The developmental evolution of avian digit homology: An update ☆

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Abstract

The identity of avian digits has been unresolved since the beginning of evolutionary morphology in the mid-19th century, i.e. as soon as questions of phylogenetic homology have been raised. The main source of concern is the persistent discrepancy between anatomical/paleontological and embryological evidence over the identity of avian digits. In this paper, recent evidence pertaining to the question of avian digit homology is reviewed and the various ideas of how to resolve the disagreement among developmental and phylogenetic evidence are evaluated. Paleontological evidence unequivocally supports the hypothesis that the fully formed digits of maniraptoran theropods are digits DI, DII, and DIII, because the phylogenetic position of *Herrerasaurus* is resolved, even when hand characters are excluded from the analysis. Regarding the developmental origin of the three digits of the avian hand the discovery of an anterior digit condensation in the limb bud of chickens and ostriches conclusively shows that these three digits are developing from condensations CII, CIII, and CIV. The existence of this additional anterior condensation has been confirmed in four different labs, using four different methods: Alcian blue staining, PNA affinity histochemistry, micro-capillary regression and *Sox9* expression. Finally, recent evidence shows that the digit developing from condensation CII has a Hox gene expression pattern that is found in digit DI of mice forelimb and chick hind limbs. The sum of these data supports the idea that digit

identity has shifted relative to the location of condensations, known as Frame Shift
Hypothesis, such that condensation CII develops into digit DI and condensation CIII
develops into digit DII, etc. A review of the literature on the digit identity of the Italian Three-
toed Skink or *Luscengola* (*Chalcides chalcides*), shows that digit identity frame shifts may not
be limited to the bird hand but may be characteristic of "adaptive" digit reduction in amniotes
(*sensu* Steiner, H., Anders, G., 1946. Zur Frage der Entstehung von Rudimenten. Die
Reduktion der Gliedmassen von *Chalcides tridactylus* Laur. Rev. Suisse Zool. 53, 537–546) in
general. In this mode of evolution two digits are lost, in the course of the adaptation of the
three anterior digits to a function that does not require the two posterior digits. This evidence
suggests that the evolution of digits in tetrapods can proceed at least on two distinct levels of
integration, the level of digit condensations and that of adult digits.

Introduction

It is a truism of evolutionary developmental biology that the evolution of
phenotypic characters has to be caused by the evolutionary modification of their
developmental pathways (Raff 1996; Hall 1998). Surprisingly, however, the
evolutionary conservation of a phenotypic character does not imply the conservation
of its developmental pathway (Wagner and Misof 1993; Hall 1994; Weiss and
Fullterton 2000). A paradigmatic case of this problem is the homology of the digits
in the bird wing. Phylogenetic evidence conclusively shows that the digits of the bird
wing are the thumb, the "index" finger and the middle finger, i.e. digits DI, DII and
DIII (Sereno 1999a). The embryological origin of these fingers, however, is identical
to that of those fingers that usually form the "index", middle and "ring" finger, i.e.
digits DII, DIII, and DIV (Hinchliffe and Hecht 1984; Müller and Alberch 1990;
Burke and Feduccia 1997). This discrepancy has been unresolved since the discovery
of the dinosaur affinities of birds in the mid-19th century and continues to fuel a
heated debate among paleontologists, ornithologists and developmental biologists
(Feduccia 1996, 1999, 2001; Prum 2002; Galis et al. 2003). Furthermore, it created a
discrepancy in the naming of the wing digits between the evolutionary literature and
the considerable number of papers in molecular developmental biology because the
chick wing is an important model system. To settle this question would thus facilitate
the integration of evolutionary and developmental biology into the nascent field of
evolutionary developmental biology, because the acid test for inter-disciplinary
integration is the ability of researchers from different fields to agree on the relevant
evidence and the inferences made from it (Nyhart 1995).

In the last 10 years the amount and the quality of evidence regarding the question
of avian digit homology has markedly increased due to novel paleontological finds
and new molecular developmental data. For this reason it may be useful to
summarize this new data and evaluate the conflict in the light of this data. It will be
argued that the preponderance of evidence pointes towards the possibility of a digit

identity frame shift, which led to a dissociation between digit identity and embryological origin of the digits.

Four ways to resolve the conflict

Above I have argued that the question of avian digit homology is essentially a conflict between two kinds of evidence associated with two ways of making scientific inferences. At the one hand there is comparative biology consisting of phylogenetics, comparative anatomy and paleontology which use the comparison of adult structures and methods of phylogenetic inference to identify the avian digits as digits DI, DII and DIII. On the other hand there is developmental biology using comparisons of early developmental stages to infer digit homology. Until very recently the developmental evidence overwhelmingly supported the hypothesis that the avian digits are digits DII, DIII and DIV. This state of affairs is quite distressing, given that the inability of resolving similar conflicts spelled the end to an otherwise successful scientific paradigm at the end of the 19th century, namely that of evolutionary morphology (Nyhart 2002). Letting conflicts like these fester convince students and academic administrators that the field is intellectual bankrupt with dire consequences for its future. We are currently experiencing a renaissance of interest in the connection between development and evolution (Hall 1998; Carroll et al. 2001). The question thus is whether we now, at the beginning of the 21st century, have the technical and conceptual means to overcome the conflicts, which haunted biologists more than a century ago, and whether we succeed in establishing conceptual continuity between evolutionary and developmental biology (Amundson 2005). I think that avian digit homology is a paradigmatic problem for evolutionary developmental biology to solve in its new molecular incarnation.

