

Limb development in the Pacific giant salamanders, *Dicamptodon* (Amphibia, Caudata, Dicamptodontidae)

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Abstract: The larvae of Pacific giant salamanders, *Dicamptodon* (Dicamptodontidae), develop in streams, display precocious limb development, and possess a full complement of digits at hatching. Limb morphogenesis was studied using cleared whole mounts stained with Alcian Blue or by immunohistochemical methods to reveal stages of limb development. Limb morphogenesis in these salamanders conforms to the general caudate pattern, i.e., preaxial dominance and precocious development of an initially isolated digital arch, despite some differences in detail from other salamander taxa. These comparisons support the hypothesis that a fundamental ground plan characterizes limb development in salamanders which transcends adaptive modifications related to ecology and life-history evolution. This ground plan differs from that which characterizes frogs and amniotes, which shows postaxial dominance and a digital arch that arises from basal elements.

Résumé : Les larves des salamandres géantes du Pacifique, *Dicamptodon* (Dicamptodontidae), se développent dans les ruisseaux, leurs membres apparaissent tôt et elles possèdent déjà tous leurs doigts à l'éclosion. La morphogénèse des membres a été étudiée par examen de montages d'animaux entiers colorés au bleu Alcian ou par des méthodes immunohistochimiques capables de montrer les stades du développement. En dépit de petites différences avec d'autres taxons, la morphogénèse des membres chez ces salamandres est typique de celle qui prévaut chez les Caudata, soit la dominance préaxiale et le développement précoce d'un arc digital d'abord isolé. Ces comparaisons confirment l'hypothèse de l'existence d'un plan de base de développement des membres chez les salamandres, plan qui transcende les modifications évolutives reliées à l'écologie ou à l'évolution de la démographie. Ce plan de base diffère de celui qui caractérise les grenouilles et les amniotes dans lequel il y a dominance postaxiale et présence d'un arc digital formé à partir d'éléments de base.

[Traduit par la Rédaction]

Introduction

Limb development in salamanders differs from that in all other tetrapods in several important respects (Shubin and Alberch 1986; Shubin and Wake 1996). However, details of limb development have been studied only in species that have pond-type larvae and in which limb development proceeds slowly, mainly taking place after hatching as the free-living larvae move about (Shubin and Alberch 1986; Blanco and Alberch 1992; Vorobyeva and Hinchliffe 1996). Thus, limbs are moved as they are developing. Limb development in anurans having free-living larvae is much delayed relative to that in salamanders, and limb development in amniotes takes place mainly within the egg or in utero; in neither instance are limbs functional as they are developing. Salamanders display a variety of life-history and development modes (Duellman and Trueb 1986). Such variation offers the possi-

bility of examining the relative impact of history (phylogeny) and ecology (and by inference, adaptation) on the evolution of limb morphogenetic patterns. In a recent study,² limb development was described in three salamanders of the family Plethodontidae that undergo intracapsular limb development, one a species with precocious development of stream-adapted larvae and the other two with direct development and no larval stage. Despite considerable divergence from the pattern of limb development in pond larvae, the major differences that distinguish salamander limb development from that in other tetrapods (Shubin and Alberch 1986) remain. In this paper, we report results of a study of limb development in a member of a family, Dicamptodontidae, that includes species which are the largest terrestrial salamanders. These salamanders have stream-type larvae rather than the pond-type larvae that characterize most members of the sister taxon, the Ambystomatidae. No studies of early development in dicamptodontids have been published previously.

The Pacific giant salamanders, *Dicamptodon* (Dicamptodontidae), are endemic to the northwestern United States and southwestern British Columbia, Canada. There are four spe-

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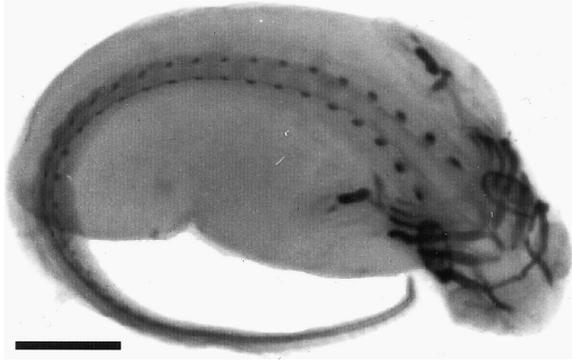
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²S.B. Marks, D.B. Wake, and N. Shubin. Limb development and evolution in the salamander genera *Desmognathus* and *Bolitoglossa*: separating hypotheses of ancestry, function and life history. Submitted for publication.

Fig. 1. Entire embryo of *Dicamptodon tenebrosus*, representing day 0 of our sequence of limb development, showing the relative size of the huge encapsulated embryo (12.2 mm SVL) stained with antibodies to type II collagen. There is a moderately strong anterior–posterior gradient of development. Staining of the hyobranchial apparatus shows that the system is completely formed. Paired neural pedicel rudiments are present for the anterior vertebrae. Rudiments of the humerus, radius, and ulna are seen in the developing forelimb. Scale bar = 2 mm.



cies, all having long-lived larvae (at least 2 years and sometimes more, with individuals that remain in a permanently larval state documented in two species) that are of the stream type (i.e., hatch at a relatively advanced stage of development with reduced fins and gills). Eggs are laid under large rocks in streams and are rarely found (Nussbaum 1969; Jones et al. 1990), so we took advantage of an unusual discovery to study the development of Pacific giant salamanders.

