and vapor, respectively. In this case, the force per unit length on the contact line due to surface tension when the contact angle was reduced to zero was $\sigma_{lv}(1 - \cos\theta) \approx 46$ mdyne/cm. The maximum pinning force must be at least as large.

Another manifestation of forces on the contact line can be seen when a superfluid drop was placed on an inclined surface. Figure 3 shows an edge-on view of a drop on a Cs surface inclined at ~10° to the horizontal; a pendant drop of fluid formed by forcing He down the capillary faster than the superfluid film on the outer surface could drain it can also be seen in the upper right corner. The most remarkable feature of the drop on the substrate is that it is stationary. Even vigorous shaking of the apparatus, which caused easily discernible waves in the drop, did not cause it to flow down the incline. The downhill edge of the drop had the same contact angle as the advancing edge of a growing drop, whereas the uphill edge had a vanishing contact angle. As more fluid was added to the drop, it eventually rolled down the incline, often with a jerky stick-slip motion. Subsequent drops immediately spread out across the path of the previous drop and rapidly flowed downhill. It seems as if the first drop, which moved across a dry substrate, left a trailing film that "lubricates" the motion of subsequent drops. This film, which persisted for hours, may be related to the metastable thick films we have observed in earlier experiments (6). The trailing film had submicroscopic thickness and was invisible in an edge-on view. It could be detected ellipsometrically and was superfluid because the local heating of a spot with a laser beam produced a thermomechanically driven bump in the film profile.

Superfluid droplets on Cs substrates have spreading and flow properties that are not simple consequences of bulk superfluid behavior. Liquid He has exceptional chemical purity, and the heterogeneity of our Cs surface is constrained by thermodynamic adsorption measurements. For these reasons, He on Cs would naïvely be expected to display nearly ideal reversible spreading behavior, because even the complications due to viscosity are negligible. In contrast, superfluid contact angles are found to be even more hysteretic than typical classical fluid drops on macroscopically heterogeneous surfaces. The hysteresis is so extreme that the superfluid contact line appears to move in only one direction, that is, so as to increase the wetted area.

It is difficult to reconcile these observations with standard models of contact-angle hysteresis. Regarded as a superfluid, droplets are remarkable because they can resist flow against a substantial chemical potential gradient. Both of these effects are presumably due to metastable configurations of the superfluid contact line, which have been inaccessible to experimental observation until very recently. In order to attribute the metastability to extrinsic defects, a mechanism that would allow small defect concentrations to cause extremely large hysteresis would need to be identified.

## REFERENCES AND NOTES

1. J. Wilks and D. S. Betts, *An Introduction to Liquid Helium* (Clarendon Press, Oxford, ed. 2, 1987).
2. E. Cheng, M. W. Cole, J. Dupont-Roc, W. F. Saam, J. Treiner, *Rev. Mod. Phys.* **65**, 557 (1993), and references therein.
3. F. Brochard-Wyart, *J. Phys. (Paris) II* **3**, 21 (1993).
4. D. Reinelt, H. Gau, S. Herminghaus, P. Leiderer [*Czech. J. Phys.* **46**, 431 (1996)] presented surface plasmon micrographs of the region of a Cs substrate covered with superfluid. These images, however, did not contain information about the contact angle.
5. J. E. Rutledge and P. Taborek, *Phys. Rev Lett.* **69**, 937 (1992).
6. P. Taborek and J. E. Rutledge, *ibid.* **71**, 263 (1993).
7. R. E. Johnson and R. H. Dettre, in *Wettability*, J. C. Berg, Ed. (Dekker, New York, 1993), pp. 1–73.
8. The contact angle has been measured by J. Klier, P. Stefanyi, and A. F. G. Wyatt [*Phys. Rev. Lett.* **75**, 3709 (1995)]. They obtained a slightly larger value at $T = 1.16$ K.
9. D. Li and A. W. Neumann, *Colloid Polym. Sci.* **270**, 498 (1992).
10. L. W. Schwartz and S. Garoff, *Langmuir* **1**, 219 (1985).

