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Amputation Level-Dependent Patterning in Urodele Limb Regeneration

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ABSTRACT Comparison of mesopodial skeletal patterns found in native and regenerated limbs of the salamander *Plethodon cinereus* reveals variant patterns unique to each group. Variant patterns in native limbs are based on fusions between laterally adjacent elements (i.e., in the anteroposterior axis). Variant patterns in the mesopodia of regenerated limbs usually exhibit fusions among proximodistally adjacent elements. Analysis of regenerates derived from limb amputation at different levels shows that the axis of fusion between regenerated mesopodial elements remains the same (i.e., proximodistal) independent of amputation level. However, the frequency of specific fusion combinations is unexpectedly sensitive to amputation level. *Proximal* (stylopodial) amputation results in mesopodial patterns with predominantly *preaxial* fusion combinations; *distal* amputation produces mesopodial patterns with predominantly *postaxial* fusion combinations. This finding is discussed in the context of other recent studies in which amputation level influenced limb regeneration patterning.

The generation of complex form during development is one of the most engaging and yet, historically, one of the most intractable of biological problems. An important model in the study of developmental patterning has been and continues to be the vertebrate limb, the ontogeny of which is documented abundantly in a wide variety of tetrapods (e.g., mammals, Milaire, '62, '65; birds, Amprino, '65; Zwilling, '72; amphibians, Stocum and Fallon, '82). However, the adult urodele limb is unique among adult tetrapod limbs, because it can recapitulate the developmental patterning process and regenerate a virtually identical replacement subsequent to limb amputation.

Empirical and theoretical studies support the hypothesis that limb development and limb regeneration are mediated by the same mechanisms (reviews by Faber, '71, '76; Stocum, '75; Amprino, '84). For example, the apical ectodermal ridge of the chick limb bud (Saunders, '48; Summerbell, '74) has a striking structural and functional analog in the apical epidermal cap of the blastema on a regenerating limb (Thornton, '60). In addition, positional dislocation of either the apex of the limb bud on its base (Maden and Goodwin, '80) or the regeneration blastema on a limb stump (Tank, '78) results in the production of supernumerary limb structures in specific locations and orientations. Most important, positional dislocation of reciprocal grafts, those between developing limb buds and regeneration blastemas in axolotl larvae, produce the same kind of supernumerary limb structures (Muneoka and Bryant, '82). Nevertheless, the actual skeletal patterns obtained from development and regeneration in the same species (or even in the same individual) may differ. We recently described an unusual instance of frequent and extensive limb skeletal variability in natural populations of the red-backed salamander, *Plethodon cinereus* (Hanken, '83; Hanken and Dinsmore, '86). Because limb regeneration researchers often find that regenerate skeletal patterns may differ slightly from the original, native pattern (e.g., Dearlove and Dresden, '76), we tested the hypothesis that skeletal variation in the natural populations was the result of regenerative events (Dinsmore and Hanken, '86). That study revealed that regenerates, produced from zeugopodial limb amputation, frequently show fusion among proximodistally adjacent mesopodial (carpal or tarsal) elements, whereas those variants found in field-collected animals and hereafter referred to as "native variants" involve fusions of laterally adjacent (anteroposterior axis) elements. Thus developmental and regenerative patterning may be regulated by fundamentally different mechanisms. Alternatively, the mechanisms may be the same but the prevailing initial epigenetic conditions (e.g., bud size vs. blastema size, developmental field volume, amputation level) may lead to divergence in patterns.

The latter hypothesis can be tested by altering the initial conditions under which regeneration is stimulated. Of the possible experimental manipulations that might influence regenerate skeletal patterning, this study explores the effect of amputation level on mesopodial patterning. Our earlier study concerned results of distal, midzeugopod amputation exclusively (Dinsmore and Hanken, '86). However, a proximal limb amputation may create a better approximation of the morphogenetic context that exists during limb development.

To test this premise, we used the red-backed salamander, a species that often has exceptional limb-skeletal variability in nature (Hanken, '83; Hanken and Dinsmore, '86) and that shows marked differences between native and regenerate mesopodial patterning as described above. Regenerate skeletal patterns produced following either proximal or distal limb amputation were compared with native skeletal patterns, both typical and variant, as well as with each other. Regenerate mesopodial patterns remained distinctly different from native variant patterns, regardless of the amputation level. However, the level of amputation was found to have a significant effect on the incidence of specific patterns of skeletal fusion and thus on mesopodial patterns of the regenerates.

