

Patterns of Variation in the Phalangeal Formulae of Land Tortoises (Testudinidae): Developmental Constraint, Size, and Phylogenetic History

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ABSTRACT Documentation of variation in phalangeal formulae in land tortoises combined with ontogenetic information from turtles in general were used, in a phylogenetic context, to infer the potential effect of size and developmental constraints upon patterns of morphological variation. A sample of 201 specimens and published illustrations of 37 tortoise species were examined, representing all but one living genera and most species of the Testudinidae. Specimens were either articulated dry skeletons or preserved animals that were x-rayed. The patterns of digital and phalangeal loss in tortoises were predicted from developmental studies of the manus and pes in other turtles. If a digit is lost, it is the first digit, which is the last one to develop. If a digit has a single phalanx, it is usually the fifth digit. The primitive phalangeal formula for land tortoises is probably 2–2–2–2–1, the most common pattern found in living testudinid species. The presence of a second phalanx in the fifth digit evolved independently many times and usually in large tortoises. Such additions were interpreted as instances of peramorphosis. Many small tortoises have a full complement of digits (five) and phalanges (two in each digit); nevertheless, phalangeal and digital loss is associated with small size. Small and medium size tortoises exhibit greater variation in phalangeal number than do large tortoises. We hypothesize that epigenetic processes, and not simply adaptation, played a major role in the evolution of the variation in phalangeal formulae in tortoises. *J. Exp. Zool. (Mol. Dev. Evol.) 302B:134–146, 2004.* © 2004 Wiley-Liss, Inc.

INTRODUCTION

Manus and pes are convenient character complexes in studies of ontogeny and phylogeny because the various phenotypes are discrete and the product of similar developmental processes shared by most tetrapods (Schmalhausen, '15; Sewertzoff, '31; Shubin and Alberch, '86). The potential relation of these developmental processes and constraints to phalangeal reduction in amniotes has been recently reviewed by Richardson and Chipman (2003).

The manus and pes of turtles are character complexes that experienced numerous transformations during their evolution, including reduction in some of the most basal stem representatives, modifications of the flippers of marine turtles, and supernumerary phalanges in some soft-shell turtles (Rabl, '10; Walker, '73; Gaffney, '90; Meylan, '96; Sheil, 2003; Rougier et al., '98). Of particular interest are land tortoises (Testudinidae), a diverse clade of living

turtles that includes numerous fossil and living taxa and a wide variation in size (Auffenberg, '74; Ernst and Barbour, '89). Documentation of variation in phalangeal formulae in land tortoises combined with ontogenetic information from turtles in general are used here, in a phylogenetic context, to infer the potential effect of size and developmental constraints upon patterns of morphological variation.

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The understanding of phalangeal formula evolution in reptiles (Greer, '91) has greatly benefited from consideration of comparative data and development at lower taxonomic levels (Shapiro, 2002; Shapiro et al., 2003), rather than comparisons among model organisms.

Studies of the ontogeny of limbs in selected tetrapods (Essex, '27; Gans, '75; Holder, '83; Hinchliffe, '85; Crumly, '90; Müller, '91; Goodwin and Trainor, '83; Oster et al., '85; Buscalioni et al., '97; Larsson and Wagner, 2002; Richardson and Oelschläger, 2002) have offered insights and predictions about the pattern of digital and phalangeal loss and gain, in most cases under the assumption that changes in phenotype are the result of heterochronic shifts (Richardson and Chipman, 2003).

In this context, recognition of the processes involved in the formation of cartilaginous limb elements and the digital arch and primary axis (Shubin and Alberch, '86) have been of fundamental importance. Shubin and Alberch ('86) identified ontogenetically dynamic processes that result in three patterns of connectivity in the formation of cartilaginous limb elements in tetrapods. A mesenchymal chondrogenetic element may form without connectivity, usually near the beginning of the process of limb formation (e.g., in the formation of the humerus or femur). A second alternative is that a single element branches resulting in two elements distal to the division. This process is called bifurcation (apparently trifurcations or other polychotomies are never observed, perhaps for the reasons suggested by Oster and Alberch, '82). Last, a single mesenchymal condensation may either bud off a new condensation distally or become subdivided into two separate elements. This last process is called segmentation. There is a conserved sequence in which these processes occur in the ontogeny of the turtle autopodials (primary axis, digital arch), so that the appearance of some elements is a

pre-requisite to the development of other subsequent elements. This hierarchy of events implies that terminal events in a sequence are expected to be lost in evolution more easily than more basal elements. This principle is expected to generate non-random patterns of homoplasy and synapomorphy in the phylogeny of the tortoise autopodials.

Size is another factor that can affect morphological outcome in autopodial development. If chondrogenesis of an element is dependent on a critical minimum number of mesenchymal cells, reduction in size can lead to reduction in number of elements (e.g., Alberch and Gale, '83, p. 193).

Evolution and development of manus and pes in turtles

Burke and Alberch ('85) demonstrated that the developmental pattern of the manus and pes of frogs and some turtles (*Chrysemys picta* and *Chelydra serpentina*, see Table 1 and Fig. 1) is similar. However, there are minor differences between anuran and chelonian manus and pes ontogeny. Concerning the manus of frogs, for example, the earliest digit to develop is the fourth followed closely by the third digit (*vide* Shubin and Alberch, '86), and then synchronously by the second and fifth digits, and finally the first digit. The fifth digit is a *de novo* unconnected structure, i.e. it shows no embryonic affiliations to more proximal elements of the manus. In contrast to this, among turtles the fourth digit develops well before the third, not at the same time. Furthermore, the fifth digit forms synchronously with the third digit, rather than the second digit as in frogs.