11. This work was supported by NSF grant DMR 9623976.

7 July 1997; accepted 15 September 1997

---

# Developmental Patterns and the Identification of Homologies in the Avian Hand

Ann C. Burke and Alan Feduccia*

Homologies of digits in the avian hand have been debated for 150 years. Cladistic analysis nests birds with theropod dinosaurs. Theropod hands retain only digits I-II-III, so digits of the modern bird hand are often identified as I-II-III. Study of the developing manus and pes in amniote embryos, including a variety of avian species, shows stereotyped patterns of cartilage condensations. A primary axis of cartilage condensation is visible in all species that runs through the humerus into digit IV. Comparison to serially homologous elements of the hindlimb indicates that the retained digits of the avian hand are II-III-IV.

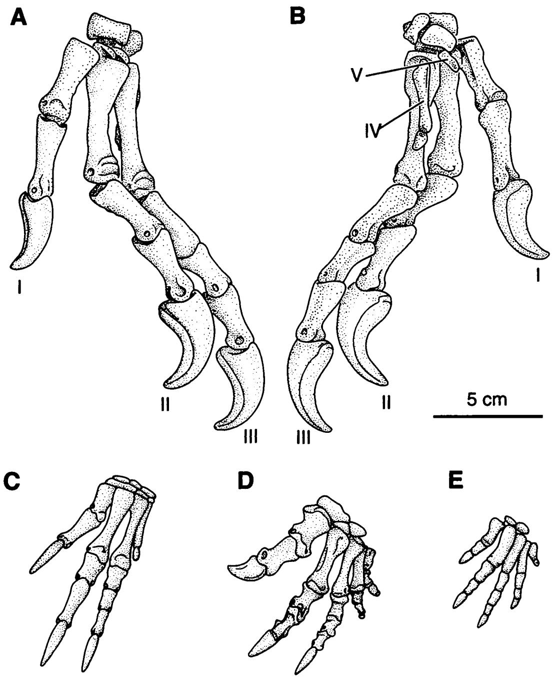
A long-standing disagreement persists in the identification of the three remaining digits in the adult avian manus. Many developmental biologists use conservation of embryonic patterning to establish homology (1, 2), while many paleontologists use the methodology of phylogenetic systematics to define homology a posteriori from cladistic analysis of multiple synapomorphies (3, 4). Cladistic analyses nest birds within the theropod dinosaurs (5). One key synapomorphy uniting theropods is a manus reduced to three digits. These digits are identified as I-II-III because of early theropods such as *Herrerasaurus* (Fig. 1) that show dramatic reduction of digits IV and V (6). A theropod origin of birds implies that the digits of the avian manus must also be I-II-III (7, 8). However, neontologists have identified the digits in the avian hand as II-III-IV in consideration of developmental anatomy. Despite several excellent descriptive studies (2, 9) and thorough reviews of the arguments (1, 8, 10, 11), no consensus on digit homology has emerged. We address the issue of avian digits, using a developmental pattern that is conserved in all amniotes examined. We examined forelimb development in turtle, alligator, and several avian embryos (12). We also compare development of the serially homologous fore- and hindlimbs in birds.

The identity of digits in modern birds as I-II-III gained acceptance because the phalangeal formula of *Archaeopteryx*, an undisputed early avian (13), coincides with digits I-II-III of the generalized archosaur hand [2-3-4-5-3 (14)]. Phalangeal formulae are widely variant among many taxa, however, and individual specimens of *Archaeopteryx* have varying phalangeal formulae in the pes (15). Furthermore, this character is developmentally plastic. For example, bone morphogenetic protein 4 (BMP4) mediates apoptosis and recent studies have shown that experimental blockage of BMP4 signaling in the avian limb bud can result in hands that are missing only the most distal phalanxes (16). Regardless, the transition to modern

---

Department of Biology, Coker Hall, Campus Box 3280, University of North Carolina, Chapel Hill, NC 27599–3280, USA.

