specimen. Richard Estes (San Diego State University) read the manuscript and disagrees with my conclusions. I thank Arnold Bell (University of California-Riverside) for taking the SEM photographs of the specimen.

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Geographic Variation in the Limb Skeleton of the Red-Backed Salamander, *Plethodon cinereus*

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Analysis of skeletal evolution is hampered by a lack of knowledge of the scope and nature of variation in natural populations. This is true even for a structure of such obvious adaptive and phylogenetic significance as the vertebrate limb. Recently, one of us documented extensive variation in the fore- and hind limb skeleton in a single population of the Red-backed Salamander, *Plethodon cinereus* (Amphibia: Plethodontidae), from Glascow Mountain, Nova Scotia, in eastern Canada (Hanken, 1983). Salamanders from this population display numerous mesopodial (i.e., wrist and ankle) and digital patterns, many of which have never been observed in any other amphibian. Interpretation of the significance of this variation, however, is made difficult by the paucity of analogous, quantitative studies of natural limb-skeletal variation in other urodele species, or even other populations of *P. cinereus*. In an attempt to rectify this problem, we quantified limb skeletal variation in samples of *P. cinereus* from three other localities. The results (1) document heretofore unreported variant mesopodial and phalangeal patterns which occur at low frequencies in more centrally-located populations, and (2) underscore the relatively extensive and exceptional nature of the variation present in Nova Scotia. We believe that this information contributes to an understanding of the origin of novel, discrete morphological characters, and of how such characters become established in natural populations.

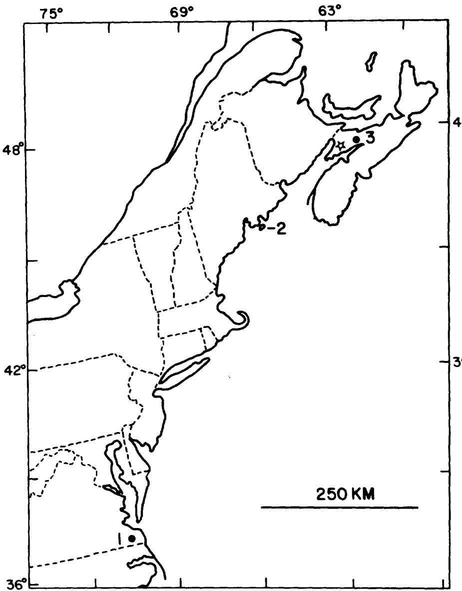

FIG. 1. Collection localities for *Plethodon cinereus* in eastern United States and Canada: 1, Dismal Swamp, Virginia; 2, Mt. Desert Island, Maine; 3, Cobequid Mtns., Nova Scotia (see text for exact locality data). Star denotes additional Nova Scotian locality studied earlier (Hanken, 1983).

Samples were obtained from the following localities: Dismal Swamp, Old Dominion University property, along north ditch adjacent to National Wildlife Refuge boundary, Suffolk, Virginia; Mt. Desert Island, Hancock Co., Maine; and Cobequid Mountains, 8 km N of Economy along River Philip Rd., Colchester Co., Nova Scotia, Canada (Fig. 1). The latter locality is approximately 45 km E of the Glascow Mountain locality sampled earlier, and lies within a similar deciduous forest.

Specimens were preserved in 10% neutral-buffered formalin, then differentially stained for bone and cartilage as whole mounts (Hanken and Wassersug, 1981). Frequencies of carpal (wrist) and tarsal (ankle) fusions, overall mesopodial patterns, and phalangeal formulae on both sides were scored as described earlier (Hanken, 1983). Only one small, poorly differentiated tarsus appeared to be regenerated; it was excluded from the analysis. Carpal fusion patterns in *P. cinereus* limb regenerates are qualitatively different from those observed in original limbs, although there is some