Given that the avian digit homology problem results from a conflict between two ways of making scientific inferences it is natural to seek to resolve the issue in two principal ways: first by questioning the inferences made from the anatomical and paleontological data, and second by questioning the inferences from the developmental data. Within each category there are currently two ideas discussed, which adds up to four possibilities that we need to consider.

Questioning the inference from paleontological data:

- Birds are not derived from theropod dinosaurs (Feduccia 1996).
- The digits in the hands of theropods are digits DII, DIII and DIV but have assumed the morphology of digits DI, DII and DIII (Pyramid Reduction Hypothesis) (Kundrát et al. 2002; Galis et al. 2003).

The first hypothesis is part of a larger controversy about the evolutionary origin of birds. It is fair to say, however, that the theropod derivation of birds is no longer seriously in doubt. Overwhelming evidence emerging from fossil finds over the last 10 years, in particular the discovery of feathered dinosaurs, have essentially settled the question (Sereno 1999a; Prum 2002). In this paper I will not consider the idea

that birds are anything else but theropod dinosaurs. More recently a more subtle point has been raised by Frietson Galis et al. (2003) that requires closer attention. It has to do with the phylogenetic position of *Eoraptor* and *Herrerasaurus* which will be considered in the next section. This observation has been advanced to support the so-called Pyramid Reduction Hypothesis (Kundrát et al. 2002), which posits that the digits DI and DV were reduced and that then digits DII and DIV assumed the phalangeal formulae of digits DI, DII and DIII.

Questioning the inferences from developmental data:

- The timing of digit development has changed (Axis Shift Hypothesis = ASH) (Shubin 1994; Chatterjee 1998; Garner and Thomas 1998).
- Digit identity is dissociated from the location of the digit anlage (Frame Shift Hypothesis = FSH) (Wagner and Gauthier 1999).

In embryological studies the most frequently used landmark to identify digit anlagen is the location of the first developing digit. This anlage usually develops into digit DIV (Shubin and Alberch 1986), and consequently the digits developing anterior to the earliest developing digit are identified as digits DI, DII and DIII. To
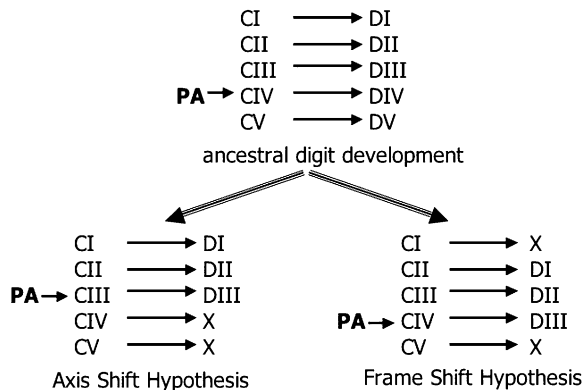

Fig. 1. Schematic comparison of ancestral digit development (top) and the modifications to avian digit develop according to the ASH and the FSH. In the ancestral digit development the primary axis (PA) gives rise to the condensation CIV and the five condensations CI–CV develop into digits of corresponding identities DI–DV. According to the ASH the digits DI, DII and DIII still develop from condensations CI, CII and CIII, but the primary axis has shifted to give rise to CIII. This hypothesis predicts that in birds two posterior condensations do not develop into definite digits. According to the FSH the primary axis remains where it was in the ancestral state but the three digits in the bird wing develop from condensations CII, CIII and CIV. This hypothesis predicts that an anterior and a posterior condensation fail to develop into definite digits. These predictions have been tested with embryological studies that visualize pre-chondrogenic condensations showing that there is an anterior condensation that does not give rise to a definite digit (Feduccia and Nowicki 2002; Kundrát et al. 2002; Larsson and Wagner 2002; Welten et al. 2005).

my knowledge Shubin (1994) was the first to question this inference suggesting that it is possible that there was heterochrony in the sequence of digit development such that in theropods the anlage of digit DIII develops first with progressive reduction of the anlage of digits DIV and DV. Chatterjee (1998) as well as Garner and Thomas (1998) elaborated this scenario (Fig. 1) in response to the paper by Burke and Feduccia (1997) using the primary axis as landmark to identify the avian digits. This scenario has been called ASH (Larsson and Wagner 2002). New developmental data regarding the ASH will be discussed in the section after the next.