*To whom correspondence should be addressed.



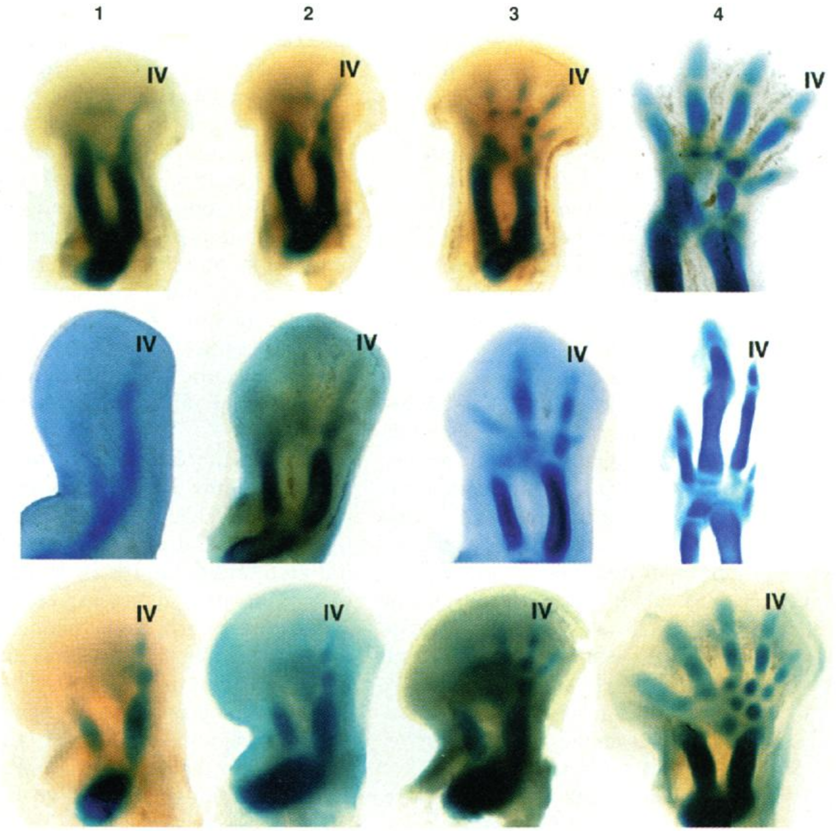


**Fig. 1 (Left).** Left carpus and manus of the late Triassic, earliest known theropod *Herrerasaurus* in dorsomedial (**A**) and ventrolateral (**B**) views. Lower, left manus of *Syntarsus/Coelophysis* (**C**), *Plateosaurus englehardti* (**D**), and *Lespthosaurus diagnosticus* (**E**), all late Triassic, to show the reduction of digits IV and V, and the elimination of V in (C). Drawn to same scale. Modified from *Journal of Vertebrate Paleontology* (*6*) and other sources. I through V indicates manual digits I through V. **Fig. 2 (right).** Dorsal views of four stages in the development of the right manus in *Alligator mississippiensis* (top row), *Gallus* (middle row), and *Chelydra serpentina* (bottom row). The stages in column 1 represent the early appearance of the primary axis. Column 2 shows the early digital arch. This pattern has been described for numerous species by many classical morphologists and more recently in chicken, turtles, alligators, lizards, and mice (*2, 9, 11, 17*). Columns 3 and 4 show subsequent development of the digits. Note the transient appearance of digit V in *Gallus*. Digit IV is labeled in each specimen. Images are not to scale.

birds from *Archaeopteryx* requires loss of additional phalanxes to reach an avian phalangeal formula of 2,2,1 whether digits are numbered I-II-III or II-III-IV. The phylogenetic and ontogenetic flexibility of phalangeal number render this a dubious criterion for identifying the homology of digits between higher taxa.