There are only a few studies of the ontogeny of the manus of turtles besides that of Burke and Alberch ('85). Rosenberg (1892) described the ontogeny of the manus of *Emys orbicularis*. Baur (1892) re-interpreted the homologies suggested by Rosenberg's results, and Burke and Alberch ('85)

TABLE 1. Differences between urodelian, anuran and chelonian digital development (taken from Alberch and Gale, '85, and Shubin and Alberch, '86)

Salamanders	Frogs	<i>Chelydra serpentina</i> and <i>Chrysemys picta</i>
I and II differentiate first	IV differentiates first	IV differentiates first
III and IV differentiate sequentially (asynchronously)	II and V differentiate next (synchronously)	III and V differentiate next (synchronously)
digits differentiate in a post-axial direction	digits differentiate in a pre-axial direction	digits differentiate in a pre-axial direction

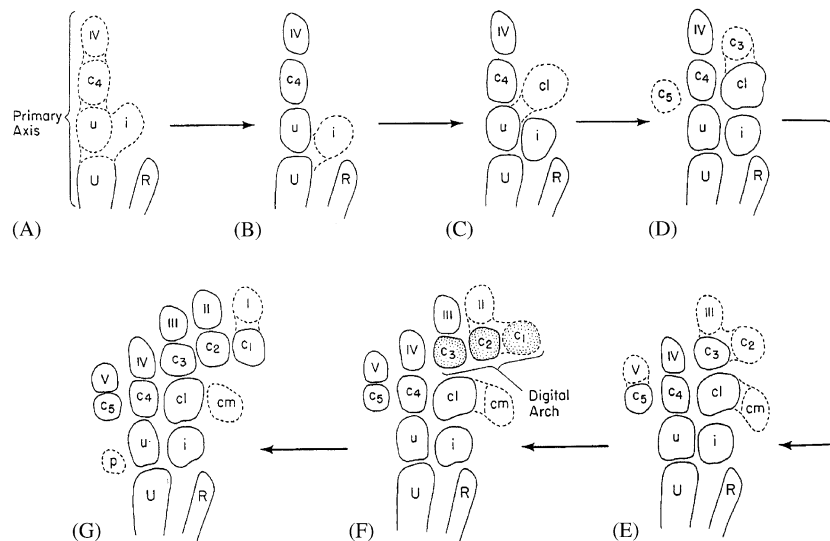


Fig. 1. A diagrammatic summary of the development of the carpus in *Chrysemys picta* and *Chelydra serpentina* (taken from Burke and Alberch, '85). The adult morphology of land tortoises suggests that they develop by means of a similar

ontogeny. Abbreviations: I-V metacarpals 1 through 5, c₁₋₅ distal carpals 1-5, cl-cm centrale, p pisiform, R radius, u ulnare, U ulna.

supported Baur's interpretation. The sequence of chondrification and ossification in the hand of some turtle species were described and discussed by Rieppel ('93, *Chelydra serpentina*) and by Sheil (2003, *Apalone spinifera*).

Auffenberg ('66) examined the adult carpus of several testudinid species and provided a functional interpretation of the observed variation. However, Auffenberg relied upon homologies now known to be incorrect (Bramble, '82) and presented illustrations so diagrammatic that independent interpretation is impossible. Homologies aside, Bramble ('82) did not corroborate many of Auffenberg's conclusions. Previous studies (e.g., Schmidt, '19; Flower, '24; '33; Hewitt, '37) provided poor or no illustrations, and usually discussed digit numbers rather than phalangeal formulae. Well-supported homologies and excellent illustrations were provided by Bramble ('82) for gopher tortoises (*Gopherus*) and other close relatives, which are specialized burrowers, but are also among the most primitive clades of the Testudinidae (Crumly, '84). Other studies of hand anatomy in turtles other than Testudinidae include Sheil (2003) for trionychids, and Gaffney ('90) and Meylan ('96), among others, for different fossil taxa.

Burke and Alberch ('85) elucidated the controversial homologies of the pes of turtles. There are no studies of the ontogeny of the pes in land tortoises. Zug ('71) is the only report of variation in the phalangeal formulae of the pes in adult

turtles. He noted that tortoises have four clawed digits, which have two phalanges each, and sometimes a fifth invisible unclawed digit that has a single phalanx. Only four species of land tortoises were examined by Zug: *Geochelone* sp., *Gopherus berlandieri*, *G. Polyphemus*, and *Kinixys erosa*. By examining a comprehensive sample of the pes of tortoises in a phylogenetic context, we expect in this work to establish the patterns of homoplasy and synapomorphy in this structure.

MATERIALS AND METHODS

Thirty-seven tortoise species were considered in this study, representing all living genera except *Acinixys* and most species of the Testudinidae (Ernst and Barbour, '89). In total, 201 specimens and published illustrations were examined. A list of specimens and the raw data are available from the senior author upon request, from the archives of the Museum of Paleontology, University of California, Berkeley. The majority of the material examined is housed at the American Museum of Natural History (AMNH), the Museum of Comparative Zoology at Harvard University (MCZ), the Natural History Museum in London (BMNH), and the National Museum of Natural History (USNM). Specimens were either articulated dry skeletons or preserved specimens that were x-rayed. Disarticulated specimens were not employed due to the difficulty of reconstructing the original pattern of elements. Many tortoise species

have dermal ossicles embedded in the skin of the forearm and heel. Thus, before X-ray photographs could be taken, specimens had to be skinned so that dermal ossicles would not be mistaken for carpal or tarsal elements. Table 2 lists the number of specimens examined for each species. Emphasis is placed on the manus, because its variation was greater than that of the pes.

Phalangeal formulae are reported as a sequence of numbers and/or letters. For example, 1-2-2-2-1 refers to a tortoise in which the first and fifth digits possess only a single phalanx, whereas the middle three digits (i.e. two, three, and four)

possess two phalanges each. An upper case 'M' is used to indicate that a digit is represented only by a metacarpal or metatarsal. In some species the phalangeal formulae vary. This is indicated by a slash. Thus, 2/1-2-2-2-1 indicates that the first and fifth digits in a particular species may possess either one or two phalanges.

