

Chapter 14 Limb Diversity and Digit Reduction in Reptilian Evolution

Michael D. Shapiro, Neil H. Shubin, and
Jason P. Downs

THE STUDY OF morphological rules, or trends, offered classical biologists the opportunity to address the mechanisms underlying the evolution of anatomical designs. Regularities in evolution suggested that common functional or developmental rules governed the transformation of structures. Parallelism is one such example. If different groups share a similar set of developmental mechanisms, then the parallel evolution of similar designs will be a frequent event in the history of life. Similarly, convergent adaptations can arise from the biomechanical or kinematic demands placed by selection on different taxa. Indeed, the selective and developmental explanations for trends are not mutually exclusive.

The reptilian limb offers an excellent opportunity to explore the persistence of, and mechanisms behind, morphological trends. The origin of new adaptive designs, from flight to cursoriality, involves changes in the shape, proportion, and number of bones in the limb. More often than not, bones are lost during the adaptive evolution of new designs. In fact, among living amniotes, only *Sphenodon* and some lizards retain the ancestral phalangeal formula (Romer 1956; Hopson 1995). Reduction is a common mode of limb evolution in other groups as well, being the predominant pattern of morphological change not only in reptiles but also in salamanders, frogs, and synapsids (including mammals). Indeed, this trend in the evolution of tetrapods is so common that it has been called a rule of morphological evolution (Sewertzoff 1931).

Here, we present an analysis of digital patterns in a phylogenetically and functionally diverse set of reptiles. These data

can serve to elucidate the possible functional and developmental mechanisms behind major transformations in reptilian limb structure, and our analysis of this database reveals regularities in the patterns of phalangeal and digital loss. This analysis further reveals qualitative differences between the reduction of digits and the loss of an entire digit. Patterns of digit reduction and loss can be clade specific and/or correlated to major differences in functional design, as revealed by the parallel acquisition of similar patterns in distantly related taxa.

What Is a Reptile?

The use of “Reptilia” to designate a monophyletic group is a recent development in a long taxonomic history marked by an ever-changing circumscription. Though Linnaeus (1758) earns credit for the name “Reptiles” and Laurentus (1768) for raising “Reptilium” to class status, both were building on the Aristotelian tradition to classify all nonmammal, nonbird tetrapods together. With the systematization of phylogenetic taxonomy (de Queiroz and Gauthier 1990, 1992, 1994), “Reptilia” was applied to the clade of amniotes stemming from the most recent common ancestor of turtles and saurians (lepidosaurs and archosaurs; Gauthier et al. 1988a, 1988c). By including Aves and excluding all of Synapsida, this usage makes “Reptilia” a monophyletic group, the most exclusive to include crown groups Testudines, Lepidosauria, and Archosauria.

Following Gauthier et al. (1988b), our usage of “reptile”

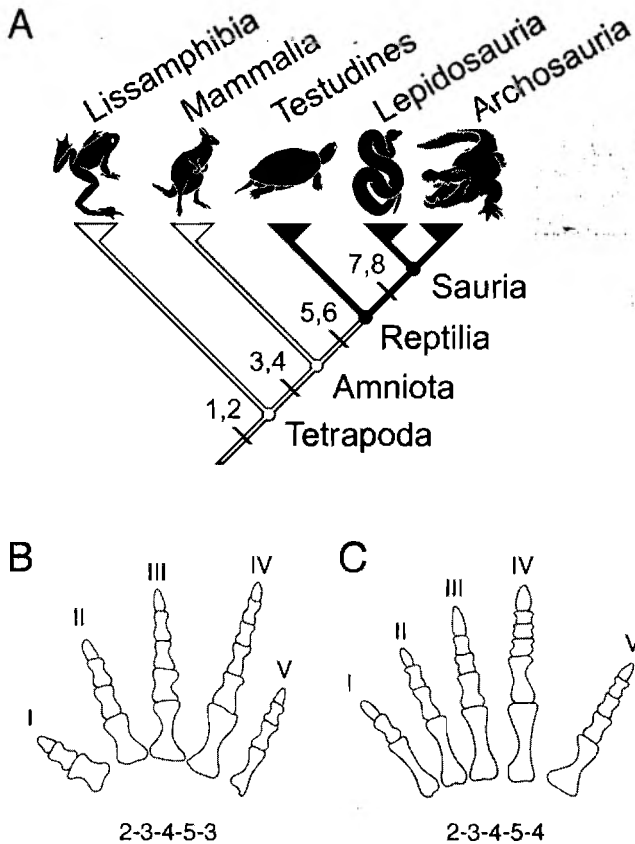


Figure 14-1 (A) Cladogram showing phylogenetic relationships of the Tetrapoda as proposed by Gauthier et al. (1988b). All names refer to a crown group. The eight numbers refer to the following characters: (1) pentadactyly; (2) dissociation of the pectoral girdle from the skull; (3) internal fertilization; (4) amniotic egg; (5) β -keratin in integumentary structures; (6) uricotelic metabolism; (7) large retroarticular process; (8) impedance-matching auditory system. Representative ancestral digit configurations of reptiles, based on the (B) manus and (C) pes of the basal amniote *Labidosaurus* (after Sumida 1997).

here refers to the species of Reptilia, the clade stemming from the most recent common ancestor of Testudinata and Sauria (fig. 14.1A). Although Aves is nested within Reptilia, this chapter will focus more specifically on Testudinata, Lepidosauria, and nonavian Archosauria. “Testudinata” refers to crown turtles and their turtle-shelled out-groups (Joyce et al. 2004); “Lepidosauria” refers to the most exclusive clade that includes Squamata (lizards, snakes, and amphisbaenians) and *Sphenodon* (Gauthier et al. 1988b); and “Archosauria” refers to the most exclusive clade that includes Crocodylia (crown crocodylians) and Aves (crown birds), and therefore also includes pterosaurs and nonavian dinosaurs (Gauthier et al. 1988b).

Primitive phalangeal formulae for testudinates, lepidosaurs, and archosaurs were assumed to match the basal amniote condition: 2-3-4-5-3 (digit I to digit V) for the manus and 2-3-4-5-4 for the pes (Romer 1956; fig. 14.1B, C).

Construction of Database and Mosaic Plots

A database of tetrapod phalangeal formulae was compiled from the neontological and paleontological literature and from museum specimens (see appendix table 14A.1). Additionally, an unpublished analysis of testudinate limb diversity was kindly made available by C. Crumly (later published in Crumly and Sánchez-Villagra 2004). We were unable to use many studies of digital formulae because the absence or reduction of a digit was not distinguished. For example, some authors use 0 in a digital formula to denote either reduction of a digit to a metacarpal or metatarsal only, or complete loss of phalanges and their supporting metapodial; such data were not useful for this analysis. As a familiar example, a phalangeal formula of 0-0-3-0-0 does not adequately describe the elements present in the manus of the modern horse because it implies that digit I, which is completely absent, and digit II, represented by a splintlike metacarpal, are equivalent. In our analysis, absent elements are denoted with X, those with metapodials only are denoted with 0, and those with phalanges are denoted by the number of phalanges present. Hence, we would have scored a horse as X-0-3-0-X.

Only cases of reduction from ancestral formulae (see below) were analyzed, and limbs with hyperphalangy of any digit, or lacking digits altogether, were excluded. Fossil forms were also excluded in cases of questionable completeness or homology. These criteria placed an emphasis on only utilizing cases in which issues of phylogenetic relationships and homology were well established. These criteria are discussed in the next section.

The frequency of different patterns of reduction is presented graphically as a set of charts known as mosaic plots (fig. 14.2). The appendage of each higher-level taxon is figured as five columns, with each digit a subdivided column. The length of the subdivisions within each column corresponds to the frequency of each kind of reduction seen in that digit. This type of plot provides a rapid visual assessment for the way in which digital reduction patterns differ between taxa and among digits in a particular taxon. Configurations listed in the appendix were reduced to a data matrix that tabulated kind of reduction with higher-level taxon. Reductions in each digit were coded in the following way (fig 14.2): one phalanx lost, all phalanges lost, entire digit lost, and unmodified.

Differences in the width of the columns in the mosaic plots are the product of an unavoidable artifact of homology assessment; not all types of variation could be factored into our coding scheme. Two kinds of coding difficulties exist, one where a single type of reduction can be coded in multiple ways, the other where a transformation cannot be coded at all. In the first instance, in a hypothetical lineage with only a

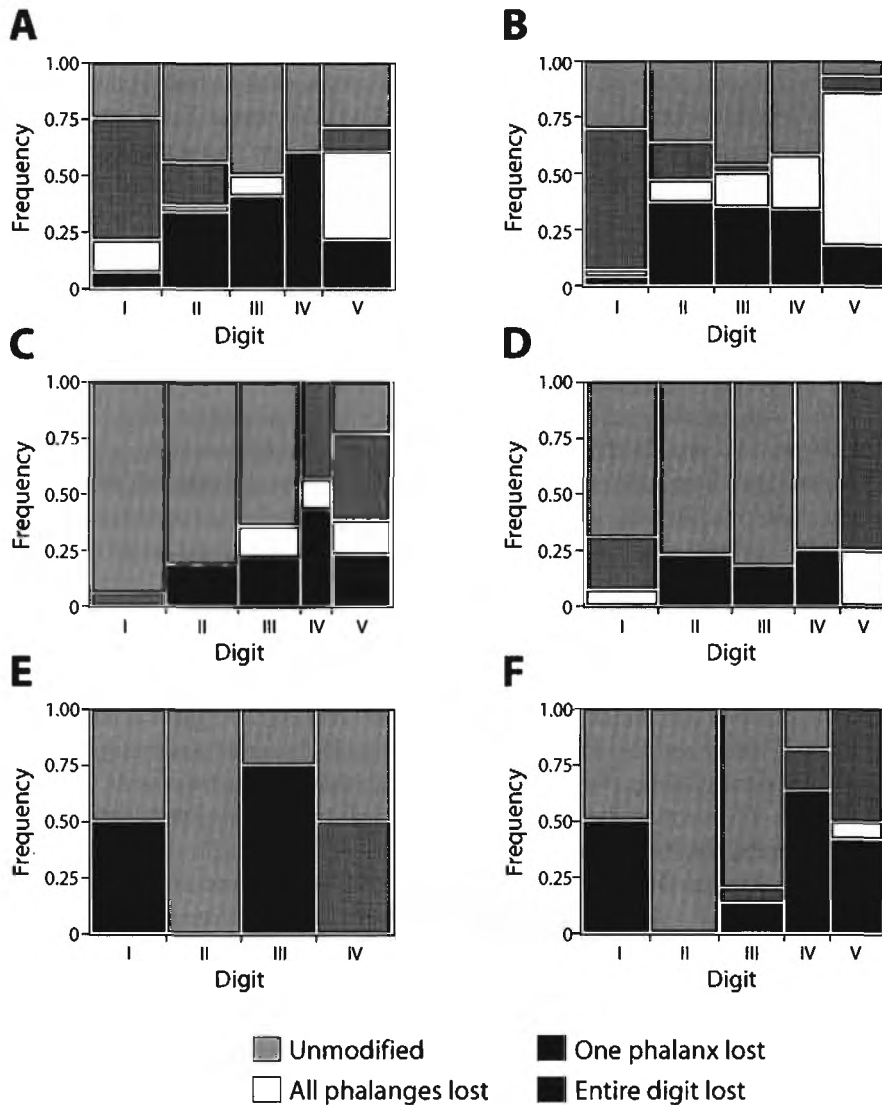


Figure 14-2 Mosaic plots of digit reduction patterns in (A) lepidosaur manus, (B) lepidosaur pes, (C) archosaur manus, (D) archosaur pes, (E) caudate manus, and (F) caudate pes. Each digit is presented as a separate column. The frequency of different reduction states (unmodified, one phalanx lost; all phalanges, but not metacarpal/metatarsal, lost; entire digit lost) is depicted for each digit.