MATERIALS AND METHODS

Adult red-backed salamanders, *Plethodon cinereus* (Plethodontidae), were collected in Colchester County, Nova Scotia, Canada, from populations that have a high incidence of native mesopodial pattern variability (Hanken, '83; Hanken and Dinsmore, '86). In the laboratory, the animals were maintained on moist paper towels in stacked fingerbowls and fed fruit flies from laboratory cultures. Prior to limb amputation, animals were anesthetized by immersion in 0.1% aqueous benzocaine. Subsequent limb amputations were performed on the stage of a dissecting microscope.

A total of 176 forelimbs and 174 hindlimbs were amputated; distal segments were preserved for analysis of native patterning. Most of the forelimbs ($N = 132$) were amputated through midradius and ulna (zeugopod), whereas the remainder ($N = 44$) were amputated at midhumerus (stylopod). Similarly, the hind limbs were amputated at either midzeugopod ($N = 132$) or midstylopod ($N = 42$). Immediately following amputation, animals were placed in petri dishes containing Holtfreter's solution, where recovery from anesthesia was rapid. They were then returned to their labeled fingerbowls for the 2–3 month period of limb regeneration and skeletal maturation. Thereafter animals were again anesthetized and placed on the stage of a dissecting microscope, where the limbs were exarticulated at the shoulder or hip joint.

All limb tissues, both native distal segments from the initial amputation and the resultant limb regenerates, were fixed in 10% neutral-buffered formalin and differentially stained for cartilage and bone as whole mounts using an Alcian blue/alizarin red technique described by Hanken and Wassersug ('81). Analysis of mesopodial fusion patterns is based on the native carpal and tarsal patterns described by Hanken ('83) and Hanken and Dinsmore ('86) and the regenerate carpal and tarsal patterns described by Dinsmore and Hanken ('86). These earlier studies found no correlation between the native mesopodial pattern, whether normal or variant, and the subsequent regenerate mesopodial pattern. Data on regenerates in the present study are therefore pooled according to amputation level and whether forelimb or hindlimb.

Data were analyzed using contingency tables correlating amputation level and the frequency of specific mesopodial patterns. The χ^2 test was used to determine statistical significance of any amputation level-dependent differences in the incidence of specific mesopodial patterns.

TERMINOLOGY

We use the term mesopodial *pattern* in reference to the overall array of mesopodial elements in any given limb. The term *fusion combination* refers to specific fusions of adjacent mesopodial elements (from a developmental perspective, "absence of fission within the precartilaginous primordia" is a more accurate but cumbersome term). Thus each limb expresses a particular *pattern*, which may contain one or more *fusion combinations*.

Mesopodial elements are abbreviated as follows: forelimb, 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; hind limb, 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. Fusion between mesopodial elements is indicated with a hypen; for example, fused centrale and intermedium, is noted "c-i."

RESULTS

Qualitatively, a vast majority of regenerate mesopodial patterns in both forelimbs and hindlimbs contain the types of fusion combinations described previously (Dinsmore and Hanken, '86). That is, fusions occur between proximodistally adjacent elements (e.g., c-i and r-cl), a phenomenon now shown to be independent of amputation level. This finding confirms the striking difference between the proximodistal axis of mesopodial fusions (and their derived patterns) in regenerated limbs and the anteroposterior axis of mesopodial fusions in the variant patterns that occur at low frequency in native limbs. Nevertheless, amputation level has a significant effect on the distribution of specific fusion combinations in regenerated limbs: *proximal* (stylopodial) amputation results in regenerate mesopodial patterns with predominantly *preaxial* fusion combinations; *distal* amputation produces regenerate mesopodial patterns with predominantly *postaxial* fusion combinations (Figs. 2, 4). This is a general finding for all the limbs in *P. cinereus*. However, since forelimbs and hindlimbs are not identical in either the native or regenerate morphologies, data for these two categories are presented separately.

Forelimb regenerates: Carpus

Four regeneration-specific fusion patterns are produced following both proximal (stylopodial) and distal (zeugopodial) amputations (r-cl, c-i, d3-d4; r-cl, d3-d4; c-i; and c-i, d3-d4; see Table 1, Fig. 1). Of the four patterns produced only by distal amputation (c-i, d4-u; dl + 2-c, d3-d4; d3-d4 only; and d4-u only), three occur at frequencies less than 5%; their absence from the much smaller sample of regenerates produced by proximal amputation may thus be a statistical artifact. Moreover, at both amputation levels, normal patterns (i.e., no fusions) were produced at the same low frequency of 13–16%; fidelity of regenerate to native patterning is poor regardless of amputation level. Similarly, four of the five most common individual fusion combinations are found in regenerates from both proximal and distal limb amputations (Table 2), and they produce essentially the same average number of fusions per limb—1.73 vs. 1.70, respectively.