Materials and methods

Our data are derived from two clutches of eggs of *Dicamptodon tenebrosus* discovered in a small stream south of Yachats, Lane County, Oregon, in 1991. The eggs were raised in the laboratory, and observations of the resulting embryos are reported in this paper. The two clutches were at advanced stages of development when collected. They were transferred to the laboratory and maintained at 14°C until hatching. Every few days, two or three eggs were preserved in 10% neutral buffered formalin or Dent's solution (80% methanol, 20% dimethylsulfoxide). Details of development and a partial table of developmental stages will be published separately.³ One of the clutches (I) produced most of the specimens reported here. After close comparison of the preserved stages, clutch II was estimated to be approximately 11 days older than clutch I.³ Selected embryos were fixed and then either cleared and stained with Alcian Blue (for chondrification centers, four specimens) or used for immunohistochemical studies. The latter specimens were stained with antibodies for type II collagen to show the developing skeleton. Successful stainings with type II collagen were obtained for seven specimens ranging in size from 11.4 to 15.9 mm (snout to posterior angle of vent, or snout–vent length (SVL)), and these proved useful in visualizing early stages of morphogenesis. This size range, which represents approximately 27 days, encompasses limb development from a stage at which two digits are present on the forelimb and none on the hind limb to one at which all four digits are present on the forelimb but only two on the hind limb. This series extends from late embryonic to early hatchling stages (hatching occurs at about 13.4 mm SVL). Time from fertilization

to hatching is not known. The first day on which embryos were preserved (August 21, 1991) is taken as day 0. This stage of development is approximately equivalent to stage 44 in the normal table for *Ambystoma maculatum* of Harrison (1969) as far as the forelimb is concerned, but not for the hind limb, which is not present until much later in development in *A. maculatum*. Comparisons were made with specimens studied by Shubin and Alberch (1986) and Marks et al. (see footnote 2) as well as with materials in the collection of the Museum of Vertebrate Zoology (MVZ) at the University of California. All specimens reported here are in the collection of MVZ. Terminology follows Shubin et al. (1995).

Results

Although we were limited by the small samples available, we obtained critical information on limb development. The limbs of *Dicamptodon* are more fully developed at hatching than those of species that develop in ponds (i.e., *Ambystoma*, *Triturus*, *Salamandrella*). At hatching, elements of the forelimb are completely differentiated, although not yet fully ossified. The hind limb contains at least two digits at hatching, but its development continues to lag behind that of the forelimbs. This lag between the differentiation of the forelimbs and hind limbs is not nearly as great as that seen in species with pond-type larvae (e.g., *A. maculatum*; Harrison 1969).

The earliest stage of limb development available to us is in an individual of 12.2 mm SVL, preserved on day 0 and stained for collagen II (Fig. 1). Externally, two digits are visible in the forelimb, while no external differentiation of the hind limb is discerned. Internally, the humerus, radius, and ulna form a deeply stained and continuous Y-shaped precartilaginous condensation. The radius is stained and is larger than the ulna. The only other precartilaginous Anlage that is evident lies distally and is discontinuous from the radius and ulna. This weakly stained region contains the Anlagen of the basal commune and digits 1 and 2. Digit 2 is distinct, but digit 1 is only weakly indicated. There is no internal staining in the hind-limb bud.

A specimen preserved on day 3 is smaller than the first specimen (11.4 mm SVL) but is more highly differentiated. Two digits are present externally in the forelimb (Fig. 2A), but the hind limb remains undifferentiated. The humerus, radius, and ulna are more deeply stained in this collagen II preparation than in the preparation from day 0. Development of the radius continues to be significantly advanced relative to the ulna (Fig. 2A). Two columns of stained matrix extend distally from the ulna. The lateral (postaxial) branch continues distally to merge with the Anlage of the third metacarpal. The medial (preaxial) branch of the ulna approaches the basale commune distally but does not connect with it. The basale commune and metacarpals 1 and 2 form a deeply stained and continuous mass in which digit 2 remains the dominant digit. Darkly stained extracellular matrix extends postaxially from the basal commune to the rudiment of the third distal carpal and metacarpal. A preaxial column extends distally from the radius; independent foci for radiale and element y are not yet present. The hind limb continues to lag behind the forelimb in development. The femur is

³R.S. Beroukhim and D.B. Wake. Embryonic development in the northern Pacific giant salamander, *Dicamptodon tenebrosus*. In preparation.

stained, but otherwise, there is only a slight darkening in the vicinity of the future basale commune.

A collagen II preparation representing day 14 measures 12.0 mm SVL and shows a more advanced stage of limb development (Fig. 2B). The distal manus now contains three distinct digits. The first two digits each contain the Anlagen of a metacarpal and two phalanges. The basale commune is a discrete focus, continuous with distal carpal 3 postaxially. From distal carpal 3, a large and well-stained rudiment of the third digit extends distally. A gap (i.e., unstained matrix) separates the ulnare from the third distal carpal. The intermedium and centrale lie within an undifferentiated column that is only weakly connected to the medial part of the fully formed ulna. A rod of stained matrix extends from the radius and contains the undifferentiated rudiments of both the radiale and element y.