single phalanx in the ancestral state, the loss of this phalanx would also involve loss of all phalanges—two different states in our coding scheme. The second type of difficulty is exemplified by testudines. Digit IV of many testudine configurations loses two phalanges relative to the ancestral amniote formula, a type of transformation that cannot be coded. Since our goal is to compare digit reduction patterns within and between taxa, we adopted a coding scheme that could be applied to all digits. Hence, the loss of two phalanges could not be coded for digit IV because such a loss is synonymous with the loss of all phalanges in digit I. Similarly, we could not score the loss of three phalanges in digit IV because digits III and V (manus only) have only three phalanges in the ancestral state. If we were to apply our mosaic

plot analysis to testudines, therefore, the column for digit IV would be very narrow because our usable sample of digit IV reductions in this group would be small. Variation among the digital patterns of reptiles is so extreme that no single coding scheme could account for their patterns of reduction. The approach that we chose maximized the comparisons between homologous elements, as discussed below.

Digit Homology and Identity

The limbs of reptiles whose digital homologies are ambiguous were excluded, and only ossified elements in adult limbs were considered; however, adult configurations may not al-

ways reflect embryonic condensation patterns (see the section "Discussion"). We did not assign identities to individual phalanges, but present the phalangeal counts for each digit in the limb. For example, using this scheme, a digit I with a single phalanx would be scored as having lost a single, unidentifiable phalanx relative to the ancestral amniote condition, not as having lost specifically the first or second phalanx of that digit. This criterion allowed comparisons between taxa.

Digit homologies are sometimes difficult to establish when fewer than five digits are present. We assigned digit homologies and identities in reduced configurations based principally on patterns of loss within a clade, adult morphology, and developmental origins of digits. In the absence of developmental data, for example, intra- and interspecific variation revealed which digit was missing in four-digit turtle limb configurations. Within several species (*Chersina angulata*, *Geochelone elephantopus*, *Homopus areolatus*, and *Testudo* spp.), pes configurations vary between 2-2-2-2-1 and four digits with two phalanges each (Zug 1971). In these cases, we determined that the four-digit formula was 2-2-2-2-X for two reasons: (1) the remaining digits had the phalangeal formula of digits I-IV in the five-digit morph, and (2) this formula was more parsimonious than the alternative configuration of X-2-2-2-2. Assuming that the ancestor of these species had five digits, the X-2-2-2-2 configuration would necessitate both loss of the first digit and hyperphalangy of the fifth.

Other configurations were difficult to evaluate without making assumptions based on known developmental data. In pentadactyl amniotes, for example, the condensation of digit IV is the first to appear along the "primary axis" (Burke and Alberch 1985; Shubin and Alberch 1986; Müller and Alberch 1990; Shapiro 2002). Hence, in lepidosaurs with only one digit, we scored digit IV as present (e.g., *Lialis*, X-X-X-0-X).

Results

Testudinata: Manus

Digit I is variable among turtle manus configurations examined. This digit is absent from one-third of configurations, while others lose one (42% of configurations; 5/12) or two (25%; 3/12) phalanges.

Digits II-V tend to be reduced to similar numbers of phalanges in all configurations. A complete (three phalanges) digit II is present in only two configurations (2-3-3-3-3 and 2-3-3-3-2), and two phalanges are lost in the most reduced configuration encountered (X-1-2-1-1; *Testudo*). More com-

monly, one phalanx is lost from digit II (75%; 9/12), yielding a total of two.

Likewise, two or fewer phalanges are typically retained by digits III-V. Digits II, III, and IV are reduced to two phalanges in all but two configurations (2-3-3-3-3 and 2-3-3-3-2), and digit V is similarly reduced in all but one configuration (2-3-3-3-3). Digit III never bears less than two phalanges, but digits IV and V are reduced to one phalanx in 33% (4/12) and 58% of (7/12) configurations, respectively.

Testudinata: Pes

Digit I is not subject to reductions in the turtle pes and always bears two phalanges.

Digit II loses a single phalanx from the ancestral three in one-third of configurations but is otherwise unaltered. Reductions of digit II occur in a variety of taxa, including *Proganochelys* (2-2-2-2-2), the earliest well-known testudinate (Gaffney 1990).

No turtle pes bears the ancestral amniote condition of four phalanges on digit III. Two-thirds of configurations lose a single phalanx, while the other one-third loses two.

Digit IV has four phalanges in the testudinate configuration with the least reductions (2-3-3-4-3, restricted to trionychids only) but is otherwise reduced to three or two phalanges.

The phalangeal count for digit V is the most variable in the testudinate pes, ranging from four phalanges to absent. Only *Dermatemys* (2-3-3-3-4) bears the ancestral four phalanges, while two configurations (2-3-3-4-3, trionychids only; and 2-3-3-3-3, many taxa) bear three. Most configurations bear two or fewer phalanges on digit V. Whether or not digit V is ever completely lost depends on the assignment homologies. According to Joyce (2000; pers. comm. 2004), the fifth metatarsal is rectangular in shape and, consequently, misidentified as a tarsal. This identification further implies that the first phalanx, when present, is commonly misidentified as the metatarsal. The phalangeal formula of 2-2-2-2-X, indicating the loss of digit V, would be revised to 2-2-2-2-0 under this interpretation. We retain the traditional assessment, pending the results of additional phylogenetic, morphological, and embryological studies on testudinate limb diversity.

Lepidosauria: Manus

Digit I loses at least one element in 75% (21/28) of reduced lepidosaurian configurations (fig. 14.2A). Most commonly, however, digit I is either complete (21%; 6/28) or absent (54%; 15/28). In 11% (3/28) of configurations, digit I is reduced to a metacarpal only, while a single phalanx remains

in only two configurations (1-3-4-5-3, *Heterodactylus*; and 1-2-3-3-2, *Bachia*). Digit I reductions are not necessarily correlated with reductions of other digits: the full range of digit I reductions is observed without any alterations of digits II-V (1-3-4-5-3, 0-3-4-5-3, and X-3-4-5-3).

Digit II reductions tend to be bimodal: either one or all phalanges are lost. The only lepidosaur encountered that loses two phalanges on digit II is the skink *Anomalopus* (X-1-2-2-0). Of the six configurations with no digit II phalanges, five also lack a metacarpal. In all but one configuration (2-2-3-3-2, *Moloch*), digit II reductions always occur with reductions of digit I only, or digit I and another digit.

Digit III retains the ancestral phalangeal count of four in 36% (10/28) of reduced configurations, and loses a single phalanx in 29% (8/28). The eight configurations in which digit III is reduced to two or fewer phalanges also exhibit severe reductions to digit I (i.e., loss of all phalanges or the entire digit) and usually to digit V. Digit III is lost entirely only in lepidosaurs lacking a manus.

Digit IV loses at least one phalanx in most reduced configurations. Nevertheless, digit IV bears phalanges in all configurations not lacking a manus. A single phalanx is lost in 29% (8/28) of reduced configurations. When two or more phalanges are lost, however, all other digits are usually reduced as well. An exception to this trend is the gekkonid *Nephrurus* (2-3-3-3-3), in which only digits III and IV are reduced. Furthermore, in configurations with three or fewer digit IV phalanges, 64% (9/14) are missing digit I and all phalanges of digit V.

Digit V undergoes frequent reductions: at least one phalanx is missing in 71% (20/28) of reduced configurations. No configuration loses two digit V phalanges, but half (14/28) lose all three. Loss of the fifth metacarpal is comparatively infrequent, however, occurring only in three of the most reduced configurations (X-X-2-3-X, X-X-0-3-X, and X-X-0-2-X). Loss of a single phalanx from digit V is not necessarily correlated with losses from other specific digits, but loss of all three phalanges is correlated with the complete loss of digit I in all but two cases (2-3-4-4-0, *Chalcides*; and 0-3-4-4-0, *Hemiergus*).

Lepidosauria: Pes

Digit I is the most frequently reduced in the lepidosaurian pes (fig. 14.2B), with 70% (21/30) of configurations exhibiting loss of at least one phalanx. Digit I is completely absent from 63% (19/30) of reduced configurations, and two other cases lose either one (1-3-4-5-0, *Anotosaura*) or both (0-3-4-4-0, *Hemiergus*) phalanges.

As in the manus, digit II reductions in the pes are bimodal, with either one or all phalanges lost. Of the latter

cases, most lose digit II entirely. Digit II reductions only occur when either digit I or V is also reduced, but reductions of the outer digits do not necessarily affect digit II. For example, the ancestral phalangeal counts of digits II-V are unaffected in the configuration X-3-4-5-0 (*Hemiergus*).

Twenty-seven percent (8/30) of reduced configurations lose a single phalanx from digit III, while 30% (9/30) lose two or more. All of the latter cases are highly reduced forms in which at least two digits other than digit III have lost all phalanges. Digit III phalanges are lost only when at least one digit—but usually more—is reduced. Metacarpal III is highly stable and is lost only in monodactyl (X-X-X-0-X) and digitless configurations.

Digit IV is the last to be lost in its entirety, but it is subject to frequent reductions. One or more phalanges are lost in 67% (20/30) of configurations, and two or more are lost in 40% (12/30). When two or more phalanges are lost from digit IV, all other digits are usually reduced as well. Three configurations restricted to two genera are the exceptions, and all retain a complete digit I: 2-2-2-0-0 and 2-2-3-3-0, *Bachia*; and 2-2-3-3-2, *Moloch*.

Digit V reductions are the most frequent in the lepidosaurian pes, characterizing 90% (27/30) of configurations. All phalanges are typically lost when this digit is reduced, but one or two are occasionally lost as well. Digit V reductions can occur independently, but the loss of all digit V phalanges co-occurs with the complete loss of digit I in 73% (16/22) of cases. Although digit V is subject to frequent reductions, it is rarely lost entirely: only the two most reduced lepidosaurian configurations (X-X-0-0-X and X-X-X-0-X; and limbless forms) lose the fifth metatarsal.

Archosauria: Manus

Digit I is remarkably stable among archosaurs (fig. 14.2C). Hadrosaurs lose this digit entirely (X-3-3-3-3), but it otherwise retains the ancestral phalangeal count of two in all configurations.

Digit II also shows little variability, with phalangeal losses occurring in 24% (4/17) of reduced configurations. These reductions occur in the graviportal stegosaurs (2-2-2-2-1) and sauropods (2-2-1-1-1 and 2-1-1-1-1), and the bipedal theropod *Compsognathus* (2-2-0-X-X).