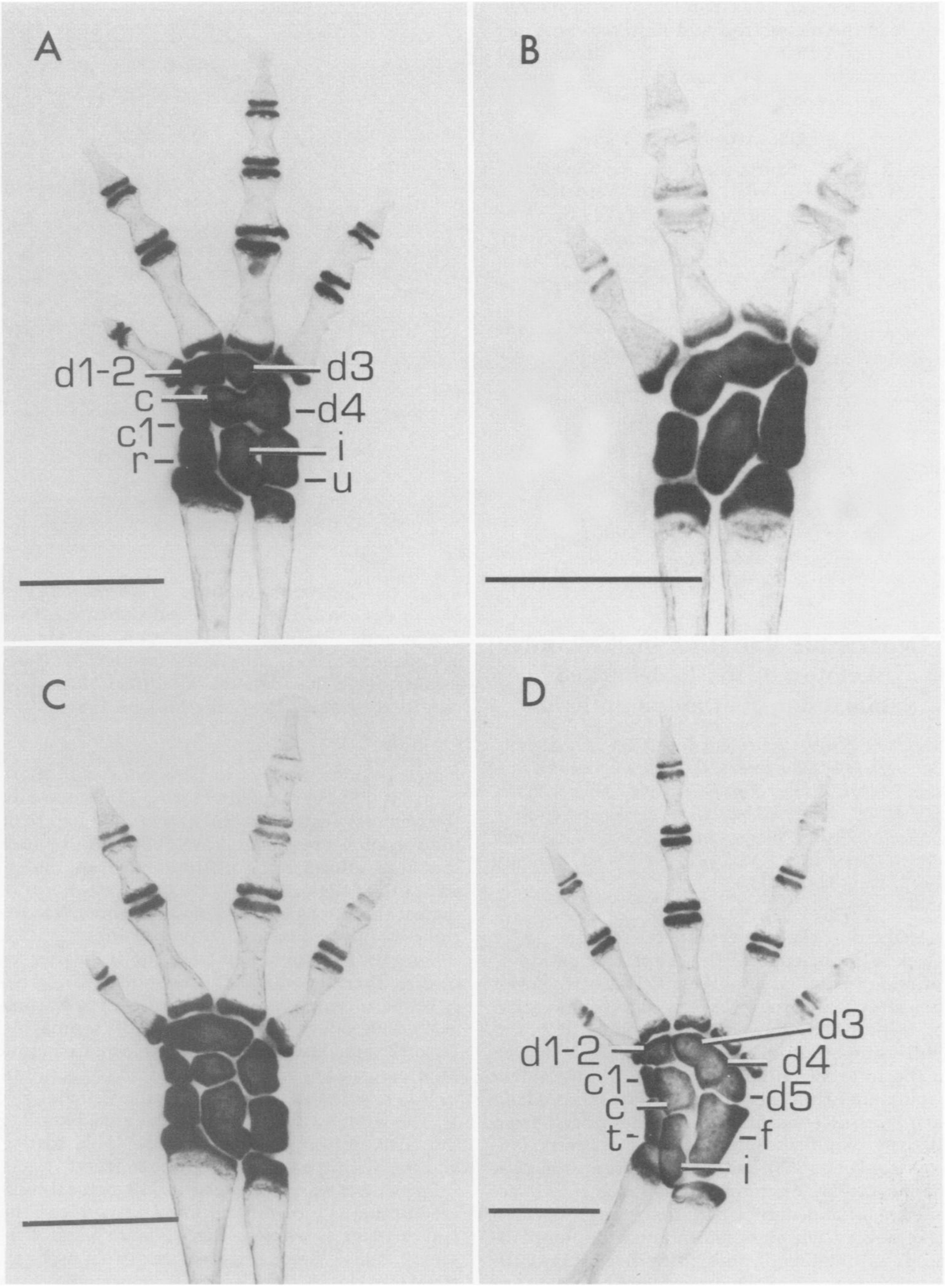

FIG. 2. Mesopodial patterns previously unreported in *Plethodon cinereus* (dorsal views). A, B, C: carpal patterns IX, X, and XI, respectively. IX and XI are right carpi, X is from the left side. All three apparently are unique to this species. Note reduced digital formula and distal articulation of metacarpals 1 and 2 in X. D: tarsal pattern VI (right side), which occurs in other plethodontid genera (see text). Abbreviations—A: i, intermedium; u, ulnare; r, radiale; c, centrale; c1, centrale 1; d1–2, distal carpals 1–2; d3, distal carpal 3; d4, distal carpal 4. D: as in A, except t, tibiale; f, fibulare; d1–2, distal tarsals 1–2; d3, distal tarsal 3; d4, distal tarsal 4; d5, distal tarsal 5. Bar equals 1 mm. Remaining patterns are illustrated in Hanken (1983).

TABLE 1. Frequency (%) of carpal fusion combinations and overall carpal patterns (numbered as in Hanken, 1983). N equals number of carpi examined. Frequency of right-left asymmetry in carpal pattern was 18%, Virginia; 18%, Maine; and 50%, Nova Scotia.

		Virginia (N = 76)	Maine (N = 90)	Nova Scotia (N = 60)
Fusion combinations[1]	(1) Intermedium, ulnare	4	7	25
	(2) Distal carpals 3, 4	—	—	2
	(3) Distal carpal 4, centrale	13	9	35
	(5) Intermedium, centrale	—	—	2
	(6) Distal carpals 1-2, 3	—	—	3
	(7) Distal carpals 1-2, 3, 4	—	—	2
	(8) Radiale, centrale 1	—	—	2
Carpal patterns[2]	I	83	86	43
	II	4	6	17
	III	—	—	2
	V	13	8	25
	VII	—	1	8
	IX	—	—	2
	X	—	—	2
	XI	—	—	2

[1] Combination (4), distal carpals 3, 4, centrale, was not observed in these populations.

