

The Digits of the Wing of Birds Are 1, 2, and 3. A Review

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ABSTRACT Fossil evidence documenting the evolutionary transition from theropod dinosaurs to birds indicates unambiguously that the digits of the wing of birds are digits 1, 2, and 3. However, some embryological evidence suggests that these digits are 2, 3, and 4. This apparent lack of correspondence has been described as the greatest challenge to the widely accepted theropod–bird link (Zhou 2004. *Naturwissenschaften* 91:455–471). Here we review the pertinent literature regarding the debate on the origin of birds and wing digital identity and the evidence in favor of a 1, 2, 3 identity of the wing digits. Recent molecular evidence shows that the expression of *Hoxd12* and *Hoxd13* in the developing wing supports the theropod–bird link. In the chicken foot and in the mouse hand and foot, digit 1 is the only digit to combine the expression of *Hoxd13* with the absence of expression of *Hoxd12*. The same is observed in the anterior digit of the wing, suggesting it is a digit 1, as expected for a theropod. Nevertheless, Galis et al. (2005. *J Exp Zool (Mol Dev Evol)* in press), argue that *Hoxd12* and *Hoxd13* expression patterns in mutant limbs do not allow distinguishing the most anterior digit in the bird wing from digit 2. They also argue that constraints to the evolution of limb development support the 2, 3, 4 identity of the wing digits. However, the case put forward by Galis et al. is biased and flawed with regard to interpretation of mutant limbs, developmental mechanisms, stages observed, and the description of the evolutionary variation of limb development. Importantly, Galis et al. do not present evidence from wild-type limbs that counters the conclusions of Vargas and Fallon (2005. *J Exp Zool (Mol Dev Evol)* 304B(1):85–89), and fail to provide molecular evidence to specifically support the hypothesis that the wing digits are 2, 3, and 4. The expression of *Hoxd12* and *Hoxd13* in the developing wing is consistent with the hypothesis that birds are living dinosaurs; this view can lead to a greater understanding of the actual limits to the evolutionary variation of limb development. *J. Exp. Zool. (Mol. Dev. Evol.)* 304B, 2005.

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Galis et al. (2005) have constructed a criticism of the molecular evidence presented in a recent article by Vargas and Fallon (2005) in favor of the hypothesis that the digits of the wing of birds are 1, 2, and 3, and defend the alternative view that the wing digits are 2, 3, and 4. In response, here we present a review on the debate on wing digital identity¹ and of the evidence in favor of a 1, 2, 3 identity of the wing digits. Answers to the

objections expressed by Galis et al. (2005) will be found within this review.

In order to provide an appropriate description of the debate on the identity of wing digits, first it is necessary to acknowledge that most discussion on this subject has been involved in the debate on the

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¹“Digit identity” is treated as a qualitative property describing the collective morphological traits of a digital ray: digit length, phalangeal number, and phalangeal morphology (Litngtung et al., 2002).

evolutionary origin of birds from the predatory, bipedal theropod dinosaurs² (Hinchliffe, '84, '97, 2002; Burke and Feduccia, '97; Thomas and Garner, '98; Chatterjee, '98; Feduccia, '99a, b, 2002a, b, 2003; Wagner and Gauthier, '99; Kundrát et al., 2002; Nowicki and Feduccia, 2002; Zhou, 2004). Although the hypothesis that birds descend from theropods was proposed as early as the 19th century (Gegenbaur, 1863; Huxley, 1868), it was commonly accepted during the mid-20th century that birds had not descended from theropods, mostly because of arguments that the observed resemblances were adaptive convergences, or due to hypothesized incompatibilities of the theropod–bird transition with evolutionary mechanisms (Heilmann, '26). These opinions, however, began to be questioned in the 1970s as new fossil theropod taxa like *Deinonychus* were found to bear striking, previously unknown similarities with *Archaeopteryx* (Ostrom, '76). Moreover, since the 1980s, most paleontologists have adopted the cladistic method of phylogenetic analysis based on algorithms of parsimony (Wiley, '91). This approach has repeatedly produced the same result: birds descend from theropods, and birds are a type of theropod in the same nested sense that humans are a type of primate (Gauthier, '86; Holtz, '98; Sereno, '99; Clark et al., 2002; Witmer, 2002; Hwang et al., 2004). Therefore, currently most scientists consider that the question of bird origins has been solved in favor of an origin from theropod dinosaurs. However, despite further accumulation of fossil taxa documenting the theropod–bird transition (Forster et al., '98; Xu et al., 2003; reviewed by Zhou, 2004), a change of scientific opinion on the subject of bird origins remains incomplete, as some researchers continue to doubt the theropod–bird link and remain skeptical on the results of phylogenetic analysis (Hinchliffe, '97; Ruben et al., '97; Thomas and Garner, '98; Dodson, 2000; Feduccia, 2002a, b, 2003). Therefore, it is desirable to confirm as many different independent sources of information that should be expected to be consistent with the theropod–bird link. The development of the digits of the bird wing is one of these sources of information (Prum, 2002, 2003; Vargas and Fallon, 2005).

Theropods, like birds, have a three-fingered hand. The hand of theropods is acknowledged as exceptional in having lost digits 4 and 5, while retaining digits 1, 2, and 3 (the thumb, index, and middle fingers; Shapiro, 2002; Burke and Feduc-

cia, '97). The general evolutionary pattern is that digit 1 is most easily lost or reduced in evolution, followed by digit 5. The most common pattern of digit reduction has been argued to reflect truncation of the development of the limb, since digit 1 is the last to initiate cartilage formation, followed by digit 5 (Shapiro, 2002; Shapiro et al., 2003).

The special condition of the hand of theropods therefore allows us to make the following assumption: as the description and knowledge of the molecular aspects of limb development continue to accumulate, and given that birds are theropods, it can be expected that molecular-developmental evidence would appear in support of the notion that birds have the exceptional theropod hand with digits 1, 2, and 3 (Prum, 2002, 2003).

