Limusaurus and bird digit identity

Limusaurus is a remarkable herbivorous ceratosaur unique among theropods in having digits II, III and IV, with only a small metacarpal vestige of digit I[1]. This raises interesting questions regarding the controversial identity of avian wing digits. The early tetanuran ancestors of birds had tridactyl hands with digital morphologies corresponding to digits I, II & III of other dinosaurs[2]. In bird embryos, however, the pattern of cartilage formation indicates that their digits develop from positions that become digits II, III, & IV in other amniotes[3]. *Limusaurus* has been argued to provide evidence that the digits of tetanurans, currently considered to be I, II and III, may actually be digits II, III, & IV, thus explaining the embryological position of bird wing digits[1]. However, morphology and gene expression of the anterior bird wing digit specifically resemble digit I, not II, of other amniotes[4,5]. We argue that digit I loss in *Limusaurus* is derived and thus irrelevant to understanding the development of the bird wing.

If the extremely reduced hand morphology of *Limusaurus* was once present in the ancestors of birds (Figure 1A), several traits of digits I, II & III must have been lost (Figure 1A, step 1) and then re-evolved on digits II, III & IV (Figure 1A, step 2)[1]. The alternative is for the extremely reduced morphology of *Limusaurus* to have evolved in Ceratosauria, while bird ancestors retained digits I, II & III (Figure 1B). Quantitative analysis only favors the II,III,IV identification of tetanuran digits when bird digits are coded as II,III,IV, a category assumption based on embryological position alone[1]. This is not a truly integrative comparison, since it excludes phalangeal and metacarpal similarities that bird digits share with digits I, II and III of other theropods. When this assumption is removed, the I,II,III identification of tetanuran digits is most parsimonious[1].

Rather than assume the priority of either morphological or embryological data, we propose that a homeotic frameshift occurred in the bird line, such that digits I, II, & III develop from embryological condensations 2, 3 & 4[6]. That hypothesis has been supported by the observed absence of expression of most HoxD genes (*HoxD-10, HoxD-11* and *HoxD-12*) only in the anterior digit of the embryonic wing, a feature diagnostic of digit I of mouse[4]; *HoxD-11* expression in alligator is also absent only in digit I[5]. Experiments applying Cyclopamine (a down-regulator of Shh signaling) to the early wing bud show a frameshift of both digit morphology and *HoxD-12* expression with regard to the pattern of cartilage formation, viz., anterior and middle digits now develop from positions 3 and 4, and the posterior digit normally developing from position 4 is lost[7]. In our scenario (Figure 1B), a similar frameshift occurred in the raptorial forelimbs of bird ancestors (Figure 1B, step 2), probably upon loss of digit IV in early Tetanurae[6,7]. The frameshift would be unrelated to digit I loss in the extremely reduced forelimbs of *Limusaurus* (Figure 1B, step 1). A few metacarpal traits of tetanurans resemble those of digits II,III & IV of other theropods[1]. The frameshift could have affected all but these few de-coupled traits, which may provide a morphological signature of the occurrence of the frameshift towards the origin of Tetanurae.

It is debatable whether digit morphologies can disappear and re-appear in a different position, and whether such a step-wise process could be considered a homeotic frameshift (as suggested by Xu et al.). While *Limusaurus* expands our knowledge of digit reduction in theropods, it does not support a strong inference that any loss and re-gain of digital morphologies has actually occurred in the lineage leading to birds.

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1. Xu, X., et al. A Jurassic ceratosaur from China helps clarify avian digit homologies. Nature 459: 940-944 (2009)

2. Gauthier, J. A. Saurischian monophyly and the origin of birds. Mem. Calif. Acad. Sci. 8: 1–55 (1986)

3. Müller, G.B., and Alberch, P. Ontogeny of the limb skeleton in *Alligator mississippiensis*: Developmental invariance and change in the evolution of archosaur limbs. J. Morphol. 203: 151–164 (1990)

4. Vargas AO, Fallon JF. Birds have dinosaur wings: The molecular evidence. J Exp Zool (Mol Dev Evol) 304B: 86–90. (2005).

5. Vargas, A.O., Kohlsdorf, T., Fallon, J. F., Brooks, J. V. & Wagner, G. P. The evolution of HoxD-11 expression in the bird wing: insights from *Alligator mississippiensis*. PLoS ONE 3, e3325 (2008).

6. Wagner, G. P. & Gauthier, J. A. 1,2,3 = 2,3,4: a solution to the problem of the homology of the digits in the avian hand. Proc. Natl. Acad. Sci. USA 96, 5111–5116 (1999).