The last hypothesis discussed here assumes that the pattern of digit development has not changed, i.e. that the primary axis in the bird wing is homologous to that in other amniotes, but that the developmental fate of the digit condensations has changed, such that condensation CII develops into digit DI and condensation CIII into digit DII, etc. (Wagner and Gauthier 1999) (Fig. 1). This scenario has been called FSH because it assumes a shift in the spatial relationship between adult digit identity and digit condensations.

Evidence regarding theropod digit identity

There are two main steps in the argument that leads to the identification of the bird digits as DI, DII and DIII. The first is the great similarity between the hand of *Archaeopteryx* and that of certain theropod dinosaurs, which are more closely related to *Archaeopteryx* and crown-group birds than to other theropods. The similarities include the phalangeal formula of 2, 3 and 4, which is the same as the plesiomorphic state for Tetrapoda in general, as well as the elongated penultimate phalange, the sharply pointed prehensile claws, as well a numerous characters concerning the shape of each element and their articulation surfaces (Gauthier 1986; Sereno 1993). These similarities imply that the digits of birds are those of the three functional digits of the non-avian maniraptorians (which is a clade in the theropos that includes the birds). The second step is the identification of the theropod digits as DI, DII and DIII based on similarity of the first metacarpal of theropods and that of basal pentadactyl dinosaurs, which has an offset head that directs the first digit away from the other fingers (Gauthier 1986) and the fact that the most basal theropod lineages, *Eoraptor* and *Herrerasaurus*, have two metacarpals posterior to the three functional fingers. These have at most one or two phalanges which have not protruded from the palm. Furthermore the trend to reduce digits IV and V is already visible in crocodiles where digits I, II and III are much stronger than digits IV and V. This pattern is also seen in some non-theropod dinosaurs, like *Heterodontosaurus tucki* (Ornithischia), the sauropodomorphs *Thecodontosaurus antiquus*, *Efraasia diagnostica* and *Massospondylus carinatus* (Gauthier, 1986). This supports the hypothesis that the digits DIV and DV have been reduced in theropods. Galis et al. (2003), however, argue that the phylogenetic relationships among basal dinosaur lineages are not clear and that there remains the possibility that the basal theropods have lost digits DI and DV rather than digits DIV and DV. If this would indeed be

the case, the basis for the theropod digit homology would be only based on the special structure of the most anterior finger (= thumb).

The phylogenetic position of the critical taxa, *Eoraptor* and *Herrerasaurus*, has been debated (Gauthier 1986; Padian 1992; Padian and May 1993; Holtz Jr. 1995; Sereno 1999a, b). While *Eoraptor* was described only in 1993 (Sereno et al. 1993), *Herrerasaurus* has been known from fragmentary evidence since 1963 (Reig 1963). Originally *Herrerasaurus* was classified as a theropod (Reig 1963; Colbert 1970; Benedetto 1973), but this classification was later found to be based on plesiomorphic characters (Novas 1997). In a cladistic re-analysis of this data this taxon was reconstructed as a sister group to the remaining dinosaurs (Gauthier 1986). After the discovery of a more complete specimen the phylogenetic position of *Herrerasaurus* was changed back again to the sister taxon to all remaining theropods (Sereno and Novas 1992; Novas 1993; Sereno 1994). The reason for this conflict between earlier and later phylogenetic reconstructions is that the 12 synapomorphic characters that unite *Herrerasaurus* with the remaining theropods have not been known until the more complete material was discovered (Sereno and Novas 1992; Novas 1993; Sereno 1993, 1994; Sereno et al. 1993). Hence the discrepancies in the literature are largely due to the incompleteness of the original specimen.

Another difficulty was that *Herrerasaurus* has an apparently plesiomorphic character state in the attachment of the pelvic girdle to the spine (two vertebrae instead of five), while both the Ornithischia and the remaining theropods have more than two. In the light of the novel data, this character is either interpreted as a reversal (Novas 1993) or a convergence, because different vertebrae are integrated into the sacrum in the Ornithischia and the Saurischia (Gauthier, pers. comm.).

For *Eoraptor* the situation is more difficult, because it seems to belong to a lineage even more basal than that of *Herrerasaurus* and thus has fewer known synapomorphies with the theropods. Sereno and collaborators (Sereno et al. 1993) list five synapomorphies, of which three are hand characters. These hand characters could be considered problematic for the question considered here, given that we want to assess the homology of these hand characters. Among the remaining two there is one, "extreme hollowing of centra and long bones," that has been considered problematic (Novas 1993) due to wide distribution of this character state in dinosaurs. Hence the *Eoraptor* affiliation is based mostly on hand characters and is problematic in the present context, where digit homology is in question.

The problem with the phylogenetic position of *Eoraptor*, however, is not critical to our argument because the affiliation of *Herrerasaurus* to theropods is supported by 12 synapomorphies excluding many problematic characters, of which only four are hand characters (Novas 1993). Hence, there are at least eight synapomorphies unrelated to hand morphology supporting the theropod nature of *Herrerasaurus*. Since *Herrerasaurus* clearly has two posterior metacarpals the identity of the functional three digits of theropods clearly is DI, DII and DIII. In conclusion, the homology of the theropod fingers is not in seriously in doubt.