Amputation level, however, clearly has an effect on the frequency of certain fusion combinations and overall carpal patterns. Thus fusions between preaxial elements r and cl predominate in regenerates from proximal amputations, whereas fusions between the postaxial elements d3 and d4 and between d4 and u are more common in regenerates from distal amputations ($P \leq 0.005$) (Table 2, Fig. 2). Fusion between central elements (c-i) is common at both amputation levels, but the difference in frequency is not statistically significant. As a consequence of the amputation level-dependent effect on the frequency of specific carpal fusion combinations, r-cl-based carpal patterns predominate in regenerates from proximal amputations, whereas d3-d4-based patterns predominate in regenerates from distal amputation (Table 1, Fig. 1).

Hindlimb regenerates: Tarsus

Results from hindlimb regenerates generally are the same as those from the forelimb. Amputation level has little if any effect on the overall array of fusion combinations or patterns regenerated or on the fidelity of regenerate patterning; normal patterns (i.e., no fusions) are produced in only approximately one-third of the regenerates at each level (Tables 3, 4). Similarly, the mean number of fusions per regenerated limb is again very close, 1.00 following proximal amputation vs. 1.05 following distal amputation. As in forelimbs, however, amputation level affects the frequency of specific fusion combinations and overall mesopodial patterns, although numerical differences between levels are not as great as in the forelimb (Tables 3, 4, Figs. 3, 4). Regenerates from proximal amputations are biased ($P \leq 0.005$) towards preaxial fusions (t-cl), whereas postaxial fusions (d3-d4) predominate ($P \leq 0.05$) in regenerates from distal limb amputations. Central (c-i) fusions are, once again, seen in regenerates from both levels, but the higher incidence following distal amputation is statistically significant ($P \leq 0.05$).

Variant patterns, i.e., those with fusions, are less frequent in the hindlimb than in the forelimb, as is the mean number of fusions per regenerated limb (see above). This parallels the earlier

TABLE 1. Incidence of specific carpal patterns in forelimb regenerates relative to amputation level[1]

	Amputation level	
Pattern[2]	Proximal (= stylopodium) (N = 44)	Distal (= zeugopodium) (N = 132)
No fusions	7 (15.9)	17 (12.9)
r-cl only	5 (11.4)	0 (0)
r-cl, c-i	10 (22.7)	0 (0)
r-cl, c-i, d3-d4	10 (22.7)	21 (15.9)
r-cl, d3-d4	3 (6.8)	4 (3)
c-i only	5 (11.4)	3 (2.3)
c-i, d3-d4	1 (2.3)	32 (24.2)
c-i, d4-u	0 (0)	6 (4.5)
(dl + 2)c, d3-d4	0 (0)	6 (4.5)
d3-d4 only	0 (0)	21 (15.9)
d4-u only	0 (0)	5 (3.8)

[1] Frequencies in parentheses denote number observed divided by sample size, expressed as a percentage.

[2] Additional patterns, which occurred only once or twice each, are not included. They represent only 20 limbs of the total of 176 examined.