Regarding the phylogeny of the taxa studied, we follow a cladogram based on a parsimony analysis of over 50 characters (mostly cranial) from 39 living tortoise species (Crumly, '84, '85). Recent phylogenetic analyses involving testudinids, both molecular and morphological, have been more

TABLE 2. Digital and phalangeal patterns observed in land tortoises

Species	N	Phalangeal formula Manus	Phalangeal formula Pes
<i>Chersina angulata</i>	10	2-2-2-2-2	2-2-2-2-1/0
<i>Geochelone carbonaria</i>	14	2-2-2-2-2/1	2-2-2-2-1
<i>Geochelone chilensis</i>	5	2-2-2-2-2	2-2-2-2-0
<i>Geochelone denticulata</i>	9	2-2-2-2-2/1	
<i>Geochelone elegans</i>	3	2/1-2-2-2-2/1	2-2-2-2-0
<i>Geochelone elephantopus</i> ssp.	9	2-2-2-2-2	2-2-2-2-1/0
<i>Geochelone gigantea</i>	3	2-2-2-2-2	2-2-2-2-1
<i>Geochelone pardalis</i>	20	2-2-2-2-2/1	
<i>Geochelone radiata</i>	5	2-2-2-2-2/1	2-2-2-2-1
<i>Geochelone sulcata</i>	2	2-2-2-2-2/1	2-2-2-2-1
<i>Geochelone yniphora</i>	2	2-2-2-2-2/1	2-2-2-?-1
<i>Gopherus agassizii</i>	9	2-2-2-2-2/1	2-2-2-2-0
<i>Gopherus berlandieri</i>	7	2/1-2-2-2/1-1	2-2-2-2-1
<i>Gopherus canyonensis</i> †	1*	?-2-2-2-1	
<i>Gopherus flavomarginatus</i>	2	2-2-2-2-1	2-2-2-2-0
<i>Gopherus laticuneus</i> †	1*	2-2-2-2-1	
<i>Gopherus polyphemus</i>	9	2/1-2-2-2-1	
<i>Gopherus uintensis</i> †	1*	2-2-2-2-1	
<i>Homopus areolatus</i>	5	0-2-2-2-2/1	2-2-2-2-1/0
<i>Homopus femoralis</i>	1	0-2-2-2-1	
<i>Homopus signatus</i>	4	2-2-2-2-2	2-2-2-2-1
<i>Indotestudo elongata</i>	8	2-2-2-2-2/1	
<i>Indotestudo forstenii</i>	1	2-2-2-2-2	
<i>Kinixys belliana</i> ssp.	14	2/1/M/0-2-2-2/1-2/1	2-2-2-2-0
<i>Kinixys erosa</i>	5	2-2-2-2-2/1	2-2-2-2-1
<i>Kinixys homeana</i>	1	2-2-2-2-2	
<i>Malacochersus tornieri</i>	11	2-2-2-2-2/1	2-2-2-2-1
<i>Manouria emys</i>	5	2-2-2-2-1	2-2-2-2-1
<i>Manouria impressa</i>	3	2-2-2-2-1	
<i>Psammobates oculifer</i>	2	2-2-2-2-1	2-2-2-2-1
<i>Pyxis arachnoides</i>	6**	2-2-2-2-2/1	2-2-2-2-0
<i>Stylemys nebrascensis</i> †	1***	2-2-2-2-2	
<i>Testudo graeca</i> ssp.	12	2/1/0-2/1-2-2-2/1	2-2-2-2-1/0
<i>Testudo hermanni</i> ssp.	3	1-2-2-2-1	2-2-2-2-1/0
<i>Testudo horsfeldii</i>	4	0-2/1-2-2/1-1	2-2-2-2-1/0
<i>Testudo kleinmanni</i>	2	1-2-2-2-2/1	2-2-2-2-0
<i>Testudo marginata</i>	1	1-2-2-2-2	2-2-2-2-1

†Fossil taxa, phalangeal formula taken from illustrations.
 *Taken from illustrations of Bramble ('82).
 **Four specimens were illustrated by Siebenrock ('06); two of these were examined by CRC.
 ***Taken from illustrations in Auffenberg ('66).

restricted in terms of the taxa studied (Lamb and Lydeard, '94; Meylan and Sterrer, 2000; van der Kuyl et al., 2002). Data for maximum size of the species examined were taken from Loveridge and Williams ('57), Williams ('60), Legler ('63), Grubb ('71), Ernst and Barbour ('72, '89), MacFarland et al. ('74), Nutaphand ('79), Pritchard ('79), Swingland and Coe ('79), Cheylan ('81), Bury ('82), Pritchard and Trebbau ('84), Juvik (pers. comm.). The nomenclature of heterochronic processes follow Alberch et al. ('79).

RESULTS

Digital and phalangeal reduction in the manus

Examination of hand phalangeal formulae mapped onto a cladogram of testudinid genera (Crumly, '84, '85) reveals several paths of evolutionary transformation (Fig. 2). No specimen was found to have more than two phalanges in either the manus or pes (this feature supports the monophyly of Testudinidae, Crumly, '84, '85)¹. In all but five specimens, the second, third, and fourth digits have two phalanges. In the specimens that did not have two phalanges in these three digits, three were *Testudo horsfieldii* (UF H-2598, USNM 14312 and ZSM 818/1920, phalangeal formula 0-2-2-1-1), one was *Gopherus berlandieri* (AMNH 94595, 1-2-2-1-1), and one was *Kinixys belliana* (MCZ 40013, 0-2-2-1-1). Also, only one of these specimens was of a taxon with five digits, i.e. *Gopherus berlandieri*. It is interesting to note that these three taxa are among the smallest species in their respective genera. Only digits two and three always had two phalanges in all specimens examined.