Evidence regarding the Axis Shift Hypothesis

As explained above, the ASH assumes a heterochronic change in the sequence of digit development in that, in the bird hand, the first digit to develop is not digit DIV but digit DIII and therefore the "primary axis" does not any longer identify the anlage of digit DIV but that of DIII (Shubin 1994; Chatterjee 1998; Garner and Thomas 1998). The main difficulty in evaluating this hypothesis is that for most of the 20th century embryologist only identified four digit anlagen in the bird wing (Hinchliffe 1977; Hinchliffe and Hecht 1984; Burke and Feduccia 1997), of which the three anterior ones form the definite digits of the bird hand. The location of the primary axis in the bird hand relative to the canonical five digits was not clear.

Embryological studies of limb skeletons in the second half of the 20th century were dominated by two staining techniques, the incorporation of radioactive sulfate (Hinchliffe and Griffiths 1983) and staining with Alcian blue (Müller and Alberch 1990; Burke and Feduccia 1997). Both techniques detect the extracellular matrix produced during chondrification. Hence these techniques identify regions of the embryo in which cartilages differentiate. It is, however, clear that the very first stage of skeletal development in the tetrapod limb is not cartilage differentiation (in contrast to the situation in paired ray finned fish (Grandel and Schulte-Merker 1998)) but the condensation of pre-chondrogenic cells (Shubin and Alberch 1986; Hall and Miyake 1995). In a leap of interpretation, skeletal elements identified with sulfate incorporation and Alcian blue staining, however, used to be identified with pre-chondrogenic condensations, which, by the nature of the staining technique, they could not be, namely pre-chondrogenic. Three different techniques have been used in recent years to overcome this technical limitation.

The first was the work by Kundrát and collaborators from Prague University (Kundrát et al. 2001, 2002) who used the fact that capillary blood vessels degenerate as pre-chondrogenic cells condense to form the first physical precursor of a skeletal element (Seichert and Rychter 1972). This phenomenon is caused by the production of an intrinsic angiogenesis inhibitor by the condensing cells (Hiraki and Shukunami 2000). Using India ink injections Kundrát and collaborators were able to demonstrate a fifth zone of capillary regression in addition to the four expected ones. The fifth capillary regression zone, indicating a possible pre-chondrogenic condensation, is located anterior to the four known digit anlagen (Kundrát et al. 2002).

Larsson and Wagner (2002) at Yale used another technique to visualize pre-chondrogenic condensations, the affinity of condensing cells to Peanut Agglutinin, a lectin specifically binding $Gal\beta1,3$–Gal3NAc residues on glycoproteins (Dunlop and Hall 1995; Zschabitz 1998). With this technique we also found evidence for an additional pre-chondrogenic condensation anterior to the three condrifying digit anlagen (Fig. 2).

Most recently Welten and collaborators at Leiden University (2005) used in situ hybridization to investigate the expression of *Sox9*, a transcription factor gene expressed in condensing skeletogenic cells, to map pre-chondrogenic condensations.
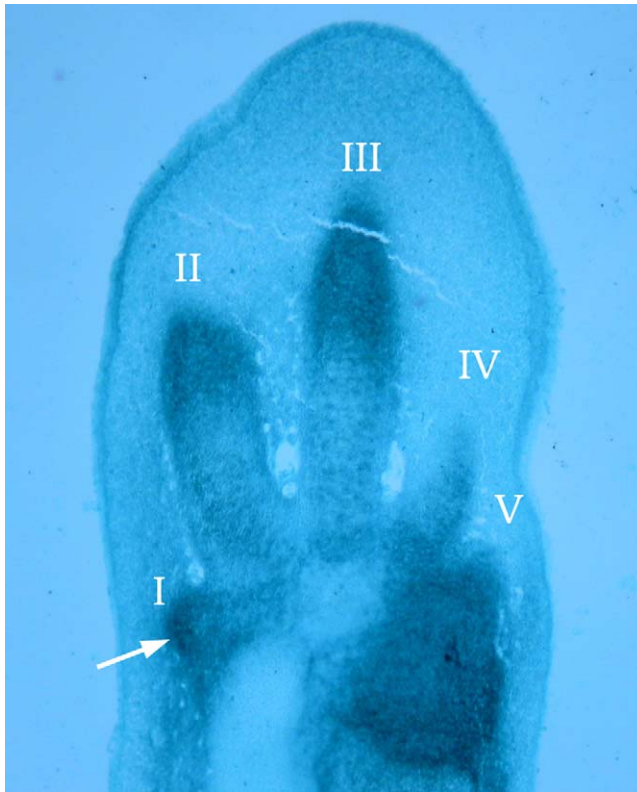
Fig. 2. Section through the chicken wing bud visualizing condensing cells with HRP-conjugated Peanut Agglutinin (H. Larsson, unpublished data, reproduced with permission). Note that there are five centers of condensation with the three middle ones developing into digits. Hence there are two condensations, one anterior and one posterior that do not develop into definite digits. This result is inconsistent with the Axis Shift Hypothesis, but consistent with the Frame Shift Hypothesis.