This assumption is especially relevant because there is a well-developed argument to the contrary, based on embryological evidence, suggesting that the wing digits are 2, 3, and 4. A highly conserved spatial and temporal sequence of cartilage formation of the skeletal elements of developing pentadactyl limbs is usually found to be related to specific digits, such that the first digit to initiate cartilage formation is digit 4. This digit is also spatially in line with the ulna in the forelimb, allowing the conceptual description of a primary axis running from the ulna into digit 4 (Shubin and Alberch, '86). In the developing wing, the fact that the posterior digit is the first to initiate cartilage formation and further is in line with the ulna has led to the embryological definition of this digit as digit 4 and, by inference, the central digit as digit 3 and the anterior digit as digit 2 (Hinchliffe, '84; Burke and Feduccia, '97). Furthermore, small mesenchymal condensations flanking the developing functional digits are present in the embryonic wing, which can be interpreted as vestiges of digital condensations 1 and 5 (Kundrát et al., 2002; Larsson and Wagner, 2002; Nowicki and Feduccia, 2002).

According to this evidence, it has been argued that birds could not have descended from theropods, but rather from an ancestor conforming to the general pattern of digit reduction that lost digits 1 and 5 (Hinchliffe, '84, 2002; Burke and Feduccia, '97; Nowicki and Feduccia, 2002). The naming of the wing digits as 2, 3, and 4 is currently a standard procedure within developmental biology, where the chicken wing is used as a classical model system for the study of limb development. Therefore, it is not surprising that the embryological evidence in favor of a 2, 3, 4 identity of the wing digits has been argued to be

²A commonly accepted most general group definition of birds includes all the descendants of the most recent ancestor shared by *Archaeopteryx* with living birds (Neornithes).

the most important reason as to why the theropod–bird link may be considered problematic (Hinchliffe, '97; Feduccia, '99a; Zhou, 2004).

MORPHOLOGICAL-PHYLOGENETIC IDENTIFICATION OF WING DIGITS

For a long time bird wing digits have been considered to be 1, 2, and 3 by comparative anatomists on the basis of morphological traits

(Fig. 1A–L; Romer and Parsons, '70). For example, the first three digits of dinosaurs, crocodiles, or lizards by default have 2, 3, and 4 phalanges, a plesiomorphic condition inherited from a remote common ancestor that is clearly present in the limbs of early amniotes of the Carboniferous such as *Paleothyris* (Carroll, '88). The three digits of the wing of early birds like *Archaeopteryx* and *Confuciusornis* also present 2, 3 and 4 phalanges, respectively (Fig. 1G), and there is no phyloge-

