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Native Variant Limb Skeletal Patterns in the Red-Backed Salamander, *Plethodon cinereus*, are not Regenerated

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ABSTRACT Species of the salamander genus *Plethodon* have a characteristically uniform morphology. Morphological conservatism at the level of interspecific comparisons, however, is not always reflected within species. Perhaps the most extreme example of intraspecific variation is the recent description of extensive variability in limb-skeletal patterning both within and between populations of the widespread species *P. cinereus*. We utilized limb regeneration following experimental amputation as a tool 1) to examine whether naturally occurring variant skeletal patterns result from limb loss and regeneration in nature, and 2) to assay the intrinsic (i.e., genetic) component of between-individual variation in mesopodial patterning. We observed the following. First, regenerate patterns are strikingly different from native patterns: interelement fusions in regenerates are typically between proximodistally adjacent cartilages, whereas interelement fusions in native variant limbs occur exclusively between laterally adjacent cartilages. Fusions also are over ten times more frequent in regenerates than in native limbs. Second, there is no strong correlation between native limb pattern (typical vs. variant) and the regenerate pattern. We conclude that variability in field-collected *P. cinereus* reflects extensive intrapopulation variation in limb-skeletal patterning during original limb development, rather than regeneration in nature, and that limb regeneration analysis provides no evidence of a strong genetic component to between-individual variation. Finally, unusual mesopodial patterns produced during limb regeneration may be related to the mechanical factors impinging on the regenerating limb in this terrestrial species.

Urodele species of the genus *Plethodon* have a remarkably uniform morphology, especially in terms of osteology (Wake, '63). Indeed, the genus has been offered as a paradigm of morphological stasis among vertebrates (Wake et al., '83). Recently, however, a Nova Scotian population of the wide-ranging species *P. cinereus* was found to contain an exceptionally high incidence and diversity of variant mesopodial (i.e., wrist and ankle) and digital skeletal patterning. The variant mesopodial patterns comprise different fusion combinations between adjacent cartilages; the variant digital patterns typically involve a reduced number of phalanges in one or more digits. Some of these patterns are found in other plethodontid genera (Hanken, '83, '85; Wake, '66), but other, less frequent patterns are unique to that population. The significance of this variability is underscored by noting that certain variant patterns in this population are as different as those that distinguish among other urodele species, or even genera. Intraspecific skeletal variation in *Plethodon* is not always as "conservative" as that between species (Hanken, '83).

We subsequently quantified limb skeletal variation in three other, geographically distant populations of *P. cinereus* from both within and outside Nova Scotia and found that variation is indeed widespread (Hanken and Dinsmore, '86). While the frequency of mesopodial variants in Nova Scotia is two to three times higher than that found elsewhere—and involves a greater diversity of

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skeletal patterns—variability is also present in populations as far away as Maine and Virginia; in these latter populations, the frequency of variant patterns in the carpus alone exceeds 10%. It is also important to note that, while their exact frequencies vary, the predominant patterns in each population are the same.

There are several possible explanations for this osteological variation; each may fall under a broadly defined principal category of either hereditary or environmental causes. In this study, we explore directly one possible environmental cause: that variant skeletal patterns in the limbs of field-collected animals (here termed *native* limbs) are in fact regenerated patterns that formed following loss of the original limb in nature. If this hypothesis is correct, then experimentally amputated limbs with the native typical (i.e., most common) skeletal pattern should produce regenerates with the naturally occurring typical or variant patterns. In addition, and based upon the previous findings that repeated limb amputation in newts increases the incidence of abnormal regenerates (Dearlove and Dresden, '76), limbs that originally bore variant skeletal patterns should produce regenerates with a higher incidence and possibly wider range of variability than occur among regenerates from native typical limbs.

In testing the hypothesis that the native variant skeletal patterns are produced during regeneration, we are also examining qualitatively the genetic component of between-individual pattern variation. For example, if the genetic component of variation is relatively high, then patterns regenerated from variant limbs should differ from those regenerated from typical limbs. Alternatively, if the genetic component is relatively low, then the frequencies and types of patterns regenerated should not differ significantly between native typical and native variant limbs.