They also found evidence for an additional anterior condensation confirming the findings of Kundrát et al. (2002), and Larsson and Wagner (2002).

Finally a different approach was used by Feduccia and Novicki at the University of North Carolina (Feduccia and Nowicki 2002) who reasoned that larger species may proceed further in the development of their rudiments and chondrify what in the chick remains completely pre-cartilagous. They investigated the development of the wing of the ostrich with standard Alcian blue staining and found a chondrified protuberance anterior to the three definite digits, consistent with the findings cited above (Feduccia and Nowicki 2002). In summary one has to conclude that the wings of birds (chicken and ostriches) contain five digit condensations of which in the chicken embryo four chondrify and three ossify.

The significance of this finding is that it gives a definite answer to the question whether the primary axis in the bird wing is different from that in other amniotes. If there was an anterior shift of the primary axis (CIII appears first instead of CIV), as hypothesized by Shubin, Chatterjee, Garner and Thomas, one would predict that there are two condensations posterior to the primary axis and two anterior. If, however, the primary axis is not shifted, then there should be three condensations anterior to the primary axis and one posterior, as in all other amniotes investigated (Burke and Alberch 1985; Shubin and Alberch 1986; Müller and Alberch 1990) (Fig. 1). The five papers cited above, clearly show that there are three condensations anterior to the primary axis implying that it has not changed in theropod phylogeny. This effectively falsifies the ASH.

While the overall picture in this area is quite clear there are a few minor issues which should be investigated to test the robustness of the conclusion about ASH. An alternative interpretation of the additional pre-chondrogenic condensation found in recent years is the possibility that is not the ancestral anlage of digit DI but a pre-pollex, i.e. an additional digit anlage which sometimes leads to a spontaneous additional digit anterior to digit DI (Braus 1906), and which is found in many anuran species (Fabrezi 2001). A critical test of this possibility would be to investigate whether a pre-chondrogenic pre-pollex exists in the limb bud of crocodilians, the closest living relatives of birds. This should be possible with the same techniques that have been used in the chick wing. Another issue is the nature of an additional condensation at the posterior limit of the limb bud, which is usually interpreted as the anlage of a pisiform bone. Some authors have speculated that this could be a digit rudiment (Vargas and Fallon 2005; Welten et al. 2005). It would be worthwhile to clarify the nature of this condensation.

Evidence regarding the frame shift hypothesis

The FSH differs from other interpretations of the evidence by rejecting the notion that digit identity is necessarily linked to the location of the digit anlage. The inspiration for this idea derives from the extensively documented fact that organ identity in many characters is not rigidly determined by the embryological origin of the character (Hall 1994). Radical forms of character identity transformations have been known since over a century and are called homeotic transformations. In evaluating the FSH, however, we need to ask specifically whether the dissociation between digit origin and digit identity has been documented.

There is much evidence from the genetic and the experimental literature (Hinchliffe and Johnson 1980) that both digit number and digit morphology can be dissociated. I do not want to attempt a comprehensive list of results to support this conclusion, but only want to point to a particularly dramatic mutation, the phenotype of knockout mutations of *Gli3*. These mutan have super-numerous digits of identical morphology, resembling digit DI (Litingtung et al. 2002; Welscher et al. 2002). The anterior–posterior differentiation of the autopod depends on a signaling

center, called the Zone of Polarizing Activity (ZPA), which produces Shh as signaling molecule (Riddle et al. 1993). *Gli3* codes for a protein necessary for the transduction of Shh signaling. In a *Gli3* mutant the Shh signal is not "read" and the morphology of all digits is identical. Clearly the position of a digit per se does not determine digit morphology, but can be influenced by the gene regulation independent of position.