[2] I: i, u, r, c, cl, d1-2, d3, d4; II: i-u, r, c, cl, d1-2, d3, d4; III: i, u, r, c, cl, d1-2, d3-d4; V: i, u, r, cl, d1-2, d3, d4-c; VII: i-u, r, cl, d1-2, d3, d4-c; IX: i, u, r, cl, d1-2-d3, d4-c; X: i-c, u, r-cl, d1-2-d3-d4; XI: i, u, r, c, cl, d1-2-d3, d4. Patterns IV, i-u, r, cl, d1-2, d3-d4-c; VI, i-u, r, c, cl, d1-2, d3-d4; and VIII, i-c, u, r, cl, d1-2, d3, d4, were not observed in these populations.

overlap in phalangeal formulae (Dinsmore and Hanken, in prep.). Thus, while limbs included in this study may include an occasional digital regenerate, we believe inclusion of limb regenerates of more proximal origin is highly unlikely.

All three populations exhibit variation in mesopodial pattern and phalangeal formula in both the fore- and the hind limbs. The Nova Scotia population is the most variable. Eight different carpal patterns were observed in the sample of 30 specimens (Table 1). The three predominant patterns—I, II, and V—also are the most common in the Glascow Mountain population sampled earlier (Hanken, 1983). Three rare variants—IX, X, and XI—are previously unreported in any plethodontid salamander (Fig. 2). The eight carpal patterns involve seven combinations of fused adjacent cartilages, including three—6, 7, and 8—previously unreported in *P. cinereus*. Three carpal patterns and one fusion combination that are rare in the Glascow Mountain population are not represented in the present sample (Table 1).

Carpal variation in Virginia and Maine comprises three and four patterns, respectively. Frequencies of the three shared patterns are remarkably similar in these two, distant populations, and are quite different from their frequencies in Nova Scotia. For example, whereas pattern I characterizes more than four-fifths of the carpi from both Maine and Virginia, it is found in fewer than one-half of the Nova Scotia specimens. The four patterns found in Maine and Virginia, however, are the four most frequent patterns in Nova Scotia.

