The structure of supernumerary limbs formed after 180° blastemal rotation in the newt Triturus cristatus

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SUMMARY

The structure of supernumerary limbs formed following 180° ipsilateral blastema rotations in the arm of the newt Triturus cristatus is analysed. Both the skeletal pattern and the muscle patterns are examined. As is the case after comparable experiments in the axolotl (see, for example, Maden & Mustafa, 1982) the extra limbs which form show a range of anatomies. Limbs symmetrical about the dorsal–ventral and anterior–posterior axis are reported as well as some limbs which were part symmetrical and part asymmetrical. It is clear that newts and axolotls appear to react in similar ways to this particular experimental procedure.

INTRODUCTION

Many surgical manipulations of developing and regenerating limb tissues result in the formation of novel limb patterns. The consistency, structure and predictability of the novel limb structures resulting from any particular surgical intervention is indicative of the rules underlying the cellular interactions creating the abnormal patterns. In recent years a considerable amount of attention has been focused on the supernumerary limb structures which emerge following transposition of limb regeneration blastemas in adult or larval urodele amphibians (see review by Tank & Holder, 1981). The main driving force behind many of these experiments was the predictions of the polar coordinate model, a theoretical framework put forward to attempt the unification of rules governing the recreation of spatial patterns in epimorphically regenerating structures (French, Bryant & Bryant, 1976; Bryant, French & Bryant, 1981).

Two experimental designs have been widely used in order to create specific types of axial misalignment between blastema and stump. The first of these, the contralateral transplantation of a blastema from a limb on one side of the body to a limb on the other allows the misalignment of only one transverse axis. Standardly the dorsal–ventral and anterior–posterior axes have been chosen for analysis. Following contralateral transplantation of the regenerating limb blastema the

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frequency of induction of extra limbs, their position of origin around the circumference of the graft–host junction, their handedness and their anatomy are remarkably consistent. For example, when the a–p axis is opposed the extra limbs grow out from the points of maximum disparity between graft and stump, have essentially normal anatomy (Maden, 1982), and have the same handedness as the stump (see, for example, Iten & Bryant, 1975; and Tank, 1978).

The consistent behaviour of contralateral transplants is readily explained by several models (see Tank & Holder, 1981), and therefore the detailed information which such grafts yield with regard to their formative patterning mechanisms is limited. In contrast, the extra limbs formed following ipsilateral rotation of a blastema upon the stump from which it originated are more varied in several ways. Blastemal rotation not only misaligns all axes of the blastema and stump, but also allows the degree of misalignment to be varied between 0° and 180° (Maden & Turner, 1978; Turner, 1981; Wallace & Watson, 1979; Stock, Krasner, Holder & Bryant, 1980).

The most striking recent observations with regard to the supernumerary limbs formed after blastemal rotation concerns the symmetry of limb tissues about the dorsal–ventral axis. The first observation of symmetrical double dorsal and double ventral supernumerary limbs was by Maden (1980, 1982), in the axolotl, using the muscle pattern as a marker. In addition to complete limbs showing dorsal or ventral symmetry, more complex structures which show partial symmetry and partial asymmetry and mixed handedness have been found (Maden, 1982; Maden & Mustafa, 1982; Tank, 1981). Mixed-handed limbs are those where part of the limb pattern is upside down with respect to the remaining limb pattern with reference to the dorsal–ventral axis. In these, and the other symmetrical cases, the anterior–posterior pattern is apparently normal. None of these complex limb anatomies have been observed following contralateral blastema grafting.