Digit III is never lost but exhibits a full range of phalangeal counts. At least one phalanx is lost in 47% (8/17) of configurations, and most of these lose at least two phalanges. In all but one of the latter cases (2-3-0-X-X, *Tarbosaurus*), digit II is also reduced.

Digit IV is missing at least one phalanx in all reduced configurations. Two phalanges are lost in 35% (6/17) of cases, while three or more are lost in 47% (8/17). In all five theropod

configurations, digit IV either has one phalanx, has no phalanges (but retains a metacarpal), or is absent. These reductions are also accompanied by severe reductions of digit V.

Digit V is highly variable. At least one phalanx is lost in 82% (14/17) of configurations, and half of these lose all digit V phalanges. Five configurations lose digit V entirely, and all but one of these (2-3-4-4-X, numerous pterosaurs) represent theropods.

No independent reductions of individual digits occur in the archosaur manus. That is, at least two digits are affected by reductions when reductions occur. However, when only two digits are affected by reductions, digits IV and V are always involved.

Archosauria: Pes

In the archosaur pes (fig. 14.2D), digit I has either all or none of its phalanges. One-third of reduced configurations have no digit I phalanges, and most of these also lose the first metacarpal. Digit I reductions are always correlated with the loss of digit V.

Digit II is never lost in the pes and has either two or three phalanges. Two of three configurations with two phalanges show reductions in all five digits; only *Camarasaurus* (2-2-2-1-1) reduces digit II and retains the ancestral two phalanges in digit I.

Digit III bears four phalanges in most configurations, but 15% (2/13) of cases lose a single phalanx, and the same proportion loses two. Phalangeal loss in digit III always entails reductions in at least three other digits.

Digit IV varies in phalangeal number from the ancestral five to a highly derived one; however, at least four phalanges are retained in most configurations. Only large dinosaurs such as sauropods (2-3-4-2-1, 2-3-3-2-1, and 2-2-2-1-1) and stegosaurs (X-2-3-3-X and X-2-2-2-X) are exceptions to this trend.

Digit V loses phalanges in all reduced pes configurations, and only rhamphorhynchoid pterosaurs (2-3-4-5-2) have more than a single phalanx. In 62% (8/13) of cases, all phalanges are lost, and most of these involve the loss of the entire digit. Digit V reductions can occur independently of reductions in other digits, as evidenced by several configurations with reductions in this digit only: 2-3-4-5-2, 2-3-4-5-1, 2-3-4-5-0, and 2-3-4-5-X.

Discussion

This study reveals different modes of digit reduction and loss that occur at distinct levels of organization. First, at the level of a single digit, some or all phalanges (but not the supporting metacarpal or metatarsal) may be lost. Alternatively, the

entire digit may be lost. As we discuss below, partial and complete losses of the digit skeleton represent qualitatively different developmental and evolutionary phenomena. Certain digit reductions or losses tend to occur in concert with others. Some of these correlations differ among groups, whereas others occur independently in unrelated lineages. Another type of change involves alteration of the absolute size and proportions of the autopod. This type of modification was addressed by Holder (1983) and cannot be inferred directly from our data. Second, at a higher level of organization, the integration of digit reductions or losses influences the configuration of the autopod as a whole, including its overall shape. For example, trends of limb reduction that emphasize the loss of external digits often yield more narrow or elongate autopodia than those that consist of uniform reductions across all digits.

The distinct trends we observe within and between clades invite further exploration of potential common mechanisms underlying the evolution of limb reduction in reptiles. Specifically, we will address the role of shared external deterministic agents, namely selection for a particular limb function, and the internal developmental parameters that may characterize each group.

Digit Reduction versus Loss

Although the distinction is seldom emphasized, the loss of all phalanges in a digit (a type of digit *reduction*) is not the same as the loss of all phalanges plus their supporting metacarpal or metatarsal (digit *loss*). Lepidosaurs, for example, frequently reduce manual and pedal digits I and V, but patterns of complete loss differ dramatically. In the lepidosaurian manus, most (15/19) of the configurations without phalanges on digit I also lose the first metacarpal. This frequency of loss differs considerably from that of digit V, however, in which less than one-quarter (3/14) of configurations lacking phalanges also include loss of the metacarpal. The disparity is even greater in the pes, wherein all cases but one (19/20) of complete phalangeal loss in digit I also exhibit loss of the whole digit, but only a small proportion of configurations (2/22) show the same pattern in digit V. In both the manus and pes, digit V is lost only in highly reduced configurations that also lose at least two other digits, whereas digit I can be lost independently. These results demonstrate that digit V is subject to frequent *reductions* but is highly resilient to complete *loss*, whereas *loss* of digit I is more common than *reduction* by one or more phalanges only.

From a developmental perspective, the loss of phalanges of a digit is qualitatively different from the loss of the entire digit. When phalanges are lost, a developing digit primordium segments into fewer elements than in the ancestral form (Storm and Kingsley 1996). Absence of a digit, on the

other hand, implies that the digit never forms in the first place (but for discussions of limb element “loss” through nonossification, fusion, or resorption see Ewart 1894; Mettam 1895; Hinchliffe and Johnson 1980; Rieppel 1992b; Galis et al. 2001; Larsson and Wagner 2002; Kundrát et al. 2002). This distinction is important because the absence of all phalanges (but presence of a metapodial) and the complete absence of a digit are frequently synonymized in the literature; however, these two phenomena are not developmentally or phylogenetically equivalent.

With this distinction in mind, we can evaluate differences in manual and pedal evolution among reptiles. For example, whereas digits tend to be *lost* from the periphery of the autopod in lepidosaurs, digits may be frequently *reduced* in the center. Indeed, in the manus, digit IV is reduced by at least one phalanx in 82% of configurations, more than any other digit. Likewise, in the pes, digit IV is reduced more often than either digit II or digit III. The frequency of loss of *all* phalanges in a digit, however, follows the order $I > V > II > III > IV$, a sequence often called “Morse’s Law” (Morse 1872). Morse based this generalization primarily on birds, but it has since been co-opted to include lizards and other tetrapod groups. Morse’s Law is usually considered to be the order of loss of complete digits, but our analysis suggests otherwise. Based on the configurations we examined, loss of entire digits, seen most commonly and completely in lepidosaurian limbs, follows the sequence $I > II > V > (III, IV)$ in the manus, and $I > II > V > III > IV$ in the pes. In the context of complete digit loss, therefore, Morse’s Law is not upheld. The observed order of loss is, however, more reflective of the sequence of digit chondrogenesis in amniotes (Mathur and Goel 1976; Burke and Alberch 1985; Shubin and Alberch 1986; Müller and Alberch 1990; Shapiro 2002). That is, digits are lost in the reverse order of digit primordia appearance. Patterns of chondrogenesis may, therefore, be a predictor of the sequence of digit loss, although not necessarily of individual digit reduction.

In summary, patterns of digit reduction and loss in lepidosaurs demonstrate that these two phenomena are not equivalent. Digit V is frequently reduced, indicating a high degree of evolutionary plasticity in its phalangeal number. In contrast, digit V is rarely completely lost, thus highlighting its remarkably conserved presence in different types of configurations.

Integrated and Correlated Digit Reductions: Shaping the Hand and Foot

Preferential reduction and loss of the outer digits is a principal mode of pedal evolution among lepidosaurs and archosaurs. Among archosaurs, for example, two pedal configurations representing theropods and bipedal ornithischians

(0-3-4-5-X and X-3-4-5-X) involve reduction or loss of outer digits with retention of the ancestral formula in the central ones. However, a different pattern of loss emerges in the archosaurian manus: reductions and losses are biased toward the postaxial digits, with only modest alterations of the preaxial digits. Digits I and II are nearly always present in these groups, whereas digits IV and V are the most frequently absent.

Another mode of reduction contrasts with the loss of digits and instead produces uniform phalangeal formulae across all digits. Such is the case among testudines, in which phalangeal counts tend to be either two or three. Furthermore, in no turtle manual configuration does the phalangeal count of one digit differ from another by more than one (except when digit I is entirely missing). A similar trend emerges among certain archosaurs in both the manus and pes. These include the sauropods (manus: 2-2-2-2-1, 2-2-1-1-1, and 2-1-1-1-1; pes: 2-2-2-1-1) and several ornithischians (manus: 2-3-3-3-3, 2-3-3-3-2, 2-2-2-2-1, and X-3-3-3-3; pes: X-2-3-3-X and X-2-2-2-X).

Thus, similar reduction trends tend to occur repeatedly among reptiles, and some of these trends are independent of phylogeny. These trends yield combinations of individual digit reductions and losses, but we have yet to examine why such correlated digit reductions are indeed correlated. The convergent appearance of reduction trends in distantly related taxa suggest that limb function may be a common factor. On the other hand, the limited number of observed trends suggests developmental constraints on the ways a limb can be reduced. Below, we consider the role of function as a target of selection in certain reduction trends, following which we discuss the potential underlying developmental mechanisms.

Functional Correlates of Digit Reduction

Reduction Patterns among Archosaurs Correlate with Functional Differences Between and Within Organisms

A single monophyletic group, Archosauria, contains taxa with three major trends in digit reduction. These different patterns are especially pronounced in the manus, where modes of reduction are seen in different functional settings.

First, in the manus of theropod dinosaurs, digits I–III are typically retained with full complements of phalanges, whereas digits IV and V are highly reduced or absent (fig. 14.3A–D). All theropods are bipedal, and their hands are often well developed with an opposable first digit, suggesting a grasping function (Romer 1956). Evolutionary retention of the preaxial digits and loss of the postaxial digits appear to preserve this specialization (Serenó 1997). Interestingly, similar digit reductions, localized to the postaxial aspect of the autopod, are also observed among salamanders (fig. 14.2E,