The development of the cartilaginous precursors of the limb skeleton is highly stereotyped among all classes of amniotes (*1, 2*). Furthermore, as serially homologous appendages, the early establishment of skeletal pattern is nearly identical between fore- and hindlimbs in a given species. Briefly, an initial condensation of precartilage cells in the forelimb bud forms a "Y" shape representing the humerus branching into the radius and ulna (Fig. 2). Following this stage, the postaxial element (the ulna) dominates, extending and branching to give rise to the intermedium and the ulnare. The postaxial condensation extends distally through the ulnare into the precursors of distal carpal IV and metacarpal IV. This postaxial, distal extension has been termed the "primary axis" (Fig. 2; column 1), and is a common feature of all amniote classes (*11, 17*). As development proceeds, digit IV forms as a linear distal development of the primary axis. Digit V appears de novo immediately posterior to the primary axis, and digits III, II, and I form in a posterior-

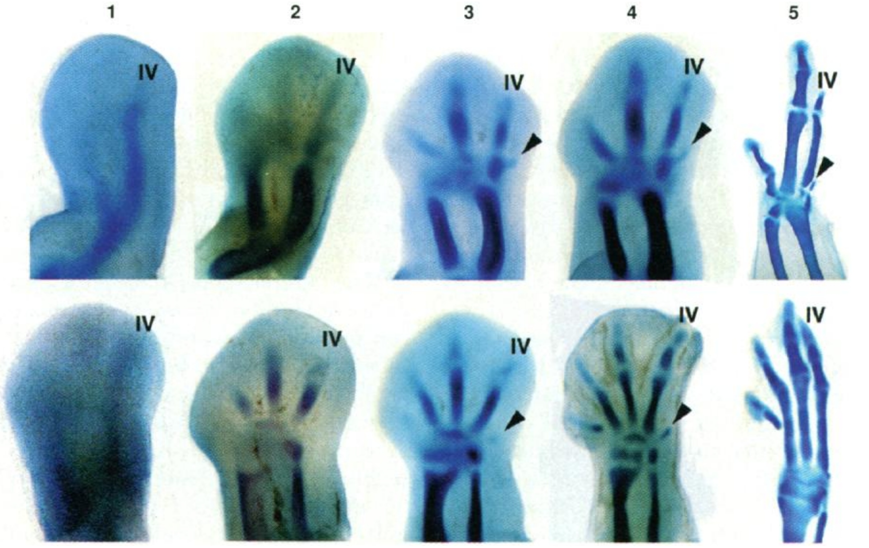


**Fig. 3.** Comparison of right manus (top row) and pes (bottom row) development in the chicken, dorsal views. Note the transient appearance of digit V in the manus and pes (arrowheads). Digit IV is labeled in each specimen. Images are not to scale.

to-anterior series known as the digital arch. The early developmental stages of alligator, turtle, and bird demonstrate the same pattern (Fig. 2) (*11*).

This pattern is repeated in the hindlimb: The femur branches into the preaxial tibia and postaxial fibula in a "Y"-shaped condensation, the postaxial fibula bifurcates into an intermedium and a fibulare, and distal tarsal IV and metatarsal IV extend distally, forming the primary axis (Fig. 3). As in the forelimb, digit V forms immediately posterior to the primary axis, and a digital arch extends anteriorly. A topographic comparison of development of the manus and pes show similar formation of the primary axis that generates digit IV (Fig. 3). In the birds we examined (*12*), the anlage of digit V appears in both the manus and pes. Metatarsal V is clear in the pes, is present in Mesozoic birds (*18*), and persists in some modern species as a bony element in adults. Digit V in the manus is a transient structure but is clearly visible at certain stages (Figs. 2 and 3). Holmgren reported the anlage of a first metacarpal in embryos of *Struthio* (ostrich) and *Larus* (gull) (*19*). We saw only very weak staining in the area anterior to digit II in *Struthio*, *Phalacrocorax* (cormorant), and *Gallus* (chicken) and could not confirm the transient presence of a first digit.