Patterns of geographic variation in the tarsus mirror on a smaller scale those in the carpus (Table 2). The predominant pattern, I, occurs in 80% of tarsi from Nova Scotia, versus 89% and 97% in Maine and Virginia, respectively. Tarsal pattern VI and fusion combination 7, both rare in the Nova Scotia sample, are not present in the samples from Maine and Virginia, nor were they detected in the Glascow Mountain sample (Fig. 2); they are, however, present in some species of three distantly-related plethodontid genera, *Bolitoglossa* (Alberch, 1981; Larson, 1983; Wake, 1966), *Eurycea* and *Typhlomolge* (Potter and Sweet, 1981).

Digital variation is present in all three populations (Table 3). Unlike the patterns of mesopodial variation, however, which distinguish the Nova Scotia population from those from Maine and Virginia, the three populations differ only slightly in the frequency of digital variants. All populations show the primitive plethodontid phalangeal formulae—1-2-3-2 (hand), 1-2-3-3-2 (foot)—at frequencies exceeding 90%. All variants represent a decrease in the number of phalanges. Three foot variants are of especial interest in that they resemble patterns seen in other urodeles. The first, 1-2-3-3-1, which is present in Virginia and Nova Scotia, characterizes two congeneric species, *P. larselli* and *P. neomexicanus* (Wake, 1966). It also has appeared, presumably independently, in the distantly-related plethodontid genus, *Thorius*, and in the salamandrid genus, *Notophthalmus* (Alberch and Gale, 1985; Hanken 1982). The second, 1-2-3-2-2, is also present in species of *Thorius* and of two other neotropical genera, *Bolitoglossa* and *Oedipina* (Alberch and Gale, 1985; Hanken, 1982; Wake, 1966). The third variant, 1-2-3-1, represents the loss of a digit. Similar four-toed patterns, albeit

TABLE 2. Frequency (%) of tarsal fusion combinations and overall tarsal patterns. N equals number of tarsi examined. Frequency of right-left asymmetry in tarsal pattern was 0%, Virgina; 19%, Maine; and 20%, Nova Scotia.

		Virginia (N = 76)	Maine (N = 44)[1]	Nova Scotia (N = 60)
Fusion combination[2]	(6) Distal tarsals 4, 5	3	11	17
	(7) Distal tarsals 3, 4, 5	—	—	3
Tarsal patterns[3]	I	97	89	80
	II	3	11	17
	VI	—	—	3

[1] One additional, regenerated tarsus was excluded.

[2] Combinations (1), intermedium, centrale, (2), tibiale, centrale 1, (3), distal tarsals 1-2, 3, (4), distal tarsals 3, 4, and (5), distal tarsals 1-2, 3, 4, were not observed in these populations.

[3] I: i, t, f, c, cl, d1-2, d3, d4, d5; II: i, t, f, c, cl, d1-2, d3, d4-d5; VI: i, t, f, c, cl, d1-2, d3-d4-d5. Patterns III, i, t, f, c, cl, d1-2-d3, d4-d5; IV, i-c, f, t-cl, d1-2, d3-d4, d5; and V, i-c, t, f, cl, d1-2-d3-d4, d5, were not observed in these populations.

with different phalangeal formulae (1-2-3-2, 1-2-3-3, or 2-3-3-2), have evolved independently at least six times in urodeles, including three plethodontid genera, *Hemidactylium, Manculus,* and *Batrachoseps* (Alberch and Gale, 1985; Wake, 1966).

There are two important implications of our findings. First, limb skeletal variation in *P. cinereus* is widespread. This species, indeed the entire genus *Plethodon,* has been considered an archetype of morphological stasis among vertebrates because of the lack of significant variation in morphology, especially osteology (Wake et al., 1983). Yet, extensive mesopodial and digital variation at three widely-separated localities indicates that the limb skeleton is anything but "conservative," although the predominant limb patterns are identical in each population. Furthermore, observed variants are phylogenetically significant: mesopodial and digital patterns that characterize other urodele species, or even genera, occur as polymorphisms within single populations or, in some cases, the same individual.