The fifth digit usually has only a single phalanx (Fig. 3D). Twenty-nine of the taxa sampled normally have five digits. Eighteen of these usually or sometimes have only a single phalanx in the fifth digit and two phalanges in the remaining four digits. In contrast, the first digit rarely has a single phalanx – in only eight of the taxa sampled. Of these eight taxa with one phalanx in the first digit, four are in the genus *Testudo*. A single phalanx in the first and fifth digits is present in seven of these same eight taxa (Fig. 3C and 3D): *Gopherus berlandieri*,

¹James Parham (pers. comm.) has found an Eocene fossil testudinid with three phalanges in one digit. We interpret this phenotype as either a returned plesiomorph, an atavism, or characteristic of a stem-group representative of this clade (pending study of the phylogenetic placement of this species).

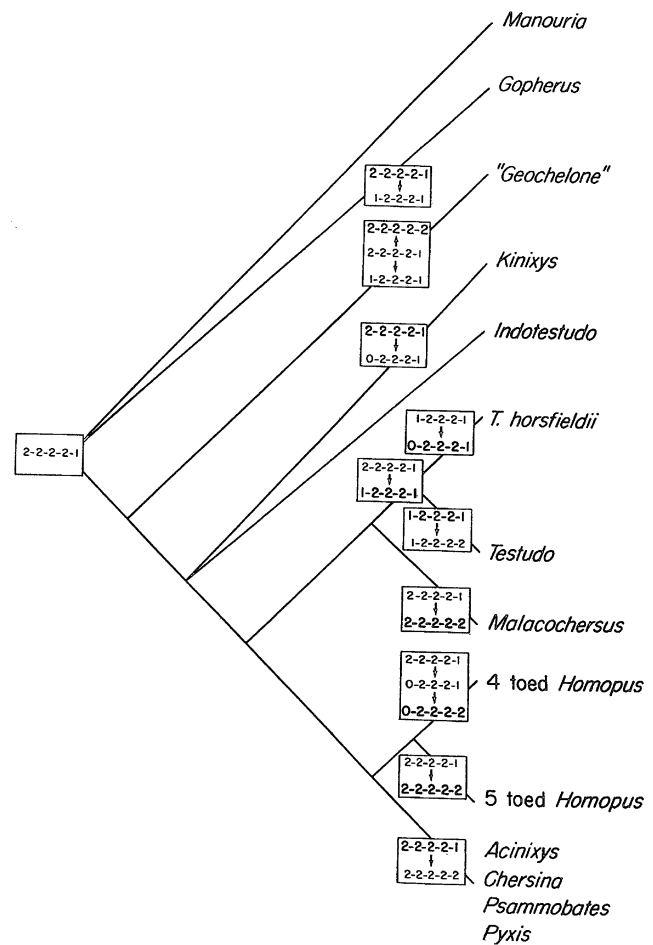


Fig. 2. Manual phalangeal formulae plotted on a cladogram for testudinid genera (Crumly, '84, '85). *Geochelone* may be paraphyletic as indicated by the quotation marks. The bold-faced formula is the most common formula observed, but not necessarily the plesiomorphic one.

Gopherus polyphemus, *Geochelone elegans*, some *Kinixys belliana*, *Testudo graeca*, *T. hermanni*, and *T. kleinmanni*. The largest and smallest extant species of *Testudo*, *Testudo marginata* and *T. kleinmanni*, respectively, are the only tortoises that ever have a single phalanx in the first digit and two phalanges in all other digits.

Many large and moderate size tortoises have two phalanges in all five digits (Fig. 3A). Examples include *Geochelone elephantopus*, *G. gigantea*, *G. radiata*, *G. sulcata*, *Chersina angulata*, some *Kinixys belliana*, and some *Geochelone chilensis*. However, even some small species have two phalanges in all digits, including *Homopus signatus*, most *Malacochersus tornieri*, and *Pyxis arachnoides*. Two of these, *Homopus signatus* and *Pyxis arachnoides*, are among the smallest of tortoises; and considering the well-known reduced