The characteristic pattern of connectivity in the skeletal anlagen in the manus and pes suggests specific morphogenetic mechanisms (*20*). A molecular genetic understanding of vertebrate limb development has begun to emerge in the last 15 years. Although direct causality between gene expression and morphology is not always clear, stereotyped expression of certain genes accompany the stereotyped pattern of skeletal formation (*21*). For instance, the expression of the *Abdominal B*–related genes in the Hox A and D clusters show consistent expression patterns in the autopod of both the fore- and hindlimbs of chickens and mice (*22*). The consistency of these gene expression patterns between mammals and birds suggests that they are primitive for amniotes and adds molecular evidence for the homology of the ontogenetic pattern.

Comparison of normal variation and reduction of digits in a wide variety of tetrapod taxa also supports a common generative pattern of limb skeletal development. The first digits lost in a lineage are always digits I or V. This is seen in amphibians, mammals, lizards, and turtles (*23*). The theropod hand is therefore unusual, because the retention of digit I and the loss of digit IV violates a consistent pattern of digital reduction in all other tetrapods. It may have evolved by strong selection for a grasping, raking hand (*6*).

The developmental evidence of homology is problematic for the hypothesized theropod origin of birds. This conflict pivots on the significance awarded to different types of data in the identification of homology. Comparative ontogenetic data suggest that a conserved developmental program is causally involved in patterning the amniote limb. The identification of early embryonic topographic landmarks and the connectivity of cartilage precursors permits the identification of specific digits as they develop in the pentadactyl hand.

It is parsimonious to assume that the theropod limb developed with a typical primary axis through distal carpal IV followed by typical development of the digital arch and digits III, II, and I, followed by a subsequent regression of the precursors of the fourth digit. Strong reduction of digit IV after its precocious appearance is seen in some lizards (*24*). The alternative would be an entirely new developmental program. It is unlikely that a shift between the typical amniote mode of development that generates digit IV through the primary axis, to a limb that develops digit III through a convergent primary axis, would maintain the pattern of cartilage condensation that is identical in avian, crocodilian, chelonian, and mammalian limbs, and the consistent patterns of gene expression between chicken and mouse limbs.

As the primary axis invariably gives rise distally to digit IV in amniotes, it serves as a consistent marker of digital identity and assigns the homologies of the reduced bird hand as digits II-III-IV. A variation of this pattern wherein the primary axis runs through digit III, would eliminate any phylogenetic significance from the morphological and molecular similarities in amniote limb development. If such a condition could be demonstrated, patterns of limb development would have to be decoupled from phylogeny, and this stereotypic pattern of development accepted as convergence.

The discrepancies that arise between different methods draw attention to a central problem of evolutionary biology, the distinction between homoplasy and synapomorphy. It remains an open question how heavily to weigh developmental characters in phylogenetic reconstruction. The inclusion of fossil characters is essential to our understanding of evolution. However, until we disqualify developmental patterns as a means of establishing homologies, the developmental patterns that identify avian digits as II-III-IV, cannot be ignored.