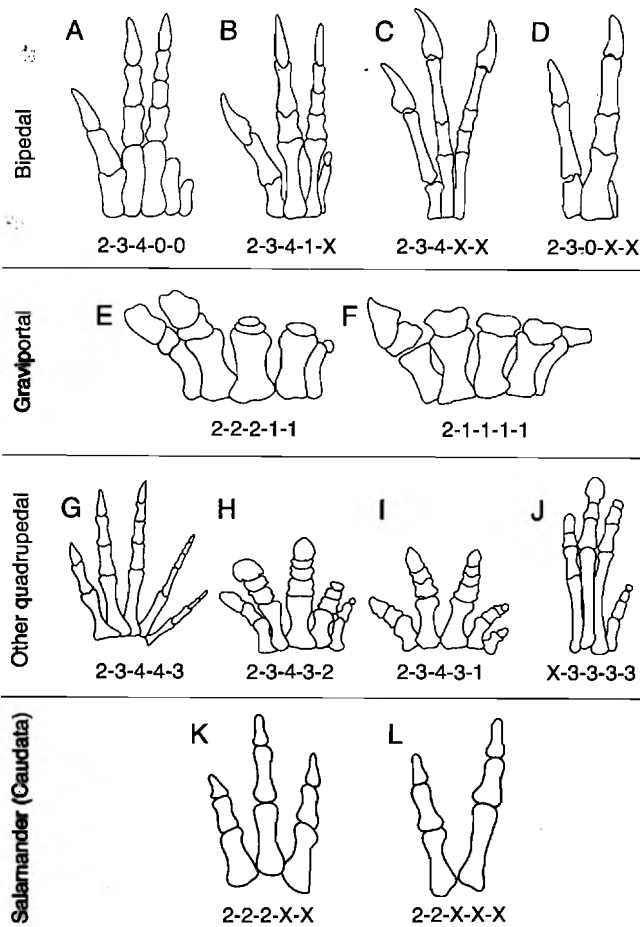


Figure 14-3 Patterns of manual digit loss and reduction among archosaurs. In all diagrams, distal is up and anterior is to the left. Phalangeal formulae are indicated below each manus. (A–D) Manual digits of the bipedal theropods: (A) *Eoraptor*, (B) *Syntarsus*, (C) *Harpymimus*, and (D) *Tarbosaurus*. The principal reduction trend within theropods involves phalangeal and digit losses from the postaxial side of the autopod. Manual digits of (E) the graviportal ornithischian dinosaur *Stegosaurus* and (F) the sauropod *Diplodocus*. In these quadrupeds, phalanges are lost essentially uniformly across all digits but the first. (G–J) Manual digits of other quadrupedal or facultatively quadrupedal archosaurs. The digits of (G) *Crocodylus*, (H) *Centrosaurus*, (I) *Leptoceratops*, and (J) *Anatosaurus* feature modest reductions, with the central digits emphasized. (K, L) Pedal digits of the salamanders *Amphiuma* (K) and *Proteus* (L). (A, E after Sereno 1997; B after Raath 1985; C after Barsbold and Osmólska 1990; D, F after Norman 1985; G after Romer 1956; H after Lull 1933; I after Brown and Schlaikjer 1940; J after Lull and Wright 1942; K, L after Shubin et al. 1995.)

F). All salamanders are obligate quadrupeds that employ a sprawling gait, so their manus and pes have a fundamentally different function from that of the manus of bipedal dinosaurs. The most reduced-limbed forms, such as *Amphiuma* (2-2-2-X-X) and *Proteus* (2-2-X-X-X; hindlimbs; fig. 14.3K, L), are elongate, undulatory swimmers with drastically shortened limbs. Despite these functional differences, salamanders and theropods are the only tetrapod groups in which the loss of postaxial digits (i.e., digits IV and V) is not accompanied by the loss of anterior digits as well.

In a second trend, exemplified by transformations in the

forelimbs of sauropod and stegosaur dinosaurs, convergent evolution of similar phalangeal formulae is seen in a different functional category. Unlike theropods, sauropod and stegosaur manus tend to be reduced to only one or two phalanges on each digit, and no digits are lost (fig. 14.3E, F). These two clades are not closely related (Sereno 1997), but both include massive, quadrupedal animals that likely assumed a graviportal posture and gait (Romer 1956). Hence, unlike theropods, their manus were regularly in contact with the ground and were primarily weight-bearing, not grasping. The uniform trend of digit reduction shared by these groups, therefore, appears to be correlated with obligate quadrupedality and a graviportal posture.

A third archosaur reduction pattern comprises intermediate functional regimes in the manus of facultative quadrupeds, nongraviportal obligate quadrupeds, and sprawling or semi-aquatic quadrupeds (fig. 14.3G–J). These taxa are characterized by modest reductions compared to the graviportal dinosaurs, and central digits tend to be the longest or bear the most phalanges, unlike the theropods. Some of these manus are clearly used for weight bearing, as evidenced by hooves on the central digits of hadrosaurs (fig. 14.3J).

Notably, only one of these three trends is also observed in the archosaurian pes. With the exception of *Apatosaurus* (2-3-4-2-1), graviportal dinosaurs tend to have nearly uniformly reduced digits. Digit losses, however, characterize the other major trend in archosaur pes reduction, occurring primarily among bipeds. Nearly two-thirds (62%) of the archosaurian pes configurations considered show loss of all phalanges from digit V, and nearly half (46%) show loss of the entire digit. Digit I can also be greatly reduced in length relative to digits II–IV, show a reduced phalangeal count, or be entirely lost (fig. 14.4A–C). Digits II–IV retain their ancestral phalangeal numbers and are likely the only functional digits in the foot. Hence, through localized reductions of the outer digits, the central digits are emphasized in these forms. Their elongate foot morphology and reduced outer digits invite functional interpretations of cursoriality (see Carrano 1999). Cursorial mammals follow a similar reduction trend. In mammals, however, the most reduced forms retain a complete digit III (the modern horse) or digits III and IV (artiodactyls, such as antelope and deer) as the functional digits, rather than digits II–IV (fig. 14.4D–F).

Localized Outer Digit Reductions in Lepidosaurs

Other reptilian groups converge upon each of the three archosaurian trends described above. First, like the hindlimbs of reduced-limbed archosaurs such as theropod dinosaurs, most reduced-limbed lepidosaurs preserve the central digits (i.e., digits II–IV; fig. 14.4G–J), but functional considerations differ. Unlike dinosaurs, which have a parasagittal posture, lizards typically maintain a sprawling posture and gait. Nev-

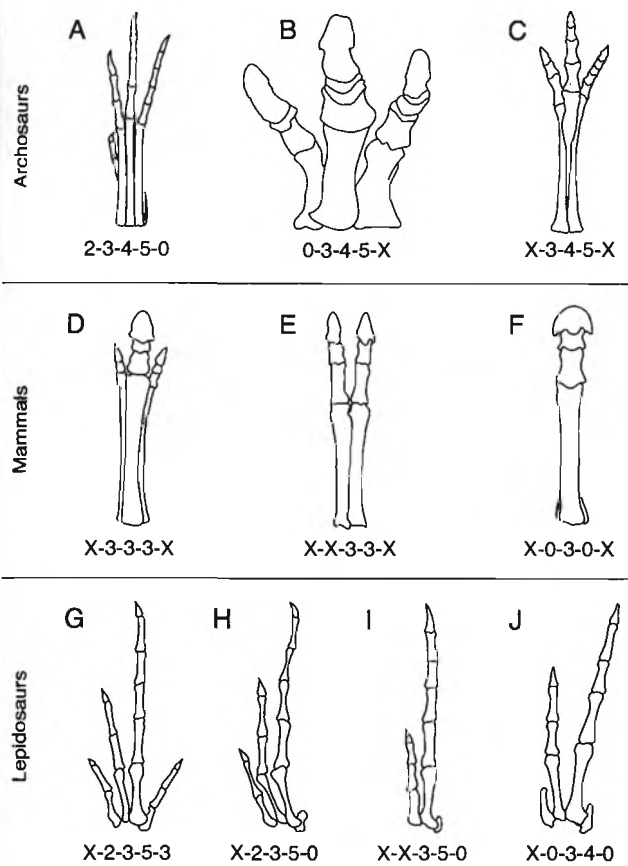


Figure 14-4 General patterns of outer digit loss in the pes of archosaurs, mammals, and lepidosaurs. In all diagrams, distal is up and anterior is to the left. Phalangeal formulae are indicated below each pes. (A–C) Pedal digits of the dinosaurs (A) *Compsognathus*, (B) *Iguanodon*, and (C) *Struthiomimus*. Each pes exhibits loss or severe reduction of one or more outer digits. Metatarsal 1 (described, but not figured, by Norman 1990) is present in *Iguanodon* (B) but is not visible in this drawing. (D–F) Pedal digits of (D) the cursorial perissodactyl *Miohippus*, (E) the artiodactyl *Poebrotherium*, and (F) the perissodactyl *Equus*. Pedal digits of (G–I) the scincid lepidosaurs *Lerista* and (J) *Hemiergis*. Both genera contain species with multiple limb morphologies (see appendix table 14A.1). In panels (G–I), digit IV is not reduced, whereas all others are either reduced or lost. In panel (J), only two digits with phalanges remain, and both are reduced in phalangeal count. (A after Ostrom 1978; B after Norman 1985; C after Osborn 1916; D after Osborn 1918; E after Scott 1940; F after Romer 1966, G–I after Greer 1990.)

ertheless, digits are typically lost or severely reduced such that digits III and IV are conserved. However, in contrast with archosaur pedal configurations, in which reductions are usually restricted to digits I and V, lepidosaurs are subject to frequent reductions of the central digits. This trend is especially notable in the Australian scincid *Lerista*, which provides the best example of graded limb reduction in any tetrapod (Greer 1987, 1989, 1990; fig. 14.4G–I). Many cases of digit reduction and loss in lizards are accompanied by body elongation and a de-emphasis on limb-powered locomotion (Gans 1975). *Lerista* species with more than three manual digits tend to move above the ground surface using their limbs, whereas forms with fewer phalanges or digits—and

typically shorter limbs—are subsurface foragers that move principally by lateral body undulation (Greer 1989).

In contrast, the phalangeal formulae of the functional digits of cursorial archosaurs are typically unaltered and are the distal components of elongate, but not shortened, limbs. Hence, the patterns of reduction shared by archosaurs (hindlimbs only) and lepidosaurs (forelimbs and hindlimbs) are superficially similar with respect to the retention of central digits, yet different with respect to the details of phalangeal loss. The retention of complete central digits in archosaurs may be related to functional constraints of parasagittal locomotion, whereas in lepidosaurs, which typically employ a sprawling gait and often utilize undulatory trunk locomotion in forms with highly reduced limbs, functional constraints for the maintenance of complete digits are likely weaker or absent. Unlike the pedes of some lepidosaurs, the reduced pedes of archosaurs are necessarily used in locomotion, and thus strongly developed central digits are retained.

Uniform Reductions in Turtles

The testudinate manus and pes both undergo uniform phalangeal reductions similar to those seen in a second functional class of archosaurs, the graviportal dinosaurs (fig. 14.5). In the manus, most (10/12) reduced turtle configurations bear only one or two phalanges on each digit. In the pes, with only two exceptions, phalangeal counts are subequal and range between one and three. While the general reduction patterns in turtles are similar to those of graviportal archosaurs, drawing functional analogies between them remains difficult; however, Zug (1971) notes that the extreme reductions in testudinoids (pond turtles and terrestrial tortoises) are associated with a shift to terrestrial locomotion.

In summary, the reduction patterns of distantly related clades—such as cursorial dinosaurs and lepidosaurs, or testudinates and graviportal dinosaurs—may show broad morphological similarities. These commonalities, however, are not always easily reconciled by functional interpretations. For example, the mode of digit loss shared between theropods and lepidosaurs is not explained by a functional similarity. Intrinsic or developmental constraints (“generative” constraints of Richardson and Chipman 2003) may also play a role in reduction patterns; therefore, we will now consider the role of development in the evolutionary transformation of digits.

