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Evolution, Vol. 49, No. 5. (Oct., 1995), pp. 874-884.

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MORPHOLOGICAL VARIATION IN THE LIMBS OF *TARICHA GRANULOSA* (CAUDATA: SALAMANDRIDAE): EVOLUTIONARY AND PHYLOGENETIC IMPLICATIONS

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Abstract.—Variation in the limb skeleton was studied in a large sample (452 individuals) of adult newts (*Taricha granulosa*) from a single population in central California. A standard morphology is found in the hands and feet in more than 70% of individuals, but there is important variation in the remainder of the sample. Although twice as many individuals express variant tarsal (18.9%) than carpal (9.3%) patterns, the carpus reveals a greater diversity of variant conditions. Only a few of the many possible arrangements (including loss, addition, and amalgamation) of the seven carpal and nine tarsal elements are encountered. Most of the observed patterns are readily classified on the basis of phylogenetic relationship and models of development. Five patterns are bilaterally symmetrical. Two of these five arrangements are atavisms that represent plesiomorphic states. The other three symmetrical variants duplicate patterns found elsewhere among derived urodele taxa. These homoplastic patterns correspond to apomorphies of other genera within the same family or apomorphies of deeply nested and highly specialized members of other families (e.g., several plethodontid genera). This variation reveals the potential of the generative system and suggests the existence of limited domains of phenotypic expression. Both the origin of novelty and the restoration of ancestral states reflect the action of design constraints that not only place limits but also provide opportunities during the morphological and phyletic diversification of urodeles.

Key words.—Atavism, caudata, constraint, homoplasy, limbs, variation.

Received October 4, 1993. Accepted August 3, 1994.

The constraints imposed by phylogenetic history and embryological development can lend a predictability to patterns of intraspecific variation. The parallels between synapomorphies, intrapopulational variability, and experimentally induced teratologies reflect the influence of internal constraints on variation (Alberch 1983; Hanken 1985; Maynard Smith et al. 1985; Wagner 1989a,b; Vogl and Rienesl 1991; Rienesl and Wagner 1992). The high frequency of atavistic morphologies in vertebrate phylogenies suggests that phylogenetic relationships can be one means of predicting patterns of intraspecific variation (Hall 1984; Wake 1991). The congruence between predictions from models of development and observations of phenotypic diversity directs attention to specific mechanisms that are involved in the generation of patterns of intraspecific variation and, by extension, morphological novelties.

The limb morphology of urodele amphibians has emerged as a promising system for analysis of the action of internal constraints on morphological diversity (Alberch 1983; Alberch and Gale 1985; Hanken 1983, 1985; Hanken and Dinsmore 1986; Shubin and Alberch 1986; Oster et al. 1988; Vogl and Rienesl 1991; Wake 1991; Rienesl and Wagner 1992). The homoplastic reduction of limb structure has been a dominant theme of urodele phylogenesis. The manus of cladistically basal urodele taxa contains a minimum of 10 carpal elements, whereas the pes contains at least 11 tarsals (fig. 1A); collectively the carpals and tarsals are termed mesopodials. Major trends in the evolution of the caudate limb are: (1) reduction in the number of mesopodials (fig. 1), (2) reduction in the number of phalanges, and (3) loss of digits (fig. 1).

Mesopodial arrangements that are homoplastic are commonly encountered in studies of intrapopulational variation. The arrangement of distal tarsals 4 and 5 is a key example.

The primitive amphibian tarsus (represented by Paleozoic temnospondyls) is similar to that of basal salamanders (represented by hynobiids and cryptobranchids, fig. 1A) in the possession of five toes and separate distal tarsals 4 and 5 (Schaeffer 1941). Several salamander taxa (e.g., *Cynops*, *Triturus*, *Nototriton*, *Bolitoglossa*, and *Parvimolge*) display the derived condition of a combined distal tarsal 4 and 5 (fig. 1C). The resulting enlarged postaxial element (dt 4 + 5) articulates with both the fourth and fifth metapodials. This amalgamation has occurred several times independently (in the families Salamandridae and Plethodontidae) and would be homoplastic in any of the hypotheses of urodele phylogeny presented by Larson and Dimmick (1993). It also is encountered in the intrapopulational variation of *Plethodon cinereus*, a species that typically has two separate elements (Hanken 1983). Atavisms also occur; in *Bolitoglossa subpalmata* (a species that normally possesses dt 4 + 5) the ancestral pattern of separate and distinct dt 4 and dt 5 is a variant observed in natural populations (Alberch 1983). This parallel between patterns of intrapopulational variability and homoplastic characters is so common as to be the expected situation in urodele limb diversity (Wake and Larson 1987; Wake 1991).

In this paper, we report on a study of variation in limb morphology in the salamandrid *Taricha granulosa*. This data base represents the largest-known series (452 individuals) from a single urodele population. Departures from a standard mesopodial arrangement are found in *Taricha*, and, at least in the hind limb, alternative patterns reach a relatively high frequency. Symmetrical patterns of variation are of special interest because they imply an organismwide cause for anomalies rather than local regenerative perturbations. We compile known patterns of urodele mesopodial diversity to examine patterns of variation at two levels. First, intrapopulational variation of *T. granulosa* is compared to “standard” patterns