Another possible result of experimental limb amputation is that regenerated patterns are qualitatively different from both native typical and native variant patterns. A substantial body of evidence, however, supports the claim that both original development and regeneration of urodele limbs are guided by the same basic mechanisms (e.g., reviews by Faber, '71; Muneoka and Bryant, '82, '84; Stocum, '75; Stocum and Fallon, '82). Furthermore, the mesopodia, on which we will focus, appear normally to arise by fission of precartilaginous or cartilaginous primordia during both native (Shubin and Alberch, '86) and regenerative (Settles and Neufeld, '80) skeletal patterning. In this context, a reasonable a priori assumption is that any deviation in regenerate skeletal patterning from the typical configuration is likely to produce one of the common variant patterns described previously (Hanken, '83; Hanken and Dinsmore, '86).

The following study is a test of the principal hypothesis that naturally occurring variant limb skeletal patterns in a population of *P. cinereus* reflect regenerative events in nature. The ramifications of the resultant data for other related issues, some of which have been noted above, will also be explored.

MATERIALS AND METHODS

Seventy-one adult red-backed salamanders, *Plethodon cinereus*, were collected principally in the vicinity of the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine. An additional 20 animals were collected south of Mount Desert Island in Boothbay, Maine, by Dr. R.J. Goss, who kindly donated them for this study. No significant differences in either the type or the frequency of limb skeletal variants were found between these populations, which are therefore treated as representing one continuous Maine population.

As *P. cinereus* is a terrestrial species, the salamanders were maintained on wet paper towelling in stacking fingerbowls with specially prepared lids and were fed ad libitum with laboratory-reared fruit flies. To obtain original limbs for skeletal analysis, animals were immobilized by immersion in 1% MS 222 (ethyl m-aminobenzoate methanesulfonate, Eastman). We have subsequently determined that immersion in 0.1% benzocaine, as recommended by Dr. Joseph Vanable at Purdue University, produces a more rapid immobilization. Animals were then placed on the stage of a dissecting microscope, and the limbs were amputated through the proximal third of both forearms and shanks, or both forearms only, with the distal segments being fixed immediately in 10% neutral-buffered formalin. A total of 182 native forelimbs and 85 native hind limbs were obtained in this manner for skeletal pattern analysis. The animals were then removed to petri dishes containing Holtfreter's solution for recovery and were subsequently maintained as described above for at least 2–3 months to allow for complete limb regeneration, especially skeletogenesis of mesopodial elements. At the end of this period, animals were again immobilized, and under the dissecting microscope the limbs were exarticulated from their girdles and fixed as above. Animals that survived the entire regeneration period provided 144 forelimb regenerates and 56 hind limb regenerates; of these, mesopodial pattern could be reliably scored in all but ten forelimb regenerates.

Both native and regenerate limbs were differentially stained for bone and cartilage as whole mounts by a variation on the alizarin red/Alcian blue technique described by Hanken and Wassersug ('81). The first 90 native forelimbs and 44 native hind limbs that were prepared and analysed in this study also provided the Maine sample data in our study of limb-skeletal geographic variation in *P. cinereus* (Hanken and Dinsmore, '86). The typical and variant carpal and tarsal patterns described by Hanken ('83) are the standards by which limbs in the present study were evaluated. Both native distal limb segments and subsequent regenerates were coded (by C.E.D.) prior to pattern analysis (by J.H.). The fidelity of mesopodial patterning in the regenerates relative to native patterns was then determined by uncoding and pair-matching each native limb with its regenerate.

RESULTS

Native mesopodial patterns

The native limbs on each animal bore either the mesopodial pattern typical of the species ("native typical": type I, Hanken, '83) or one of several variant patterns that involve fusion of adjacent cartilages that are separate in the typical patterns ("native variant"; Figs. 1, 2). In this population, 9.9% of native forelimbs (N = 182) have at least one fusion combination (Table 1). This represents an incidence of 6.0% with d4-c fusion only, 2.7% with i-u fusion only, and 1.1% with both d4-c and i-u fusions. Native hind limbs (N = 85) have a higher incidence of interelement fusion, with 15.3% expressing a complete or partial d4-d5 fusion; this, however, is the only combination observed (Table 2). Each fusion combination involves only central and postaxial cartilages; preaxial cartilages are never involved. Animals with variant forelimb or hind limb patterns typically have them on one side only; overall, the frequency of right-left asymmetry is 6.0% (forelimb) and 10.6% (hind limb).