The hind limb of the day 14 specimen consists of a small pad that only weakly shows the tips of digits 1 and 2 (Fig. 3A). The hind-limb preparation from day 14 is generally similar to that of the forelimb on day 3. The basale commune and the rudiments of digits 1 and 2 are joined together in a continuous undifferentiated mass with the tips of the digits widely separated from each other. A rudimentary digital arch extends posteriorly from the basale commune toward the fibulare as a zone of stained matrix, and a second medial zone of staining extends between these two regions, forming a more or less continuous ring surrounding a zone of unstained matrix. The undifferentiated rudiments of the centrale and intermedium are contained within the medial zone of staining extending from the fibula. Preaxially, there is a discrete fibula from which a lightly stained rudiment extends distally. This preaxial column is well separated from the ring and from the basale commune.

A collagen II preparation of a specimen preserved on day 22 reveals all four digits of the forelimb (Fig. 2C). Two phalanges are evident in digits 1 and 2, and the second phalanx in the digit 2 is attenuated and elongated. Only a single phalanx is present in digit 3. The fourth digit is differentiated but continuous with the rudiment of distal carpal 4. The digital arch is complete and distal carpal 4 is separated by a zone of weak staining from the fibulare. The radiale and ulnare are well formed and there is a distinct element y. The preaxial column remains well differentiated from both the medial series and the basale commune. The rudiment of the intermedium is deeply stained but remains continuous with an expanded rudiment of the centrale. The centrale has two foci, one of which extends postaxially toward distal carpal 4 (Fig. 2C).

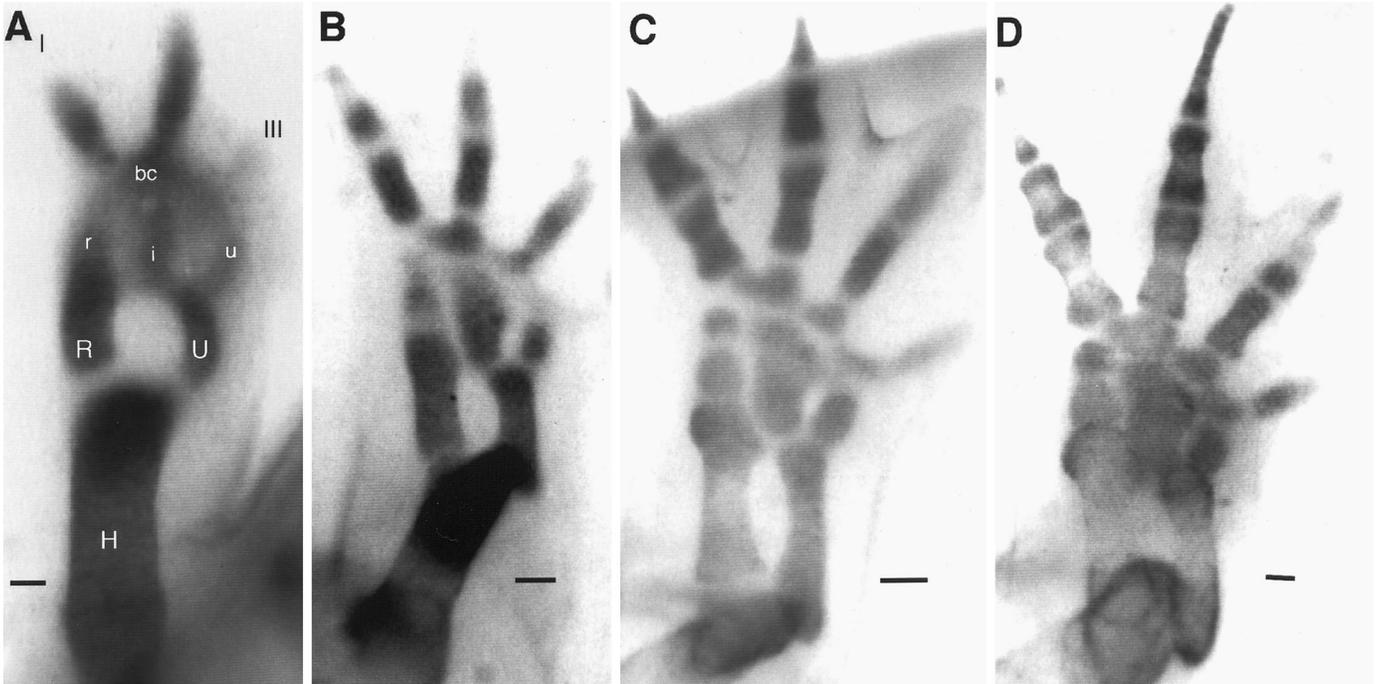
The hind limb of the preparation from day 22 now contains three digital rudiments (Fig. 3B), but these are barely evident externally. A deeply stained basale commune is continuous with distal tarsal 3. The first two digits remain as single elements that show a pattern of weak proximodistal differentiation. Digit 3 is represented by a lightly stained rod that is continuous with distal tarsal 3. The femur, tibia, and fibula are all separate elements. The most prominent feature in the proximal tarsus is a heavily stained column of matrix that contains the future intermedium and centrale. Proximally this column is continuous with the tibia and fades distally as it reaches the basale commune. The postaxial branch

of the fibulare is weakly stained and poorly bounded; it represents the future fibulare.