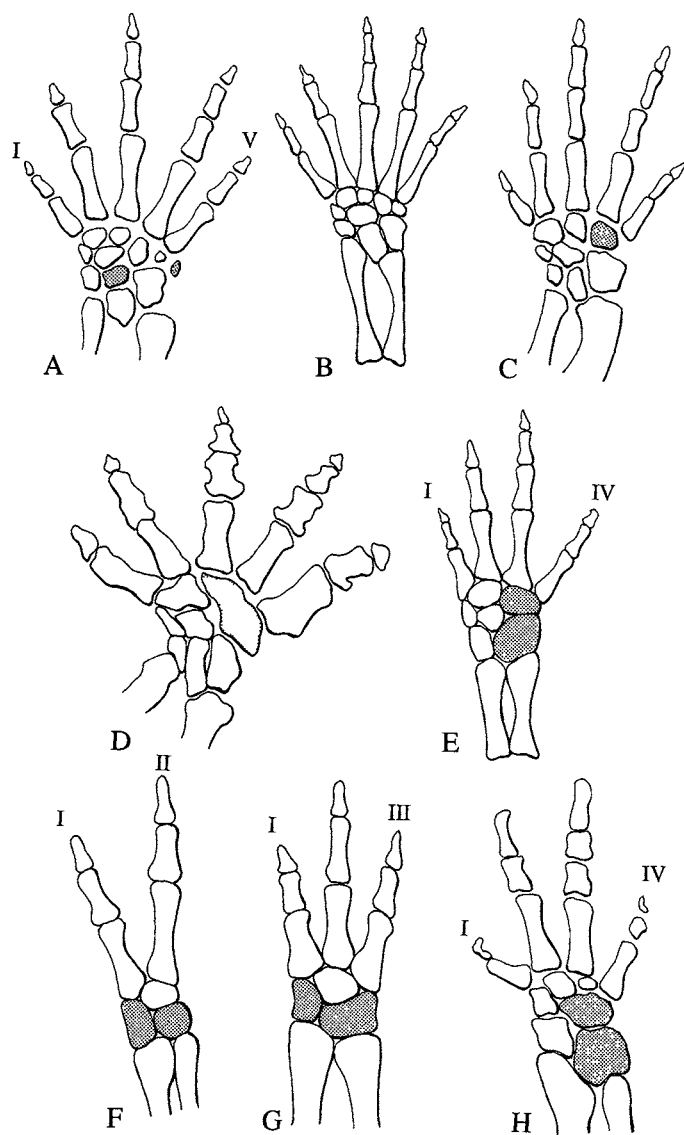


FIG. 1. The tarsus of selected urodeles. Compare with *Taricha* (fig. 3). Preaxial (anterior) is on the left. No distinction is made between cartilage and bone. A. Plesiomorphic urodeles such as hynobiids, (e.g., *Liua*, shown) possess "extra elements" (stippled) such as po (right) and an extra c (left). B. *Ambystoma* has a generalized pattern common to many nonhynobiid urodeles. C. A combined dt 4 and dt 5 (stippled) has evolved independently in salamandrids (e.g., *Cynops*, shown) and plethodontids. D. Some species of *Bolitoglossa* (see table 5) possess the pattern in C and some others (e.g., *B. dolfeini*, shown) have a combined dt 3, dt 4, and dt 5 (stippled). E. *Necturus* has a combined dt 3 + 4 (stippled, top) and a combined f + i (stippled, bottom); in addition, the fifth digit has been lost. F. The reduced hind limbs of *Proteus* have but two digits and two proximal tarsals; t and y (stippled, left) form a single unit and f and i (stippled, right) are fused. G. *Amphiuma* has small limbs with three digits but its tarsus is otherwise similar to *Proteus*. H. The plethodontid *Thorius* has a combined dt 4, dt 5 and c (stippled, top) and a combined i and f (stippled, bottom). This pattern also is seen in the plethodontid *Oedipina*. Lists of the abbreviations are in Appendixes 1-3.

of other taxa. Second, variants of *Taricha* are compared to intrapopulational patterns described in other urodeles. We give a phylogenetic interpretation of deviations from the general pattern to gain an understanding of regularities of phylogenetic transformation and to hypothesize possible developmental causes of variation. This phylogenetic approach suggests that patterns of variation are predictable from knowledge of the history of lineages and their patterns of morphogenesis.

MATERIALS AND METHODS

We obtained more than 500 individuals of *Taricha granulosa* from a single population in Marin County, California (Point Reyes National Seashore). All of these individuals perished in a mass kill (sudden and complete freeze of a small, shallow pond) during December 1991 and were collected as part of a salvage of the site. Several badly decomposed individuals were not included in this study. We prepared 452 individuals by clearing and double-staining whole mounts for bone and cartilage (Hanken and Wassersug 1981). All specimens are catalogued in the collection of the Museum of Vertebrate Zoology (MVZ). Mesopodial morphology and phalangeal formulae were scored for all limbs. We noted both unilateral and bilateral patterns of variation and were able to score all but one forelimb and two hind limbs (which were badly damaged or so bizarre as to present unrecognizable elements). Our total sample was 903 forelimbs and 902 hind limbs.

MORPHOLOGY OF THE URODELE LIMB SKELETON

The development of the urodele limb (Shubin and Alberch 1986; Blanco and Alberch 1992) enables us to classify anatomical variation. Anlagen of skeletal elements mainly arise as extensions of existing cartilage elements that later separate from one another. Three major patterns of developmental connectivity occur: bifurcation, segmentation, and de novo condensation (Oster et al. 1988). Bifurcation occurs when two anlagen arise within a Y-shaped extension from an existing element, whereas segmentation occurs when a single element divides from an existing extension. An element that arises in isolation is a de novo condensation. In *Ambystoma mexicanum*, two major axes of development are observed: a proximodistal axis initiates at the humerus/femur, radius/tibia, ulna/fibula and extends to other mesopodials, while an anteroposterior (digital) axis arises on the anterior (preaxial) border and development of both digits and distal mesopodials progresses posteriorly (postaxially) (fig. 2B). The two axes of development terminate in the region of the centrale and ulnare/fibulare, where they converge and merge.

The forelimb includes a proximal humerus (H) which extends distally to a bifurcation that contains the anlagen of the radius (R) and the ulna (U). The anlage of R in turn extends distally and segments the radiale (r) and a more distal element (variously termed a mediale or a centrale; we refer to it as y). The anlage of U extends distally and postaxially to a bifurcation that contains the anlagen of the intermedium (i) and the ulnare (u); i then segments to produce a centrale (c). The centrale may bifurcate, segment or both; but, it neither bifurcates nor segments in the more derived urodele

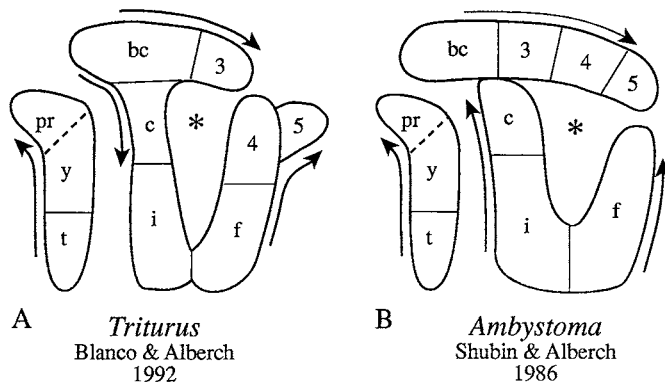


FIG. 2. Two modes of urodele limb development, based on studies by Blanco and Alberch (1992) for *Triturus marmoratus* (A) and Shubin and Alberch (1986) for *Ambystoma mexicanum* (B). In both taxa, individual elements are typically connected to one another in early development and separate during later stages. Carpal and tarsal variation in urodeles can be classified by their correspondence to these patterns. Expected patterns of variation are the amalgamation of elements that are connected during embryogenesis (e.g., $i + c$, $dt\ 4 + 5$, $y + t$). The fusion of elements that are not connected during embryogenesis (e.g., $dt\ 4 + 5 + c$) is not explained by these developmental patterns. An asterisk denotes the central region that is the site of diverse patterns of amalgamation and condensation (fig. 4; tables 1, 3, 5; Wake 1966, 1991). The proximodistal and anteroposterior axes of differentiation (arrows) often appear to merge at this zone in later stages.

lineages (most families). The anlage of the basale commune (bc) forms as a de novo condensation that is distal to and separate from the other carpal rudiments. The bc and metapodials one and two are the first elements of the digital arch to appear. The digital arch segments and bifurcates in a postaxial sequence to produce the distal carpals (dc), normally two in number, and the metacarpals (mc), four in number. Each metacarpal segments to produce the final phalangeal formula of 1-2-3-2 (from pre- to postaxial).