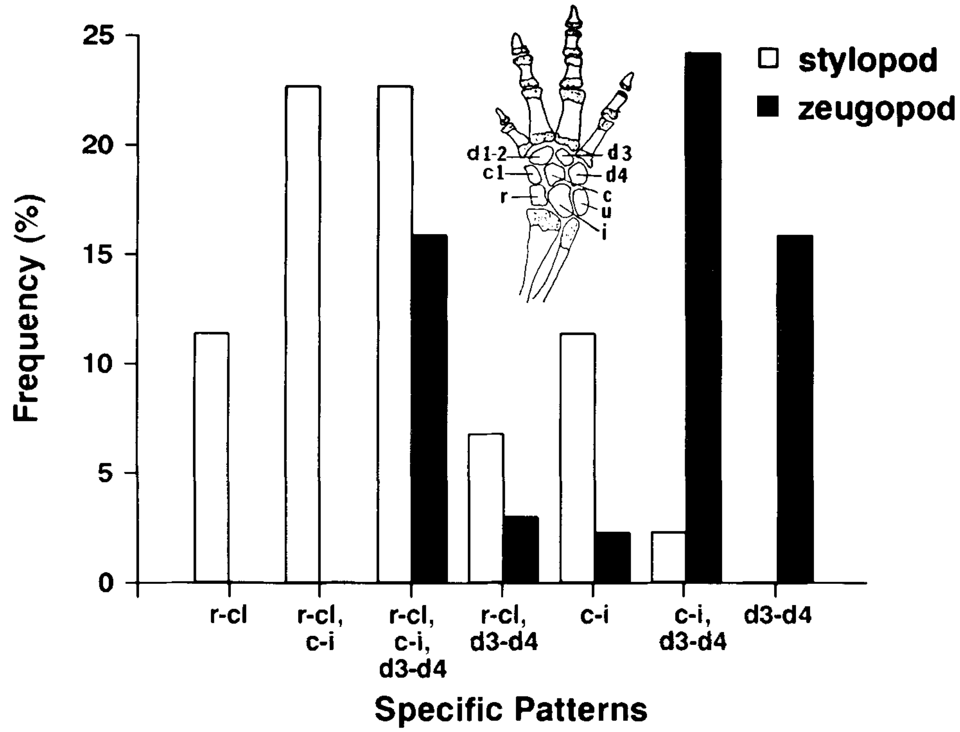

Fig. 1. Frequency of specific carpal patterns in regenerates produced following proximal (stylopod) amputation (open bars) vs. distal (zeugopod) amputation (solid bars). Characteristic fusion combinations of each pattern are indicated.

observation that native forelimbs have a higher incidence of variant patterns than native hindlimbs (Hanken and Dinsmore, '86).

DISCUSSION

Amputation level-independent mesopodial fusions

In *Plethodon cinereus,* native variant patterns of mesopodial elements represent viable, yet limited, alternatives to the typical ontogenetically produced skeletal pattern (Hanken, '83; Dinsmore and Hanken, '86). Together with the typical morphs, they may denote the limits of a bounded domain defined by local developmental constraints (Maynard Smith et al., '85). Limb amputation, however, initiates a secondary unfolding of morphogenetic tissue interactions that produce a new limb. As outlined in the Introduction, there are many similarities between the developing limb bud and the regenerating limb blastema, yet there are fundamental differences with respect to the resulting skeletal pattern. These differences involve the major axis of interelement mesopodial fusion. The axis of mesopodial fusion in the vast majority of native variant limbs is the anteroposterior axis in both carpus and tarsus. Interelement fusions in regenerates, however, almost invariably involve proximodistally adjacent mesopodial elements (Dinsmore and Hanken, '86) (Tables 1, 2). Moreover, as this study has shown, these differences are indepen-

TABLE 2. Incidence of individual carpal fusion combinations in forelimb regenerates relative to amputation level[1]

	Amputation level	
Combination[2]	Proximal (=stylopodium) (N = 44)	Distal (=zeugopodium) (N = 132)
(dl + 2)-c	0 (0)	10 (7.6)
r-cl	30 (68.2)	34 (25.8)
c-i	29 (65.9)	70 (53.0)
d3-d4	14 (31.8)	89 (67.4)
d4-u	2 (4.5)	13 (9.8)
(dl + 2)-d3-d4	1 (2.3)	1 (0.8)

[1] Frequencies in parentheses denote number observed divided by sample size, expressed as a percentage. A given limb may contain more than one combination.

[2] Five additional combinations, which occurred only once or twice following zeugopodial amputation, have not been included. They represent only seven instances of carpal fusion of the total of 224.