7. Vargas, A.O. & Wagner GP. Frame-shifts of digit identity in bird evolution and Cyclopamine-treated wings. Evolution & Development 11: 163-169 (2009)

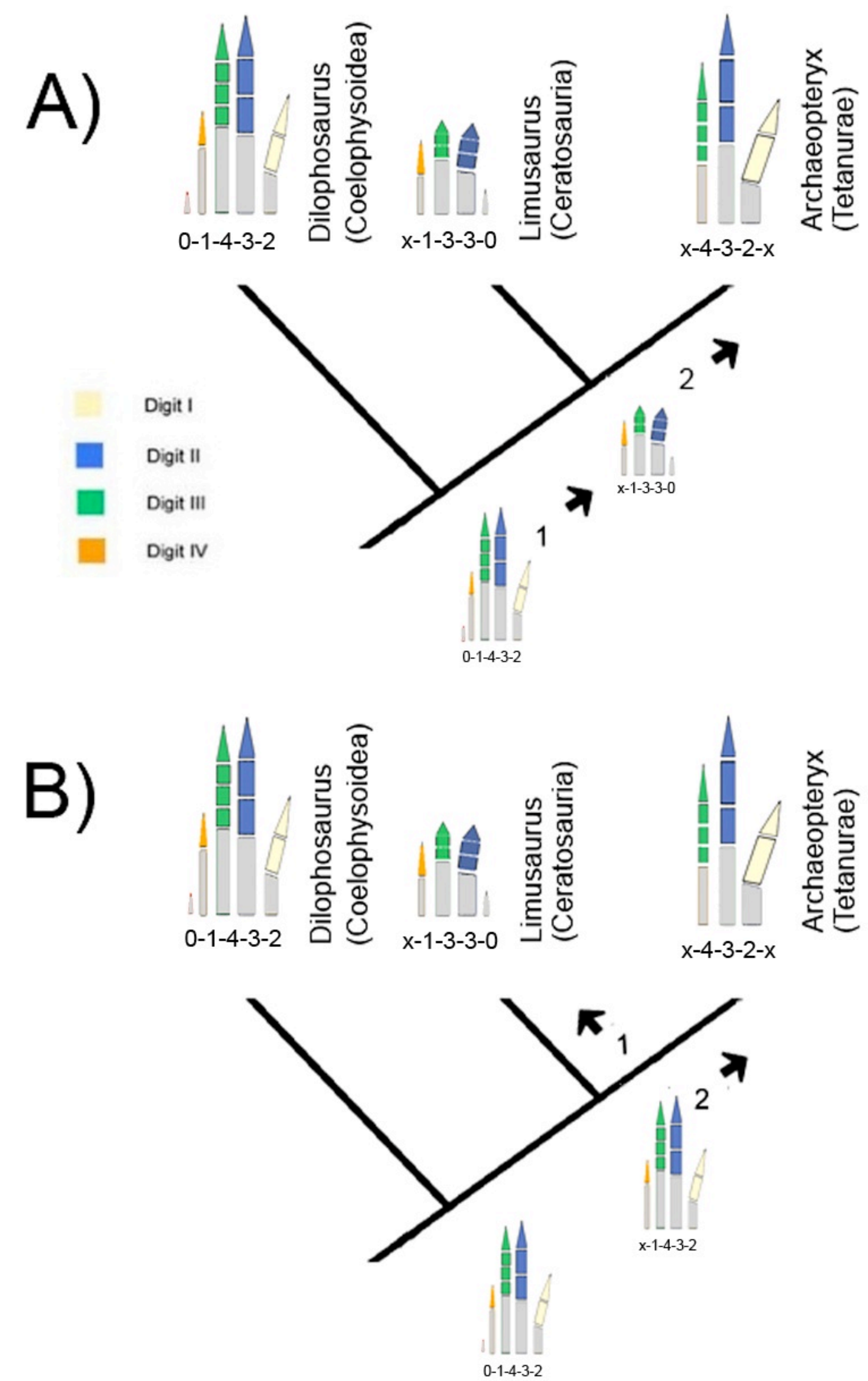

Figure 1. Alternative interpretations (A and B) of *Limusaurus* and the evolution of bird digit morphology. A) *Limusaurus* represents the morphology of bird ancestors; this implies extreme digit reduction occurred (Step 1: loss of digit I, loss and shortening of phalanges), but thereafter normal I, II, III morphologies re-appeared on digits II, III and IV (Step 2). B) *Limusaurus* does not represent the morphology of bird ancestors. Extreme digit reduction occurred only in Ceratosauria (Step 1); we propose that a homeotic frameshift accompanied the loss of digit IV (orange) in Tetanurae (Step 2), such that morphology and gene expression of digits I, II and III occur at embryological positions 2, 3 and 4[6]. Colours indicate digit identity according to number of phalanges, morphology and gene expression. The number of phalanges developing at each inferred embryological position is indicated under each hand (x means complete loss of the adult digit). Image modified from Xu et al.[1]