A cleared and stained preparation (MVZ 215981, not shown) from the second clutch appears to represent a later stage of development, and we estimate that it represents approximately a day 24 stage in the first clutch. The differences in resolution between immunohistological and cartilage-stained preparations are vividly demonstrated by this specimen. Far less detail is evident than in the day 22 specimen that was stained by immunohistochemistry. In the day 24 example the hind limb shows staining only in the femur. Much more staining is present in the forelimb, where the humerus, radius, and ulna are apparent. The ulna is considerably smaller than the radius and is bifurcated distally. The ulnare is separate from the ulna. The intermedium is the largest of the three basal units of the manus, and it remains in continuity with the ulna. A distinct, distally pointed radiale is about the same size as the ulnare and is separated from the radius. There is a large unstained gap across the center of the manus between the basale commune and the weakly differentiated Anlagen of the centrale. The ulnare and radiale are both separated from the distal elements by a gap of unstained matrix. The basal commune has the shape of a deep crescent with each point connected to a metacarpal representing each of the first two digits. Both of these digits have two distinct phalanges, and in each case the second is elongate and sharply pointed distally. A small, weakly stained element postaxial to the basale commune represents distal carpal 3. Extending distally from it is a small, weakly stained rudiment of the third digit.

A specimen from the second clutch (15.6 mm SVL) that is immunologically stained (collagen II) represents the next stage of limb morphogenesis. Using the 11-day correction factor, this embryo is expected to be at a stage approximately equivalent to day 31 of the first clutch. Limb morphogenesis is somewhat less advanced than in a slightly larger specimen from the first clutch that is at day 29 (see below). There has been relatively little change from the day 22 embryo. No more phalanges are present, but the main carpus is somewhat more consolidated and the elements are denser. The centrale and intermedium remain continuous in a relatively massive condensation, in contrast with what is seen in the cartilage preparation from day 24. The ulnare now contacts distal carpal 4, which is larger than in the earlier preparations, and this carpal is continuous with the rudiment of the fourth digit. The first two digits are larger than previously, and the phalanges are thicker and more compact. More significant changes are evident in the hind limb (Fig. 3C). Three digits are represented, the third containing only a single element representing mainly the rudiment of the metatarsal. The first and second digits have one phalanx on one side, and there is a second phalanx in the second digit on the other side. The basal commune is distinct and separated from all other elements. Distal tarsal 3 is also distinct. A weakly stained digital arch bridges the gap between the distinct fibulare, which is well separated postaxially from the fibula. Proximally, a large medial column is continuous with the medial side of the distal end of the fibula. This column is weakly differentiated into an intermedium and a centrale, the latter being, in turn, well separated from the basale com-

Fig. 2. Forelimb development of *D. tenebrosus* visualized with immunohistochemistry (stained with antibodies to type II collagen). (A) Right forelimb of an embryo, day 3. The humerus (H), radius (R), and ulna (U) are well stained. The basale commune (bc) and the bases of digits 1 (I) and 2 are joined, and the digital arch extends posteriorly and bifurcates to connect to the base of digit 3 (III). A ring of staining extends from the ulna (u) mediodistally in the direction of the basale commune and posterodistally in the direction of the digital arch and surrounds an unstained area. The medial portion represents the rudiment of the intermedium (i). r, radiale. Scale bar = 0.8 mm. (B) At day 14, the manus now contains discrete elements: radiale, intermedium-centrale, and ulnare from preaxial to postaxial and basal, basale commune, digit 1 (containing metacarpal and phalanges 1 and 2), digit 2 (containing metacarpal and phalanges 1 and 2, the latter very weakly stained), and digit 3 (consisting solely of an undifferentiated rudiment) distally. Scale bar = 0.12 mm. (C) At day 22 of limb development, four digits are now represented. The digital arch is complete and the condensations of distal carpals 3 and 4 are evident. A complex of intermedium and centrale elements forms a medial condensation. Both radiale and ulnare are distinct, as is element y. Distal carpal 3 is separate from digit 3 but remains connected to distal carpal 4. Scale bar = 0.12 mm. (D) The day 29 forelimb is more fully differentiated and the digits are longer and stouter. Scale bar = 0.16 mm.



There is also a distinct preaxial column extending from the distal end of the tibia. This column is weakly differentiated into the rudiments of the tibiale and the distally pointed element y.