How to Deconstruct a Limb: Developmental Origins of Limb Reduction

Among reptiles, and amniotes in general, digits I and V are most susceptible to complete loss or severe reductions (e.g., the loss of all phalanges). However, as described above,

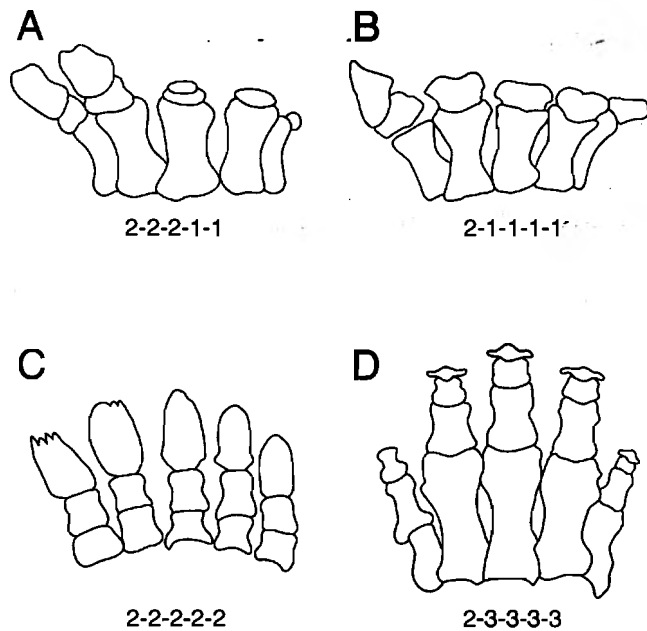


Figure 14-5 Uniform manual digit reductions among reptiles. In all diagrams, distal is up and anterior is to the left. Phalangeal formulae are indicated below each drawing. (A, B) Manual digit morphologies of the archosaurs (A) *Stegosaurus* and (B) *Diplodocus*. (C) Manual digits of the testudinate *Testudo*, which shows a broadly similar pattern of uniform reductions across all digits. (D) Manual digits of the graviportal mammal *Elephas*. In contrast with (A) and (B), the elephant does not lose phalanges from any digit relative to the ancestral mammalian formula (2-3-3-3-3; Flower 1870; Jenkins 1971), and the central manual digits are the most prominent. (Credits for A, B listed in figure 14.3. C after Williston 1925; D after Cornwall 1956.)

digit I is lost much more frequently than is digit V, and this morphological pattern has developmental implications. First, this pattern implies that digit I either condenses and develops two phalanges, condenses and soon regresses, or does not condense at all. This tendency contrasts with patterns in other digits, especially digits II–IV, which often exhibit modest reductions of one or two phalanges. Second, digit V nearly always develops, but subsequently does not segment to form phalanges in many taxa. As a result, only a metapodial persists into adulthood. The mechanism underlying nonsegmentation of digit primordia is unknown but may involve localized tissue shortages (i.e., not enough mesenchyme to support additional chondrogenesis; see Alberch and Gale 1983; 1985; Raynaud 1990; Shapiro et al. 2003) or changes in the regulation of joint formation (see Storm and Kingsley 1996, 1999; Hartmann and Tabin 2001).

The evolutionary conservation of digit V is intriguing, and its developmental persistence may be even greater than adult limb skeletal morphologies indicate. For example, the presence of small splints fused to the postaxial aspects of metatarsal 4 in single specimens of the dinosaurs *Iguanodon*

(fig. 14.4B) and *Stegosaurus* (Galton 1990b) hint at the transient presence of digit V in these species. This phenomenon may extend beyond reptiles as well. Although most birds (pes only) and mammalian ruminants lack any trace of digit V postnatally, a digit V metapodial appears in the embryos of some species but later fuses to digit IV (Mettam 1895; Romanoff 1960; Hinchliffe 1977). The frequency and phylogenetic distribution of this phenomenon is difficult to assess, however, due to the paucity of basic embryological studies of reduced-limbed species. The frequency is even more difficult to assess in fossil forms, which usually provide very few ontogenetic data.

What are the changes in development that bring about the patterns of reduction observed in reptiles? Among living taxa, reptiles offer a multitude of candidate model systems to study the developmental origins of limb reduction. For example, lizards exhibit varying degrees of evolutionary limb reductions, ranging from the loss of a single phalanx to complete limblessness, even at low taxonomic levels (Greer 1991). While the adult morphologies of many reduced-limbed lizards have been studied in detail, the developmental and molecular mechanisms producing these morphologies have not been explored, with a few exceptions.

In some limbless reptiles, for instance, embryonic cessation of limb outgrowth appears to result from a loss of distal signals, which in turn leads to an arrest of mesenchymal proliferation and patterning. In particular, limb bud degeneration in some serpentiform lizards (Raynaud 1962; 1963; Rahmani 1974; Vasse et al. 1974; Raynaud et al. 1975) and a snake (Cohn and Tickle 1999) is known to result from early breakdown of the apical ectodermal ridge (AER), an ectodermal thickening in the distal limb bud that is critical for its outgrowth (Saunders 1948). AER breakdown, in turn, leads to a loss of normal patterning and proliferation of the underlying mesenchyme, resulting in a dramatically truncated (or absent) limb (Raynaud 1985; 1990; Cohn and Tickle 1999).

Early decline or arrest of limb bud proliferation can also result in modest limb reductions, including the loss of digits. Raynaud and colleagues (Raynaud 1986, 1989, 1990, 1991; Raynaud and Clergue-Gazeau 1986; Raynaud and Kan 1988) show that experimental treatment of lizard embryos with the mitotic inhibitor cytosine-arabinofuranoside decreases cell proliferation in the limb buds and can lead to a loss of digits. Moreover, these experimental digit reductions and losses occur in the same order as evolutionary digit loss in many lepidosaurs, with peripheral digits lost before central ones (Morse 1872; Sewertzoff 1931; Greer 1987; Raynaud 1987; Greer 1989; 1990; 1991). Similarly, Alberch and Gale (1983, 1985; Alberch 1985b) demonstrate that experimentally induced (also by mitotic inhibition) patterns of salamander digit loss mimic naturally occurring ones. The striking simi-

larities between experimental and evolutionary patterns of digit loss suggest a common mechanism in both phenomena (Raynaud 1990), and this mechanism may be common to multiple tetrapod lineages. Like the complete loss of limbs in serpentine reptiles, the loss or reduction of individual digits may be linked with a shortage of tissue in the developing limb.

In both lizard and salamander experiments, the loss of a single digit is usually not accompanied by widespread reductions of the remaining digits. Similarly, some natural populations and species of lepidosaurs and archosaurs in this study show single digit losses without major (if any) reductions of other digits. Based on known chondrogenesis sequences, these reductions do not represent simple truncations of pentadactyl skeletal developmental programs. If they did, we would expect them to match, or at least closely resemble, intermediate embryonic configurations of pentadactyl developmental sequences of other amniotes (table 14.1); instead, they represent novel configurations.

This observation is further supported by developmental studies of limb reduction among natural populations of reptiles. The Australian skink genus *Hemiergis*, for example, provides an ideal model organism in which to study developmental aspects of evolutionary digit reduction and loss. Shapiro (2002; Shapiro et al. 2003) detailed the limb skeleton development sequences of *Hemiergis* populations and species with two, three, four, and five digits, and concluded that limbs with fewer than five digits did not result from simple developmental truncations of a pentadactyl (or any other) chondrogenesis sequence (fig. 14.6). Rather than yielding fewer numbers of complete digits, truncations of a five-digit developmental program in *Hemiergis* would yield a series of incomplete digits (fig. 14.6, intermediate configurations).

The archosaur manus, especially that of theropods, follows an evolutionary pattern of reduction that is superficially similar to the common lepidosaurian mode seen in *Hemiergis*. Nevertheless, the sequence of digit loss in the theropod manus differs from that of other reptiles and, indeed, all other amniotes. Theropods lose digits IV and V but never completely lose digits I–III (the enigmatic monodactyl theropod *Mononykus* was excluded from our analysis due to ambiguous digit homologies). Hence, theropods appear to defy the digit loss trend seen in all other reptile groups. This difference may reflect an anterior shift in the primary axis to digit III, which could potentially increase the stability of the anterior digits. Alternatively, digit IV—the “primary axis” digit that is first to appear in known amniote developmental sequences—may have been the first to form in these organisms, only to later obtain the identity of a digit III during osteogenesis (G. P. Wagner and Gauthier 1999), or to regress

or fuse with digit III. The latter scenario is observed in the “missing” metatarsals 2 and 5 of bovid mammals, which fuse embryonically with metatarsals 3 and 4, respectively (Mettam 1895). Similarly, in the avian foot a cartilaginous metatarsal 5 fuses with metatarsal 4 (Hinchliffe 1977).

Notably, the identity of the earliest digits to develop differs among tetrapod lineages. Specifically, salamanders differ from all other groups for which data are available (Shubin and Alberch 1986; table 14.1). In amniote (and anuran) sequences, metapodials generally appear in the order $IV > (III, V) > II > I$. Phalanges are added essentially uniformly across all digits, beginning with the digits that develop first, until the adult configuration for each digit is attained. Digits that appear first may add phalanges before other digits begin chondrogenesis, but phalangeal addition is completed nearly simultaneously across all digits. In salamanders, however, digits appear in the order $(I, II) > III > IV > V$ (Shubin and Alberch 1986; Blanco and Alberch 1992). Moreover, in contrast to amniotes, chondrification of the phalanges is not uniform across the autopod, and in some cases each digit completes phalangeal condensation before the next one begins (Blanco and Alberch 1992). Thus, salamander digits emerge from the limb and chondrify one at a time (or nearly so), whereas the digits of other groups develop essentially simultaneously. The earliest-appearing digits—I and II in salamanders, III and IV in all other groups—are also the most evolutionarily stable. The theropod manus is a possible exception to this rule. In general, however, patterns of digit stability and loss are dependent on developmental properties of each group (Alberch and Gale 1983).