The hind limb includes a proximal femur (F), which extends to a bifurcated, Y-shaped zone that contains the anlagen of the tibia (T) and the fibula (Fi). The anlage of T extends distally and segments successively to include the tibiale (t) and then y. The anlage of Fi bifurcates postaxially to form i and the fibulare (f); i later segments to form c. The anlage of bc gives rise to the digital arch, which segments and bifurcates to produce the series of distal tarsals (dt), normally three, and the metatarsals (mt), normally five. Each metatarsal segments to produce the final phalangeal formula of 1-2-3-3-2.

Blanco and Alberch (1992) noted that limb development in *Triturus marmoratus* (Salamandridae) differs from that of *A. mexicanum*. In this species, c and i arise within a proximal extension of bc and the digital arch includes only three digits (I–III) in the hand and four digits in the foot (I–IV). The development of the postaxial mesopodium differs from *Ambystoma*; dc 4 is originally connected to the ulnare, whereas the primordium of dt 5 initially extends distally and postaxially from the fibulare. The developmental pattern of *Taricha* is currently undescribed, but material in the collection of the Museum of Vertebrate Zoology suggests that it shares features with both *Triturus* and *Ambystoma* (Shubin and Wake

in press). *Taricha* has proximodistal and anteroposterior axes of limb development but differs from *Triturus* in not having a distal to proximal sequence of development of the central region. The digital arch of *Taricha* definitely includes bc and, dc 3/dt 3, and dt 4. Our series of *Taricha* does not allow us to determine whether dc 4 and dt 5 develop within the digital arch (as in *Ambystoma*) or connected to the the ulna/fibula (as in *Triturus*).

Reductions in numbers of mesopodial elements in urodeles result from: (1) failure of an element to form during development, (2) formation of an element but a failure to separate early developmental connections (typically resulting in an enlarged “combined” element) and (3) fusion of elements that are separate developmentally. Extra elements (supernumerary elements of various authors) arise from extended sequences of segmentation or bifurcation, or from novel patterns of de novo condensation.

RESULTS

The normal limb morphology of *Taricha granulosa* is shown in figure 3. Variant patterns were encountered more commonly in the hind limb (11.1% of limbs, occurring in 18.9% of individuals) than in the forelimb (4.8% of limbs, occurring in 9.3% of individuals). Tables 1, 2, 3, and 4 list all patterns of variation; all bilateral patterns encountered are illustrated in figure 4. Two or more anomalous patterns were rarely encountered in the same limb.

Forelimb

There are 21 different mesopodial arrangements, and each variant condition is rare. Extra carpal elements are found in 2.1% of the limbs (4.2% of the individuals). The frequencies of these conditions are all less than 1%. Independence of u and i is seen in five individuals (probable failure of an expected fusion during development). Five limbs have an extra c (table 1). Three limbs possess an element between u and dc 4. An extra postaxial dc was observed in three limbs. There is one example of po, a tiny element situated postaxial and proximal to dc 4.

Amalgamations are seen in 2.7% of limbs (5.1% of individuals) and 14 different patterns are observed (table 1). The frequencies do not exceed 1% of limbs or individuals. A case of symmetry involves $dc\ 4 + c$ (there is also an additional asymmetrical case) (fig. 4A).

Variation in the number of phalanges is found in 3.5% of forelimbs (table 2). The distal phalanx on the third digit is lost in 1% of the limbs (table 2). Other digital anomalies are present on fewer than 1% of the limbs.

Hind Limb

Twelve different mesopodial arrangements are observed. Supernumerary elements are more common in the tarsus (6.4% of limbs, 10.6% of individuals) than in the carpus (table 3). The most common extra element, m, is found in 5.4% of limbs (fig. 4D). There are nine symmetrical cases (2.0% of individuals), and m is found in 8.9% of all individuals. There are five instances of a pr (one symmetrical) (fig. 4B), two instances of an asymmetrical double c, and one

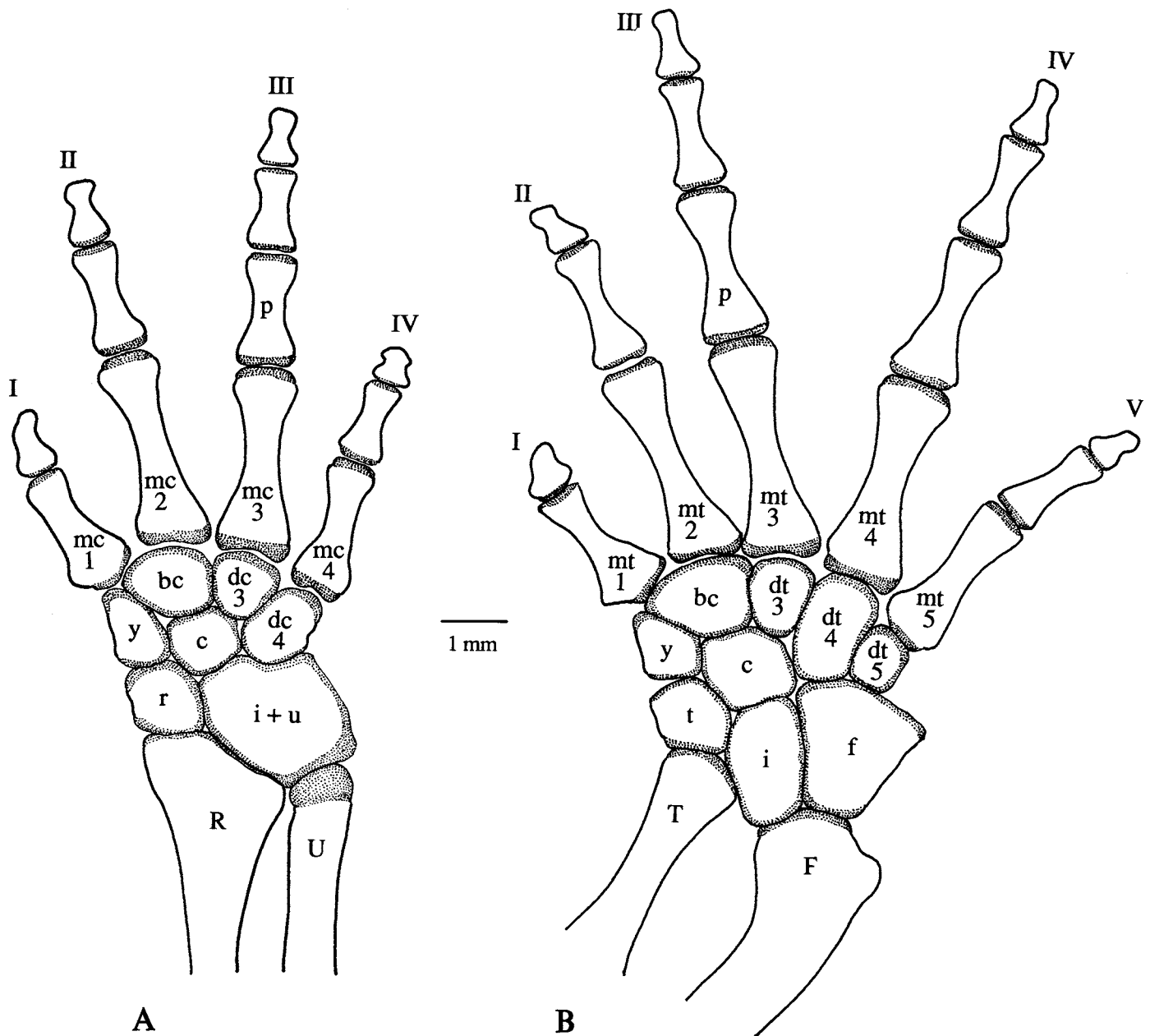


FIG. 3. Standard pattern of the right forelimb (A) and right hind limb (B) of *Taricha granulosa* (based on MVZ 216255). Cartilage is stippled.

triple c. There is one instance of po, and one instance of a preaxial proximal element lying between t and i.