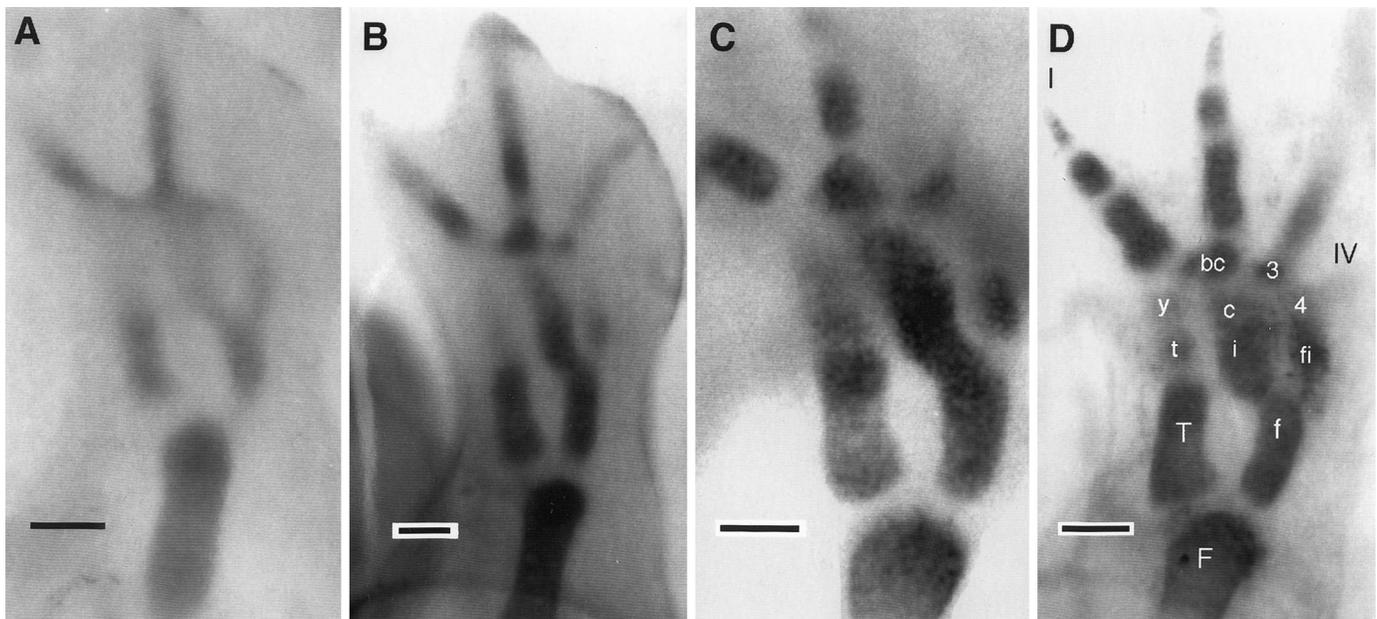
The latest stage of development in our series is a collagen II preparation of a day 29 stage of limb development (15.7 mm SVL). Although morphogenesis of the forelimb is well advanced (Fig. 2D), the medial column containing the rudiments of the centrale and intermedium remains consolidated into a mass that contains three main concentrations. One of these foci (the intermedium) lies basally, while the other two lie distally (these appear to be separate rudiments of the centrale). Distal carpals 3 and 4 and the rudiment of the metacarpal of the fourth digit are distinct. There are two distinct phalanges in both the first and second digits, and the terminal phalanx of the second gives evidence of further regional differentiation. Four centers of differentiation are present in the third digit, but only the metacarpal and the first phalanx are distinct. The preaxial column is differentiated into a radiale and element y. The postaxial column, consisting only of the ulnare, is separated from distal carpal 4.

Four digits are evident in the hind limb at day 29 (Fig. 3D), although the fourth is weakly stained. The third and fourth

are undifferentiated regionally and are in continuity with their associated distal tarsals. Each of these digits contains a distinct metatarsal and two phalanges. The basale commune is distinct and separate from other elements. There are three distinct columns at the base of the tarsus. The preaxial column is differentiated into a tibiale and a weakly stained and distally pointed element y. The intermedium and centrale are distinct elements. The centrale appears to consist of two foci, a preaxial focus and a more postaxial rudiment that may represent element m (see Shubin et al. 1995). The postaxial column consists of a deeply stained fibulare that appears to become continuous distally with a weakly stained digital arch extending postaxially and proximally from the fourth distal carpal.

Limbs of adult *Dicamptodon ensatus* (a closely related species) were studied in cleared and stained preparations (Fig. 4). There are four digits and five toes, the standard pattern for salamanders, with phalangeal formulas of 2-3-2-2 from preaxial to postaxial in the manus and 2-2-3-3-2 in the pes. The phalanges and metapodial elements are ossified, but with large cartilaginous ends (save for the terminal phalanges, which are finished in bone). The mesopodial elements remain cartilaginous, but in some large individuals

Fig. 3. Hind-limb development of *D. tenebrosus* visualized with immunohistochemistry (stained with antibodies to type II collagen). (A) At day 14 of limb development, rudiments of the first two digits are present. The femur, tibia, and fibula are all stained, and a weak ring of staining extends from the tibia to the condensation that marks the site of the basal commune, the rudiments of the digits, and the digital arch. Scale bar = 0.15 mm. (B) Day 22 hind limbs show three digits. A basale commune and rudimentary digital arch are evident. The femur, tibia, and fibula are all present, and the tibial rudiment bifurcates distally into a medial and a postaxial column. Scale bar = 0.16 mm. (C) This specimen is estimated to be equivalent to specimens of day 31 of the clutch that produced the other specimens in this series. Rudiments of three digits are present. The tibia and fibula are well stained. A medial column of condensation is continuous with the distal, medial end of the tibia. This column shows weak differentiation into rudiments of the intermedium and the centrale, but the centrale falls short of the distinct basale commune. The fibulare is small and isolated distally from the fibula in the postaxial region. A preaxial column is weakly differentiated into rudiments of the tibiale and element y. Scale bar = 0.24 mm. (D) At day 29 of limb development, there are now four digits (I–IV), although the fourth is only weakly stained. Two phalanges are present in the first two digits, and the metacarpals are distinct, but only a single rudiment is present in digits 3 and 4 and it is continuous with the associated distal tarsal (3 and 4) in each instance. The basale commune (*bc*) is well formed and discrete. An anterior column is differentiated into a tibiale (*t*) and element y (*y*). The medial column is relatively massive and only weakly differentiated regionally into an intermedium (*i*) and centrale (*c*). The postaxial column contains a well-differentiated fibulare (*fi*) that is in contact with the developing digital arch distally. The femur (F), tibia (T), and fibula (*f*) are all deeply stained. Scale bar = 0.12 mm.