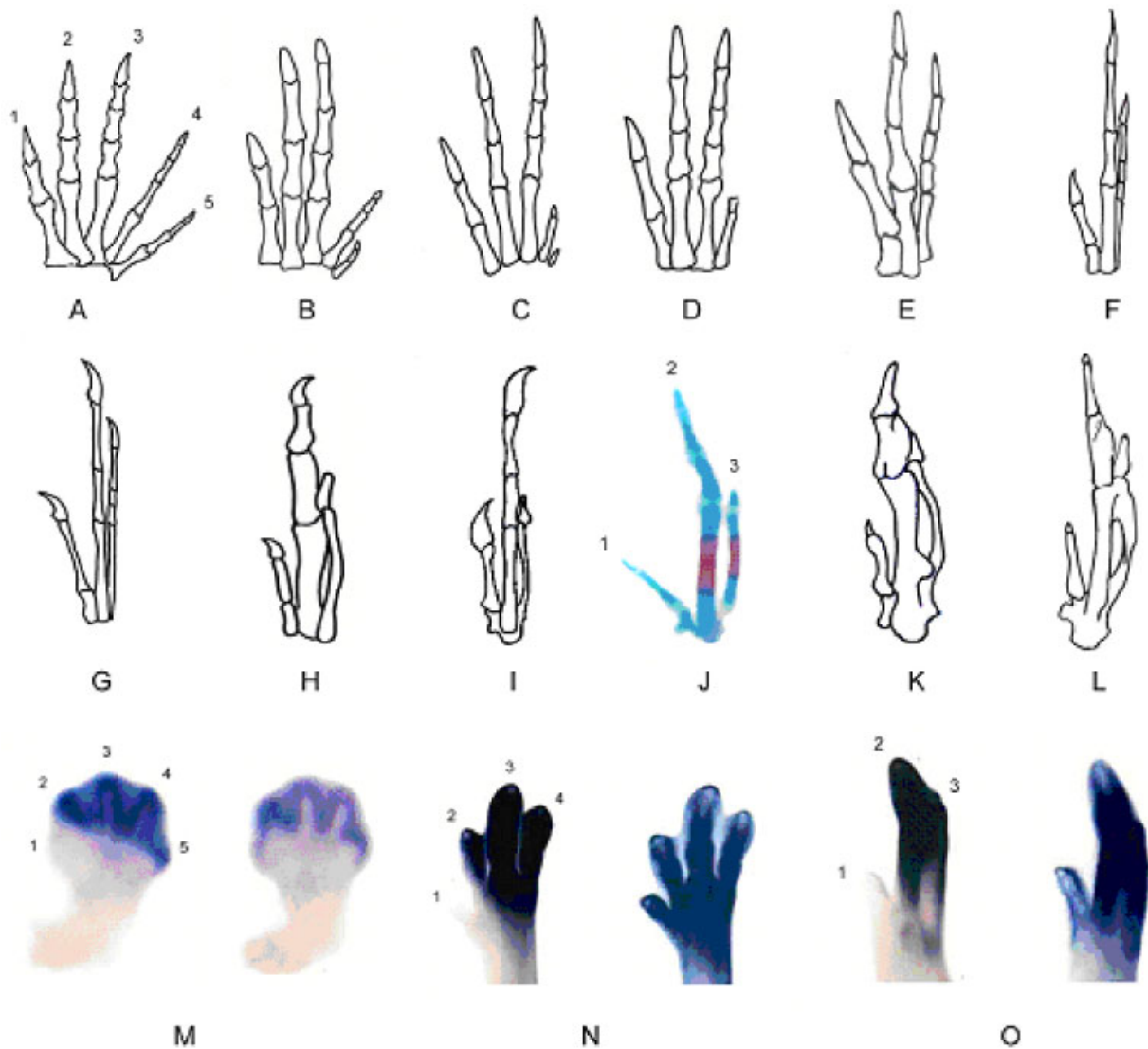


Fig. 1. Evidence for a 1, 2, 3 identity of wing digits (A–L). Phylogenetic homology of the digits of the wing to digits 1, 2, and 3. Successive taxa share a more recent common ancestor with modern birds. (A) *Alligator*, (B) the ornithischian dinosaur *Heterodontosaurus*, (C) the early theropod *Herrerasaurus*, (D) the neotheropod *Coelophysis*, (E) the tetanuran *Allosaurus*, (F) the early maniraptoran *Ornitholestes*, (G) the early bird *Archaeopteryx*, (H) the enantiornithine bird *Sinornis*, (I) hatchling of the living bird *Opisthocomus*, (J) 12.5 day embryo of the duck *Anas*, (K) the adult chicken *Gallus*, (L) adult *Opisthocomus*. (M–O) Expression of *Hoxd12* (left) and *Hoxd13* (right) in developing wild-type limbs. (M) Mouse hand at 12.5 dpc, (N) chicken foot at day 8 (stage 34), (O) chicken wing at day 8 (stage 34). Expression of *Hoxd13* in the absence of *Hoxd12* expression in the wing is consistent with a digit 1 identity of the anterior digit as expected for a theropod dinosaur with digits 1, 2, and 3.

netic–morphological evidence to consider that the phalangeal formula of digits 1, 2, and 3 has not conserved the default condition for reptiles. The advent of cladistic phylogenetic analysis and the accumulation of intermediate fossil taxa have further supported the hypothesis that birds descend from theropod dinosaurs. The early theropod *Herrerasaurus* further presents vestiges of digits 4 and 5, a fact that is consistent with the identification of the three remaining functional digits of the hand of theropods (including birds) as digits 1, 2, and 3 (Fig. 1C, Sereno, '93).

Along the theropod–bird transition, the fact that the digits of the wing of birds are 1, 2, and 3 is unambiguously indicated by the morphological resemblances between digits 1, 2, and 3 of maniraptoran theropods like *Deinonychus* to the digits of the wing of early birds like *Archaeopteryx* and *Confuciusornis* (Fig. 1F and G; Padian and Chiappe, '98; Wagner and Gauthier, '99). Along with the presence of the 2–3–4 phalangeal formula, other resemblances include a reduced metacarpal 1, a central digit longest with digit 3 of intermediate length between that of digits 1 and 2, and two short proximal phalanges on digit 3 (Wagner and Gauthier, '99). Further recent fossil discoveries of Mesozoic birds have provided new information on the transition from early birds like *Archaeopteryx* to modern birds (Neornithes). Among the Mesozoic birds, the Ornithotoraes (including the extinct Enantiornithes and the ancestors of modern birds) have reduced the number of phalanges on digit 3 from four phalanges to one (Fig. 1G and H; Chiappe, 2002; Sereno et al., 2002). However, birds retain the presence of two and three phalanges on the first and second digits, respectively, as can be observed in the embryo of the duck (Fig. 1J), reported in the chicken embryo (Parker, 1888) and most evident in the clawed wings of the hatchlings of the Hoatzin (*Opisthocomus*, Fig. 1I). The fact that the two anterior digits of the wing retain the default phalangeal formula for digits 1 and 2 of reptiles is obscured in the adult by fusion of the distalmost phalanx to the next proximal (Parker, 1888) or by artifactual loss of small distal phalanges in skeleton preparation, leading to the appearance of the first two digits of the adult wing with one and two phalanges (Wagner and Gauthier, '99). This contradicts the argument of Galis et al. (2005), based only on observation of adults, that the phalangeal count of the anterior and middle wing digits stands against evidence in support of a 1, 2, 3 identity of the wing digits.

IS THE PRIMARY AXIS ALWAYS RELIABLE TO ASSESS DIGITAL IDENTITY?

As explained in the introduction, the embryology of the wing seems to contradict the morphological–phylogenetic assessment of digital identity as 1, 2, and 3. However, birds are not the only case where such an apparent contradiction can be observed: in the three-toed skink *Chalcides chalcides*, the position of the primary axis suggests that the three digits are 2, 3, and 4, while their morphology suggests they are 1, 2, and 3 (Raynaud, '85; Shapiro, 2002; Shapiro et al., 2003). Similarly, the position of the primary axis in the hand of anurans suggests a 2, 3, 4, 5 identity of the digits (Oster et al., '88), but classic textbooks of comparative anatomy refer to these digits as digits 1, 2, 3, and 4 (Romer and Parsons, '70). Although the sequence of cartilage formation has been considered to reflect events of branching and segmentation of precartilaginous condensations, the alleged mechanistic relevance of this pattern can be questioned by developmental evidence showing that certain elements can develop out of sequence, without the formation of the elements that they are assumed to branch from. A particularly evident case is exemplified by the fact that digit 1 can form in the absence of any other digits (Chiang et al., 2001; Ros et al., 2003), contrary to the assumption that digit 1 branches off from the precursors of posterior digits, as inferred from the sequence of cartilage formation. Further evidence has currently led to a discussion on the actual developmental and evolutionary relevance of the primary axis of cartilage formation (Cohn et al., 2002). Although the position of the primary axis will mark the position of digit 4 in most amniotes, caution should be taken before considering this as the main criterion for the assessment of digital identity.