In this context a rare human disorder, tri-phalangeal thumb (TPT) is of particular interest. A TPT is a malformation of the first digit of the human hand which can be associated with various other symptoms of variable pathogenic origin (Qazi and Kassner 1988) (Fig. 3A) . Among the various kinds of TPT is a distinct form which is, in its strongest expression, a perfect homeotic transformation of the thumb into an index finger, called non-opposable tri-phalangeal thumb (no-TPT) [MIM#190600, from the Online Mendelian Inheritance in Man[TM]: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = OMIM&itool = toolbar].   The morphology of the no-TPT resembles that of the posterior digits 2–5 not only in the number of phalanges, but also in many other fundamental morphological characters (Fig. 3). The metacarpal I (MC-I) is sub-equal to the MC-II and gracile, unlike the normal MC-I which is short and robust (Fig. 3B). In normal hands the MC-I has a proximal epiphysis, while MCs II to V have a distal epiphysis (Heiss 1957). The MC-I of the no-TPT has a distal epiphysis. The proximal joint of the MC-I in primates is saddle shaped allowing the characteristic opposability. The proximal joint of the no-TPT is like that of MCs II to V and therefore not opposable. The pisiform bones normally present at the distal joint of the MC-I (Fig. 3B) are absent in the no-TPT, and the characteristic muscles associated with the thumb are also absent (*Mm. abductor/opponens/adductor pollicis*). There is no question that no-TPT represents a homeotic transformation of digit identity as first hypothesized by Joachimsthal
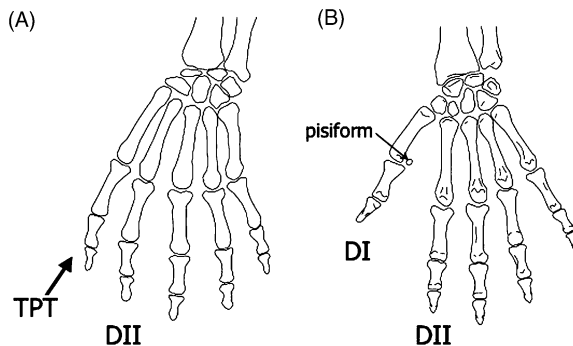

Fig. 3. Drawings of skeletal elements from radiographs of the left human hand viewed from dorsal. (A) the radiograph from a patient with a condition known as Triphalangeal Thumb (TPT) (from Heiss 1957). (B) a radiograph of a normal hand. Note that the first digit in the TPT patient has many characteristics similar to the digit DII. It is likely that this condition is due to a homeotic transformation of DI into DII (Joachimsthal 1900; Warm et al. 1988). Drawing courtesy of Casey Dunn.

(1900) and confirmed by Warm et al. (1988). In 90% of cases the no-TPT is found on both hands and inherited in a dominant autosomal mode (Heiss 1957; Swanson and Brown 1962; Warm et al. 1988). Hence, there are genetic mutations which can change digit identity without compromising the mental ability and the fertility of the affected individuals, otherwise extensive families with this disorder, as studied by Swanson and Brown (1962) and Warm et al. (1988) would not exist. The patient described by Heiss (1957) reported to have an advantage playing the piano also indicating a normal mental capacity. It seems thus plausible that mutations affecting digit identity do not need to have widespread pleiotropic effects and might thus be fixed by natural selection (Fig. 4).

Another piece of evidence relevant to the FSH is that digit identity remains labile until late in development. Dahn and Fallon have shown that, in the chicken, digit morphology is influenced by signaling from the interdigital mesenchyme (IDM) posterior to the developing digit (Dahn and Fallon 2000). Manipulations of the IDM affect digit morphology long after the metacarpals have chondrified, i.e. long after the stage of development that is used as embryological evidence for digit identity by embryologists. This implies that the patterning of digit number and digit identity are temporally dissociated and may thus also evolve independently.

Most recently Vargas and Fallon (2005) have used Hox gene expression patterns to test the FSH. They observed that in late stages of development the digit DI is
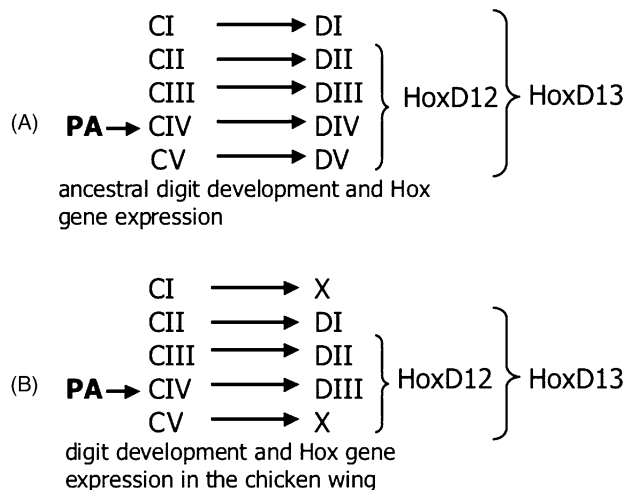

Fig. 4. Schematic comparison of Hox gene expression in the ancestral hand (A) and the bird wing (B) according to Vargas and Fallon (2005). In mice hands and bird hind limb, taken to represent the ancestral condition, the digit which develops from condensation CI, expresses HoxD13 but not HoxD12, while the DII expresses both, HoxD12 as well as HoxD13. In the chick wing, however, the digit developing from the condensation CII expresses HoxD13 but not HoxD12. This is consistent with the Frame Shift Hypothesis which proposes that, in the bird wing, the digit developing from condensation CII assumes the adult identity of digit DI and the digit developing from CIII assumes identity of DII.

distinguished from the other digits by expression of *HoxD13* but the absence of *HoxD12* gene expression while the digits DII–DV express both genes. This was found to be the case in the chick hind limb and in the mouse, where digit identity is not in question. They further tested the association between Hox gene expression and digit identity in the Silkie mutant in chicken, where a digit DII develops anterior to the digit DI in the foot. Again DI was distinguished by the presence of *HoxD13* and absence of *HoxD12* expression from the rest of the digits even though, in this mutant, digit DI is not the most anterior digit in the limb. Hence this expression pattern is not just a marker of the anterior edge of the limb bud, but could be specific to digit DI, at least in mouse and chick hind limb. They then reported the expression pattern in the wing finding that the anterior most definite digit expresses *HoxD13* but not *HoxD12*, consistent with the hypothesis that this digit is in fact DI. This is the first developmental evidence consistent with the paleontological data on digit identity, and also suggests that changes in the Hox gene expression patterns specific to the hand could have effected the digit identity shift as proposed by the FSH (Wagner and Gauthier 1999).