they become mineralized to a varying extent. The number and arrangement of the mesopodials is in the typical pattern of derived salamander families such as the Ambystomatidae and Salamandridae (Shubin and Wake 1996). In the manus there are the three basal elements, the preaxial radiale, the medial intermedium, and the postaxial ulnare. Element y is present distal to the radiale. There is a single large centrale in the middle of the manus, distal to the intermedium. The basale commune and two distal carpals complete the mesopodium. In the pes there are again three basal elements, the preaxial tibiale, the medial intermedium, and the postaxial fibulare. Element y is present distal to the tibiale. There is a single large centrale in the middle of the pes, distal to the intermedium. The basale commune and three distal tarsals complete the mesopodium.

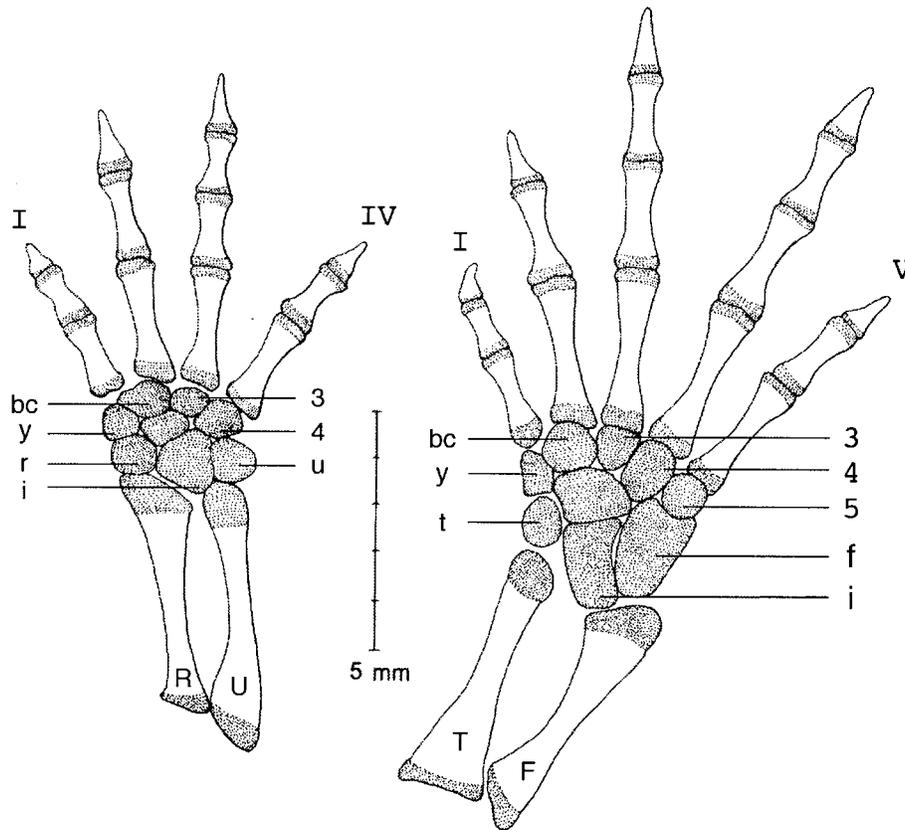
Discussion

Dicamptodon has a generalized morphology, and three of the four species metamorphose as fully as any other salamander with a metamorphic life history. Metamorphosed dicamptodontids are large, well-ossified organisms with

large, well-developed limbs. These traits make them appropriate for comparing with related taxa, especially Paleozoic amphibians. There are features of *Dicamptodon* that make them attractive for studies of development and evolution, including an extended period of intracapsular embryonic development. They have stream-adapted larvae, characterized by advanced development at hatching (the limbs are relatively well developed at this point), perhaps associated with survival in rapidly flowing streams where they hatch. In these respects, dicamptodontids differ from the three species of salamanders in which limb development has been studied most thoroughly to date, *Ambystoma mexicanum* (Shubin and Alberch 1986) (family Ambystomatidae), *Triturus marmoratus* (Blanco and Alberch 1992) (family Salamandridae), and *Salamandrella keyserlingii* (Vorobyeva and Hinchliffe 1996) (family Hynobiidae), all of which have pond-type larvae in which limb development proceeds slowly and continues well after hatching (typically, only forelimb buds are present at hatching).

Marks et al. (see footnote 2) investigated limb development in two species from remotely related genera in the family Plethodontidae that display direct development and

Fig. 4. Skeletons of the hand (left) and foot (right) of a young adult (approximately 65 mm SVL) *D. ensatus* from Jenner, Sonoma County, California. Bone is outlined and cartilage is stippled. For an explanation of abbreviations see Figs. 2 and 3.