HYPOTHETICAL RESTRICTIONS ON THE EVOLUTION OF LIMB DEVELOPMENT

The main argument to consider that the embryology of the wing of birds cannot be derived from a hand with digits 1, 2, and 3 is that the development of limbs is highly constrained in general terms (Feduccia, 2002a, b, 2003; Galis et al., 2003, 2005). For example, genetic changes required for polydactyly have been hypothesized to have negative pleiotropic effects on fitness, such

as a greater susceptibility to cancer, constraining the evolutionary occurrence of polydactyly at an interspecific level (Galis et al., 2001). Similar negative pleiotropic effects have also been argued to be a possible constraint to deriving the embryology of the bird wing from that of a limb with digits 1, 2, and 3 (Feduccia, 2002a, b, 2003). However, in the case of preaxial polydactyly, the pleiotropic effect hypothesis is not supported by developmental evidence. Recently, the limb-specific *Sonic hedgehog* (*Shh*) long-range enhancer has been described in a highly conserved region of intron 5 of the *Lmbr1* gene that is located about one megabase upstream of the *Shh* locus (Lettice et al., 2003; Sagai et al., 2004, 2005). Single base pair changes in the highly conserved sequence are correlated with preaxial polydactyly (contra Galis et al., 2005). There is now evidence that the single base pair change found in *Hemimelic extra toes* (*Hx*) polydactylous mouse mutant is the genetic basis of the polydactylous phenotype (Maas and Fallon, 2005). Galis et al. (2005) state that polydactyly and oligodactyly have never been documented in amniotes at the evolutionary level of interspecific variation, despite their common occurrence within species and despite the possible adaptive value of these changes (Galis et al., 2001). However, this is not the case, according to the recent discovery of an unambiguously polydactyl species of aquatic amniote from the Triassic of China (Wu et al., 2003). Limb development is certainly not constrained such that evolutionary digit loss (oligodactyly) cannot occur. Rather, interspecific oligodactyly is extensively documented in evolution within several groups and lifestyles (skink lizards, ostriches, and ungulates, to mention a few). Hypothesized constraints cannot be assumed to be general and pervasive to question the occurrence of developmental change in the bird wing, especially given that the alleged evidence upon which these hypothesized constraints are constructed upon can be questioned according to actual developmental mechanisms and evolutionary variation.

THE FRAMESHIFT HYPOTHESIS

As implied from the discussion above, the case of the bird wing is especially interesting for the discussion of the actual evolutionary plasticity of limb development. Specifically, it has introduced

the possible occurrence of homeotic transformation of digits in evolution (Wagner and Gauthier, '99). As a way of reconciling the embryological and paleontological evidence, the hypothesis of a homeotic frameshift acknowledges that the spatial position of the digital condensations of the embryonic bird wing is in fact the same as that of condensations that develop into digits 2, 3, and 4 in other amniotes, but it assumes that a frameshift homeotic transformation of digital identity has occurred along the theropod–bird transition, such that condensations 2, 3, and 4 actually develop into digits 1, 2, and 3 (Wagner and Gauthier, '99; Larsson and Wagner, 2002). It is broadly acknowledged that homeotic transformations are commonplace in evolution, and within vertebrates are well known in the axial skeleton (for an example, see evidence for dorsal to cervical transformation adding vertebrae to the long neck of sauropod dinosaurs; Wilson and Sereno, '98). The homeotic transformation of digits poses an identical challenge to that of the occurrence of homeotic transformation of vertebrae: homologies are best judged by shared molecular-developmental pathways and morphology, rather than simple numbering according to spatial position (Burke et al., '95).

The development of the limbs of the three-toed skink and of anurans discussed above also suggests the possibility of further cases of frameshift homeotic transformations of digits in evolution. Homeotic transformation of digits is also supported by its developmental plausibility: digital condensations remain undetermined until late stages, and homeotic transformations can be experimentally achieved (Dahn and Fallon, 2000). The natural occurrence of a homeotic transformation of the thumb into an index finger can even be occasionally observed in humans (Fig. 2A; Heiss, '57; Pérez-Cabrera et al., 2002), a fact that suggests that *Hoxd12* may be mis-expressed in this digit (see Knezevic et al., '97; Vargas and Fallon, 2005).

The argument that a homeotic frameshift would require losing digit 1 and at the same time re-acquiring a previously reduced or lost digit 4 (Galis et al., 2005) bypasses the important distinction between undetermined early digital condensations and digits with a fully developed identity. Until late stages (HH stage 29), digital condensations retain a potential to develop into any digit (Dahn and Fallon, 2000). A shift in the position of the mechanisms that confer digital identity could therefore