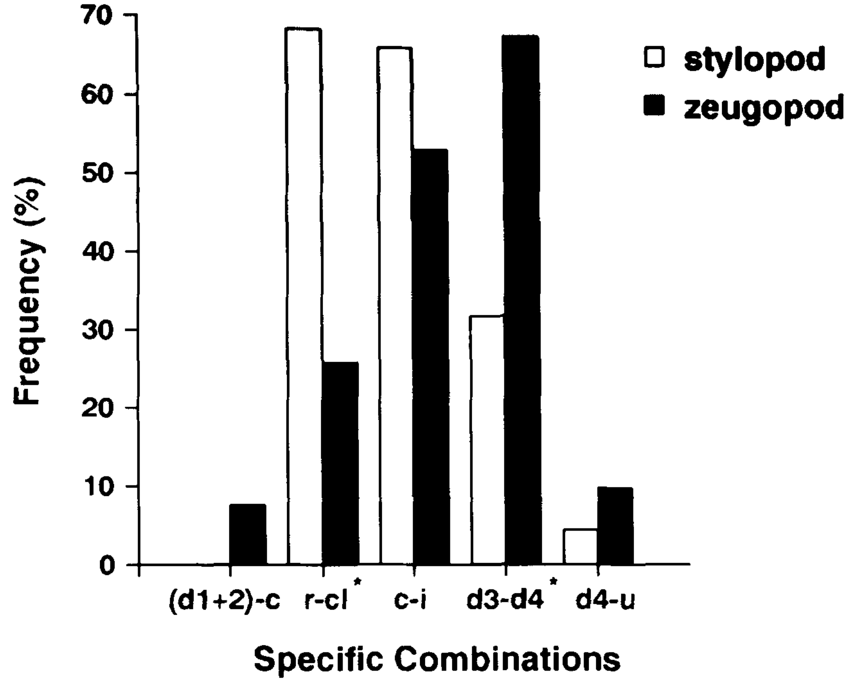

Fig. 2. Frequency of specific carpal fusion combinations in regenerates produced following proximal (stylopod) amputation (open bars) vs. distal (zeugopod) amputation (solid bars). Asterisks denote fusion combinations for which amputation level made a significant difference ($P \leq 0.05$) in their occurrence.

dent of the amputation level. Experiments designed to elucidate the basis of these differences between developing and regenerating limbs must take into account, among other alternatives, the possibilities that they lie either in the nature of certain physical parameters (e.g., size-dependent mechanical pressures) or in the differential responsiveness of embryonic tissues relative to stump-derived blastema cells.

Differential effects of amputation level

The concept of positional information (Wolpert, '69, '71) may provide a mechanistic explanation for the differential effects of amputation

TABLE 3. Incidence of specific tarsal patterns in hindlimb regenerates relative to amputation level[1]

	Amputation level	
Pattern[2]	Proximal (=stylopodium) (N = 42)	Distal (=zeugopodium) (N = 132)
No fusions	15 (35.7)	44 (33.3)
t-cl only	8 (19.0)	6 (4.5)
t-cl, c-i	4 (9.5)	7 (5.3)
t-cl, c-i, d3-d4	2 (4.8)	9 (6.8)
t-cl, d3-d4	3 (7.1)	4 (3.0)
c-i only	0 (0)	15 (11.4)
c-i, d3-d4	1 (2.4)	9 (6.8)
c-i, d4-d5	0 (0)	3 (2.3)
d3-d4 only	2 (4.8)	24 (18.2)
d4-d5 only	2 (4.8)	5 (3.8)

[1] Frequencies in parentheses denote number observed divided by sample size, expressed as a percentage.
[2] Additional patterns, which occurred only once or twice each, are not included. They represent only 11 hindlimbs of the total of 174 examined.

TABLE 4. Incidence of individual tarsal fusion combinations in hindlimb regenerates relative to amputation level[1]

	Amputation level	
Combination[2]	Proximal (=stylopodium) (N = 42)	Distal (=zeugopodium) (N = 132)
(dl + 2)d3	2 (4.7)	0 (0.0)
t-cl	20 (47.6)	29 (22.0)
c-i	8 (19.0)	48 (36.4)
d3-d4	8 (19.0)	47 (35.6)
d4-d5	3 (7.1)	9 (6.8)
d3-d4-d5	0 (0)	5 (3.8)

[1] Frequencies in parentheses denote number observed divided by sample size, expressed as a percentage. A given limb may contain more than one combination.
[2] One additional combination that occurred only once following stylopodial amputation is not listed.