TABLE 1. Incidence of typical and variant carpal patterns in native forelimbs (N = 182)[1]

Carpal pattern[2]	No.	Frequency (%)
Typical (no fusions)	164	90.1
d4-c	11	6.0
i-u	5	2.7
d4-c, i-u	2	1.1

[1] Twenty-three animals (24.7%) bore all of the mesopodial variants. Each had only one forelimb or one hind limb variant pattern, with the following exceptions: three animals had bilateral forelimb variants (symmetric in two), one had bilaterally symmetric hind limb variants, one had bilaterally symmetric forelimb and hind limb variants, and one had one forelimb and one hind limb variant.

[2] Abbreviations of carpal patterns are as in Figure 1.

TABLE 2. Incidence of typical and variant tarsal patterns in native hind limbs (N = 85)[1]

Tarsal pattern[2]	No.	Frequency (%)
Typical (no fusions)	72	84.7
d4-d5	13	15.3

[1] See footnote for Table 1.

[2] Abbreviation of tarsal pattern is as in Figure 2.

Regenerate mesopodial patterns

One hundred thirty-four forelimb and 56 hind limb regenerates were obtained from animals that survived the entire regeneration period, and they were scored for mesopodial pattern. We will first consider regenerate mesopodial patterns with respect to the native patterns described above, and then we will compare the patterns regenerated from typical limbs with those regenerated from variant limbs.

Native vs. regenerate patterning

Most regenerate forelimbs (93.3%) and hind limbs (98.2%) produced variant mesopodial patterns, with fewer separate cartilages than the typical patterns (Tables 3 and 4). Clearly, a reduced number of skeletal elements following limb regeneration is the norm in this species. The extent and frequency of reduction, however, far exceeds that observed following repeated limb amputation in newts (Dearlove and Dresden, '76). Furthermore, the regenerate patterns are qualitatively dif-

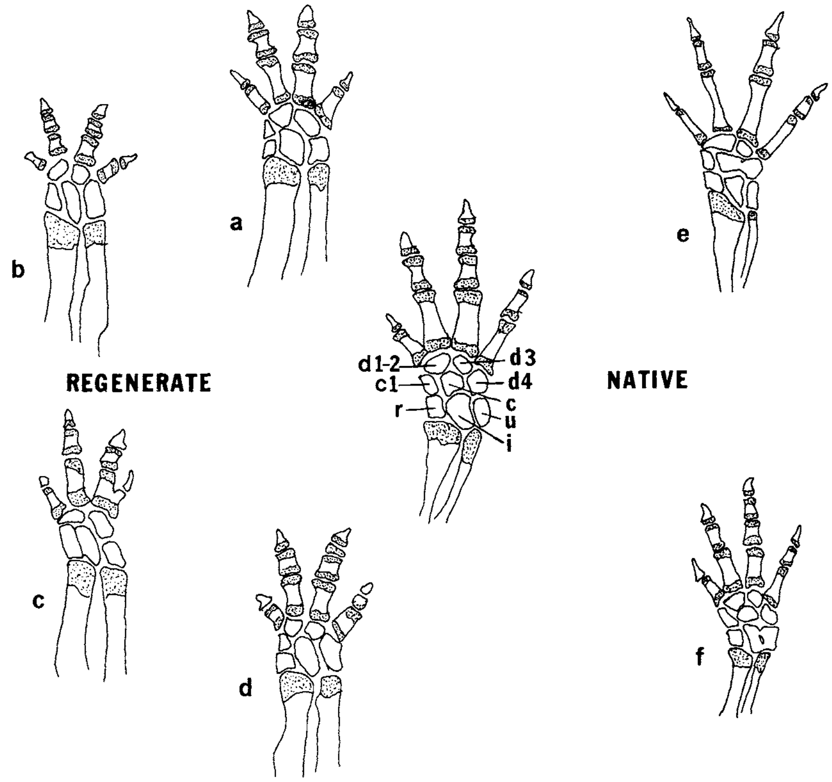

Fig. 1. Typical (central diagram) and some variant regenerate (a–d) and native (e and f) forelimb mesopodial patterns: (a) d3-d4, c-i combination; (b) d4-u, c-i, r-c1; (c) d3-d4, c-i, r-c1; (d) d4-u, c-i; (e) d4-c; (f) i-u. Abbreviations: d1–2, distal carpal 1–2; d3, distal carpal 3; d4, distal carpal 4; c, centrale; c1, centrale 1; r, radiale; i, intermedium; u, ulnare. Figures are drawn from photomicrographs; stippling indicates noncarpal cartilage.