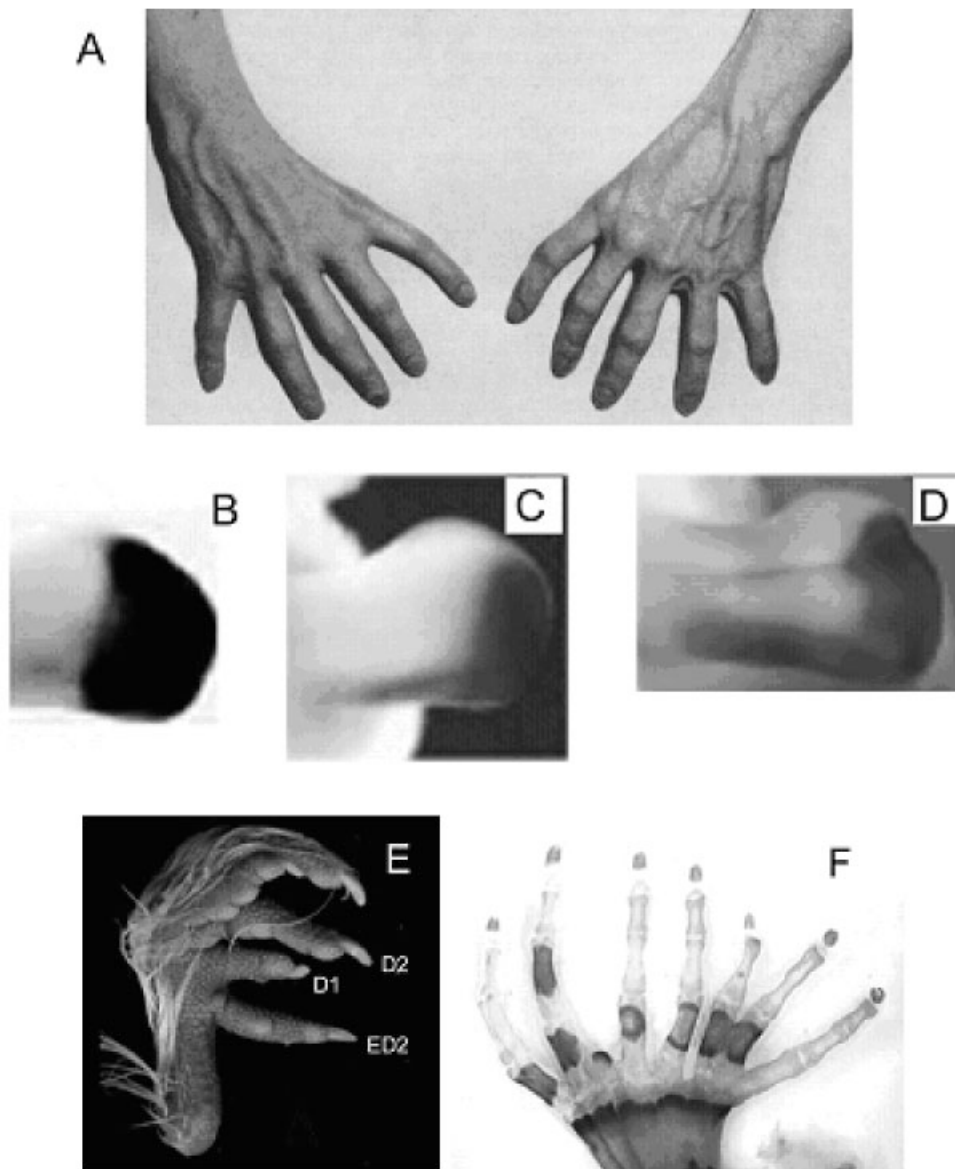


Fig. 2. Mutant limbs. (A) Homeotic transformation of a thumb (digit 1) into an index in a woman. From Heiss ('57). (B) Expression of *Hoxd13* in the wild-type chicken foot at stage 27. From Ros et al. (2003). (C) Expression of *Hoxd12* in the wild-type chicken foot at stage 27. From Suzuki et al. (2004). (D) Misexpression of *Tbx2* leads to an anterior expansion of *Hoxd12* but it does not encompass precursor cells of digit 1. From Suzuki et al. (2004). (E) Foot of the Silkie breed of chicken. D1: digit 1; D2: digit 2; ED2: ectopic digit 2. (F) Foot of the chicken mutant *talpid*². Triphalangeal, long digits are posteriorized. (From Litingtung et al., 2002).

conceivably lead to the development of digital condensations 2, 3, and 4 into digits 1, 2, and 3. In contrast with the hypothesis of a homeotic frameshift, the proposal that birds have digits 2, 3, and 4 requires the unparsimonious assumption that morphological resemblances of the digits of *Archaeopteryx* to digits 1, 2, and 3 of dinosaurs are the result of convergences between the adult morphology of these digits.

CAN THE DIGITS OF THEROPODS BE 2, 3, AND 4?

Galis et al. (2003), while expressing some doubt on the theropod–bird link, alternatively proposed a solution to the problem of digit identity that accepts the evidence that birds are theropods, but instead argues that the digits in the hand of theropods may in fact be 2, 3, and 4 rather than 1,

2, and 3. However, current paleontological evidence effectively discards this possibility, despite the affirmation of Galis et al. (2005) to the contrary.

The argument that theropods have digits 2, 3, and 4 is only possible by ignoring the morphological resemblances between the three functional digits of theropods to digits 1, 2, and 3 of other dinosaurs (Fig. 1; Larsson and Wagner, 2003). If the digits of theropods were 2, 3, and 4, this would imply the further unparsimonious assumption that digits 2, 3, and 4 convergently acquired traits of digits 1, 2, and 3, such as the phalangeal formula and the presence of an enlarged claw in digit 1. No paleontological evidence exists that such convergence actually occurred (such as transitional intermediate morphologies) or for the loss of digit 1 in theropods (such as an early theropod presenting reduction of this digit). Moreover, recent phylogenetic analysis has confirmed that *Herrerasaurus*, whose hand presents reduced digits 4 and 5, is in fact an early theropod (Serenó, '93, '99).

Therefore, the proposal that theropods have digits 2, 3, and 4 is truly hopeless as an end to the debate of wing digital identity, because paleontologists will continue to follow the unambiguous fossil evidence in naming the digits of theropods (including birds) as digits 1, 2, and 3.

WING DIGITS ARE 1, 2, AND 3: MOLECULAR EVIDENCE

According to the assumption presented in the introduction, molecular evidence on wing development has been recently presented in favor of the 1, 2, and 3 identification of wing digits. The evidence is straightforward. In the forelimbs and hindlimbs of wild-type mouse and in the hindlimb of wild-type chicken (where the identity of digits is uncontroversial), digit 1 and other digits express *Hoxd13*, but only digit 1 does not express *Hoxd12* (Fig. 1M and N). When observed in the wild-type wing, the anteriormost digit expresses *Hoxd13*, but does not express *Hoxd12* (Fig. 1O). Therefore, the best inference according to this molecular evidence is that this digit is digit 1, as expected for the tridactyl hand of a theropod with digits 1, 2, and 3³ (Vargas and Fallon, 2005).