## REFERENCES AND NOTES

1. J. R. Hinchliffe and M. K. Hecht, *Evol. Biol.* **18**, 21 (1984).
2. N. Shubin and P. Alberch, *ibid.* **20**, 319 (1986).
3. J. A. Gauthier, *Mem. Calif. Acad. Sci.* **8**, 1 (1986); J. M. Clark, *J. Vertebr. Paleontol.* **12**, 532 (1992).
4. G. Nelson, in *Homology: The Hierarchical Basis for Comparative Biology*, B. K. Hall, Ed. (Academic Press, San Diego, CA, 1994), pp. 101–149.
5. L. M. Witmer, in *Origins of the Higher Groups of Tetrapods*, H.-P. Schultz and L. Trueb, Eds. (Cornell Univ. Press, Ithaca, NY, 1991), pp. 427–466; L. M. Chiappe, *Nature* **378**, 345 (1995); K. Padian, *ibid.* **382**, 400 (1996); L. M. Witmer, *Science* **276**, 1209 (1997).
6. P. C. Sereno, *J. Vertebr. Paleontol.* **13**, 425 (1993); ______ and F. E. Novas, *Science* **258**, 1137 (1992).
7. C. McGowan, *Make Your Own Dinosaur out of Chicken Bones* (Harper Perennial, New York, 1997); K. Padian, *Am. Sci.* **85**, 178 (1997); C. Zimmer, *Discover* **18** (no. 6), 68 (1997).
8. N. Shubin, in *Homology, the Hierarchical Basis for Comparative Biology*, B. K. Hall, Ed. (Academic Press, San Diego, CA, 1994), pp. 249–269.
9. W. Montagna, *J. Morphol.* **76**, 87 (1945); J. R. Hinchliffe, in *Vertebrate Limb and Somite Morphogenesis*, D. Ede, J. R. Hinchliffe, M. Balls, Eds. (Cambridge Univ. Press, Cambridge, 1977), pp. 293–309; J. R. Hinchliffe and P. Griffiths, in *Development and Evolution*, B. C. Goodwin, N. Holder, C. C. Wylie, Eds. (Cambridge Univ. Press, Cambridge, 1983), pp. 99–121.
10. M. K. Hecht and B. M. Hecht, *Acta Palaeontol. Pol.* **38**, 329 (1994).
11. G. B. Müller and P. Alberch, *J. Morphol.* **203**, 151 (1990).
12. We examined a series of embryos of turtles (*Chelydra serpentina* and *Chrysemys picta*), an alligator (*A. mississippiensis*), and birds from diverse orders including *Struthio*, *Phalacrocorax*, *Anas*, and *Gallus*. Embryos were fixed in 10% formalin, stained with alcian blue, and cleared in glycerol.
13. *Archaeopteryx* is considered a member of a monophyletic class Aves on the basis of the following synapomorphies: (i) presence of feathers with rachis, barbs, and barbules; (ii) reflexed hallux; (iii) opisthopubic pubis with hypopubic cup; (iv) scapula articulating with coracoid at 90° angle.
14. A. S. Romer, *Osteology of the Reptiles* (Univ. of Chicago Press, Chicago, 1955).
15. P. Wellnhofer, *Nat. Hist. Mus. Los Angel. Cty. Sci. Ser.* **36**, 3 (1992).
16. H. Zou and L. Niswander, *Science* **272**, 738 (1996).
17. A. C. Burke and P. Alberch, *J. Morphol.* **186**, 119 (1985).
18. L. Hou, L. D. Martin, Z. Zhou, A. Feduccia, *Science* **274**, 1164 (1996).
19. N. Holmgren, *Acta Zool.* **36**, 243 (1955).
20. G. F. Oster, N. Shubin, J. D. Murray, P. Alberch, *Evolution* **42**, 862 (1988).
21. R. L. Johnson, R. D. Riddle, C. Tabin, *Curr. Opin. Genet. Dev.* **4**, 535 (1994); N. Shubin, C. Tabin, S. Carrol, *Nature* **388**, 639 (1997).
22. C. E. Nelson *et al.*, *Development* **122**, 1449 (1996).
23. E. Morse, *Ann. Lyc. Nat. Hist. N.Y.* **10**, 3 (1872); amphibians: P. Alberch and E. Gale, *Evolution* **39**, 8 (1985); lizards: A. E. Greer, *J. Herpetol.* **25**, 166 (1991); (*24*); lizards and mammals: R. Lande, *Evol.* **32**, 73 (1978); mammals: J. R. Hinchliffe and D. R. Johnson, *The Development of the Vertebrate Limb* (Clarendon Press, Oxford, 1980).
24. A. Raynaud, in *Biology of the Reptiles*, C. Gans and F. Billet, Eds. (Wiley, New York, 1985), vol. 15, pp. 61–148.

25. We thank N. Shubin and L. Witmer for valuable discussion on the manuscript. S. Whitfield did the illustrations and R. Cheeks helped with photography. Permission to modify drawings for Fig. 1 courtesy of *Journal of Vertebrate Paleontology*. A.C.B. thanks P. Alberch for early support of these ideas.

30 June 1997; accepted 15 September 1997