Second, limb skeletal variation in Nova Scotia clearly is more extensive than in other parts of the species' range surveyed to date. A total of 11 carpal and 6 tarsal patterns are now known from two Nova Scotia localities. Of these, 7 carpal and 4 tarsal patterns are unique to one or both of these localities. The frequency of variant patterns also is substantially higher in samples taken from Nova Scotia. There are no data available that describe limb skeletal variation in populations of *P. cinereus* between central coastal Maine and northwestern Nova Scotia. Defining the nature of the transition between areas of high and low frequency thus awaits additional sampling.

What is the developmental basis of the observed patterns of limb skeletal variation? Frequent asymmetry, especially in Nova Scotia populations (Tables 1–3), suggests a dominant role of nongenetic factors (McGrath et al., 1984). However, the widely-ranging frequencies of carpal fusion combinations at each locality, and the predominance of identical arrangements at different localities (Table 1), indicate directionality in the expression of morphological variability in the limb (discussed in Hanken, 1983). This directionality may represent a bounded domain limited or defined by developmental (epigenetic) constraints; variability within this domain, and its evolution, are products of developmental perturbations (Alberch, 1980, 1983; Alberch and Gale, 1985). Thus, even if much of the variation is environmentally

TABLE 3. Frequency (%) of alternate phalangeal formulae.

	Virginia (N = 76, 74)[1]	Maine (N = 88, 44)	Nova Scotia (N = 59, 60)
Hand			
1-2-3-2	100	98	93
1-2-2-2	—	1	—
1-1-3-2	—	1	—
1-2-3-1	—	—	3
1-1-2-1	—	—	2
1-0-3-2[2]	—	—	2
Asymmetry[3]	— (38)	5 (44)	14 (29)
Foot			
1-2-3-3-2	96	95	92
1-2-2-3-2	—	2	2
1-2-3-2-2	—	2	—
0-2-3-3-2[4]	1	—	—
1-2-3-3-1	1	—	5
1-1-3-3-2	1	—	—
1-2-3-1	—	—	2
Asymmetry[3]	8 (37)	5 (21)	7 (30)

[1] Hands and feet, respectively.

[2] Metacarpal 2 present; digit 1 small.

[3] Frequency of different phalangeal formulae on right and left sides; number of specimens examined is in parentheses.

[4] Metatarsal 1 present, unossified.

induced, the limb-development program apparently is restricting the range of variants produced. Moreover, as demonstrated by Waddington's (1957) model of "genetic assimilation," even environmentally-induced changes of the phenotype may, given the proper selective regime, become genetically "determined" and no longer require the original environmental stimulus for their formation.

We suggest that in this instance perturbations of either genetic or environmental origin may inhibit completion of carpal and tarsal ontogeny during which a small number of cartilaginous primordia would ordinarily divide to produce a greater number of adult cartilages. Accordingly, the variant fusion combinations in *P. cinereus* may reflect precocious arrest of ontogeny producing a smaller-than-normal number of mesopodial elements. Alternatively, perturbations might reduce element number indirectly by decreasing the size of the limb primordium; Alberch and Gale (1983, 1985) recently proposed a similar model to explain digital reduction and loss in amphibians. Since urodele limb regeneration is widely accepted as a high fidelity analog of initial limb development (Fallon and Caplan, 1983), we are presently exploring these hypotheses by analyzing skeletal patterning in *P. cinereus* limb regenerates which recapitulate the ontogenetic sequences of tissue interactions and histogenesis. Questions related to this study that we are pursuing currently include: Why do carpal and tarsal patterns occur in the same relative abundance at each locality, whereas absolute frequencies differ widely between some localities? Are interlocality differences in the frequency of initial limb variants associated with differences in the frequency of regenerate pattern variants? Are variant limb patterns reproduced during limb regeneration?

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