³Although our molecular evidence only marks the development of digit 1, alternative identification of the wing digits as 1, 2, 4 or 1, 3, 4, is not supported by paleontological evidence (Fig. 1), nor by the fact that no case is currently known for the evolutionary loss of a middle digit while retaining those that flank it (Holder, '83).

The expression pattern associated with the development of digit 1 becomes established at late stages. For instance, precursor cells of digit 1 do not express *Hoxd13* at early stages, and only acquire the expression of this gene at late stages. This differs from the expression of *Hoxd13* in the precursor cells of other digits, which is present since earlier stages. The early expression of *Hoxd13* in the precursor cells of posterior digits has been argued to reflect the expansion of a posterior population of *Hoxd13*-expressing cells, by its coincidence with cell-fate mapping, such that no cells acquire *Hoxd13* expression (Vargesson et al., '97). This situation could differ from the expression of *Hoxd13* in digit 1, which may be secondarily acquired. A different mechanism of *Hoxd13* expression in digit 1 is also supported by evidence that a *Hoxd13*-expressing digit 1 can develop in the absence of *Shh* and in the absence of the formation of any other digit (Chiang et al., 2001; Ros et al., 2003). This suggests that a digit 1-specific enhancer of *Hoxd13* expression is present. Another fact suggesting a unique differential enhancer of *Hox* gene expression in digit 1 is that this is the only digit where there is no activity of the digit enhancer described by Spitz et al. in mouse (see Fig. 3B in Spitz et al., 2003).

The expression pattern associated with the development of digit 1 becomes established around chick stage 27 (day 5). Although individual digital condensations are not easily distinguished at this stage, expression of *Hoxd13* is expanded anteriorly in respect to that of *Hoxd12*, suggesting that the anterior region of cells that express *Hoxd13* but not *Hoxd12* are precursors of digit 1 (Fig. 2B and C). At stage 29, individual condensations are morphologically evident and the expression pattern related to the development of digit 1 has become unambiguously established both in the wing and the foot. This coincides with the stage at which digital condensations become determined. Thereafter, expression of *Hoxd13* in the absence of *Hoxd12* in digit 1 remains evident at stage 31 and as late as stage 34 (day 8, Fig. 1N and O).

Galis et al. (2005) present cases of experimental mutant limbs in which they argue that the expression of *Hoxd12* is not absent in the developing digit 1 (Suzuki et al., 2004), or where posterior digits develop in the absence of *Hoxd12* expression (Selever et al., 2004). However, in these cases *Hox* expression was observed at early stages before the actual determination of digital identity (Suzuki et al., 2004) or even before any individual

condensations are distinguishable (Selever et al., 2004). Moreover, the anterior expansion of *Hoxd12* by misexpression of *Tbx2* in the developing foot of the chicken does not encompass the precursor cells of digit 1 (Fig. 2D; Suzuki et al., 2004). Given the dynamic expression pattern of *Hox* genes along limb development, the late determination of digital identity, and the difficulty identifying individual condensations at early stages, these cases cannot be considered as evidence to discard an expression pattern that must be observed at a later stage. Galis et al. (2005) also presume that no changes in *Hoxd12* and *Hoxd13* expression occur when overexpression of *Hoxd11* leads to posteriorization of the identity of digit 1 (Morgan et al., '92; Goff and Tabin, '97). Needless to say, presumptions cannot substitute for actual evidence. Chicken mutants presenting altered antero-posterior patterning of digits provide accessory evidence on the reliability of the expression pattern of *Hoxd12* and *Hoxd13* to identify digit 1. Given that mutants may present abnormal development, it is necessary to discuss the specific traits used to identify digits. In the polydactylous limbs of *talpid*² mutants, digits can be considered abnormal, but it is possible to state that no digit 1 is formed, because the digits of *talpid*² share traits with the posterior digits that are not shared by digit 1. The digits of *talpid*² mutants surviving into late stages are considerably longer than digit 1 and present three phalanges (Fig. 2F; Litingtung et al., 2002). *talpid*² limbs also show high Gli3-190:Gli3R levels across the A/P axis (Wang et al., 2000; Litingtung et al., 2002). Contrary to the argument of Galis et al. (2005), this is consistent with the fact that all digits of *talpid*² express both *Hoxd12* and *Hoxd13*, like posterior digits.

In the Silkie breed, an ectopic digit 2 develops anterior to digit 1. This digit can be identified as digit 2 on morphological grounds, such as the presence of three phalanges, and a length intermediate between that of digits 1 and 3 (Fig. 2E). Regardless of whether Silkie is interpreted as a mirror-image duplication or not, the fact that the ectopic digit is a digit 2 is also consistent with the expression of both *Hoxd12* and *Hoxd13* in this digit. We conclude that the evidence provided by the developing limbs of Silkie and *talpid*² is truly compelling, because the late expression pattern related to digit 1 formation is conserved even under conditions in which the antero-posterior patterning of the digital arch becomes altered.

The well-known evidence for functional redundancy among *Hox* genes also provides no clear reason to consider that the expression pattern of *Hoxd12* and *Hoxd13* is not reliable to identify the development of digit 1 (contra Galis et al., 2005). The mild effect of the knockout of *Hoxd12* (Davis and Capecchi, '96) serves to reject the oversimplification of considering that lack of *Hoxd12* expression is the only requisite to determine the development of a digit 1 identity (in which case a transformation of all digits to digit 1 should have occurred). The development of a digit 1 identity has additional requirements, which is also suggested by the evidence discussed above for differential timing and enhancing of *Hox* gene expression in this digit. Absence of *Hoxd12* expression can be an important requisite (albeit not the only) for the development of a digit 1 identity. This is suggested by the fact that in the mouse, misexpression of *Hoxd12* in this digit leads to its homeotic transformation into a digit 2 (Knezevic et al., '97). Despite the functional redundancy between *Hox* genes, it is important to point out that according to the mechanistic relevance of colinearity, functional differences among these genes depend on their 5'-3' position along the *Hoxd* cluster, which determines differences in the pattern and intensity of expression (Kmita et al., 2002). *Hoxd12* expression in the development of a digit 1 identity is intrinsically related to its normal position along the *Hoxd* cluster, which also shows phylogenetic conservation. This is consistent with the unexpected consequences of alleged deletion of *Hoxd* gene loci that alter the cluster structure, as compared with the loss of function of these genes by disruption of their coding sequence. Within such gene clusters, the "function" of a given gene cannot be considered on its own, but instead must be integrated into the larger context of the cluster itself (Kmita et al., 2002).