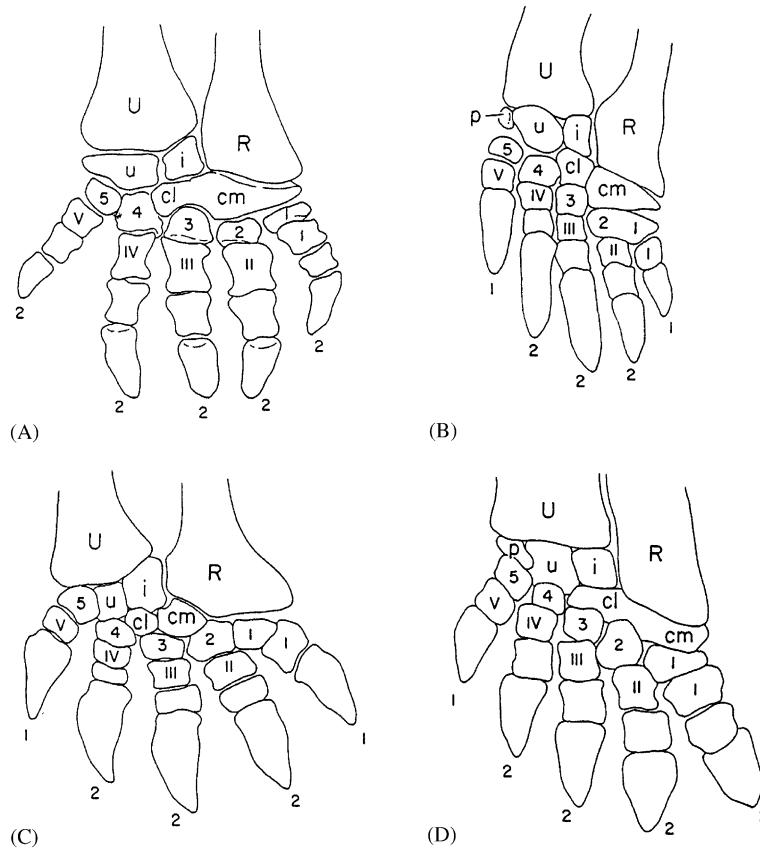


Fig. 3. Left manus of **A**) *Geochelone elephantopus epihippium* (USNM 222493) taken from an X-ray photograph of a dry specimen- Note the 2-2-2-2-2 phalangeal formula. **B**) *Testudo hermanni* (MCZ 39652) taken from a 35 mm color transparency. **C**) *Gopherus polyphemus* (USNM 7555) taken from a X-ray photograph. Note that in *G. polyphemus* as in

T. hermanni the first and fifth digits possess a single phalanx and that the phalangeal formula is 1-2-2-2-1. **D**) *Kinixys erosa* (USNM 33484) taken from a X-ray photograph. Note that the fifth digit has only a single phalanx and the phalangeal formula is 2-2-2-2-1.

shell ossification of *Malacochersus*, it is interesting that all five digits have two phalanges.

In addition to variation in phalangeal number, some tortoises have only four digits. *Homopus areolatus*, *H. femoralis*, *Testudo horsfieldii*, and *Kinixys belliana nogueyi* usually have only four digits with claws. In these taxa, there are no carpal elements representative of a fifth digit. The presence of only four digits evolved independently in each genus, as indicated not only by the phylogeny (Fig. 2), but also by the very different patterns of carpal elements. The first digit is always the one which has apparently failed to develop. This is supported by the mutual contacts of individual carpal elements (Table 3, Fig. 4) and is suggested by the ontogenetic studies of Burke and Alberch ('85), which showed the first digit as the last one to start chondrification in the digital arch. Specimens of *Kinixys belliana nogueyi* have a pisiform in the carpus, whereas both four-fingered

Homopus species and *Testudo horsfieldii* lack a pisiform. In contrast, the intermedium and ulnare are fused (or never differentiated into separate elements) in *T. horsfieldii*, whereas they are separate elements in *Homopus* and in *Kinixys*.

As stated above, tortoises with only four digits are not closely related. *Kinixys* is certainly a monophyletic taxon, based on its unique carapacial hinge (Crumly, '84, '85; Loveridge and Williams, '57; Meylan and Auffenberg, '86). Only *Kinixys belliana nogueyi* is characterized by the possession of four fingers (Loveridge and Williams ('57) observed the four-fingered condition occasionally in other *K. belliana*). *Testudo* is also a monophyletic genus as indicated by the posterior plastral hinge and dorsally concealed prootic (Loveridge and Williams, '57; Crumly, '84, '85). *Testudo* is the only genus that may be characterized (in combination with other characters) by its phalangeal formula: 1-2-2-2-1; and *T. horsfieldii*

TABLE 3. The morphological relationships of the carpus of the Testudinidae with only four digits

	<i>Homopus areolatus</i> and <i>H. femoralis</i>	<i>Testudo horsfeldii</i>	<i>Kinixys belliana nogueyi</i>
Digit lost	first	first	first*
5th distal carpal and distal ulna	no contact, ulnare separates them	contact	no contact, ulnare separates them
4th distal carpal and distal intermedium/ulnare	contact	contact	no contact
2nd distal carpal and distal radius	sometimes no contact, separated by centralia	contact	variable, sometimes fused to 3rd carpal
Intermedium and ulnare	separate	fused	separate
Lateral centrale and intermedium/ulnare	not fused	sometimes fused	not fused
Medial centrale	present and fused to lateral centrale	lost or reduced	reduced and fused to lateral centrale
Pisiform	absent	absent	present

*In some *Kinixys belliana spekkii* (i.e. MCZ 40011), the first digit is reduced, but a metacarpal is still present.

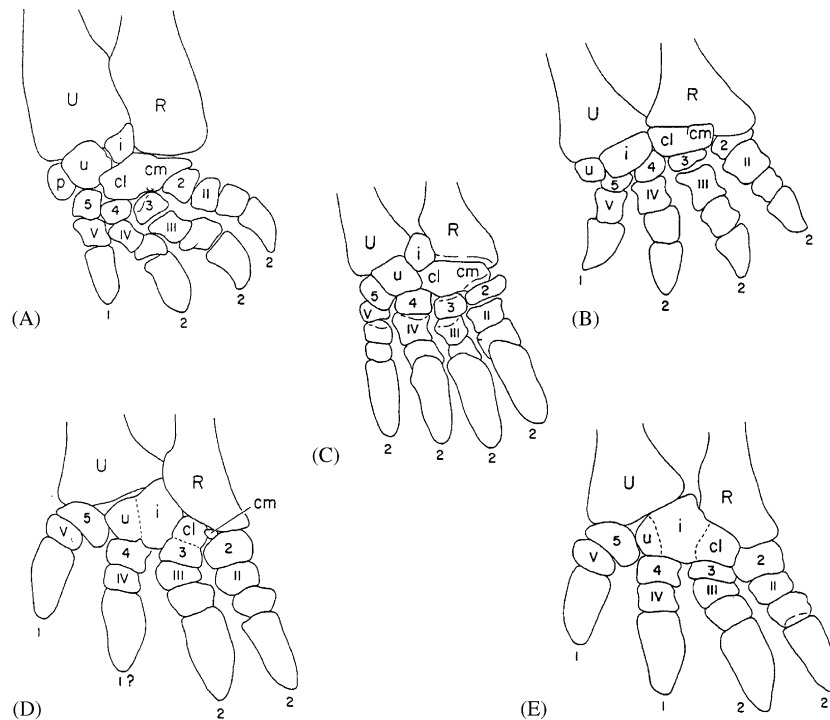


Fig. 4. **A**) The right manus of *Kinixys belliana nogueyi* (MCZ 54118) taken from an X-ray photograph. Note that the pisiform is present and that the phalangeal formula is 0-2-2-1. **B**) The right manus of *Homopus femoralis* (MCZ 42215) taken from an X-ray photograph. The pisiform is absent and the phalangeal formula is 0-2-2-1. **C**) The right manus of *Homopus areolatus* (MCZ 4057). Note that the pisiform is