TABLE 3. Incidence of carpal patterns in forelimb regenerates[1]

	Native pattern (No./%)		Overall (typical and variant)
Regenerate pattern[2]	Typical (N = 123)	Variant (N = 11)	No./%
Typical (no fusions)	8/6.5	1/9.1	9/6.7
d3-d4, c-i	31/25.2	2/18.2	33/24.6
d4-u, c-i, r-c1	14/11.4	3/27.3	17/12.7
d3-d4, c-i, r-c1	17/13.8	0	17/12.7
d4-u, c-i	12/9.8	2/18.2	14/10.4
d3-d4	11/8.9	2/18.2	13/9.7
d3-d4-u, c-i, r-c1	10/8.1	1/9.1	11/8.2
d3-d4-u, c-i	5/4.1	0	5/3.7
Others[3]	15/12.2	0	15/11.2

[1] The number and frequency of each regenerate pattern (column 1) are presented both with respect to the native patterns (typical vs. variant) that preceded the regenerates (columns 2 and 3) and as overall measures (native typical and variant measures combined; column 4).

[2] Abbreviations of regenerate patterns are as in Figure 1.

[3] Eleven additional patterns each occurred only once or twice.

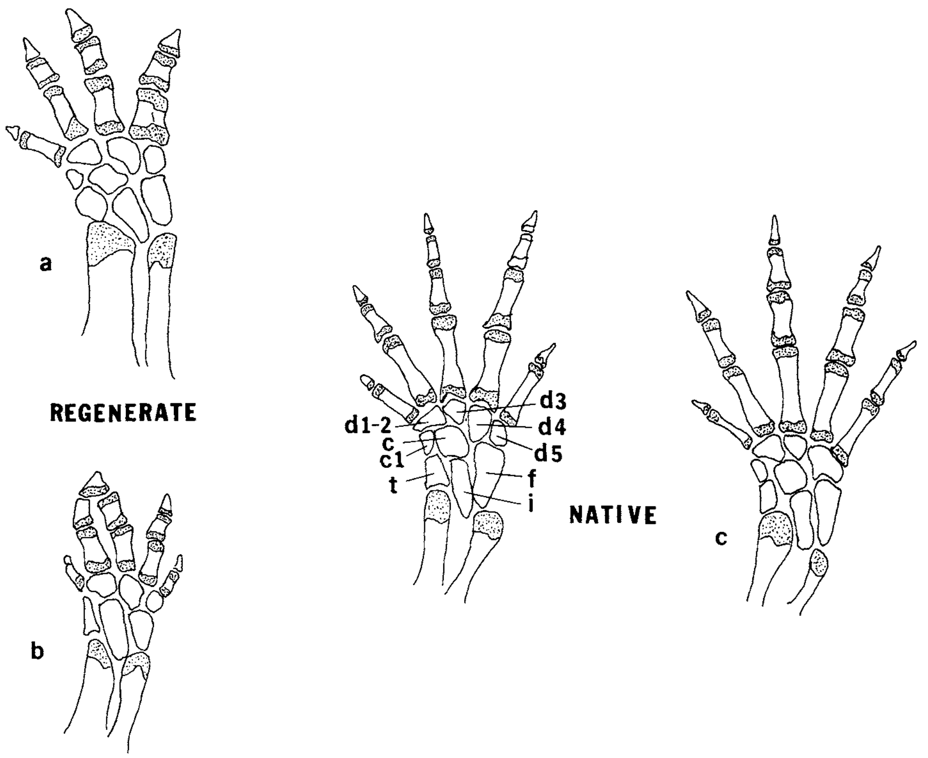

Fig. 2. Typical (central diagram) and some variant regenerate (a and b) and native (c) hind limb mesopodial patterns: (a) d3-d4 combination; (b) d3-d4, c-i, t-c1; (c) d4-d5. Abbreviations: d1-2, distal tarsal 1-2; d3, distal tarsal 3; d4, distal tarsal 4; d5, distal tarsal 5; c, centrale; c1, centrale 1; t, tibiale; i, intermedium; f, fibulare. Figures are drawn from photomicrographs; stippling indicates noncarpal cartilage.