In contrast to the localized reduction and loss of outer digits described above, relatively uniform reductions occur across all digits in several lepidosaurs (e.g., *Nephrurus* manus, 2-3-3-3-3; *Moloch* manus, 2-2-3-3-2), graviportal archosaurs (e.g., *Stegosaurus* manus, 2-2-2-2-1), and most reduced testudinate configurations. In many of these configurations, all five digits are retained, and some of these formulae closely resemble intermediate stages of pentadactyl chondrogenesis sequences. Hence, since the last phalanges to form are the first to be lost, these configurations may indeed represent truncations of ancestral chondrogenetic sequences (also see fig. 10.10 of Shapiro and Carl 2001; Shapiro 2002). This trend is distinguished from those described earlier because all five digits typically persist. Condensation of all five digit primordia, an early event in digit morphogenesis, often occurs in taxa showing uniform reductions, but all phalanges do not necessarily undergo segmentation within the digital ray, a later event in morphogenesis. Therefore, the morphogenetic mechanisms that lead to loss of a digit must act earlier than those that result in a reduction in the number of phalanges. Some uniform truncations of this type are

Table 14-1 Pentadactyl developmental sequences for several tetrapod species

Taxon	Manus	Pes	Reference
Testudinata (Chelydridae) <i>Chelydra</i>	2-3- 3 -3-3 ^a 0-0-1-1-1 X-X-0-0-0		Burke and Alberch 1985
Testudinata (Emydidae) <i>Chrysemys</i>		2-3- 3 -3-4 ^a 0-0-1?-1-1? X-X-0-0-0	Burke and Alberch 1985
Lepidosauria (Agamidae) <i>Calotes</i>	2-3-4-5-3 2-3-4-4-3 1-2-4-3-2 0-1-3-2-1 0-1-2-2-1 0-0-1-1-0	2-3-4-5-4 1-2-3-4-3 1-2-3-3-3 1-2-2-2-2 0-1-1-1-1 0-0-1-1-1	Mathur and Goel 1976 ^b
Lepidosauria (Scincidae) <i>Hemiergis</i>	2-3-4- 4 -3 1-3-3-3-2 1-2-3-3-2 1-2-2-2-2 0-1-1-1-1 X-0-0-1-0 X-X-0-0-X	2-3-4- 4 -3 1-3-3-3-2 1-2-3-3-2 1-2-2-2-2 0-1-1-2-1 X-0-0-1-0 X-X-0-0-X	Shapiro 2002
Archosauria (Crocodylidae) <i>Alligator</i>	2-3-4-5-4 1-2-2-2-1 1-1-1-1-1 0-1-1-1-0 X-1-1-1-0 X-X-X-0-X	2-3-4-5- 0 2-3-3-2-0 1-1-1-1-0 X-0-1-0-0 X-X-X-0-X	Müller and Alberch 1990
Archosauria (Phasianidae) <i>Gallus</i>		2-3-4-5- X ^c 0-1-2-2-0 0-1-2-1-0 0-1-1-1-0 X-1-1-1-0 X-X-0-0-0	Hinchliffe 1977
Archosauria (Anatidae) <i>Anas</i>		2-3-4-5- X ^c 1-2-3-4-0 1-2-3-3-0 0-1-1-1-0 X-X-0-0-X	Romanoff 1960
Mammalia (Hominidae) <i>Homo</i>	2-3-3-3-3 ^d 2-2-2-2-2 0-1-1-1-1 X-X-0-0-X	2-3-3-3-3 ^d 2-2-2-2-2 1-1-1-1-1 0-0-1-1-0 0-0-0-0-0 X-0-0-0-0	O'Rahilly et al. 1957
Caudata (Abystomatidae) <i>Ambystoma</i>		2-2-3-4-2 2-2-3-4-1 2-2-3-3-0 2-2-3-1-X 2-1-1-0-X	Alberch and Gale 1985

Table 14-1 (continued)

Taxon	Manus	Pes	Reference
		1-0-0-X-X 0-0-X-X-X	
Anura (Pipidae) <i>Xenopus</i>		2-2-3-4-3 2-2-3-3-2 1-1-3-2-1 0-1-2-2-1 0-0-1-1-0 X-0-1-1-0 X-0-0-0-0 X-X-0-0-X	Alberch and Gale 1985

NOTE: Adult phalangeal formulae are in the top row for each sequence, with progressively earlier (principally cartilaginous) embryonic configurations listed below. Boldface numbers in adult formulae indicate reductions relative to ancestral formulae, and italics indicate hyperphalangy.

^aAdult formulae from Zug 1971.

^bFrom Greer 1991.

^cMetatarsal 5 never supports phalanges at any embryonic stage and fuses with metatarsal 4 while still cartilaginous; metatarsal 5 thus appears to be missing in the adult.

^dThese formulae represent the ancestral configurations for Mammalia but reduced configurations for Synapsida (manus: 2-3-4-5-3; pes: 2-3-4-5-4).

coincident with evolutionarily miniaturized limbs (Lande 1978), suggesting that this mode of reduction, too, has a tissue availability component. Miniaturization is not an issue with the gigantic sauropods that follow this trend, however.

Molecular Control of Limb Reduction

Our understanding of the genetic control of limb development has increased manifold in recent years (for reviews, see Tickle 1995; R. L. Johnson and Tabin 1997; Shubin et al. 1997), but the molecular mechanisms underlying limb reduction remain poorly understood. In amniotes, the zone of polarizing activity (ZPA) and the AER coordinate mesenchymal proliferation and patterning through a positive feedback loop involving *Sonic hedgehog* (*Shh*), which encodes a secreted intracellular signal expressed in the ZPA, and fibroblast growth factors (FGFs), expressed in the AER (Laufer et al. 1994; Niswander et al. 1994; Zúñiga et al. 1999). Notably, reduced-limbed morphologies in *Hemiergus* are associated with decreased Shh protein expression (plate 14.1; Shapiro et al. 2003). Furthermore, these naturally occurring digit configurations are strikingly similar to some experimental morphologies generated by Raynaud's mitosis inhibition experiments. Such similarities are not surprising, however, since mitotic inhibition of limb mesenchyme and decreased Shh expression should have similar effects: both decrease proliferation and limit the quantity of tissue available for digit condensations. Consequently, differences in proliferation mediated by Shh (or another signal in its feedback loop with the AER, including FGFs) or changes in digit identity and

quantity regulated by Shh (Harfe et al. 2004) and Shh-Gli3 interactions (Aoto et al. 2002; Litingtung et al. 2002; te Welscher et al. 2002a, 2002b) may regulate digit number. The prevalence of outer digit reduction and loss among reptiles, and tetrapods in general, further suggests that this may be a common developmental mechanism of limb reduction across several major clades.

"Natural experiments" such as *Hemiergus*, in which a variety of morphologies occur among closely related taxa, are underrepresented in the evolutionary studies of vertebrate development. A comparative approach complements experimental work on traditional model species: experimental manipulations using chicks, mice, and frogs can help unravel the mechanisms and pathways of development, but they cannot always predict how evolution modifies these mechanisms to produce novel morphologies. While important for understanding the molecular basis for limb reduction, studies of nontraditional model organisms tend to be correlative. In the case of *Hemiergus*, for example, Shh is implicated in the loss of digits. However, based on available evidence, we cannot determine whether a regulatory or coding change in *Shh* is a primary cause of limb reduction, or whether *Shh* is downregulated in response to another molecular cue. A crucial next step in studies of evolution and development will entail the integration of genetics in studies of morphological diversity among natural populations of vertebrates. To date, no such studies focus on reptile limb diversity, but limb (fin) studies in other vertebrates suggest the feasibility of such experiments in the near future (Peichel et al. 2001; Shapiro et al. 2004).

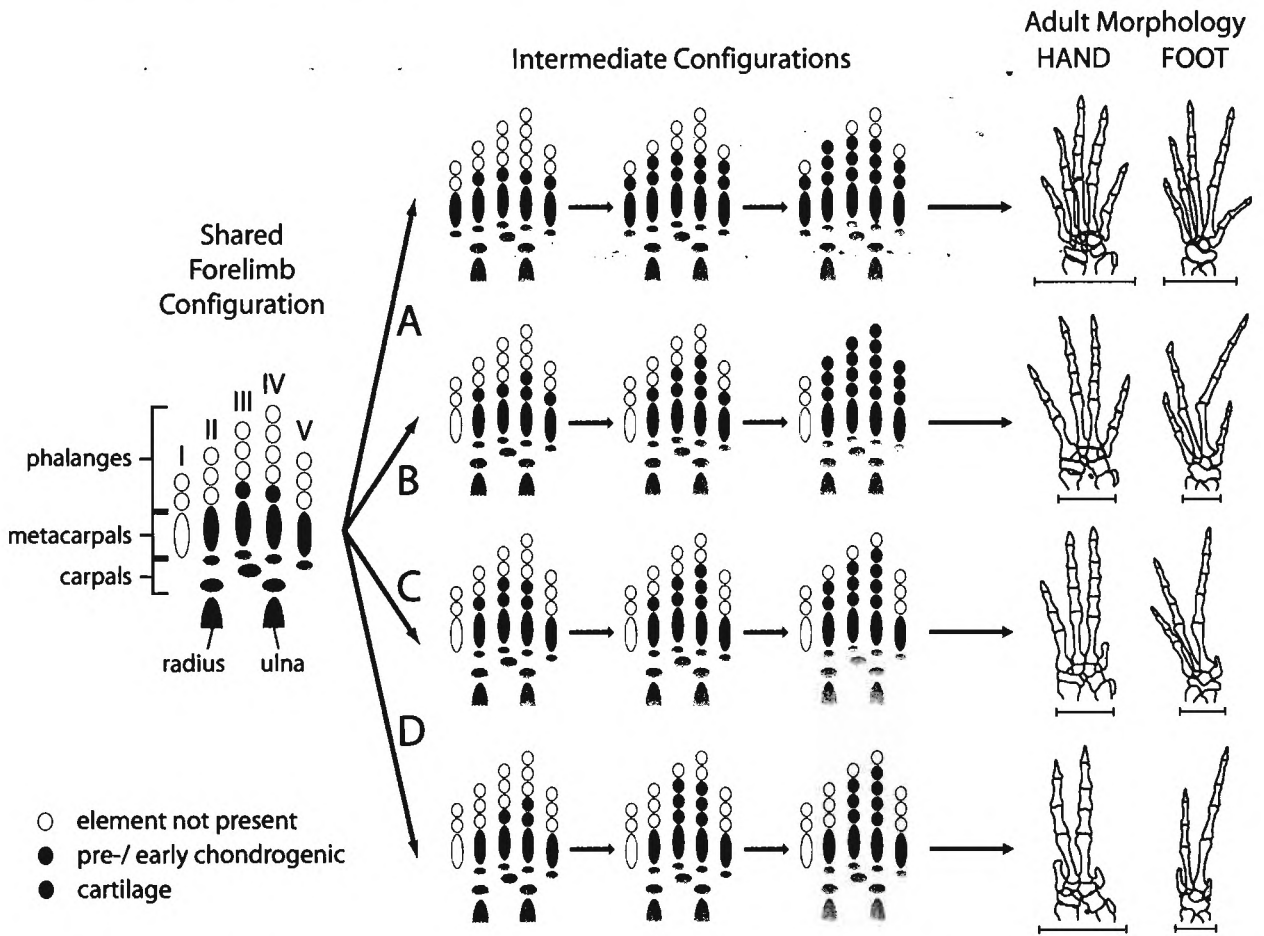


Figure 14-6 Limb skeletal development in *Hemiergis*. In all diagrams, distal is at the top and anterior is to the left. Following shared, early skeletal configurations (left), the developmental trajectories of (A) *H. initialis* (5/5), (B) *H. peronii* (4/4), (C) *H. peronii* (3/3), and (D) *H. quadrilineata* (2/2) autopodia diverge, culminating in different adult morphologies (right). The shared and intermediate stages depict forelimb configurations only, but hindlimb data are virtually identical. Intermediate configurations are based on data from whole mounts and serial sections, and do not necessarily represent identical embryonic stages among all four morphs. Scale bars = 1 mm for adults. (After Shapiro et al. 2003.)

Reducing the Limb: A Two-Tiered Hierarchy

As outlined above, tetrapod limb reduction operates at distinctly different levels of organization. Individual digits can be either reduced in phalangeal count or lost altogether, and correlated patterns of reduction and loss determine the overall autopod shape and phalangeal configuration. Limbs are functional organs used by organisms to move about in their environments and to manipulate their surroundings. Thus, we should not be surprised to find functional correlates of limb reduction trends among, for example, cursorial or graviportal tetrapods in our analysis. That these trends span phylogenetic boundaries lends support to the notion that function is a primary determinant of overall autopod shape.