Here we wish to emphasize that although the identity of the digits in mutants must be discussed, current analysis of wild-type limbs of mouse and chicken where digital identity is uncontroversial clearly supports the 1, 2, 3 identification of the wing digits. Experimental conditions such as gene misexpression in developing limbs are informative but may not be representative of the mechanisms of change actually available in nature. Moreover, no positive molecular evidence has been presented either in wild-type or mutant limbs to specifically support the hypothesis that the wing digits are 2, 3, and 4,

which continues to rely completely on embryological evidence. The original developmental 2–3–4 hypothesis depends entirely on the assumption that position is equivalent to identity. But this classic notion is entirely falsified by recent evidence on the development of digit identity (Dahn and Fallon, 2000; Litingtung et al., 2002; Suzuki et al., 2004). Given that there is no additional molecular evidence to support the notion of 2–3–4, the hypothesis should be rejected.

Here we propose that the expression pattern related to digit 1 formation was probably already present in both forelimbs and hindlimbs of the most recent common ancestor of all amniotes, and has been conserved in both mouse and chicken. We therefore expect this pattern to be conserved in most amniotes as a default condition.

MESENCHYMAL CONDENSATIONS IN THE CHICKEN WING

The early pantetrapod Devonian ancestors of birds were polydactylous, with up to eight fingers on both forelimbs and hindlimbs (Clack, 2002). In the embryos of several modern tetrapods, it is possible to observe the presence of mesenchymal condensations other than those of digits 1–5 that may represent vestiges of the additional digits of early pan-tetrapods. A mesenchymal condensation in front of digit 1 is called a prepollex, and a mesenchymal condensation found posterior to digit 5 is called a postminimus. Some have interpreted the presence of a mesenchymal condensation in front of the anterior digit of the wing as a demonstration that the anterior digit is digit 2 (Feduccia and Nowicki, 2002; Galis et al., 2003). However, Vargas and Fallon (2005) have emphasized the possibility that this condensation may in fact be comparable to a prepollex, an interpretation consistent with the 1, 2, 3 identity of wing digits. Although Galis et al. (2005) argue that the prepollex was lost in the line of birds and reptiles, this is in fact unknown. Only some fossil and living amphibian taxa show an ossified prepollex that can be easily observed in the adult (Romer, '70; Fabrezi, 2001). Otherwise, the prepollex described in the embryonic hand of several mammals is a small, transitory mesenchymal condensation that does not even initiate cartilage formation. Before any statements can be made on the evolutionary loss of the prepollex in reptiles, the actual presence of a prepollex must be correctly assessed by methods allowing the visualization of mesenchymal condensations that are

difficult to detect, pretty much in the same way in which the presence of an anteriormost mesenchymal condensation of the chicken wing was uncertain, until visualized by means of peanut agglutinin (Larsson and Wagner, 2002), absence of stained vascularization (Kundrát et al., 2002), and *Sox9* gene expression (Welten et al., 2005). Future assessment of the presence of a prepollex may reveal its generalized persistence in the embryos of reptiles.

Although only three digits develop in the wing of the chicken, recent work on the expression of the *Sox9* gene allowing the visualization of mesenchymal condensations has revealed that six mesenchymal condensations can be found in the developing wing that can be compared to digital condensations (Welten et al., 2005). Remarkably, it is possible to interpret these condensations as consistent with the proposal that the anteriormost condensation may be a prepollex, the following three digital condensations are digits 1, 2 and 3, the next condensation is a vestige of digit 4, and the posteriormost condensation (named “element X” by Welten et al., 2005) can be assumed to be a vestige of digit 5. It is critical that these authors also note the absence of BMP receptor B (BMPR1B) in the anteriormost *Sox9* region (Welten et al., 2005). It has been reported that loss of function of BMPR1D in the mouse specifically affects the proximal two phalanges of all digits, but not the distal phalanx (see, e.g., Baur et al., 2000; Yi et al., 2000). In fact, there is no evidence of phalangeal initiation of any kind in the brief *Sox9*-expressing region anterior to the first digit in the wing. It remains plausible that this is a prepollex. However, it is necessary to point out that the numbering of the mesenchymal condensations along the wing does not explain how the primary axis of cartilage formation comes to be in line with digit 3, rather than digit 4 as in other amniotes. This fact requires an arguably large scale of developmental change in the wing, such as the homeotic frameshift (Wagner and Gauthier, '99), or a shift in the position of the primary axis (Chatterjee, '98). If we consider that the embryological position of the digital condensations of the wing is actually the same as digits 2, 3, and 4 of other amniotes, as currently accepted within developmental biology, then we must assume that a homeotic frameshift has occurred. In this case the posteriormost condensation (element “x”) may be compared to a postminimus. It is important to point out that the newly described anteriormost and posteriormost condensations of

the wing appear at a much later stage than the digital condensations of the functional digits, are very small sized, and only transiently detectable. Therefore, these mesenchymal condensations cannot be compared with much certainty to specific digits that were lost in the adult.

IS NEUTRAL DEVELOPMENTAL CHANGE IMPOSSIBLE?