absent and the phalangeal formula is 0-1-2-2-1. **D**, **E**) The left manus of two specimens of *Testudo horsfeldii* (**E** is USNM 14312 and **D** is BMNH 1920.1.20.628). **D** was redrawn from a sketch of the central carpus using the outline of **E**; **E** was taken from a X-ray photograph. Note that the phalangeal formulae for both specimens is 0-2-2-1-1.

is the only species of *Testudo* characterized by having only four fingers. The evidence for the monophyly of *Homopus* is not as clear cut; most of the evidence is either from reversals or reduction. For example, the large forearm scales are underlaid by osteoderms. These osteoderms

were lost by more basal tortoises and re-appeared in *Homopus*. *Homopus* is divided in two groups, those with four fingers and those with five. Both groups lack synapomorphies that would ally *Homopus* to either *Testudo* or *Kinixys* (Crumly, '84, '85).

Further examination of the pattern of phalangeal variation in a hypothesis of phylogeny of testudinids reveals many examples of parallelism (Fig. 2). The phalangeal formula 2-2-2-2-2 appears in unrelated species of *Geochelone*, in *Malacochersus*, *Kinixys*, and *Chersina*. *Gopherus polyphemus*, a very specialized tortoise that is part of a basal clade, shares the same phalangeal formula with many species of *Testudo*, 1-2-2-2-1. Of course, four-fingered tortoises often have the same phalangeal formula, 0-2-2-2-1.

Although the phalangeal formula of land tortoises is variable, the formula almost never varies in one respect: tortoises never have more than two phalanges per digit. A single specimen of a Galápagos tortoise possesses a small lenticular bone in the third digit between the ungual and penultimate phalanx. If this intercalary is considered a phalanx, the phalangeal formula for this specimen is 2-2-3-2-2. In contrast, emydids and chelydrids usually have three phalanges in the second, third, and fourth digits (Rabl, '10).

Observations on the hand can be summarized as follows:

1. Although all tortoises of all sizes can have two phalanges on all five digits, larger tortoises never have fewer than two phalanges in all five digits. Only smaller tortoises may lose an entire digit.
2. On average, tortoises with a single phalanx in any digit are smaller than those with two phalanges on all digits. However, this difference is not statistically significant.
3. If a digit possess only a single phalanx, it is usually the fifth digit.
4. If two phalanges are lost, it happens typically in the first and fifth digits.
5. Tortoises with only four digits have lost the first one and are among the smallest members of their respective genera.

Phalangeal reduction in the pes

Tortoises possess only four clawed digits, each with two phalanges, in the pes. However, in many species, there is a phalanx distal to the fifth metatarsal. Otherwise, there is no variation in the number of phalanges in each digit.

Tortoises with a single fifth digit phalanx appear to be larger than those without. The mean Maximum Shell Length (MSL) for tortoises without a fifth metatarsal phalanx is 0.36 meters (± 0.36); whereas tortoises with the phalanx

are larger (MSL=0.44 \pm 0.38). However, a t-test was performed and no significant difference was detected (critical $t_{05(2)(29)}=2.045$, calculated $t=0.564$).

DISCUSSION

The effect of size

Three of the parameters that effect digital ontogeny are the size of the limb bud, the rate of limb bud growth (or rate of cell death in the limb bud), and certain cellular and/or extracellular properties (Alberch and Gale, '85). Although there is no information regarding any of these three parameters for land tortoises, there is information on maximum adult size, which can be used to infer the effect of size upon the ultimate range of morphological patterns. From Figure 5 it is obvious that small tortoises above a certain threshold size do not exhibit reduced digital and phalangeal patterns. For instance, tortoises that achieve over half a meter in length always have

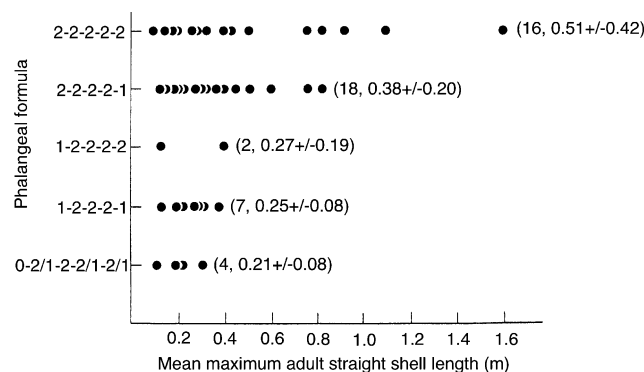


Fig. 5. Phalangeal formula *versus* Mean maximum adult straight shell length MASSL (m). Individual points represent species, and numbers in parentheses indicate number of species, mean, and standard deviation of MASSL for each phalangeal formula. Taxa with variable phalangeal formulae are included in all appropriate categories. Small size is not necessarily accompanied by the loss of phalanges and digits. Rather, large size constrains morphology as suggested by the reported standard deviation. Tortoises of larger size elevate the standard deviations for those formulae at the top of the list, because they do not possess phalangeal formulae near the bottom of this list. A t-test was performed for the mean adult straight shell length for those tortoises with a 2-2-2-2-2 phalangeal formula and those tortoises with only four digits. Differences in size were not statistically significant (critical $t_{05(2)(18)}=2.101$, calculated $t=0.936$). This further suggests that small size does not induce an obligatory response. Data for maximum size were taken from Loveridge and Williams ('57), Williams ('60), Legler ('63), Grubb ('71), Ernst and Barbour ('72, '89), MacFarland et al. ('74), Nutaphand ('79), Pritchard ('79), Swingland and Coe ('79), Cheylan ('81), Bury ('82), Pritchard and Trebbau ('84) and Juvik (pers. comm.).