TABLE 4. Incidence of tarsal patterns in hind limb regenerates[1]

Regenerate pattern[2]	Native pattern (No./%) Typical (N = 48)	Native pattern (No./%) Variant (N = 8)	Overall (typical and variant) (N = 56)
Typical (no fusions)	1/2.1	0	1/1.8
d3-d4	15/31.3	2/25.0	17/30.4
d3-d4, c-i, t-c1	6/12.5	2/25.0	8.14.3
c-i	3/6.3	—	3/5.4
d3-d4, c-i	0	2/25.0	2/3.6
d4-d5	2/4.2	0	2/3.6
d5 lost	10/20.8	0	10/17.9
d3-d4-d5, t-c1	0	1/12.5	1/1.8
d3-d4, d5-f, t-c1	0	1/12.5	1/1.8
Others[3]	11/22.9	0	11/19.6

[1] The number and frequency of each regenerate pattern (column 1) are presented both with respect to the native patterns (typical vs. variant) that preceded the regenerates (columns 2 and 3) and as overall measures (native typical and variant measures combined; column 4).

[2] Abbreviations of regenerate patterns are as in Figure 2.

[3] Eleven additional patterns each occurred only once.

ferent from the native variant patterns, contradicting the fundamental premise of the regeneration hypothesis (cf. Figs. 1, 2; Tables 1 and 3, 2 and 4). Native variant patterns are characterized by interelement fusions between laterally adjacent cartilages in the postaxial half of the limb (Figs. 1e,f, 2c). Most regenerate patterns, however, contain at least one fusion between proximodistally adjacent cartilages (Figs. 1b,c, 2b), including preaxial cartilages in a large number of cases (e.g., r-c1; Table 5). Indeed, none of the combinations of fused adjacent cartilages that are present in native variant forelimbs is present in regenerates, which have an entirely different complement of fusion combinations (Table 5). Similarly, in the hind limb, the lone fusion combination present in native variant limbs occurs at very low frequency in regenerates, which have several, more frequent fusions that are not present in native limbs (Table 6).

Regenerates from typical vs. variant limbs

Forelimb

Most limbs that originally bore the typical mesopodial pattern regenerated a variant pattern (Table 3). Of the 123 regenerates from native typical limbs, 77.2% produced one of six common variant patterns not seen in native limbs; only 6.5% of the limbs regenerated the typical pattern. Several additional variant patterns, also not seen in native limbs, appeared at very low frequencies.

Similar results were obtained from native variant limbs. All but one of these regenerates produced one of the six common patterns regenerated from typical limbs; the one remaining native variant limb regenerated the typical pattern. Conversely, only one of the six patterns common among regenerates from typical limbs was not seen among regenerates from variant limbs. This pattern (d3–d4, c-i, r-c1), which is not seen among the regenerates from variant limbs, accounted for 12.8% of regenerates from typical limbs. Given the small sample of 11 regenerates from variant limbs, we do not consider its absence from this group, and other differences that involve the frequencies of variants regenerated in the two groups, to be significant.

The weak correlation between native and regenerate mesopodial patterns is underscored by matching individual native variant forelimb patterns with the regenerates from the same limbs (Table 7). Each type of native variant limb produced at least two different

TABLE 5. Carpal fusion combinations common in native and regenerate forelimbs[1]

	Native limbs (N = 182)		Regenerate limbs (N = 134)	
Fusion combination	No.	Frequency (%)	No.	Frequency (%)
d4-c	13	7.1	0	0
i-u	7	3.8	0	0
d3-d4	0	0	63	47.0
r-c1	0	0	45	33.6
d4-u	0	0	31	23.1

[1] A given limb may contain more than one combination.

TABLE 6. Tarsal fusion combinations common in native and regenerate hind limbs[1]

	Native limbs (N = 85)		Regenerate limbs (N = 56)	
Fusion combination	No.	Frequency (%)	No.	Frequency (%)
d4-d5	13	15.3	2	3.6
d3-d4	0	0	28	50.0
c-i	0	0	13	23.2
t-c1	0	0	10	17.9
d5 lost	0	0	10	17.9

[1] Regenerate limbs may contain more than one combination.

TABLE 7. Forelimb patterns regenerated from native variant limbs[1]

Native pattern	Regenerate pattern	No./%[2]
d4-c (N = 6)[3]	d4-u, c-i, r-cl	3/50.0
	d3-d4, c-i	2/33.3
	d3-d4-u, c-i, r-cl	1/16.7
i-u (N = 3)[3]	d4-u, c-i	2/66.7
	d3-d4	1/33.3
d4-c, i-u (N = 2)	Typical	1/50.0
	d3-d4	1/50.0

[1] Patterns regenerated from native typical limbs are listed in Table 3.