If function determines *what* a reduced limb looks like, then development determines *how* reductions are effected. Developmental processes dictate the order of digit formation

and therefore which digits are most susceptible or resistant to reduction and loss. The last digits to form are typically the first to be lost, but these losses are not consistent with a simple model of heterochronic truncation at the level of limb chondrogenesis (for an extended discussion, see Shapiro and Carl 2001; Shapiro 2002). Nevertheless, studies of comparative patterns of limb skeletal chondrogenesis provide insight and predictability regarding the sequence of reductions. Amniotes form the most preaxial digits last, and these are the first digits to be lost (except, apparently, in theropod dinosaurs). In cases of relatively uniform reductions across all digits, the resulting phalangeal formulae often resemble intermediate developmental configurations, and thus phalanges that fail to form are those that would otherwise form last. Hence, this type of reduction is predictable based on known developmental sequences. Importantly, uniform reductions across multiple digits are limited to amniotes (and

anurans) because they assemble all digits essentially simultaneously. Truncations at intermediate developmental stages, therefore, may produce a series of incomplete digits. Salamanders, on the other hand, typically complete development of one digit before beginning the next, and thus any simple truncation would produce fewer, but more complete, digits.

Reptiles are a group of organisms whose phylogenetic, anatomical, and functional diversity can be used to assess the mechanisms behind morphological trends. Some trends in reptilian limb morphology are unique to specific groups, while others are more general and appear to evolve convergently. Reptilia exhibits a morphological diversity that makes the clade an especially attractive subject for the study of the developmental and genetic bases of morphological change. Despite this fact, reptiles have not received a level of

attention to match their high potential for discovery. A major challenge for the future of reptilian limb studies will be to build upon the molecular, developmental, and genetic tools and techniques that are widely used in studies of “traditional” model organisms (e.g., the laboratory mouse, the chicken, and the frog *Xenopus laevis*). In doing so, we can hope to determine whether convergence in reptilian limb morphology is the product of common developmental and genetic mechanisms. Are convergent morphologies in different taxa caused by changes in the same genes? Are similar numbers of genetic changes required to effect similar morphological changes among different lineages of reptiles? Answers to these questions will only come from the comparative molecular analysis of reptile limb evolution and development.

Appendix

Table 14A-1 Phalangeal formulae for reptiles and salamanders used in this study

Configuration	Taxon	Source
Testudinata: manus		
2-3-4-5-3	<i>Captorhinus</i>	Gaffney 1990
2-3-3-3-3	<i>Macroclemys</i>	Gaffney 1990
	<i>Podonemis</i>	Gaffney 1990
2-3-3-3-2	<i>Thalassochelys</i>	Romer 1956
2-2-2-2-2	<i>Chersina</i>	Crumly and Sánchez-Villagra 2004
	<i>Geochelone</i>	Crumly and Sánchez-Villagra 2004
	<i>Gopherus</i>	Crumly and Sánchez-Villagra 2004
	<i>Homopus</i>	Crumly and Sánchez-Villagra 2004
	<i>Indotestudo</i>	Crumly and Sánchez-Villagra 2004
	<i>Kinixys</i>	Crumly and Sánchez-Villagra 2004
	<i>Malacochersus</i>	Crumly and Sánchez-Villagra 2004
	<i>Meiolania</i>	Gaffney 1990
	<i>Proganochelys</i>	Gaffney 1990
	<i>Pyxis</i>	Crumly and Sánchez-Villagra 2004
	<i>Stylenys</i>	Crumly and Sánchez-Villagra 2004
	<i>Testudo</i>	Williston 1925
2-2-2-2-1	<i>Geochelone</i>	Crumly and Sánchez-Villagra 2004
	<i>Gopherus</i>	Crumly and Sánchez-Villagra 2004
	<i>Indotestudo</i>	Crumly and Sánchez-Villagra 2004
	<i>Kinixys</i>	Crumly and Sánchez-Villagra 2004
	<i>Malacochersus</i>	Crumly and Sánchez-Villagra 2004
	<i>Manouria</i>	Crumly and Sánchez-Villagra 2004

- continued

Table 14A-1 (continued)

Configuration	Taxon	Source
	<i>Psammobates</i>	Crumly and Sánchez-Villagra 2004
	<i>Pyxis</i>	Crumly and Sánchez-Villagra 2004
	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
2-2-2-1-1	<i>Kinixys</i>	Crumly and Sánchez-Villagra 2004
1-2-2-2-2	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
1-2-2-2-1	<i>Gopherus</i>	Crumly and Sánchez-Villagra 2004
	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
1-2-2-1-1	<i>Gopherus</i>	Crumly and Sánchez-Villagra 2004
X-2-2-2-2	<i>Homopus</i>	Crumly and Sánchez-Villagra 2004
X-2-2-2-1	<i>Homopus</i>	Crumly and Sánchez-Villagra 2004
	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
X-2-2-1-1	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
X-1-2-1-1	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
Testudinata: pes		
2-3-4-5-4	<i>Captorhinus</i>	Gaffney 1990
2-3-3-4-3	<i>Lissemys</i>	Zug 1971
	<i>Pelochelys</i>	Zug 1971
	<i>Trionyx</i>	Zug 1971
2-3-3-3-4	<i>Dermatemys</i>	Zug 1971
	<i>Malaclemys</i>	Zug 1971
	<i>Chrysemys</i>	Zug 1971
2-3-3-3-3	<i>Carettochelys</i>	Zug 1971
	<i>Casichelydia</i>	Gaffney 1990
	<i>Claudius</i>	Zug 1971
	<i>Clemmys</i>	Zug 1971
	<i>Damonina</i>	Zug 1971
	<i>Kinosternon</i>	Zug 1971
	<i>Macrolemys</i>	Gaffney 1990
	<i>Podocnemis</i>	Gaffney 1990
	<i>Staurotypos</i>	Zug 1971
	<i>Terrapene</i>	Zug 1971
2-3-3-3-2	<i>Casichelydia</i>	Gaffney 1990
	<i>Claudius</i>	Zug 1971
	<i>Platysternon</i>	Zug 1971
	<i>Rhinoclemys</i>	Zug 1971
2-3-3-2-2	<i>Terrapene</i>	Zug 1971
2-3-3-2-1	<i>Terrapene</i>	Zug 1971
2-2-2-2-2	<i>Proganochelys</i>	Gaffney 1990
2-2-2-2-1	<i>Chersina</i>	Crumly and Sánchez-Villagra 2004
	<i>Geochelone</i>	Zug 1971
	<i>Gopherus</i>	Zug 1971
	<i>Homopus</i>	Crumly and Sánchez-Villagra 2004
	<i>Kinixys</i>	Zug 1971
	<i>Malachochersus</i>	Crumly and Sánchez-Villagra 2004
	<i>Manouria</i>	Crumly and Sánchez-Villagra 2004
	<i>Psammobates</i>	Crumly and Sánchez-Villagra 2004
	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
2-2-2-2-X	<i>Chersina</i>	Crumly and Sánchez-Villagra 2004
	<i>Geochelone</i>	Crumly and Sánchez-Villagra 2004
	<i>Gopherus</i>	Zug 1971
	<i>Homopus</i>	Crumly and Sánchez-Villagra 2004
	<i>Kinixys</i>	Crumly and Sánchez-Villagra 2004
	<i>Meiolania</i>	Gaffney 1990
	<i>Pyxis</i>	Crumly and Sánchez-Villagra 2004
	<i>Testudo</i>	Crumly and Sánchez-Villagra 2004
Lepidosauria: manus		
2-3-4-5-3	Ancestral saurian	Romer 1956
2-3-4-5-2	<i>Chamaesaura</i>	Greer 1991

Table 14A-1 (continued)

Configuration	Taxon	Source
2-3-4-4-3	<i>Anotosaura</i>	Kizirian and McDiarmid 1998
	<i>Chalcides</i>	Caputo et al. 1995
	<i>Ctenophorus</i>	Greer 1989
	<i>Hemiergus</i>	Choquenot and Greer 1989
	<i>Moloch</i>	Greer 1989
	<i>Nephurus</i>	Stephenson 1960
	<i>Rhynchoedura</i>	Greer 1989
2-3-4-4-2	<i>Chalcides</i>	Caputo et al. 1995
2-3-4-4-0	<i>Chalcides</i>	Caputo et al. 1995
2-3-3-4-3	<i>Hemidactylus</i>	Haake 1976
2-3-3-3-3	<i>Nephurus</i>	Stephenson 1960
2-2-3-3-2	<i>Moloch</i>	Greer 1989
1-3-4-5-3	<i>Heterodactylus</i>	Kizirian and McDiarmid 1998
1-2-3-3-2	<i>Bachia</i>	Kizirian and McDiarmid 1998
0-3-4-5-3	<i>Colobodactylus</i>	Kizirian and McDiarmid 1998
	<i>Colobosaura</i>	Kizirian and McDiarmid 1998
0-3-4-4-3	<i>Bachia</i>	Kizirian and McDiarmid 1998
0-3-4-4-0	<i>Hemiergus</i>	Choquenot and Greer 1989
0-2-2-2-2	<i>Bachia</i>	Kizirian and McDiarmid 1998
X-3-4-5-3	<i>Hemiergus</i>	Choquenot and Greer 1989
X-3-4-5-0	<i>Hemiergus</i>	Choquenot and Greer 1989
X-2-4-5-3	<i>Lerista</i>	Greer 1989
X-2-3-4-2	<i>Lerista</i>	Greer 1990
X-2-3-4-0	<i>Lerista</i>	Greer 1989
X-2-3-3-0	<i>Coeranoscincus</i>	Greer and Cogger 1985
	<i>Chalcides</i>	Caputo et al. 1995
X-2-3-2-0	<i>Anomalopus</i>	Greer and Cogger 1985
X-2-2-2-0	<i>Chalcides</i>	Caputo et al. 1995
X-1-2-2-0	<i>Anomalopus</i>	Greer and Cogger 1985
X-0-3-4-0	<i>Hemiergus</i>	Choquenot and Greer 1989
X-X-2-3-0	<i>Chalcides</i>	Caputo et al. 1995
X-X-2-3-X	<i>Lerista</i>	Greer 1989
X-X-1-3-0	<i>Chalcides</i>	Caputo et al. 1995
X-X-0-3-X	<i>Lerista</i>	Greer 1989
X-X-0-2-X	<i>Lerista</i>	Greer 1989
Lepidosauria: pes		
2-3-4-5-4	Ancestral saurian	Romer 1956
2-3-4-5-3	<i>Anotosaura</i>	Kizirian and McDiarmid 1998
	<i>Chalcides</i>	Caputo et al. 1995
	<i>Ctenophorus</i>	Greer, 1989
	<i>Heterodactylus</i>	Kizirian and McDiarmid 1998
	<i>Rankinia</i>	Greer, 1989
2-3-4-5-0	<i>Heterodactylus</i>	Kizirian and McDiarmid 1998
	<i>Chalcides</i>	Caputo et al. 1995
2-3-4-4-4	<i>Nephurus</i>	Stephenson 1960
2-3-4-4-3	<i>Agama</i>	Greer 1991
	<i>Chalcides</i>	Caputo et al. 1995
	<i>Hemiergus</i>	Choquenot and Greer 1989
	<i>Moloch</i>	Greer 1989
2-3-4-4-0	<i>Bachia</i>	Kizirian and McDiarmid 1998
2-3-3-4-3	<i>Hemidactylus</i>	Haake 1976
	<i>Stenpdactylus</i>	Haake 1976
2-2-3-3-2	<i>Moloch</i>	Greer 1989
2-2-3-3-0	<i>Bachia</i>	Kizirian and McDiarmid 1998
2-2-2-0-0	<i>Bachia</i>	Kizirian and McDiarmid 1998
1-3-4-5-0	<i>Anotosaura</i>	Kizirian and McDiarmid 1998
0-3-4-4-0	<i>Hemiergus</i>	Choquenot and Greer 1989

continued

Table 14A-1 (continued)

