezquerro JJ, Nikbakht N, McMahon AP, Hofmann C, Tickle C. 2000. A model for anteroposterior patterning of the vertebrate limb based on sequential long- and short-range Shh signalling and Bmp signalling. *Development* 127:1337–1348.
- Dunlop L-LT, Hall BK. 1995. Relationships between cellular condensation, preosteoblast formation and epithelial-mesenchymal interactions in initiation of osteogenesis. *Int J Dev Biol* 39:357–371.
- Emery C. 1890. Zur morphologie des hand-und fussskeletts. *Anat Anz* 5:283–294.
- Feduccia A, Nowicki J. 2002. The hand of birds revealed by early ostrich embryos. *Naturwissenschaften* 89:391–393.
- Ferguson MW. 1985. Reproductive biology and embryology of the crocodylians. In: Gans G, Billet F, Maderson PFA, editors. *Biology of the reptilia*. London: Academic Press. p 329–491.
- Galis F. 1999. Why do almost all mammals have seven cervical vertebrae? Developmental constraints, Hox genes, and cancer. *J Exp Zool (Mol Dev Evol)* 285B:19–26.
- Galis F, Alphen JMv, Metz JAJ. 2002. Digit reduction: via repatterning or developmental arrest? *Evol Dev* 4:249–257.
- Galis F, Kunderát M, Metz JAJ. 2005. Hox genes, digit identities and the theropod/bird transition. *J Exp Zool (Mol Dev Evol)* 304B:198–205.
- Galis F, Kunderát M, Sinervo B. 2003. An old controversy solved: bird embryos have five fingers. *TREE* 18:7–9.
- Galton PM. 1971. Manus movements of the coelurosaurian dinosaur *Syntarsus* and opposability of the theropod hallux. *Arnoldia* 15:1–8.
- Gauthier JA. 1986. Saurischian monophyly and the origin of birds. *Mem Calif Acad Sci* 8:1–55.
- Gehring WJ. 1994. A history of the homeobox. In: Duboule D, editor. *Guidebook to the homeobox genes*. Oxford: Oxford University Press. p 1–10.
- Holder N. 1983. Developmental constraints and the evolution of vertebrate digit patterns. *J Theor Biol* 104:451–471.
- Holmgren N. 1933. On the origin of the tetrapod limb. *Acta Zoologica* 14:187–248.
- Hughes CL, Kaufman TC. 2002. Hox genes and the evolution of the arthropod body plan. *Evol Dev* 4:459–499.
- Irish VF. 2003. The evolution of floral homeotic gene function. *Bio Essays* 25:637–646.
- Ji Q, Currie PJ, Norell MA, Ji S-A. 1998. Two feathered dinosaurs from northeastern China. *Nature* 393:753–761.
- Kükenthal W. 1893. Zur entwicklung des handskelettes des krokodils. *Morph Jahrb* 19:42–55.
- Kunderát M, Seichert V, Russell AP, Smetana Jr K. 2002. Pentadactyl pattern of the avian wing autopodium and pyramid reduction hypothesis. *J Exp Zool (Mol Dev Evol)* 294:152–159.
- Larsson HCE. 2001. The endocranial anatomy of *Carcharodontosaurus saharicus* (Theropoda: Allosauroidea) and its implications for theropod brain evolution. In: Tanke D, Carpenter K, editors. *Mesozoic vertebrate life*. Bloomington: Indiana University Press. p 19–33.
- Larsson HCE, Sereno PC, Wilson JA. 2000. Forebrain enlargement among nonavian theropod dinosaurs. *J Vert Paleontol* 20:615–618.
- Larsson HCE, Wagner GP. 2002. The pentadactyl ground state of the avian wing. *J Exp Zool (Mol Dev Evol)* 294:146–151.
- Larsson HCE, Wagner GP. 2003. Old morphologies misinterpreted. *TREE* 18:10.
- Larsson HCE, Wagner GP. Testing inferences in developmental evolution: The forensic evidence principle. *J Exp Zool (Mol Dev Evol)*; in review.
- Laurin M. 1998. A reevaluation of the origin of pentadactyly. *Evolution* 52:1476–1482.
- Lewontin RC. 1978. Adaptation. *Scientific American* 239:212–228.
- Livezey BC, Zusi RL. 2007. Higher-order phylogeny of modern birds (Theropoda, Aves: Neornithes) based on comparative anatomy. II. Analysis and discussion. *Zool J Linn Soc* 149:1–95.
- Motani R. 2005. Evolution of fish-shaped reptiles (Reptilia: Ichthopterygia) in their physical environments and constraints. *Annu Rev Earth Planet Sci* 33:395–420.
- Müller GB, Alberch P. 1990. Ontogeny of the limb skeleton in *Alligator mississippiensis*: developmental invariance and change in the evolution of archosaur limbs. *J Morphol* 203:151–164.