Amalgamations occur in 4.4% of limbs (7.5% of individuals), and there are six different patterns (table 3). The most common patterns are dt 4 + 5 (possibly incorporating the anlage of m) (2.9% of limbs; 4.7% of individuals; 5 cases of symmetry) (fig. 4C) and what we interpret to be a fusion of dt 5 with m (0.8% of limbs; 1.3% of individuals; 1 case of symmetry) (fig. 4E).

Phalangeal variation was found in approximately 3.6% of the limbs (table 4). Individual anomalies occur at a frequency of less than 1%.

DISCUSSION

Comparative Morphology

Patterns of variation typically involve: (1) union of adjacent proximal carpals/tarsals (r/t, i, u/f); (2) amalgamation of y and the more proximal r or t; (3) union of adjacent distal carpals/tarsals (e.g., dt 4 + 5); (4) fusions between postaxial distal mesopodials and c (e.g., dc 4 + c); (5) additional centralia; (6) additional postaxial distal mesopodials, including po; (7) pr; and (8) atavistic separation of amalgamated elements (a symplesiomorphy for the taxon in question; e.g., separation of u from i in *Taricha*, or separation of dt 4 and

TABLE 1. Variant carpal patterns encountered in the sample of *Taricha granulosa* from Point Reyes National Seashore. Total FL are the total numbers of forelimbs coded. Symm FL are bilateral conditions that are found in the left and right limbs of a given individual. The slash demarcates the percentage of limbs with a particular variant condition (left) from the percentage of limbs that have the variant pattern bilaterally symmetrical (right). See Appendix 2 for abbreviations.

| | Total FL | Symm FL | % |
|------------|----------|---------|-------|
| Typical | 860 | | 95.7 |
| +2c | 1 | 0 | .1/0 |
| +c | 5 | 0 | .5/0 |
| +dc | 3 | 0 | .3/0 |
| +u | 3 | 0 | .3/0 |
| bc + 3 | 1 | 0 | .1/0 |
| bc + 3 + 4 | 1 | 0 | .1/0 |
| bc + 3 + c | 1 | 0 | .1/0 |
| bc + c | 1 | 0 | .1/0 |
| dc2 + c | 1 | 0 | .1/0 |
| dc3 + 4 | 4 | 0 | .4/0 |
| dc4 + c | 3 | 1 | .3/.1 |
| i + c | 1 | 0 | .1/0 |
| m | 1 | 0 | .1/0 |
| po | 1 | 0 | .1/0 |
| r + i + u | 1 | 0 | .1/0 |
| R + r | 1 | 0 | .1/0 |
| sep. u/i | 5 | 0 | .5/0 |
| u + i + c | 3 | 0 | .3/0 |
| U + u | 1 | 0 | .1/0 |
| y + r | 4 | 0 | .4/0 |
| y + r + c | 1 | 0 | .1/0 |

5 in *Triturus*). These eight arrangements encompass most of the patterns described in urodeles (table 5).

Variation in the forelimb is less common in *Taricha* than in other urodeles studied to date (plethodontids, *Plethodon*: Hanken 1983; *Thorius*: Hanken 1985; *Bolitoglossa*; Alberch 1983. Salamandrids, *Triturus cristatus* and *Triturus marmoratus*: Rienesl and Wagner 1992; *Triturus carnifex*: Zaffaroni et al. 1992. Rhyacotritonids: Good and Wake 1992). There were numerous kinds of carpal arrangements even though

TABLE 2. Digital variation in the manus in the sample of *Taricha granulosa* from Point Reyes National Seashore. Phalangeal formulae are shown with departures from the standard arrangement depicted in bold. The total represents the number of limbs with a particular pattern.

| Phalangeal formula | Total | % |
|--------------------|-------|------|
| 1—2—3—2 | 836 | 96.5 |
| 1—2— 2 —2 | 13 | 1.5 |
| 1—2— 4 —2 | 1 | .1 |
| 1—2—3— 3 | 2 | .2 |
| 1— 3 —3—2 | 4 | .5 |
| 2 —2—3—2 | 1 | .1 |
| 1 —3—2 | 1* | .1 |
| 1—2— 3 | 1* | .1 |
| 0 —1—2—2 | 1 | .1 |
| 1—2— — —2 | 1* | .1 |
| 1—2—3—2 | 1 | .1 |
| 1—2—3—2— 2 | 2 | .2 |
| 1—2— — —3 | 1 | .1 |
| 1—2—3— 2 | 1† | .1 |

* Conditions for which digital homologies could not be safely drawn.

† A case in which the distal phalanx of the fourth digit is bifurcated distally.

TABLE 3. Variant tarsal patterns encountered in the sample of *Taricha granulosa* from Point Reyes National Seashore. Total HL are the total numbers of scoreable hind limbs in the sample. Symm HL are bilateral conditions that are found in the left and right limbs of a given individual. The slash demarcates the percentage of limbs with a particular variant condition, on the left, from the percentage of limbs that have the variant pattern in a bilaterally symmetrical state, on the right. See Appendix 3 for abbreviations.

| | Total HL | Symm HL | % |
|------------|----------|---------|---------|
| Typical | 802 | | 88.9 |
| +2c | 1 | 0 | .1/0 |
| +c | 2 | 0 | .2/0 |
| +y (or pr) | 5 | 1 | .5/.1 |
| -dt5 | 2 | 0 | .2/0 |
| bc + 3 | 2 | 0 | .2/0 |
| bc + c | 1 | 0 | .1/0 |
| dt3 + 4 | 3 | 0 | .3/0 |
| dt4 + 5 | 26 | 5 | 3.0/0.5 |
| dt5 + m | 7 | 1 | .7/0.1 |
| f + i | 1 | 0 | .1/0 |
| m | 49 | 9 | 5.4/1 |
| preaxial c | 1 | 0 | .1/0 |

this variation was not common. Most carpal anomalies occur at low frequency (<1%) and only one is present bilaterally. The only symmetrical pattern, an amalgamation of dc 4 and c (dc 4 + c; fig. 4A, table 1), is standard for the plethodontid genera *Thorius* (Hanken 1985; Hanken and Wake 1994) and *Oedipina*, both of which have miniaturized limbs. Distal carpal 4 + c has been described as a common variant in populations of *Plethodon cinereus* (Hanken 1983; Dinsmore and Hanken 1984, 1986; Hanken and Dinsmore 1986).