[2] Percentages denote the frequency of each regenerate pattern within a given class of native pattern.

[3] Seven additional salamanders with native variant patterns—five with d4-c fusions, two with i-u fusions—did not survive to produce regenerate limbs.

TABLE 8. Hind limb patterns regenerated from native variant limbs[1]

Native pattern	Regenerate pattern	No./%
d4-d5 (N = 8)[2]	d3-d4	2/25.0
	d3-d4, c-i, t-cl	2/25.0
	d3-d4, c-i	2/25.0
	d3-d4-d5, t-cl	1/12.5
	d3-d4, d5-f, t-cl	1/12.5

[1] Patterns regenerated from native typical limbs are listed in Table 4.

[2] Five additional salamanders with native d4-d5 fusions did not survive to produce regenerate limbs.

regenerate patterns; one limb, as noted above, produced the typical pattern. Thus, differences in original carpal patterning (typical vs. variant) are not associated with differences in regenerate patterning. Instead, variation in regenerate patterning is similar in both groups.

Hind limb

Observations and conclusions presented above for the forelimb also hold generally for the hind limb. Native hind limbs with the typical pattern usually did not regenerate this pattern, producing instead 16 additional variant patterns (Table 4). Three variant patterns account for 64.6% of the regenerates from typical limbs; only one limb (2.1%) regenerated the typical pattern. Nearly one-fourth (22.9%) of the regenerates from typical limbs had patterns that were seen only once. This frequency is much higher than in the forelimb, in which only 5.2% of limbs with native typical patterns regenerated a unique pattern.

Eight of 13 regenerates from native variant hind limbs—all d4-d5—were recovered. These eight regenerates defined five variant tarsal patterns; none showed either the native typical pattern or the native variant pattern (Tables 4, 8). Two of the variant patterns—d3-d4, and d3-d4, c-i, t-ci—were commonly seen in regenerates from typical limbs. The three remaining patterns, each found in one or two limbs, were not present among regenerates from typical limbs. However, because of the high incidence of unique variants in hind-limb regenerates from typical limbs, we consider it inappropriate to regard this as strong evidence of different patterning tendencies between native typical and native variant limbs.

DISCUSSION

Are naturally occurring variants native or regenerated?

The primary hypothesis that we are investigating is that variant (i.e., non-typical) mesopodial patterns observed in field-collected salamanders are the product of limb regeneration. Clearly, they are not. In the forelimb, not one of the naturally occurring variant patterns or fusion combinations was seen in any regenerate (cf. Tables 1, 3, 5). Indeed, even the typical pattern, which characterizes over 90% of native limbs, was produced only rarely in regenerates. Instead, regenerated patterns, as well as the particular combinations of fused adjacent cartilages that they comprise, differ from both the typical pattern and naturally occurring variants (cf. Fig. 1). In the hind limb, the single native variant pattern—d4-d5—constituted less than 4% of regenerates, which displayed a total of 18 additional variant patterns not seen in limbs of field-collected animals (Tables 4, 6). We conclude, therefore, that variability previously documented in field-collected *P. cinereus* (Hanken, '83; Hanken and Dinsmore, '86) reflects extensive intrapopulation variation in limb-skeletal patterning during original limb development.

Is there a strong genetic component to between-individual variation?

A secondary question of interest is what is the genetic component of between-individual variation in native mesopodial pattern? That is, to what extent is the variation in mesopodial pattern among individuals attributable to intrinsic differences among these individuals? One alternative view, for example, is that the variation is primarily environmentally induced on a relatively homogeneous (with respect to limb development) genetic background. The array of native patterns observed would in this case define a bounded domain of feasible developmental options (Alberch, '80; Alberch and Gale, '85) that reflect plasticity in the developmental program (sensu Oster and Alberch, '82). Unfortunately, plethodontid salamanders are poor subjects for standard analyses of heritability, which entail controlled matings and analysis of sib-sib and parent-sib character correlations; they typically do not breed readily in the laboratory, and early posthatching stages are difficult to rear and grow slowly even under the best conditions. Instead we can use our data on limb regenerates as one alternative qualitative assay of the genetic component of variation.