TABLE 4. A list of possible single-step transitions of phalangeal formula. All transitions follow two general rules:

1. If a digit is lost entirely (i.e. a plesiomorphic condition of two phalanges to a derived condition of no phalanges or other carpal elements), it is inferred it is the first digit; and
2. otherwise phalanges are lost or gained at a time. Abbreviations: 2 = two phalanges in the digit; 1 = one phalanx in the digit; M = digit represented by a metacarpal; 0 = digit absent and no associated carpal elements

Plesiomorph	Apomorph	Hypothesized ontogenetic process	Genera with both apomorph and plesiomorph conditions	Genera with plesiomorph condition only
2-2-2-2-1	2-2-2-2-2	Peramorphosis	<i>Geochelone, Indotestudo, Kinixys, Malacochersus, Pyxis</i>	<i>Manouria, Psammobates</i>
	1-2-2-2-1	Paedomorphosis	<i>Geochelone, Gopherus, Kinixys, Pyxis, Testudo</i>	
	2-2-2-1-1	Paedomorphosis	<i>Kinixys</i>	
	2-2-2-2-M	Paedomorphosis	None	
	0-2-2-2-1	Paedomorphosis	<i>Kinixys</i>	
1-2-2-2-1	1-2-2-2-2	Peramorphosis	<i>Testudo</i>	
	2-2-2-2-1	Peramorphosis	<i>Testudo</i>	
	0-2-2-2-1	Paedomorphosis	<i>Testudo</i>	
	M-2-2-2-1	Paedomorphosis	None	
	1-2-2-2-M	Paedomorphosis	None	
	1-2-2-1-1	Paedomorphosis	<i>Gopherus</i>	
2-2-2-2-2	2-2-2-2-1	Paedomorphosis	<i>Geochelone</i>	<i>Chersina</i>
	1-2-2-2-2	Paedomorphosis	None	
	0-2-2-2-2	Paedomorphosis	<i>Homopus</i>	
1-2-2-2-2	2-2-2-2-2	Peramorphosis	None	
	M-2-2-2-2	Paedomorphosis	None	
	1-2-2-2-1	Paedomorphosis	<i>Testudo</i>	
	0-2-2-2-2	Paedomorphosis	None	
2-2-2-1-1	2-2-2-2-1	Peramorphosis	<i>Kinixys</i>	
	1-2-2-1-1	Paedomorphosis	None	
	2-2-2-1-M	Paedomorphosis	None	
	0-2-2-1-1	Paedomorphosis	None	
1-2-2-1-1	2-2-2-1-1	Peramorphosis	None	
	1-2-2-2-1	Peramorphosis	<i>Gopherus</i>	
	M-2-2-1-1	Paedomorphosis	None	
	1-2-2-1-M	Paedomorphosis	None	
	0-2-2-1-1	Paedomorphosis	None	
0-2-2-2-1	0-2-2-2-2	Peramorphosis	<i>Homopus</i>	
	1-2-2-2-1	Peramorphosis	<i>Testudo</i>	
	0-1-2-2-1	Paedomorphosis	None	
	0-2-2-2-M	Paedomorphosis	None	
	0-2-2-1-1	Paedomorphosis	<i>Kinixys, Testudo</i>	
0-2-2-2-2	1-2-2-2-2	Peramorphosis	None	
	0-1-2-2-2	Paedomorphosis	None	
	0-2-2-2-1	Paedomorphosis	<i>Homopus</i>	
0-1-2-2-2	1-1-2-2-2	Peramorphosis	None	
	0-1-2-2-1	Paedomorphosis	None	
0-M-2-2-2		Paedomorphosis	None	
0-2-2-1-1	1-2-2-1-1	Peramorphosis	None	
	0-2-2-2-1	Peramorphosis	<i>Testudo</i>	
	0-1-2-1-1	Paedomorphosis	<i>Testudo</i>	

TABLE 4 Continued.

Plesiomorph	Apomorph	Hypothesized ontogenetic process	Genera with both apomorph and plesiomorph conditions	Genera with plesiomorph condition only
	0-2-2-1-M	Paedomorphosis	None	
0-1-2-1-1	1-1-2-1-1	Peramorphosis	None	
	0-1-2-2-1	Peramorphosis	None	
	0-2-2-1-1	Peramorphosis	<i>Testudo</i>	
	0-1-2-1-M	Paedomorphosis	None	
	0-M-2-1-1	Paedomorphosis	None	

five digits, four of which always possess two phalanges. Furthermore, tortoises longer than three quarters of a meter have five digits with two phalanges in each. In essence, the morphological variability in the hand of testudinids is constrained by large size. Of interest here is the Bell's hinge-back tortoise, *Kinixys belliana*, the smallest species of *Kinixys* and a moderate sized tortoise. This taxon has the most variable phalangeal formula, 2/1/M/0-2-2-2/1-2/1. Small tortoises exhibit any of the major phalangeal formulae; whereas larger tortoises exhibit fewer patterns and the largest tortoises exhibit but a single formula. Thus, morphological constraint (as estimated by adult variability) is the most severe in larger tortoises. The prominent trend towards dwarfism in some tortoise lineages (Crumly, '84) may somehow relax this constraint, thereby contributing to higher levels of adult variability. As in the plethodontid salamander *Thorius* (Hanken, '85), miniaturization in tortoises is associated with an increase in variation in the hand. This is the reverse of the pattern observed in domestic dogs. Alberch ('85) modeled heterochronic processes by noting that large breeds often (but not necessarily) had a dew claw, whereas smaller breeds lacked such a claw. In *Thorius* and tortoises, small size and increased variation are correlated. In dogs, large size and variation are correlated. Heterochrony is associated with both patterns.