Some asymmetrical variants correspond to patterns that are standard in other taxa (table 5). Many of these variants restore plesiomorphic states. For example, an extra centrale (+c, table 1) is encountered in Paleozoic temnospondyls (fig. 5), some hynobiids (e.g., *Liua*, fig. 1A), and *Andrias* (Cryptobranchidae). Distinct and separate u and i (sep. u/i, table 1)

TABLE 4. Digital variation in the pes of *Taricha torosa*. Phalangeal formulae are depicted with the departures from the standard arrangement shown in bold. The total is the number of limbs with the specified pattern.

| Phalangeal formula | Total | % |
|-----------------------------|-------|------|
| 1—2—3—3—2 | 838 | 96.4 |
| 1—3—3—3—2 | 3 | .4 |
| 1—2— 4 —3—2 | 5 | .6 |
| 1—2— 4 — 4 —2 | 2 | .2 |
| 1—2—3—3— 1 | 5 | .6 |
| 1—2— 2 —3—2 | 2 | .2 |
| 1— 1 —3—3—2 | 2 | .2 |
| 1—2—3— 4 —2 | 2 | .2 |
| 0 —2—3—3—2 | 4* | .5 |
| 1—2—3—2 | 1† | .1 |
| 1— 3 —3—3—3 | 1 | .1 |
| 1—2—3—3— 0 | 1* | .1 |
| 1—2— 2 — 3 —2 | 1‡ | .1 |
| 1—2— 2 —3— 1 | 1‡ | .1 |
| 1—2—3—3—3— 1 | 1§ | .1 |

* Conditions in which a metatarsal is present without any phalanges.

† Case in which a digit was missing altogether.

‡ Cases in which metatarsals are fused (all in bold).

§ Case in which a digit is intercalated between two others.

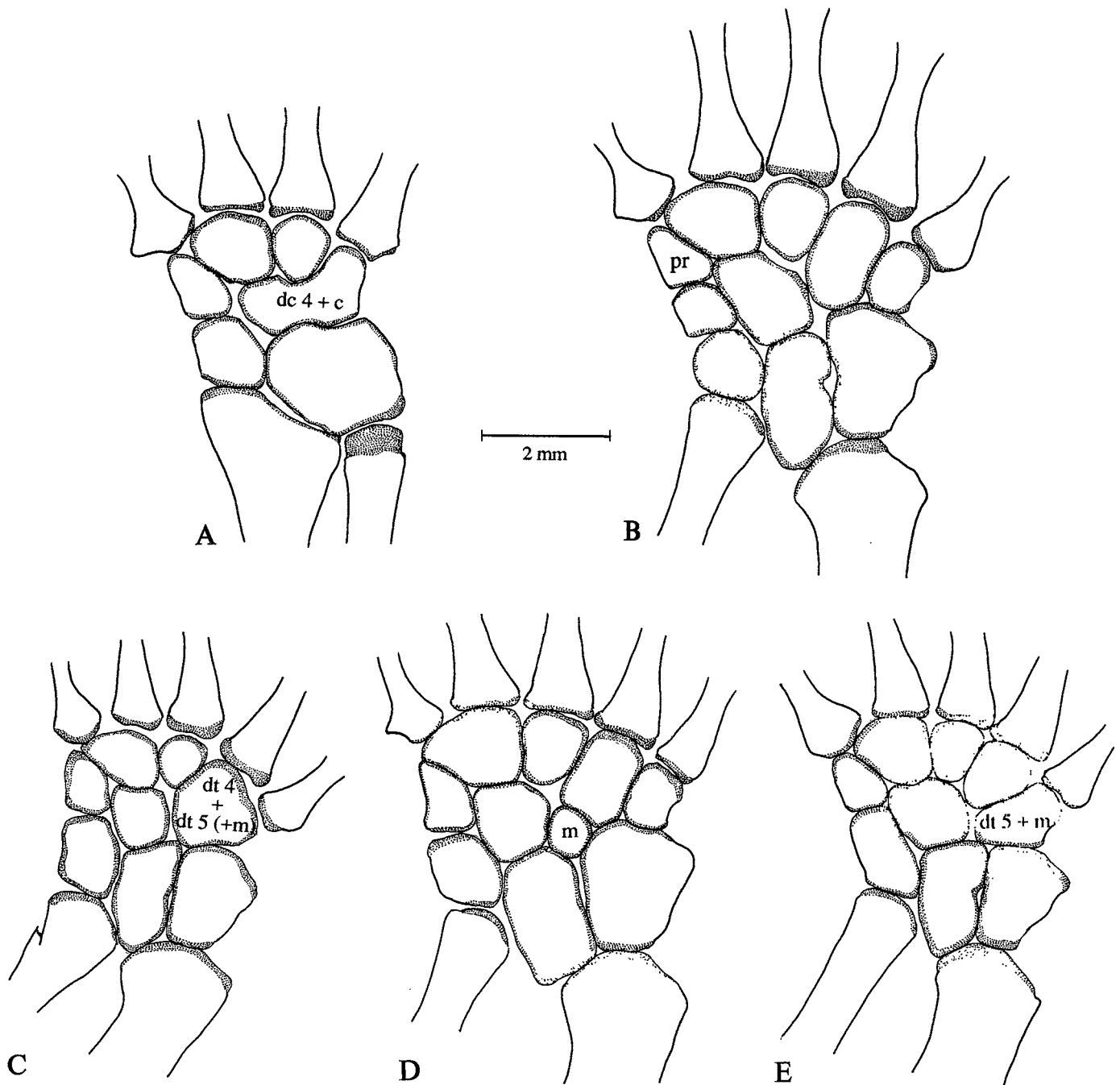


FIG. 4. Five bilateral patterns of mesopodial variation occur in limbs of *Taricha granulosa*. A. Amalgamation of dc 4 and c in the forelimb (MVZ 216442). B. Extra element (pr) distal to y (MVZ 216402). C. Amalgamation of dt 4 and dt 5 (plus m?) (MVZ 216171). D. Schmalhausen's m, the commonest variant pattern found, (MVZ 216358). E. Amalgamation of dt 5 and m (MVZ 216582).

are found in other salamandrid genera (e.g., *Salamandra*), and in most other urodeles, with the exception of taxa with reduced (e.g., Amphiumidae, Sirenidae, Proteidae, which have lost digits and even entire limbs) or miniaturized limbs (e.g., *Thorius*, *Nototriton*, *Bradytriton*, *Parvimolge*, and *Oedipina*). An additional element (po, table 1) at the postaxial end of the digital arch may represent an atavistic postminimus; such an element is standard in many Paleozoic amphibians (e.g., *Eryops*), some hynobiids, and the cryptobran-

chid *Andrias*. Other variants are standard conditions in taxa in which limbs are reduced. For example, the amalgamation of dc 3 and 4 (dc3 + 4, table 1) is standard in the proteid *Necturus* and commonly observed in the natural patterns of intraspecific variation of *Triturus* (Rienesl and Wagner 1992). A combined y and r (r + y, table 1) is standard in *Andrias*, *Rhyacotriton*, sirenids, and proteids (table 5).