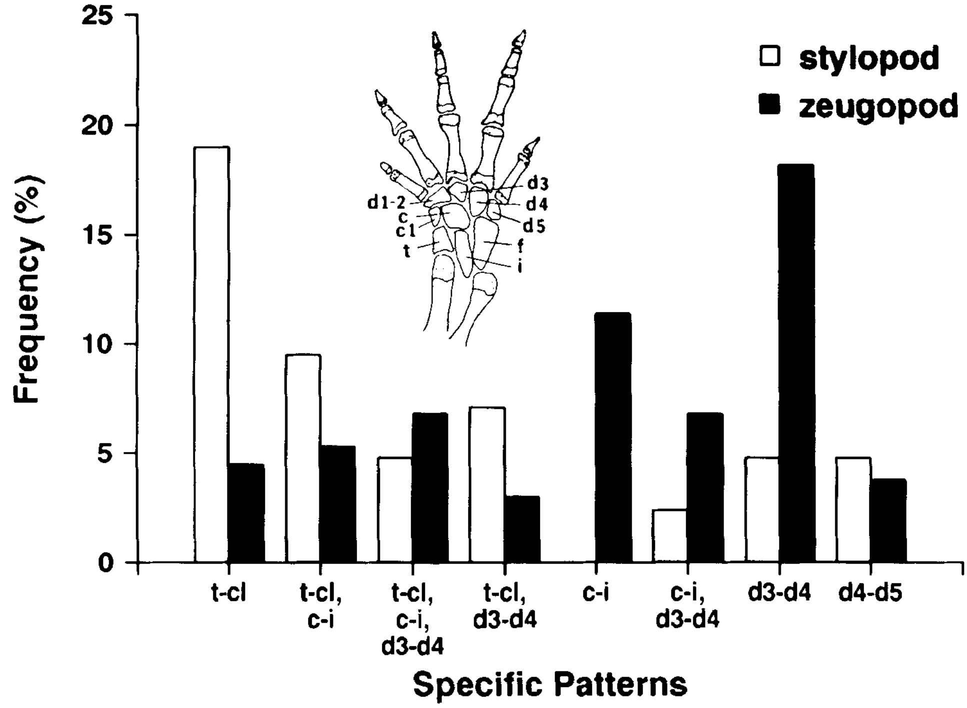

Fig. 3. Frequency of specific tarsal patterns in regenerates produced following proximal (stylopod) amputation (open bars) vs. distal (zeugopod) amputation (solid bars). Characteristic fusion combinations of each pattern are indicated.

level on regenerate mesopodial patterning. There is some evidence that positional values, associated with the idea of positional information, are distributed differentially along and around the limb's primary axes. In this context, our data agree with those of other studies that reveal regenerate patterning differences following proximal vs distal levels of amputation. Significantly, the other studies include experimental protocols that are distinctly different from ours, e.g., surgical and biochemical interventions.

Half limbs

Several earlier studies using surgically constructed half and double-half (symmetrical) limbs provide evidence for an asymmetrical circumferential as well as proximodistal distribution of positional values regulating regenerate morphogenesis. For example, surgically created half limbs are able to regenerate distal structures (Goss, '57; Wigmore and Holder, '85). However, the regulative ability of these half limbs, that is, their capacity to regenerate the complete distal pattern, depends not only on the particular half that remains intact (anterior, posterior, dorsal, or ventral) but also on the level of amputation. A proximal amputation level typically results in regenerates with a higher average number of digits (Wigmore and Holder, '85) than those produced by distal amputation (Wigmore, '86). These studies are also consistent with investigations of regenerate pattern regulation in variously constructed double-half (symmetrical) limbs (Stocum, '78; Tank and Holder, '78; Holder et al., '80). In addition, they support Maden's ('79) suggestion that positional values are clustered posterodorsally in the upper limb, yielding a greater regulative capacity at that level, but are more uniformly distributed in the lower, zeugopodial level, where mosaic behavior is noted.

It therefore appears that the skewed distribution of positional values in the limb affects not only the number and type of digits in the regenerate, and the muscle distribution in the limb (Wigmore and Holder, '86), but also specific aspects of mesopodial patterning. How amputation level-dependent differences in positional information influence the frequency of preaxial vs. postaxial mesopodial fusions, however, is not yet known.

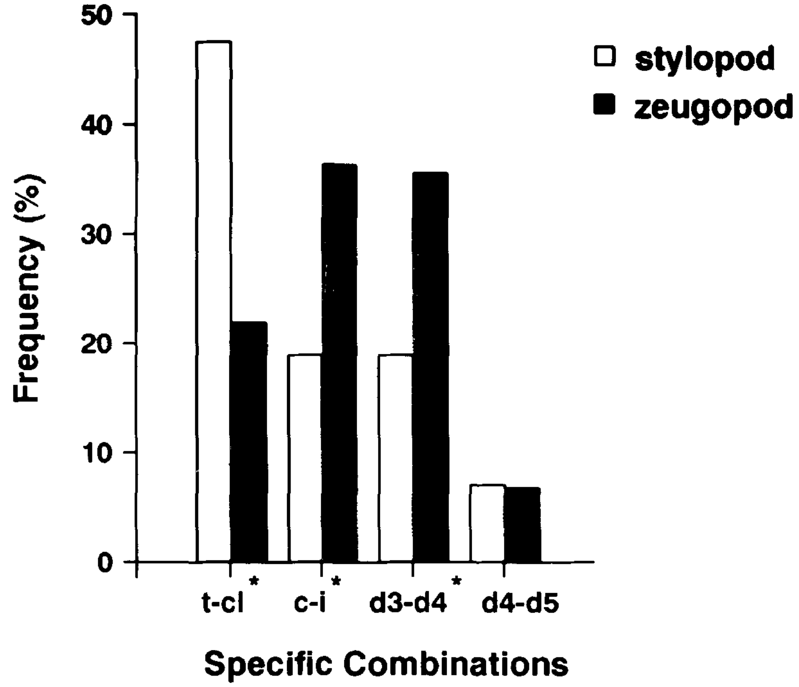

Fig. 4. Frequency of specific tarsal fusion combinations in regenerates produced following proximal (stylopod) amputation (open bars) vs. distal (zeugopod) amputation (solid bars). Asterisks denote fusion combinations for which amputation level made a significant difference ($P \leq 0.05$) in their occurrence.