The salamanders clearly did not regenerate the same pattern they had originally. Thus, we cannot use the extent to which animals regenerate their native pattern as our measure of the genetic component. We can, however, ask if animals with native typical patterns as a class regenerate a different set of patterns from those animals with native variant patterns. A positive result would suggest that there is a strong intrinsic component. A negative result would mean either that there is no strong intrinsic component, or that, if there is a strong intrinsic component, it is not manifest during limb regeneration in the same way that it is during original limb development.

We interpret our data comparing patterns regenerated from native typical vs. variant limbs (Tables 3, 4) as showing no significant difference between the two groups. In other words, limb regeneration analysis provides no evidence of a strong genetic component to the naturally occurring limb skeletal variation between individuals. Instead, the extensive native mesopodial variability apparently reflects a predominantly environmental modulation of a plastic developmental system. As was discussed earlier, the small sample of patterns regenerated from native variant limbs precluded statistical analysis of frequency differences between this group and those regenerated from native typical limbs; we are basing our claim primarily on the fact that the *types* of patterns regenerated in the two groups are, with few exceptions, identical. This result is also in agreement with the extremely high frequency of right-left asymmetry observed in field-collected animals, variation that implicates a large role for nongenetic factors (cf. McGrath et al., '84).

Native vs. regenerate patterning

In answering the above questions we have identified extensive and clear-cut differences between native and regenerate patterning in this species. This finding was unanticipated for at least two reasons: first, the demonstrated morphological similarities between the developing limb bud and the regeneration blastema, and second, comparable experimental studies on both developing (e.g., Eichele et al., '85; Saunders, '48; Tickle et al., '85) and regenerating (e.g., Maden et al., '85; Muneoka and Bryant, '82, '84; Thoms and Stocum, '84; Thornton, '57, '58) limbs, which have documented that the same fundamental processes govern morphogenesis in both systems. Admittedly, the occasional appearance of abnormal regenerates, that is, those that differ morphologically from the original structure, is familiar to anyone studying urodele limb regeneration. For example, in an earlier study of the red-spotted newt, *Notophthalmus viridescens*, approximately 10% of amputated limbs produced abnormal regenerates with respect to gross and skeletal morphology, a frequency that increased with repeated amputation (Dearlove and Dresden, '76). (Mesopodial patterns and fusion combinations were not recorded in this study, thus precluding direct comparisons with our results.) In our study, however, the vast majority—over 90%—of both forelimb and hind limb regenerates were qualitatively different from native limbs both in the principal axis of mesopodial fusions (anteroposterior vs. proximodistal) and in the incorporation of preaxial cartilages in fusion combinations. Why should regenerate mesopodial patterns be so dramatically different from native patterns in *Plethodon*? More specifically, why should a significant morphogenetic theme in native variant limbs—fusion between laterally adjacent elements—undergo an axial change in regenerated limbs—fusion between proximodistally adjacent elements?

We suggest one possible explanation, which concerns the local mechanical environment within which limb regeneration occurs. Most regeneration studies have used aquatic and semiaquatic urodele species (e.g., axolotls and newts), whereas *P. cinereus*, lacking an aquatic larval stage, is completely terrestrial (Dunn, '26). While it has not been demonstrated empirically, it is reasonable to presume that the nature and magnitude of mechanical stresses and strains experienced by the regenerating limb differ substantially between an aquatic salamander that approaches neutral buoyancy and a terrestrial salamander that must support much of its body weight with its limbs. At the same time, the powerful influence of mechanical forces on form and its development have been long appreciated (e.g., Murray, '36; Smith, '81; Thompson, '69); indeed, the local mechanical environment is a critical component in some recent models of limb pattern formation (Oster et al., '83). We suggest that the different mechanical influences to which a regeneration blastema is subjected in a terrestrial environment may alter the sequence of condensation and fission that characterizes limb chondrogenesis, thereby producing different patterns from those observed in aquatic species. Similarly, differences between native and regenerate patterns in *P. cinereus* may be due to the fact that the mechanical influences experienced by the limb during development in ovo—when the limb is not yet used in body support—differs significantly from that experienced by a regenerating limb.

This model, which is admittedly speculative, is not inconsistent with the view that mechanisms of pattern formation during limb development and regeneration are the same; instead, it simply posits that the actual pattern generated by these mechanisms is influenced by prevailing mechanical factors. It does, however, imply that differences between native and regenerate patterning should be typical of terrestrial species, a prediction that can be analyzed by direct testing of species other than *P. cinereus.*

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