Conflict between anatomical and embryological evidence on digit identity in other animals: *lo strano caso della Luscengola*

Digit reduction is a common mode of evolution and has been studied extensively since the advent of evolutionary theory (Fürbringer 1870). An interesting taxon for the reconstruction of the stepwise reduction of digits and limbs to the complete loss of these structures are the skinks (Greer 1991). Within the skinks at least one case is known where a similar conflict exists between anatomical and embryological evidence as in birds, namely the Italian Three-toed Skink or *Luscengola* (*Chalcides chalcides*) (Orsini and Cheylan 1981). This case has not been discussed in the most recent literature, may be because the primary literature on this problem is entirely in German and French (see below). I want to summarize the relevant results here for the first time in English to make this fascinating segment of the scientific literature more accessible.

Ch. chalcides has three well formed digits in both the forelimb as well as the hindlimb with a rudimentary metatarsal posterior to the three toes. Based on anatomical evidence Fürbringer (1870) (in his time: *Ch. chalcides* was called *Seps tridactylus*) identified the three fully formed digits as digits DI, DII, and DIII, a conclusion confirmed by other investigators looking at the structural similarities between *Ch. chalcides* limbs and that of species with five digits, like *Chalcides ocellatus* (Steiner and Anders 1946; Renous-Lecuru 1973). In contrast all the embryological studies agree that the three digits of *Luscengola* develop from anlagen that normally give rise to digits DII, DIII, and DIV in the lizards with five digits (Sewertzoff 1931; Raynaud et al. 1986), i.e. these digits develop from condensations CII, CIII, and CIV. This is exactly the same situation as in avian digits.

Within the taxon *Chalcides* most species have either five or three digits (Caputo et al. 1995; Greer et al. 1998) with the exceptions of two species with four digits, *Ch. mionecton* and *Sphenops sphenopsiformis*, which is nested within the taxon *Chalcides* according to a new phylogenetic analysis of skinks (Brandley et al. 2005). These two species have a rudimentary metapodial (MP, being either a metacarpal or a metatarsal) MP-V showing that the reduction in these species is clearly postaxial, unlike the tendency in mammals where digit DI tends to be reduced first. Unfortunately, there is no species of *Chalcides* described that, like *Herrerasaurus* in theropods, has three fully formed digits and two metapodials in the adult skeleton. The position of these rudimentary metapodials would be very interesting in terms of the phylogenetic history of digit loss in this taxon.

The discrepancy between the expected pattern of digit reduction based on mammalian examples and the results from *Luscengola* did not escape the attention of the investigators starting with Fürbringer (1870). In this context an interesting point was raised by Steiner and Anders (1946). These authors noted that one needs to distinguish between various forms of digit loss. At the one hand there is the case of rudimentation, basically a melting away of the limb when it loses its function completely. But Steiner and Anders point out that the limb of *Luscengola* is not functionless, in particular the three digits are not reduced but well formed. The limbs are used to balance the body when the animal is at rest (Fig. 5), and to push through vegetation when it is moving slowly (Bruno and Maugeri 1976; Orsini and Cheylan 1981). The limbs, though small, are also used for burrowing in those *Chalcides*

Fig. 5. *Chalcides chalcides* at rest. Note that the small limbs are used to stabilize the body at rest. Hence the much reduced limbs are not functionless, and have been reported to be also used during slow crawling through grass.

species that live in sandy soils. That also explains why the three digits of the *Luscengola* are fully formed and strong, not reduced except in size. Steiner and Anders (1946) propose that the digit loss in *Ch. chalcides* is not a case of rudimentation but due to an adaptive modification of the limb during which some digits get lost. Steiner and Anders call that mode of evolution "adaptive reduction" (p. 545 in Steiner and Anders 1946). Note that in theropods digit loss is also not a case of rudimentation *sensu* Steiner and Anders, because it happens during an adaptive modification of the hand for prey capture (Sereno 1999a; Wagner and Gauthier 1999). Hence it is possible that, in the taxon *Chalcides*, we witness a neontological case of digit identity shift similar to the one in avian digits. The difference, though, is that, in the case of *Chalcides*, we have many closely related species with five, four and three digits in the Recent fauna.