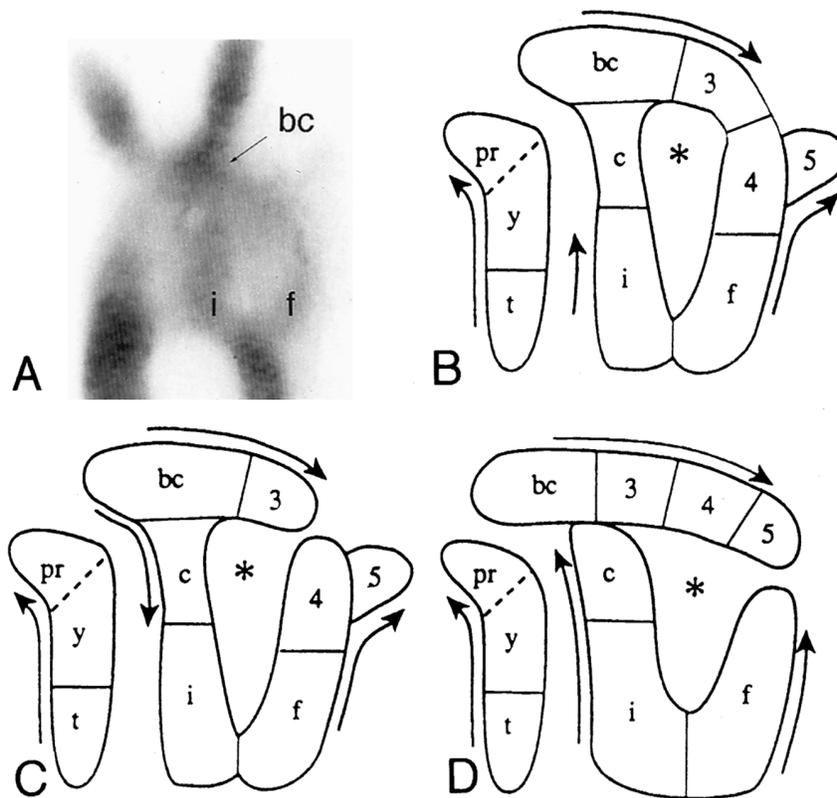


from one member of that family that has an extended period of intracapsular development and a stream-type larva (see footnote 2). These plethodontids resemble other salamanders and differ from frogs and amniotes in showing an early preaxial dominance in development, with the limb axis extending through digit 2 rather than through digit 4, and in showing precocious development of the basale commune in an isolated distal position. However, they differed in having a less pronounced digital arch and more simultaneous development of distal mesopodial elements. In these respects, plethodontids displayed more amniote-like and frog-like characteristics. In amniotes, limbs develop during the embryonic period and they are not used as they are forming. In frogs, the forelimbs, in particular, are enclosed within the gill chamber and form slowly, during which time they are not used. The direct-developing salamanders and those with extended intracapsular development have similar development. Accordingly, *Dicamptodon* offers an opportunity to test the hypothesis (Marks et al., see footnote 2) that features of limb development in salamanders with pond-type larvae represent larval adaptation and that species with intracapsular development will show more similarities to frogs and amniotes with respect to some features of limb development.

The most appropriate taxon for comparative analysis is the sister taxon of the Dicamptodontidae, the Ambystomatidae (Larson and Dimmick 1993). *Dicamptodon* lays eggs in streams, undergoes much intracapsular development, hatches with both forelimbs and hind limbs relatively well devel-

oped, and has larvae that stay in streams for at least 2 years. In contrast, *A. mexicanum* (and most other members of this speciose genus) lays eggs in ponds, has relatively little intracapsular development, has pond-type larvae, and hatches with incompletely developed forelimbs and almost no hind limbs. The larvae of most species of *Ambystoma* metamorphose within a few months of hatching. *Dicamptodon* differs substantially from *Ambystoma* in the overall degree of limb development achieved prior to hatching. It also differs in that its limb buds form small paddles rather than being bullet-shaped. However, with respect to details of internal limb development, *Dicamptodon* resembles *A. mexicanum* (Shubin and Alberch 1986). Development of the limb in all salamanders is a series of bifurcation and segmentation events. The primordia of the proximal element of the limbs (humerus and femur) bifurcate to connect to paired distal elements (radius and ulna and tibia and fibula, respectively). The preaxial member of each pair gives rise to a preaxial column, which then segments twice, producing the radiale and element y in the manus and the tibiale and element y in the pes. The postaxial member of each pair bifurcates, giving rise to medial and postaxial columns. The medial column starts to bifurcate into a centrale and element m, but this process is aborted and the column essentially segments to form the definitive centrale, which incorporates element m (Fig. 5; see also Fig. 1 in Shubin and Wake 1996). The postaxial column ceases its independent growth without either segmenting or bifurcating and becomes either the ulnare

Fig. 5. Patterns of connectivity, both primary and secondary, in the development of the limb of diverse salamander species. (A) From Fig. 2A, a preparation stained with antibodies to type II collagen, showing connectivities. (B) General model of pattern of limb development in the family Dicamptodontidae, based on data given for *Dicamptodon* in this paper. (C) General model of the pattern of limb development in a member of the family Salamandridae, based on *Triturus marmoratus* (from Blanco and Alberch 1992; Shubin et al. 1995). (D) General model of the pattern of limb development in a member of the family Ambystomatidae, based on *Ambystoma mexicanum* (from Shubin and Alberch 1986; Shubin et al. 1995). *pr*, prepollex; for an explanation of other abbreviations see Figs. 2 and 3.