Hind-limb variants are more common than are those of the forelimb, and they, too, can be classified as either additions

TABLE 5. Classification of all of the patterns of variation of *Taricha granulosa* according to their occurrence in other taxa.

| Variants | |
|--|---|
| Atavistic variants | |
| Forelimb | |
| +c | Paleozoic temnospondyls, Cryptobranchidae (<i>Andrias</i>) |
| po | Paleozoic temnospondyls, Hynobiidae, Cryptobranchidae |
| sep. u/i | Paleozoic temnospondyls, Hynobiidae, Cryptobranchidae, Plethodontidae, Ambystomatidae, Dicamptodontidae, Rhyacotritonidae |
| Hind limb | |
| +c | Paleozoic temnospondyls, Hynobiidae (<i>Liua</i> , <i>Salamandrella</i> , <i>Ranodon</i>), Cryptobranchidae (<i>Andrias</i>) |
| m | Paleozoic temnospondyls, Cryptobranchidae (<i>Andrias</i>) |
| pr | Paleozoic temnospondyls, Cryptobranchidae (<i>Cryptobranchus</i>), Hynobiidae (<i>Salamandrella</i>) |
| Variants that correspond to apomorphies of other urodeles | |
| Forelimb | |
| dc3 + 4 | Proteidae (<i>Necturus</i>) |
| dc4 + c | Plethodontidae (<i>Thorius</i> , <i>Oedipina</i>) |
| y + r | Rhyacotritonidae (<i>Rhyacotriton</i>), Proteidae (<i>Proteus</i>), Sirenidae (<i>Pseudobranchus</i>) |
| i + c | Rhyacotritonidae (<i>Rhyacotriton</i>) |
| Hind limb | |
| dt5 + m | Plethodontidae (<i>Aneides</i> , <i>Chiropterotriton</i>) |
| dt3 + 4 | Proteidae (<i>Necturus</i>) |
| dt4 + 5 | Plethodontidae (<i>Bolitoglossa rostrata</i> , <i>B. subpalmata</i> , <i>B. adspersa</i> , <i>B. altamazonica</i> , <i>B. peruviana</i> , <i>Parvimolge</i> , <i>Nototriton</i> , <i>Haideotriton</i> , <i>Bradytriton</i>), Salamandridae (<i>Cynops</i> , <i>Triturus</i> , <i>Euproctus</i>) |
| f + i | Proteidae (<i>Proteus</i> , <i>Necturus</i>), Plethodontidae (<i>Thorius</i> , <i>Oedipina</i>), Amphiumidae (<i>Amphiuma</i>) |
| Variants that correspond to intrapopulational conditions in other taxa | |
| Hind limb | |
| -dt5 | Plethodontidae (<i>Eurycea</i> , <i>Batrachoseps</i>), Salamandridae (<i>Salamandra</i>) |
| bc + 3 | Plethodontidae (<i>Plethodon</i> , <i>Thorius</i>), Salamandridae (<i>Triturus</i>) |
| dt4 + 5 | Plethodontidae (<i>Bolitoglossa dofleini</i> , <i>Plethodon</i>) |
| dt5 + m | Plethodontidae (<i>Bolitoglossa adspersa</i> , <i>B. macrinii</i>) |
| m | Plethodontidae (<i>Pseudoeurycea</i> , <i>Aneides</i> , <i>Bolitoglossa</i>), Salamandridae (<i>Triturus</i>) |
| Forelimb | |
| bc + 3 | Salamandridae (<i>Triturus</i>) |
| bc + 3 + c | Plethodontidae (<i>Thorius</i>) |
| bc + c | Plethodontidae (<i>Plethodon</i>) |
| u + i + c | Salamandridae (<i>Triturus</i>) |
| dc3 + c | Salamandridae (<i>Triturus</i>) |
| r + i + u | Salamandridae (<i>Triturus</i>) |
| dc4 + c | Plethodontidae (<i>Plethodon</i>) |
| Variants not encountered in other urodeles | |
| Forelimb | |
| r + R | (standard in many mammals) |
| c + dc2 | |
| u + U | |
| y + r + c | |
| +u | |
| +2c | |
| Hind limb | |
| preaxial c | |
| bc + c | |

or amalgamations. There are fewer kinds of tarsal arrangements than carpal arrangements, despite this higher frequency of tarsal variation (tables 1, 3). By far the most common symmetrical addition is the presence of m (m, table 3). This element was first described as an intrapopulational variant in *Ranodon sibericus* (Schmalhausen 1917). Subsequently, the

element was found in the standard tarsal pattern in *Trematops* (fig. 5) and the cryptobranchid *Andrias*. This element is an intrapopulational variant in the salamandrid *Triturus cristatus* and the plethodontids *Pseudoeurycea* and *Aneides* (table 5).

One symmetrical pattern is an extra element, possibly a prehallux (pr, table 3), on the distal end of the preaxial side.

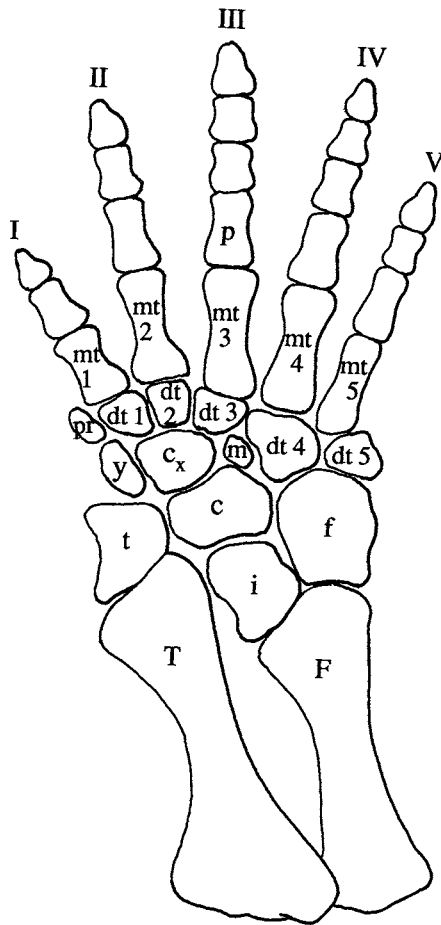


FIG. 5. Right hind limb of *Trematops milleri* (modified from Schaeffer 1941).

A prehallux is present in Paleozoic temnospondyls and is known in some hynobiids and cryptobranchids (Schaeffer 1941; fig. 5). This pattern is also seen as a variant in populations of *Triturus cristatus* (Rienesl and Wagner 1992).

There are two symmetrical patterns of amalgamation. A combined dt 4 + 5 (dt4 + 5, table 3) is a low-frequency symmetrical pattern. This arrangement is found as the standard pattern (table 5) in a number of plethodontid (Wake and Larson 1987) and salamandrid genera (Rabl 1910; Rienesl and Wagner 1992). Distal tarsal 4 + 5 is seen in the intrapopulational variation of several taxa: *Plethodon cinereus* (Hanken 1983; Hanken and Dinsmore 1986; Dinsmore and Hanken 1986), *Plethodon neomexicanus* (Dwyer and Hanken 1990), species of *Rhyacotriton* (Good and Wake 1992), and *Bolitoglossa dofleini* (Alberch 1983). A final low frequency bilateral pattern is an enlarged dt 5 that articulates with c (dt 5 + m, fig. 4E, interpreted in the manner of Wake 1991). This is a standard pattern in the plethodontid genera *Aneides* and *Chiropterotriton* (Wake 1966, 1991) and an intrapopulational variant in *Bolitoglossa adspersa* and *B. macrinii* (Wake and Shubin unpubl. data).