Facts without the force of evidence

In the previous Sections I discussed recent empirical findings pertaining to the question of avian digit homology. The issues raised in these sections do not cover all the arguments that have been fielded in this debate. I have not discussed these remaining arguments because they differ from the results summarized above in that they do not have the force of evidence in this context. They are questions about patterns of digit reduction and mechanistic plausibility (see below) and highlight important issues to be addressed in future research but have not the scientific standing to decide the problem at hand, namely theropod/avian digit identity. I am quick to acknowledge that it is not easy to say exactly what evidence is. But it is also clear that making the distinction between evidence and non-evidence is important for any science. For that reason I want to start this section with a short account of how I make the distinction between evidence and non-evidence.

Evidence consists of empirical facts together with a well-established theoretical framework that allow scientific inferences. Furthermore, the empirical facts need to be directly pertaining to the question at hand, in this case avian digit homology, i.e. observations on birds and their close relatives, the non-avian archosaurs. An obvious example is fossil data which is used with accepted phylogenetic inference methods to establish the phylogenetic relationships of *Eoraptor*, *Herrerasaurus* and the other dinosaurs. This data counts as evidence because the osteology of the hand of these animals is directly relevant for determining the homology of the fully formed digits of theropods and by implication that of birds.

In contrast, facts that do not have the force of evidence are at least of two kinds. At the one hand there are empirical generalizations that do not directly address the question of avian digit homology. The most often raised fact of this kind has to do with patterns of digit reduction in other taxa, in particular lizards and mammals (Sewertzoff 1931; Steiner 1934; Montagna 1945; Raynaud and Clergue-Gazeau 1986; Müller and Alberch 1990; Kundrát et al. 2002). However, findings on say mammals cannot falsify hypotheses regarding theropods. There is no reason to believe that the

generalizations from digit reduction patterns in one taxon, i.e. mammals, have to apply to another taxon. The perceived difference might as well be real and it is important to understand the biological reasons for this difference, if it exists, but one cannot argue that something did not happen in taxon A because it did not happen in taxon B. This certainly cannot be considered as evidence for the problem at hand.

The second type of argument, which cannot be admitted as evidence, are claims of impossibility based on incomplete or fragmentary knowledge. In the context of avian digit identity these arguments come in two flavors. One argument is that a digit identity frame shift is implausible (read ''impossible''), given our current mechanistic knowledge (Feduccia 1999; Galis et al. 2003). The other pertains to mechanisms of evolutionary change, claiming that the inferred changes in digit development cannot be true, because we do not know what the adaptive advantage of them might have been (Galis et al. 2003, 2005). Both arguments point to true facts, namely that our mechanistic knowledge is incomplete and there is no mechanistic explanation of what we see in bird evolution, and it is true that there is no adaptive scenario which explains the differences in digit development between birds and alligators. But these facts are clearly irrelevant, because one is derivative of a very weak knowledge base and the other is only negative. Even though our knowledge of limb development has dramatically improved in the last 20 years we still do not know the developmental-genetic basis of limb evolution. An inference cannot be stronger than the mechanistic knowledge from which it extrapolates. A historical example that shows the dangers of this type of argument is the history of continental drift and plate tectonics. The idea that continents are not nailed to the surface of the earth, proposed by Alfred Wegener in 1912, was largely based on fossils found in various parts of the world, the shape of continents and similarities of rock formations on different continents. The greatest weakness of his theory was its mechanistic explanation. Wegener assumed that continents are pieces of the earth crust ''swimming'' on a sea of magma and driven by centrifugal-, Coriolis- and convection forces. Geo-physicists were quick to point out that this is physically impossible and they were right. What was fallacious, however, was the conclusion that for that very reason the continents did not move at all and thus the continental drift hypothesis is wrong (Schwarzbach 1980). Clearly, what was wrong was Wegener's *explanation* of the phenomenon and not the hypothesis that continental drift has occurred and still is occurring. Similarly the questions how digits and digit development evolved in theropods is a question that needs to be considered independently of whether we have the knowledge and the tools to find out what the mechanisms were that caused that change. For instance it is not controversial that organ rudimentation happens in evolution, but we still do not know whether it is caused by mutation accumulation and genetic drift or by natural selection. A failure to explain what happened has no implications on whether an event occurred or not. This failure only reflects on the limits of our current knowledge.

Of similar structure is the argument that FSH cannot be correct because there is no adaptive reason for this change to happen (Galis et al. 2003). But again, this argument is one of the lack of explanation, not one of relevant empirical evidence. A

historical fact can be true whether we can explain it or not. Hence, one should first focus on the question which hypothesis of fact, ASH, FSH, etc. is correct and only then we should focus on how to explain it. The sum of current evidence is pointing towards the possibility of a digit identity frame shift in the stem lineage of birds and possibly also within *Chalcides* skinks. Furthermore, it is possible that this change was caused by a change in the expression of *Hox* genes (Vargas and Fallon 2005). Now the challenge is to find out whether this mechanistic explanation is true, and what evolutionary forces may have caused the digit identity frame shift.

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