Hypotheses of heterochrony in the evolution of the testudinid hand

With the phylogeny of testudinids as background (Crumly, '84, '85), we suggest hypotheses on the changes in developmental timing or heterochronies that were associated with the evolutionary changes here described (see Table 4).

The primitive phalangeal formula of the tortoise hand is likely 2-2-2-2-1 (Fig. 2), the most

common pattern found in living testudinid species (nearly 60%). Outgroup comparison supports this hypothesis; but not because the phalangeal formulae in emydid turtles is directly comparable. Rather, in most emydids, the fifth digit has fewer phalanges than the first four digits. We suggest that this relationship is conserved and probably the consequence of maintained developmental parameters within tortoises. If this hypothesis is true, then the addition of a phalanx in the fifth digit (i.e. a phalangeal formula of 2-2-2-2-2) would be peramorphic (Alberch et al., '79). A 2-2-2-2-2 phalangeal formula is common in the large species of *Geochelone* (e.g., *G. elephantopus* and *G. gigantea*), where hypermorphosis would be a reasonable expectation for an evolutionary process. But this formula is also found in medium size tortoises (e.g., *Chersina angulata*) and even small tortoises (e.g., *Homopus signatus* and *Pyxis arachnoides*). In these smaller taxa, acceleration of the development is a more reasonable hypothesis of an evolutionary process. In most medium and small tortoises, which commonly exhibit the 2-2-2-2-2 phalangeal formula, the only frequent variant is the 2-2-2-2-1 formula. This further suggests that 2-2-2-2-1 is the primitive phalangeal formula for land tortoises. Furthermore, many of these small species with the 2-2-2-2-2 formula also have well developed osteoderms underlying the scales of the brachium, which may be symptomatic of some peramorphic process.

It seems unlikely that the testudinid phalangeal formula was achieved through the sequential loss of individual phalanges; otherwise one might expect the occasional atavism (*sensu* Hall, '84) of a tortoise with three phalanges in one of its digits. It is more reasonable to assume that a single evolutionary event precipitated the tortoise phalangeal formula. We can only speculate as to what developmental parameter (e.g., size of limb bud, etc.) may have been altered by such an event. This question could be addressed by comparative

developmental studies of testudinid and emydid ontogenies, which may reveal crucial aspects associated with the origin of the Testudinidae.

CONCLUSIONS

The patterns of digital and phalangeal loss in land tortoises are predicted from developmental studies of the manus and pes in other turtles and frogs. If a digit is lost, it is the first digit, which is the last one to develop. If a digit has a single phalanx, it is usually the fifth digit. The primitive phalangeal formula for land tortoises is probably 2-2-2-2-1. Outgroup comparisons and ontogenetic information support this interpretation. The presence of a second phalanx in the fifth digit evolved independently many times and usually in large tortoises. Such additions are interpreted as instances of peramorphosis. Many small tortoises have a full complement of digits (five) and phalanges (two in each digit); nevertheless, phalangeal and digital loss is associated with small size. Variation in phalangeal formula is constrained by large size. Small and medium size tortoises exhibit greater variation in phalangeal number than do large tortoises. With the exception of one phalangeal formula found in only one specimen, all observed phalangeal formulae can be derived from single-step transformations that follow two rules:

1. If a digit is lost entirely (including its carpal and metacarpal) in one step, it is always the first digit;
2. otherwise phalanges are lost one at a time.

Following these rules, many transformations are possible that are never observed. Although observed and unobserved patterns can be interpreted as peramorphic or paedomorphic, data are not available to support any of the possible processes (i.e. acceleration, hypermorphosis, neoteny, progenesis) by which digits and/or phalanges could be lost or gained.

We hypothesize that epigenetic processes, and not simply adaptation, played a major role in the evolution of the variation in phalangeal formulae in land tortoises. Auffenberg ('66, p.181) interpreted the high levels of variation in the carpus of tortoises as related to '...failure of the component elements to serve a definite mechanical need.' However, in order to test for the potential functional significance of phalangeal/digit loss in testudinids, the relative length of the structures

interacting with the locomotory substrate (digits/hand) should be measured, analyzed, and compared across species (Shapiro, 2002). Perhaps some of the observed variation in phalangeal formulae is produced by mechanical demands of terrestrial locomotion. One could speculate that loss of phalanges does not occur in large species because manus and pes have to sustain a heavier body, and changes in the number of elements in digits may influence body stability during locomotion. Concerning reduction, Hildebrand ('85, p. 104) suggested a functional hypothesis to explain the evolution of a 2-2-2-2-1 phalangeal formula in fossorial (*Gopherus*) tortoises. He claimed that '...rigidity may be achieved by the loss or fusion of bones. Gopher tortoises have the reduced phalangeal formula 2-2-2-2-1 (but non-fossorial testudinids have the reduced formula 2-2-2-2-2)...' Our analysis of variation in phalangeal formulae in Testudinidae, taking into account phylogenetic and developmental aspects, shows that functional considerations alone are, however, not enough to explain variation in this character complex in tortoises. This study is an example of an examination of 'patterns of phenotypic evolution' in the research agenda of developmental evolution, as summarized by Wagner et al. (2000, p. 820), providing an example of how 'developmental mechanisms can either constrain or facilitate evolutionary change.'

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