It is argued that the developmental change required to derive the wing from a hand with digits 1, 2, and 3 (such as the homeotic frameshift) can be doubted because such a change would have no adaptive value and as such would not be selected (Galis et al., 2003; Feduccia, 2003). Under this adaptationist point of view, non-adaptive evolutionary change is considered to be so improbable that the combination of phylogenetic, morphological, and molecular evidence is discarded before accepting the possibility that even a "rare" exception could have occurred. This argument is remarkable because of the well-known opposition to the "panglossian" notion that all (or most) evolutionary change must be adaptive (Gould and Lewontin, '79). Non-adaptive evolutionary change is exemplified by genetic drift and neutral molecular change (Kimura, '68). In fact, the evolution of development in the avian wing provides yet another countless example of how variation can exist among developmental pathways of homologous structures (Wagner and Misof, '93). Since in these cases phenotypic traits are identical to selection, regardless of the fact that they develop by different means, the adaptively neutral variation of developmental pathways has been described as "phenogenetic drift" (Weiss and Fullerton, 2000). Moreover, current conceptual frameworks for evolution have argued that the concept of adaptively neutral drift, widely accepted at the molecular level, is also applicable to the level of phenotypic traits (Maturana and Mpodozis, 2000; Weiss and Buchanan, 2003). Therefore, it must be understood clearly that the absence of a selective advantage for the developmental change in the wing does not imply that it could not have occurred.

Digit 1 in dinosaurs was functionally specialized, as suggested by an enlarged, medially deflected claw in early ornithischian, sauropodomorph, and theropod dinosaurs. According to the general pattern of digit reduction by truncation of limb development, it would seem difficult to lose digit 4 if digit 1 could not be lost for functional

reasons. However, once the shift in the correspondence of digital identity to the sequence of cartilage formation had occurred, truncation of the sequence of cartilage formation would lead to postaxial reduction. After the shift, the main axis no longer ran through digit 4, and the condensation developing into digit 4 may well have initiated cartilage formation after the condensation that developed into digit 1, which could explain how it was possible to lose digit 4 without losing digit 1.

From this point of view, the shift in digital identity could have occurred as a selectively neutral change (phenogenetic drift, according to Weiss and Fullerton, 2000), and thereafter digit loss would proceed as usual, by truncation of the sequence of cartilage formation.

CONCLUDING REMARKS: THE DEBATE ON BIRD ORIGINS

The hypothesis of a theropod–bird link has proven to be notably successful (Prum, 2002, 2003). Predictions of the theropod–bird link that have been carried through are the discovery of medially fused clavicles in several theropod taxa (Chure and Madsen, '96; Makovicky and Currie, '98; contrary to the classical objection that theropods did not have adequate precursors of the avian furcula, Heilmann, '26); the receding of the gap in the fossil record of the maniraptora (Jensen and Padian, '89; Xu et al., 2001a; Hwang et al., 2004); and the discovery of theropods preserved with unambiguous feathers (Ji et al., '98; Ji et al., 2001; Xu et al., 2001b, 2003; as predicted by Bakker, '86; Paul, '88). Aside from the classic use of skeletal morphological characters, recent additional evidence is also provided by the similarity of eggshell structure (Grellet-Tinner and Chiappe, 2004) and the amazing preservation of theropods in brooding and sleeping positions identical to those currently observed in living birds (Norell et al., '95; Clark et al., '99; Xu and Norell, 2004).

Despite the fact that the theropod–bird transition is well documented by the fossil record (Zhou et al., 2004), the continuity of the debate on bird origins stems greatly from the skepticism on cladistic phylogenetic analysis as a method capable of solving the problem (Dodson, 2000). By disregarding the results of phylogenetic analysis, it is argued that the resemblances among birds and theropods can be the result of "massive" convergence (Burke and Feduccia, '97), and that

feathered dromaeosaurids and oviraptorids may not be theropods but secondarily terrestrial birds that resemble theropods by convergence (Jones et al., 2000a; Feduccia, 2002a, b). Further confusion has stemmed from the claim that the long flight scales of the Triassic non-dinosaur archosauromorph *Longisquama* are in fact feathers (Jones et al., 2000b), despite evident structural differences (Reisz and Sues, 2000; Prum, 2001; Unwin and Benton, 2001), and phylogenetic analysis indicating that birds are closer to theropods than to *Longisquama* (Sumida and Brochu, 2000). Finally, there is new fossil and molecular-developmental evidence that demonstrates that feathers are homologous with scales at the early placode stage, but that the feather bud and all subsequent feather structures are evolutionary novelties that are not homologous with a scale or parts of a scale, effectively discarding the hypothesis that feathers evolved from enlarged flight scales (Xu et al., 2001; Prum and Brush, 2002; Harris et al., 2002; Prum, 2003).

Several recent arguments against the theropod–bird link (Ruben et al., '97; Jones et al., 2000a) have been adequately contested (Hicks and Farmer, '99; Paul, 2001; Perry, 2001; Christiansen and Bonde, 2002), but the embryological objection on the identity of the wing digits has proven to be long-standing and continues to be discussed (Nowicki and Feduccia, 2002; Feduccia, 2003; Galis et al., 2003; Zhou, 2004). However, the molecular evidence provided by the expression of *Hoxd12* and *Hoxd13* supports the prediction of phylogenetic analysis that the digits of the wing of birds are 1, 2, and 3 (Vargas and Fallon, 2005), and is further consistent with the *Sox9* and *BMPR1b* expression data (Welten et al., 2005). It also contradicts the argument that the development of the wing does not support the theropod–bird link.

Taking into account that the “embryological objection” is frequently considered the greatest challenge to acceptance that birds are living theropods (Feduccia, '99; Zhou, 2004), the new molecular evidence must be considered yet another significant support of the hypothesis of a theropod–bird link, which it is hoped will lead to broader acknowledgment of its implications. Additional molecular studies can provide further evidence to the satisfaction of both the developmental and paleontological scientific communities that may end the debate on wing digital identity and, as necessary, supersede theoretical speculation.

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