Retinoids

The second example in which amputation level-dependent differences have been described concerns the effect of retinoids. The principal morphogenetic effect of retinoids on limb regenerate patterning is serial duplication of structures, particularly skeletal elements, in the proximodistal axis (Niazi and Saxena, '78; Maden, '82, '83; Thoms and Stocum, '84). More recent studies have shown that the positional information for the anteroposterior axis also is modified (Kim and Stocum, '86a), as is typical in chick limb buds (Tickle et al., '82; Summerbell, '83), and that the effects of retinoids vary according to amputation level (Kim and Stocum, '86b). Low doses of retinoids induce duplication of the zeugopodia from distal, zeugopodial amputation, whereas higher doses are required to produce equivalent duplication from proximal, stylopodial amputation. The nature of the mechanism underlying the retinoid-induced morphogenetic changes remains to be elucidated. However, a recent finding that the expression of a homeobox gene transcript in regenerating newt limbs varies according to amputation level (Savard et al., '88) suggests a molecular mechanism.

Summarizing our study, mesopodia regenerating after proximal or stylopodial amputation have predominantly preaxial fusions, whereas distal, zeugopodial amputations produce regenerates with a higher incidence of postaxial fusions. This amputation level-dependent, preaxial-to-postaxial shift in fusion sites is particularly striking in the forelimb. In both groups of regenerates, however, fusions are between proximodistally adjacent mesopodial elements unlike those characteristic of native limbs. This patterning difference, which is dependent on amputation level, may represent another consequence of the unequal proximodistal distribution of circumferential positional information in urodele limbs. Amputation level-dependent morphogenetic differences in limb regenerates illustrate real differences whose underlying mechanistic cause remains unknown.

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LITERATURE CITED

Amprino, R. (1965) Aspects of limb morphogenesis in the chicken. In R.L. Dehaan and H. Ursprung (eds.): Organogenesis. New York: Holt, Rinehart and Winston, pp. 255–281.

Amprino, R. (1984) The development of the vertebrate limb. Clin. Orthop. Rel. Res. *188:*263–284.

Dearlove, G.E., and M.H. Dresden (1976) Regenerative abnormalities in *Notophthalmus viridescens* induced by repeated amputation. J. Exp. Zool. *196:*251-262.

Dinsmore, C.E., and J. Hanken (1986) Native variant limb skeletal patterns in the red-backed salamander, *Plethodon cinereus,* are not regenerated. J. Morphol. *190:*191–200.

Faber, J. (1971) Vertebrate limb ontogeny and limb regeneration: Morphogenetic parallels. In M. Abercrombie, J. Brachet, and T.J. King (eds.): Advances in Morphogenesis, Vol. 9. New York: Academic Press, pp. 127–147.

Faber, J. (1976) Positional information in the amphibian limb. Acta Biotheor. *25:*44–65.

Goss, R.J. (1957) The relation of skin to defect regulation in regenerating half limbs. J. Morphol. *100:*547–564.

Hanken, J. (1983) High incidence of limb skeletal variants in a peripheral population of the red-backed salamander, *Plethodon cinereus* (Amphibia: Plethodontidae), from Nova Scotia. Can. J. Zool. *61:*1925–1931.

Hanken, J., and C.E. Dinsmore (1986) Geographic variation in the limb skeleton of the red-backed salamander, *Plethodon cinereus.* J. Herpetol. *20:*97–101.

Hanken, J., and R.J. Wassersug (1981) The visible skeleton. Funct. Photog. *16:*22–24, 44.

Holder, N., P.W. Tank, and S.V. Bryant (1980) Regeneration of symmetrical forelimbs in the axolotl, *Ambystoma mexicanum.* Dev. Biol. *74:*302–314.

Kim, W.S., and D.L. Stocum (1986a) Retinoic acid modifies positional memory in the anteroposterior axis of regenerating axolotl limbs. Dev. Biol. *114:*170–179.

Kim, W.S., and D.L. Stocum (1986b) Effects of retinoids on regenerating limbs: comparison of retinoic acid and arotinoid at different amputation levels. Roux Arch. Dev. Biol. *195:*455–463.