Some asymmetrical additions correspond to standard patterns of other taxa (table 5). An extra centrale (+c, table 3)

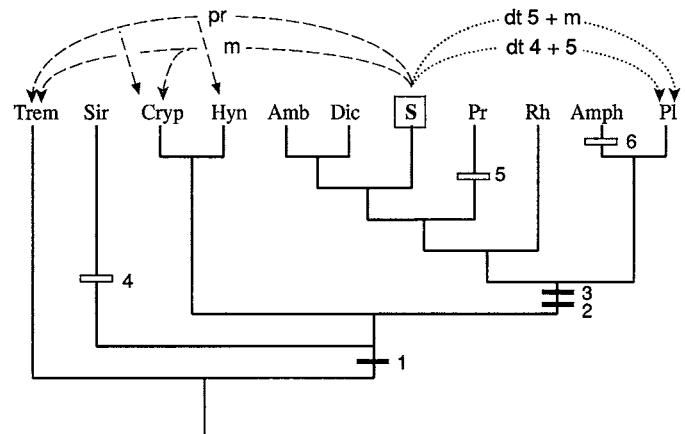


FIG. 6. All four bilateral patterns of tarsal variation either correspond to plesiomorphic conditions (Schmalhausen 1917; Wake 1991) or apomorphies of highly specialized urodeles (Wake 1966). Our working phylogenetic hypothesis is derived from the studies of Trueb and Clouthier (1991), Larson and Dimmick (1993), and Good and Wake (1992). Paleozoic temnospondyls (Trem) are used as the outgroup. Dashed lines indicate two atavistic variant patterns (elements pr and m). Dotted lines indicate two variant patterns that correspond to apomorphies in highly nested taxa—dt 4 + 5 (occasionally incorporating m, see text), and dt 5 + m. Plethodontidae (Pl) is highly speciose and both dt 5 + m and dt 4 + 5 have arisen (from two to five) times within highly nested species of the family (Wake and Elias 1983). Numbered solid bars indicate synapomorphies. Numbered open bars indicate autapomorphies for three families with reduced limbs. The synapomorphies are as follows: (1) amalgamation of dc 1 and dc 2, and dt 1 and dt 2, to form bc; this occurs in both the manus and the pes and should be considered to constitute two synapomorphies; (2) reduction to one c; (3) loss of "external" mesopodial elements (po and pr), which may also be considered to constitute two synapomorphies; (4) loss of hind limbs; (5, 6) loss of digits and mesopodial reductions (see text). Amb, Ambystomatidae; Amph, Amphiumidae; Cryp, Cryptobranchidae; Dic, Dicaeodontidae; Hyn, Hynobiidae; Pl, Plethodontidae; Pr, Proteidae; Rh, Rhyacotritonidae; S, Salamandridae; Sir, Sirenidae; Trem, *Trematops*, representing a temnospondyl outgroup taxon.

is standard in *Trematops* (fig. 5), some hynobiids (fig. 1A), and the cryptobranchid *Andrias*.

There are two asymmetrical mesopodial reductions that appear elsewhere in taxa with reduced limbs (table 5). A fusion of dt 3 and dt 4 (dt 3 + 4, table 3) is present in the proteid *Necturus*, and an amalgamation of f and i (f + i, table 3) is the standard pattern for the Proteidae (Schaeffer 1941), Amphiumidae (Rabl 1910), and several plethodontid genera with miniaturized limbs (Wake and Elias 1983; Hanken 1985).

Phylogenetic Perspectives on Skeletal Variation

Most of the anomalous patterns seen in the limbs of *Taricha* are encountered in other urodeles. About 75% of the anomalous patterns in this sample of *Taricha* are either standard patterns in other taxa or correspond to variant conditions in other urodele species (table 5). Of the patterns seen in *Taricha*, 40% are standard patterns of other taxa (table 5). All four of the bilateral patterns of variation encountered in the hind limb of *T. granulosa* are standard patterns of other taxa (fig. 6, table 5). The most frequent variant (m, fig. 4D) may also be the most significant phylogenetically. Element m ap-

pears to be atavistic in that it reconstitutes the arrangement of the postaxial central region of the tarsus found in the outgroup *Trematops* (figs. 5, 6) and the primitive cryptobranchid *Andrias*. In addition, a bilateral pr can be interpreted as an atavism that restores the condition seen in Paleozoic temnospondyls and cryptobranchids. Another symmetrical pattern of variation (dt4 + 5, possibly including m, fig. 4C) found in *Taricha* corresponds to apomorphic patterns of other salamandrid (Rienesl and Wagner 1992) and plethodontid (Wake and Elias 1983) genera (fig. 6, table 5). In *Bolitoglossini* (a highly nested plethodontid clade) this arrangement is homoplastic and may have independently arisen in two to five different genera (Wake and Elias 1983). Thus, two of the four bilateral variants essentially "look backward," in a phylogenetic sense, whereas the other two "look forward," in that they are apomorphic novelties of highly nested clades of other urodeles (fig. 6).

In the hind limb, three of four cases of symmetrical variation involve element m; we postulate that this element (or the underlying developmental processes that may produce it) is a central player in the phylogenetic history of the tarsus. Schmalhausen (1917) and Holmgren (1933) noted that primitive tetrapods have more than one central element in the mesopodium and that this primitive pattern is a frequent intrapopulational atavism in derived taxa (which typically possess only a single centrale). Atavistic centralia typically are found on the postaxial side of c (Holmgren 1939). Schmalhausen noted that this central region of the tarsus can be filled in several different ways, by (1) an independent element (m) situated between c, dt 4, and dt 5 (fig. 5); (2) a proximal extension of dt 4; or (3) a preaxial extension of dt 5. Schmalhausen (1917) argued that m (his m3) fills this space and that it either remains separate (as a rare variant now known to restore the ancestral state, see above) or fuses to dt 4. When m also was discovered as an intrapopulational variant in plethodontids, Schmalhausen's reasoning was extended and placed into a context whereby novel and possibly adaptive tarsal arrangements can be explained (Wake 1991). Salamanders appear to retain the generative mechanisms necessary to produce m, and this capacity apparently has been used in the phylogenesis of the cluster of "advanced" salamander families (Larson and Dimmick 1993; Good and Wake 1992). Rhachitomes and basal urodeles share the primitive arrangement that consists of small, spherical elements dt 4 and 5 and a variable number of centralia. One of these centralia, Schmalhausen's m (fig. 4), lies in the region proximal to dt 4 and 5. Most extant urodeles retain a primitive pattern that consists of an oblong dt 4 that extends proximally to the region posterior to the centrale. In these taxa, dt 5 remains small and spherical and is separated from the centrale by the enlarged dt 4. The plethodontid genera *Aneides* and *Chiropterotriton* have independently evolved an arrangement that appears to be associated with climbing. In these taxa, dt 5 is enlarged and dt 4 is reduced in size. This alteration in size has been interpreted as the result of a rudiment of m joining either dt 4 or dt 5 (Wake 1991, following similar arguments made by Schmalhausen 1917). Alternate arrangements are seen in many plethodontids and salamandrids in which dt 4 and dt 5 are fused into a single element (one of our symmetrical variants). The plethodontid genus *Bolitoglossa* typ-