Configuration	Taxon	Source
X-3-4-5-3	<i>Hemiergis</i>	Choquenot and Greer 1989
X-3-4-5-0	<i>Hemiergis</i>	Choquenot and Greer 1989
X-3-4-4-0	<i>Hemiergis</i>	Choquenot and Greer 1989
X-2-4-5-4	<i>Lerista</i>	Greer 1989
X-2-4-5-0	<i>Lerista</i>	Greer 1987
X-2-3-5-3	<i>Lerista</i>	Greer 1990
X-2-3-5-0	<i>Lerista</i>	Greer 1989
X-2-3-4-0	<i>Chalcides</i>	Caputo et al. 1995
X-2-3-3-0	<i>Coeranoscincus</i>	Greer and Cogger 1985
	<i>Chalcides</i>	Caputo et al. 1995
X-2-2-2-0	<i>Chalcides</i>	Caputo et al. 1995
X-2-2-0-0	<i>Anomalopus</i>	Greer and Cogger 1985
X-0-3-4-0	<i>Hemiergis</i>	Choquenot and Greer 1989
	<i>Chalcides</i>	Caputo et al. 1995
X-0-2-2-0	<i>Alcys conciana</i>	Greer 1989
X-0-0-0-0	<i>Delma</i>	Greer 1989
	<i>Pygopus</i>	Stephenson 1962
X-X-3-5-0	<i>Lerista</i>	Greer 1989
X-X-0-3-0	<i>Lerista</i>	Greer 1989
X-X-0-0-0	<i>Paradelma</i>	Greer 1989
X-X-0-0-X	<i>Anomalopus</i>	Greer and Cogger 1985
X-X-X-0-X	<i>Lerista</i>	Greer 1990
	<i>Lialis</i>	Stephenson 1962
Archosauria: manus		
2-3-4-5-3	Ancestral saurian	Romer 1956
2-3-4-4-3	<i>Crocodylus</i>	Romer 1956
2-3-4-4-2	<i>Anchisaurus</i>	Carroll 1987
	<i>Geosaurus</i>	Romer 1956
2-3-4-4-X	Numerous pterosaurs	Wellnhofer 1991
2-3-4-3-2	<i>Centrosaurus</i>	Lull 1933
	<i>Heterodontosaurus</i>	Norman 1985
	<i>Plateosaurus</i>	Galton 1990a
	<i>Protoceratops</i>	Brown and Schlaikjer 1940
2-3-4-3-1	<i>Leptoceratops</i>	Brown and Schlaikjer 1940
2-3-4-3-0	<i>Hypsilophodon</i>	Norman 1985
2-3-4-1-X	<i>Ceratosaurus</i>	Norman 1985
	<i>Syntarsus</i>	Raath 1985
2-3-4-0-0	<i>Eoraptor</i>	Sereno 1997
2-3-4-X-X	<i>Allosaurus</i>	Molnar et al. 1990
	<i>Deinonychus</i>	Ostrom 1969
	<i>Dromeceiomimus</i>	Barsbold and Osmólska 1990
	<i>Scipionyx</i>	Dal Sasso and Signore 1998
	<i>Struthiomimus</i>	Barsbold and Osmólska 1990
2-3-3-3-3	<i>Iguanodon</i>	Norman and Weishampel 1990
2-3-3-3-2	<i>Camptosaurus</i>	Norman and Weishampel 1990
	<i>Pinacosaurus</i>	Coombs and Maryanska 1990
2-3-0-X-X	<i>Tarbosaurus</i>	Norman 1985
2-2-2-2-1	<i>Shunosaurus</i>	McIntosh 1990
	<i>Stegosaurus</i>	Galton 1990b, Sereno 1997
2-2-1-1-1	<i>Apatosaurus</i>	McIntosh 1990
2-2-0-X-X	<i>Compsognathus</i>	Norman 1990
2-1-1-1-1	<i>Brachiosaurus</i>	McIntosh 1990
	<i>Diplodocus</i>	Norman 1985
X-3-3-3-3	Hadrosauridae	Weishampel and Horner 1990
Archosauria: pes		
2-3-4-5-4	Ancestral saurian	Romer 1966
2-3-4-5-2	Most Rhamphorhynchoidea	Wellnhofer 1991

Table 14A-1 (continued)

Configuration	Taxon	Source
2-3-4-5-1	<i>Pterodactylus</i>	Wellnhofer 1991
	<i>Anchisaurus</i>	Romer 1956
2-3-4-5-0	<i>Alligator</i>	Kuhn-Schnyder and Rieber 1986
	<i>Camptosaurus</i>	Norman and Weishampel 1990
	<i>Centrosaurus</i>	Lull 1933
	<i>Ceratosaurus</i>	Norman 1985
	<i>Compsognathus</i>	Ostrom 1978; Norman 1990
	<i>Deinonychus</i>	Ostrom 1969
	<i>Dromeceiomimus</i>	Barsbold and Osmólska 1990
	<i>Plateosaurus</i>	Galton 1990a
	<i>Protoceratops</i>	Brown and Schlaikjer 1940
	<i>Protosuchus</i>	Romer 1956
	Most Pterodactyloidea	Wellnhofer 1991
	<i>Syntarsus</i>	Rowe and Gauthier 1990
2-3-4-5-X	<i>Allosaurus</i>	Molnar et al. 1990
	<i>Heterodontosaurus</i>	Norman 1985
	<i>Hypsilophodon</i>	Norman 1985
	<i>Leptoceratops</i>	Brown 1914
	<i>Tarbosaurus</i>	Norman 1985
2-3-4-4-0	<i>Geosaurus</i>	Romer 1956
2-3-4-4-X	<i>Talarurus</i>	Coombs and Maryanska 1990
2-3-4-2-1	<i>Apatosaurus</i>	Gilmore 1936
	<i>Diplodocus</i>	McIntosh 1990
2-3-3-2-1	<i>Diplodocus</i>	McIntosh 1990
	<i>Janenschia</i>	McIntosh 1990
2-2-2-1-1	<i>Comarasaurus</i>	Norman 1985
0-3-4-5-X	<i>Iguanodon</i>	Norman and Weishampel 1990
X-3-4-5-X	<i>Edmontosaurus</i>	Norman 1985
	<i>Euoplocephalus</i>	Coombs and Maryanska 1990
	<i>Struthiomimus</i>	Osborn 1916
X-2-3-3-X	<i>Huayangosaurus</i>	Galton 1990b
X-2-2-2-X	<i>Stegosaurus</i>	Galton 1990b
Caudata: manus		
2-2-3-2-X	<i>Cryptobranchus</i>	Cope 1889
	<i>Karaurus</i>	Ivachnenko 1978
	<i>Lipoxitriton</i>	Shubin and Wake 2003
	<i>Laccotriton</i>	Gao and Shubin 2001
2-2-2-2-X	<i>Siren</i>	Shubin and Wake 2003
2-2-2-X-X	<i>Pseudobranchus</i>	Shubin and Wake 2003
1-2-3-2-X	<i>Taricha</i>	Shubin and Wake 2003
	<i>Triturus</i>	Shubin and Wake 2003
	<i>Sinerpeton</i>	Gao and Shubin 2001
1-2-2-X-X	<i>Amphiuma</i>	Cope 1889
Caudata: pes		
2-2-3-4-3	<i>Karaurus</i>	Ivachnenko 1978
2-2-3-4-2	<i>Ambystoma</i>	Alberch and Gale 1985
	<i>Laccotriton</i>	Gao and Shubin 2001
2-2-3-3-2	<i>Ambystoma</i>	Shubin et al. 1995
	<i>Cryptobranchus</i>	Alberch and Gale 1985
	<i>Dicamptodon</i>	Alberch and Gale 1985
	<i>Hynobius</i>	Alberch and Gale 1985
	<i>Liua</i>	Shubin et al. 1995
	<i>Tylototriton</i>	MVZ 219764
2-2-3-3-1	<i>Echinotriton</i>	Alberch and Gale 1985
2-2-3-3-0	<i>Echinotriton</i>	Alberch and Gale 1985

continued

Table 14A-1 (continued)

Configuration	Taxon	Source
2-2-3-3-X	<i>Echinotriton</i>	Alberch and Gale 1985
2-2-3-2-X	<i>Batrachuperus</i>	CAS 152088
	<i>Hynobius</i>	Alberch and Gale 1985
	<i>Necturus</i>	Shubin et al. 1995
2-2-2-X-X	<i>Amphiuma</i>	Shubin et al. 1995
2-2-X-X-X	<i>Proteus</i>	Shubin et al. 1995
1-2-3-4-2	<i>Sinerpeton</i>	Gao and Shubin 2001
1-2-3-3-2	<i>Taricha</i>	Shubin et al. 1995
	Most Plethodontidae, some Salamandridae	Alberch and Gale 1985
1-2-3-3-X	<i>Batrachoseps</i>	Alberch and Gale 1985
	<i>Eurycea</i>	Alberch and Gale 1985
	<i>Hemidactylum</i>	Alberch and Gale 1985
1-2-3-3-1	<i>Thorius</i>	Alberch and Gale 1985
1-2-3-2-2	<i>Bolitoglossa</i>	Alberch and Gale 1985
1-2-3-2-X	<i>Salamandrina</i>	MVZ 184845
	<i>Batrachoseps</i>	Alberch and Gale 1985
	<i>Thorius</i>	Shubin et al. 1995
1-2-2-1-1	<i>Bolitoglossa</i>	Alberch and Gale 1985
1-2-1-1-1	<i>Bolitoglossa</i>	Alberch and Gale 1985

Note: In many cases, formulae listed are present in additional taxa as well. Institutional abbreviations: CAS, California Academy of Sciences, San Francisco, CA; MVZ, Museum of Vertebrate Zoology, Berkeley, CA.

Acknowledgments

We thank A. W. Crompton, J. Hanken, J. A. Hopson, F. A. Jenkins Jr., W. Joyce, N. Rosenthal, S. Scott, and C. Tabin for helpful comments and discussion on earlier drafts of the manuscript. Portions of this work were supported by an R. A. Chapman Memorial Fellowship, a Helen Hay Whitney postdoctoral fellowship, and grants from the National Science Foundation, the Society for Integrative and Comparative Biology, and Sigma Xi to Michael D. Shapiro.