Maden, M. (1979) Regulation and limb regeneration: The effect of partial irradiation. J. Embryol. Exp. Morphol. *52:*183–192.

Maden, M. (1982) Vitamin A and pattern formation in the regenerating limb. Nature *295:*672–675.

Maden, M. (1983) The effect of vitamin A on the regenerating axolotl limb. J. Embryol. Exp. Morphol. *77:*273–295.

Maden, M., and B.C. Goodwin (1980) Experiments on developing limb buds of the axolotl, *Ambystoma mexicanum.* J. Embryol. Exp. Morphol. *57:*177–187.

Maynard Smith, J., R. Burian, S. Kauffman, P. Alberch, J. Campbell, B. Goodwin, R. Lande, D. Raup, and L. Wolpert (1985) Developmental constraints and evolution. Q. Rev. Biol. *60:*265–287.

Milaire, J. (1962) Histochemical aspects of limb morphogenesis in vertebrates. In M. Abercrombie and J. Brachet (eds.): Advances in Morphogenesis. Vol. 2. New York: Academic Press, pp. 183–209.

Milaire, J. (1965) Aspects of limb morphogenesis in mammals. In R.L. Dehaan and H. Ursprung (eds.): Organogenesis. New York: Holt, Rinehart, and Winston, pp. 283–300.

Muneoka, K., and S.V. Bryant (1982) Evidence that patterning mechanisms in developing and regenerating limbs are the same. Nature *298:*369–371.

Niazi, I.A., and S. Saxena (1978) Abnormal hind limb regeneration in tadpoles of the toad, *Bufo andersoni,* exposed to excess vitamin A. Folia Biol. (Krakow) *26:*3–8.

Saunders, J.W., Jr. (1948) The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. J. Exp. Zool. *108:*363–403.

Savard, P., P.B. Gates and J.P. Brockes (1988) Position dependent expression of a homeobox gene transcript in relation to amphibian limb regeneration. EMBO J. *7:*4275–4282.

Stocum, D.L. (1975) Outgrowth and pattern formation during limb ontogeny and regeneration. Differentiation *3:*167–182.

Stocum, D.L. (1978) Regeneration of symmetrical hindlimbs in larval salamanders. Science *200:*790–793.

Stocum, D.L., and J.F. Fallon (1982) Control of pattern formation in urodele limb ontogeny: A review and a hypothesis. J. Embryol. Exp. Morphol. *69:*7–36.

Summerbell, D. (1974) Interaction between the proximodistal and anteroposterior coordinates of positional value during the specification of positional information in the early development of the chick limb-bud. J. Embryol. Exp. Morphol. *32:*227–237.

Summerbell, D. (1983) The effect of local application of retinoic acid to the anterior margin of the developing chick limb. J. Embryol. Exp. Morphol. *78:*269–289.

Tank, P.W. (1978) The occurrence of supernumerary limbs following blastema transplantation in the regenerating forelimb of the axolotl, *Ambystoma mexicanum.* Dev. Biol. *62:*143–161.

Tank, P.W., and N. Holder (1978) The effect of healing time on the proximodistal organization of double-half forelimb regenerates in the axolotl, *Ambystoma mexicanum.* Dev. Biol. *66:*72–85.

Thoms, S.D., and D.L. Stocum (1984) Retinoic acid-induced pattern duplication in regenerating urodele limbs. Dev. Biol. *103:*319–328.

Thornton, C.S. (1960) Influence of an eccentric epidermal cap on limb regeneration in *Amblystoma* larvae. Dev. Biol. *2:*551–569.

Tickle, C., B. Alberts, L. Wolpert, and J. Lee (1982) Local application of retinoic acid to the limb bond mimics the action of the polarizing region. Nature *296:*564–568.

Wigmore, P. (1986) Regeneration from half lower arms in the axolotl. J. Embryol. Exp. Morphol. *95:*247–260.

Wigmore, P., and N. Holder (1985) Regeneration from isolated half limbs in the upper arm of the axolotl. J. Embryol. Exp. Morphol. *89:*333–347.

Wigmore, P., and N. Holder (1986) The effect of replacing different regions of limb skin with head skin on regeneration in the axolotl. J. Embryol. Exp. Morphol. *98:*237–249.

Wolpert, L. (1969) Positional information and the spatial pattern of cellular differentiation. J. Theor. Biol. *25:*1–47.

Wolpert, L. (1971) Positional information and pattern formation. Curr. Topics Dev. Biol. *6:*183–224.

Zwilling, E. (1972) Limb morphogenesis. Dev. Biol. *28:*12–17.