or the fibulare. This pattern is essentially identical in *Dicamptodon* and *Ambystoma* and appears to be fundamental to all salamanders that have been studied to date. Another fundamental feature associated with caudate limb development is the precocious development of digits 1 and 2 and the associated basale commune in both the forelimb and the hind limb. Thus, the primary axis of the limb extends through digit 2, rather than through digit 4 as is typical of frogs and amniotes (Shubin and Alberch 1986). Furthermore, there is a distinct digital arch that develops in a preaxial to postaxial gradient. Where the digital arch approaches the postaxial column, the last one or two distal carpals or tarsals form and the last two digits appear. The last digit to appear is the most postaxial, and as in other salamanders, there are only four digits on the forelimb. However, *Dicamptodon* differs from *Ambystoma* in the formation of a ringlike series of condensations in the postaxial portion of the manus and pes (Fig. 5) (a similar arrangement was illustrated for *S. keyserlingii* by Schmalhausen 1910). Although there is early separation of the basale commune from more basal elements, later there develops a continual dense matrix out of which form distal carpal 4 and the fourth digit in the manus and distal tarsals 4 and 5 and the fourth and fifth digits in the pes. Accordingly, the digital arch of *Dicamptodon* is trun-

cated and limited to the basal commune and distal element 3 (as in *T. marmoratus*; Blanco and Alberch 1992) (Fig. 5).

In some respects, limb development in *Dicamptodon* is more similar to that found in plethodontids (*Desmognathus* and *Bolitoglossa*; see footnote 2) than in ambystomatids. These include the degree of differentiation prior to hatching, the weak postaxial extension of the digital arch, and the absence of a bullet-shaped external form. These differences may reflect adaptive changes associated with life-history evolution. Hatchlings of species with pond-type larvae are altricial, with tiny or no limb buds. Such limbless larvae survive well. They have paired anterior appendages, balancers, that function partially as limbs until forelimbs appear (reviewed by Crawford and Wake 1998). By contrast, in species having larvae that hatch in streams, there is high survival value in having well-developed limbs at the time of hatching in order that the organisms will be better able to maintain their position in streams as they move about on the bottom. Nevertheless, such fundamental salamander features as preaxial dominance are strongly manifest in *Dicamptodon* and appear to have evolved early in the history of the Caudata.

Dicamptodontidae and Ambystomatidae are sister taxa, so the several differences between them need to be partitioned as to whether they are derived or ancestral. Certainly, there

has been homoplasy because ambystomatids resemble hynobiids and salamandrids in several respects, while dicamptodontids resemble plethodontids. We are presently engaged in a broad comparative study of limb development in the Caudata (e.g., Shubin and Wake 1996), but the absence of close outgroups makes assessment of synapomorphies problematic. For example, the transient appearance of element m in both dicamptodontids and ambystomatids probably represents a retained ancestral trait, but the expression of element m in occasional adult plethodontids and its association with distal mesopodials in several unrelated plethodontid lineages (Wake 1991; Shubin and Wake 1996) represent apomorphic change and homoplasy. Whether the general pattern of limb development in dicamptodontids is convergent with that in other stream-dwelling taxa or represents retention of an ancestral trait is uncertain.

We believe that several of the features found in *Dicamptodon* as well as in other salamanders are synapomorphies at the level of the Caudata. These include those traits in which salamanders differ from anurans and amniotes: preaxial dominance in development, precocious development of the basale commune and digits 1 and 2, a limb axis that goes through digit 2, and a digital arch that grows from preaxial to postaxial. In addition, all salamanders have a unified basale commune (an amalgamation of the first two distal elements) in both the manus and the pes, and no salamander has more than four digits on the forelimb (Shubin et al. 1995; Shubin and Wake 1996). However, while these features are constant, there is reason to believe that some of them (e.g., preaxial developmental dominance and precocious development of the first two digits) may have become accentuated by adaptive processes in those species with pond-type larvae. In such larvae, especially those in the family Salamandridae, the second digit becomes extremely elongated (e.g., Blanco and Alberch 1992) and may play a sensory role. In larvae of the family Hynobiidae, a curious filamentous structure that has the external form of a spine is present between the first and second digits of the manus (e.g., Vorobyeva and Hinchliffe 1996); it may function in locomotion and maintenance of balance. These specializations appear after hatching, and whatever function they serve starts prior to full limb development. Thus, they function as the limbs are developing, and this may be a proximal selective factor leading to preaxial dominance and precocious distal development of the basale commune and digits 1 and 2. If these features are synapomorphies of the Caudata, retention of them in the dicamptodontids, in a more subdued form than in the salamandrids, ambystomatids, and hynobiids, may represent a recapitulatory aspect of limb development.

The new data on *Dicamptodon* further buttress the basic division, between salamanders on the one hand and frogs and amniotes on the other, that has long been noted and discussed (Shubin and Alberch 1986). However, we do not envision these differences as having the significance they were given in the past, when they led to such extreme interpretations as the diphyletic origin of tetrapods (Holmgren 1933). The fact that frogs resemble amniotes in most of the features of limb development discussed here might be taken as support for these features being symplesiomorphic features of

tetrapods, with the salamanders displaying apomorphic traits. If one accepts that the metapterygial axis of sarcopterygian fishes gave rise to the digital arch, then one might argue that the proximodistal sequence of development of lungfish fins corresponds to the posterior to anterior pattern of digital arch expansion in amniotes and frogs, and the salamander anterior to posterior pattern represents an apomorphy of salamanders. However, the paucity of outgroup taxa that would permit a proper phylogenetic analysis makes this suggestion problematic. We further explore the balance between adaptive modifications and phylogenetic effects in a comprehensive review of limb development in Caudata that is in preparation.

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