ically has such an arrangement, but some species of the genus show an extension of this pattern so that a combined dt 3 + 4 + 5 is present. The most common intrapopulational variant condition in *Bolitoglossa* involves an apparent atavistic state, dt 5 + m (Alberch 1983, unpubl. data), which argues in favor of the view that a rudiment of m is present in the other fusions (dt 4 + 5, dt 3 + 4 + 5) as well. An independent m is not seen in any individual (of any urodele species) with an expanded dt 4, an expanded dt 5, or any combination of these distal tarsals that extends into the central region.

Shubin and Alberch (1986) and Oster et al. (1988) hypothesize that generative rules of cartilage formation may serve to bias anatomical variation. These analyses provide a developmental context to predict morphological variation; many amalgamations include elements that are physically connected to one another during embryogenesis (cf. fig. 2 and table 5). Exceptions to the generative rules involve fusions of elements that are wholly separate during development, for example: r + i in the miniaturized limbs of *Thorius*, dc 3 + 4 in *Triturus* (Rienesl and Wagner 1992), and the amalgamation of dt 4 + c in various plethodontids. In *Taricha* (and other urodeles, Schmalhausen 1917), the central region of the mesopodium is a highly variable region (see above); a diversity of conditions is found (fig. 4, tables 1, 3, 5). The position, size, and patterns of amalgamation of m, c, and dc 4 in the manus (tables 1, 5) and m, c, dt4, and dt 5 in the pes (tables 3, 5) are highly variable (Schmalhausen 1917; Wake 1966; Wake 1991). The proximodistal and anteroposterior axes of chondrogenesis converge at this region (asterisk in fig. 2) in later stages and the high diversity of morphological conditions may correlate to the dynamic interaction that occurs when these developmental axes converge.

General Model of Limb Parallelism

Bilateral patterns of variation in *Taricha* both restore ancient structures and "anticipate" derived conditions that arise in parallel within highly nested taxa. These regularities suggest that the same processes that underlie the expression of atavistic characters are involved in the origin of evolutionary novelties.

We interpret the dual anteroposterior and proximodistal axes of development (and the resulting cartilage connections; Shubin and Alberch 1986; Blanco and Alberch 1992) as a synapomorphy of urodeles. Taxa that retain these patterns of development are hypothesized to show congruent patterns of variability. Phylogenetic analysis reveals that fixation of individual patterns occurs in parallel in both basal and highly nested taxa. Underlying developmental influences on anatomical variation may exert their effect on cladistic topology because of the structural hierarchy of the urodele limb. The function of the mesopodium is directly related to its articular arrangements; the number of skeletal elements is only peripherally related to this functional organization (Schaeffer 1941). Particular mesopodial arrangements may be subject to weak selection (such as dt 5 + m, Wake 1966) or may be selectively neutral. We hypothesize that apomorphies in the arrangement of limb cartilages result from fixation of individual patterns from a reservoir of variability that is biased by phylogenetically conserved generative mechanisms. The

similarity of patterns of intrapopulational variation in diverse clades and their correspondence to taxic apomorphies suggests that the structural and generative properties that produce limb variation have a major influence on the phylogenetic diversification of the urodele limb.

ACKNOWLEDGMENTS

M. J. Blanco, J. Hanken, and G. Wagner provided helpful reviews of the manuscript. Gary Fellers (National Park Service) and A.-M. Tan provided assistance in obtaining specimens. This research was supported by National Science Foundation grant BSR 9006800.

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APPENDIX 1

List of abbreviations of “standard” mesopodials.

| | |
|--------|------------------------------|
| bc | basale commune (dc/dt 1 + 2) |
| c | centrale |
| cx | supernumerary centrale |
| dc3–4 | distal carpals 2–4 |
| dt3–5 | distal tarsals 2–4 |
| F | femur |
| f | fibulare |
| Fi | fibula |
| H | humerus |
| i | intermedium |
| I–V | digits I–V |
| m | Schmalhausen’s M3 |
| mc 1–4 | metacarpals 1–4 |
| mt 1–5 | metatarsals 1–4 |
| p | phalanx |
| po | postminimus |
| pr | prepollex/prehallux |
| R | radius |
| r | radiale |
| T | tibia |
| t | tibiale |
| U | ulna |
| u | ulnare |
| y | element Y |

APPENDIX 2

List of abbreviations of variant carpal conditions.

| | |
|------------|---|
| +2c | two supernumerary c situated along c-i-bc axis |
| +c | supernumerary c that articulates with c and bc |
| +dc | supernumerary dc situated on the postaxial to dc 4 |
| +u | supernumerary proximal element postaxial to u |
| bc + 3 | amalgamation of bc and dc 3 |
| bc + 3 + 4 | amalgamation of bc and dc 3 and dc 4 |
| bc + 3 + c | amalgamation of bc and dc 3 and c |
| bc + c | amalgamation of bc and c |
| dc3 + 4 | amalgamation of dc 3 and dc 4 |
| dc2 + c | amalgamation of c and dc 2 |
| dc4 + c | amalgamation of dc 4 and c |
| i + c | separate i and u; i amalgamated with c |
| m | Schmalhausen's m; small mesopodial inserted between c, dc 3, and dc 4 |
| po | postminimus; supernumerary element postaxial and distal to dc 4 |
| r + i + u | amalgamation of r and i and u |
| R + r | amalgamation of R and r |
| sep. u/i | separate u and i |
| U + u | amalgamation of U and u |
| y + i + c | amalgamation of y and i and c |
| y + r | amalgamation of y and r |
| y + r + c | amalgamation of y and r and c |

APPENDIX 3

List of abbreviations of variant tarsal patterns.

| | |
|------------|---|
| +2c | two supernumerary c situated along c-i-bc axis |
| +c | supernumerary c articulating with c and bc |
| +y (or pr) | supernumerary mesopodial distal to y (often identified as prehallux) |
| -dt5 | absence of dt 5, no evidence of amalgamated dt 4 and 5 |
| bc + 3 | amalgamation of bc and dt 3 |
| bc + c | amalgamation of bc and c |
| dt3 + 4 | amalgamation of dt 3 and dt 4 |
| dt4 + 5 | amalgamation of dt 4 and dt 5 |
| dt5 + m | amalgamation of dt 5 and m, dt 5 expanded proximally and preaxially |
| f + i | amalgamation of f and i |
| m | Schmalhausen's m; small mesopodial inserted between c, dt 4, and dt 5 |
| preaxial c | supernumerary c situated between c and y |