- Ostrom JH. 1969. Osteology of *Deinonychus antirrhopus*, an unusual theropod from the Lower Cretaceous of Montana. *Bull Peabody Mus Natl Hist, Yale U* 30:1–165.
- Padian K, Chiappe LM. 1998. The origin and early evolution of birds. *Biol Rev* 73:1–42.
- Prum RO. 1999. Development and evolutionary origin of feathers. *J Exp Zool (Mol Dev Evol)* 285:291–306.
- Prum RO. 2002. Why ornithologists should care about the theropod origin of birds. *Auk* 119:1–17.
- Prum RO, Brush AH. 2002. The evolutionary origin and diversification of feathers. *The Q Rev Biol* 77:261–295.
- Richardson MK, Allen SP, Wright GM, Raynaud A, Hanken J. 1998. Somite number in vertebrate evolution. *Development* 125:151–160.

- Richardson MK, Oelschläger HHA. 2002. Time, pattern, and heterochrony: a study of hyperphalangy in the dolphin embryo flipper. *Evol Dev* 4:435–444.
- Romer AS. 1956. *Osteology of the reptiles*. Chicago: University of Chicago Press.
- Ros MA, Dahn RD, Fernandez-Teran M, Rashka K, Caruccio NC, Hasso SM, Bitgood JJ, Lancman JJ, Fallon JF. 2003. The chick oligozeugodactyly (ozd) mutant lacks sonic hedgehog function in the limb. *Development* 130:527–537.
- Schmidt-Ehrenberg EC. 1942. Die Embryogenese des Extremitätenskelettes der Säugetiere. *Rev Suisse Zool* 49:33–132.
- Sereno 1994. The pectoral girdle and forelimb of the basal theropod *Herrerasaurus ischigualastensis*. *J Vert Paleontol* 13:425–450.
- Sereno PC. 1999. The evolution of dinosaurs. *Science* 284:2137–2147.
- Steiner H. 1921. Hand und Fuß der Amphibien, ein Beitrag zur Extremitätenfrage. *Anat Anz* 53:513–542.
- Towers M, Mahood R, Yin Y, Tickle C. 2008. Integration of growth and specification in chick wing digit-patterning. *Nature* 452:882–886.
- Vargas AO, Fallon JF. 2005a. Birds have dinosaur wings: the molecular evidence. *J Exp Zool (Mol Dev Evol)* 304B:86–90.
- Vargas AO, Fallon JF. 2005b. The digits of the wing of birds are 1, 2, and 3. A review. *J Exp Zool (Mol Dev Evol)* 304B:206–219.
- Vargas AO, Kohlsdorf T, Fallon JF, VandenBrooks J, Wagner GP. 2008. The evolution of *HoxD-11* expression in the bird wing: insights from *Alligator mississippiensis*. *PLoS ONE* 3:e3325.
- Vargas AO, Wagner GP. 2009. Frame-shifts of digit identity in bird evolution and cyclopamine-treated wings. *Evol Dev* 11:163–169.
- Wagner GP, editor. 2001. *The character concept in evolutionary biology*. San Diego, CA: Academic Press.
- Wagner GP. 2005. The developmental evolution of avian digit homology: an update. *Theory Biosci* 124:165–183.
- Wagner GP, Gauthier JA. 1999. 1,2,3 = 2,3,4: A solution to the problem of the homology of the digits in the avian hand. *PNAS* 96:5111–5116.
- Wagner GP, Larsson HCE. 2003. What is the promise of developmental evolution? III. The crucible of developmental evolution. *J Exp Zool (Mol Dev Evol)* 300B:1–4.
- Wellnhofer P. 1985. Remarks of the digit and pubis problems of *Archaeopteryx*. In: Hecht MK, Ostrom JH, Viohl G, Wellnhofer P, editors. *The beginnings of birds: Proceedings of the International Archaeopteryx Conference, Eichstätt*. Willibaldsburg, Eichstätt: Freunde des Jura-Museums Eichstätt. p 113–122.
- Welten MCM, Verbeek FJ, Meijer AH, Richardson MK. 2005. Gene expression and digit homology in the chicken embryo wing. *Evol Dev* 7:18–28.
- Williston SW. 1925. *The osteology of the reptiles*. Chicago: University of Chicago Press.
- Young RL, Caputo V, Giovannotti M, Kohlsdorf T, Vargas AO, May GE, Wagner GP. 2009. Evolution of digit identity in the three-toes Italian skink *Chalcides chalcides*: a new case of digit identity frame shift. *Evol Dev* 11:647–658.
- Zschäbitz A. 1998. Glycoconjugate expression and cartilage development of the cranial skeleton. *Acta Anat* 61:254–274.