It is apparent from these initial reports that blastemal rotations yield far more diverse results than contralateral grafts. Furthermore, the only published data, which all pertain to the axolotl, show striking variations between laboratories from apparently very similar experiments. In his recent paper, Tank (1981) observed no completely symmetrical double dorsal or double ventral structures but instead saw either normal asymmetrical or mixed-handed patterns. Maden's results (Maden, 1980, 1982; Maden & Mustafa, 1982; Tank, 1981) on the other hand, show a similar frequency for all kinds of observed limb anatomy. It is our view that these different structures and results merely reflect the spectrum of anatomies that can be made by interactions between cells following blastemal rotation. It seems likely that differences in frequency of specific limb types occur for trivial reasons in terms of patterning mechanisms. It is possible therefore that these bizarre limb structures will prove informative with regard to understanding patterning mechanisms when sufficient numbers of supernumerary limbs formed from 180° rotations have been examined histologically and a statistical analysis
The frequency of formation of particular types of supernumerary limbs has been carried out. To this end we present in this paper the results of a series of 45 180° ipsilateral blastema rotations in which both the skeleton and muscle patterns have been analysed. The experiment was performed on the forelimbs of the newt *Triturus cristatus* and is therefore the first such analysis on any animal other than the axolotl. This is important because many previous studies of blastemal rotations in which only the skeletal pattern was analysed were performed on newts (see, for example, Iten & Bryant, 1975; Bryant & Iten, 1976; and Stock et al. 1980). Our results demonstrate that limbs symmetrical about the dorsal–ventral axis do occur in newts. In addition, the frequencies of formation of particular classes of limb anatomies differ from both the previous studies, although no new class of limb structure was seen.

**Materials and Methods**

All of the experiments were carried out on the forelimbs of the Italian crested newt *Triturus cristatus*, which were obtained from Gerrard Haig Ltd. Animals were maintained together in large tanks prior to experimentation. Following surgery animals were kept in separate plastic containers in tap water and were fed raw liver twice weekly. During any experimental procedure all animals were anaesthetised in MS222 (Sigma).

**Experimental design**

Both the right and left forelimbs of selected animals were amputated through the upper limb midway between the elbow and shoulder. The amputation surface was trimmed flat with scissors. Animals were then returned to their containers and were allowed to regenerate their limbs to the stage of palette or early digits (Iten & Bryant, 1973). At this stage appropriate animals were selected and the blastemas on both forelimbs were cut off at the initial level of amputation. Any protruding skeletal tissue of either blastema or stump was removed and the blastema was then replaced upon the stump rotated through 180°. Animals were returned to cold water and kept overnight in a 4°C refrigerator to facilitate healing while the animals were inactive. Animals were then returned to room temperature where they remained for the duration of the experiment. In some cases when the graft detached, the stump was allowed to regenerate again and the second blastema was rotated. This treatment had no effect on the regenerative ability of the rotated blastema because only one regenerate out of 45 successfully rotated limbs had any abnormality, and this limb had one missing digit.

**Control operations**

These involved the same procedure as above except that the blastemas were replaced upon the stump without rotation.
Analysis of results

The animals were examined carefully during the first week after surgery to ensure that any lost grafts were documented. Animals were then examined once a week thereafter. Approximately three months after the initial surgery the limbs were cut off at the shoulder and fixed in Bouin's fluid. The limbs were then decalcified in EDTA for about a week, dehydrated and stained with Victoria blue, and cleared in methyl salicylate to reveal the skeleton (Bryant & Iten, 1974). Once the skeletal pattern, position, and apparent handedness of the supernumerary limbs were ascertained, methyl salicylate was removed from the specimens with several changes of absolute alcohol and they were then processed for wax sectioning. Transverse sections, 10 μm thick, were cut on a rotary microtome, mounted and stained with haematoxylin and eosin.

In some cases the supernumerary limbs were removed from the host limb prior to wax embedding to make cutting more straightforward.

RESULTS

1. Derotation and the time of supernumerary limb formation

Supernumerary limbs appeared at widely different times following rotation. The first additional blastema appeared after 2 weeks and the last to form did so 8 weeks after rotation.

In other experiments (see Maden & Turner, 1978; Stock et al. 1980; Wallace & Watson, 1979; and Tank, 1981) the phenomenon of blastemal back rotation following rotation has been observed. In this study it was not possible to mark the blastema clearly with ink or some other suitable dye because the skin of Triturus is a dense black colour. The only way of assessing whether back rotation had occurred was retrospective. The form of the forearm bones at the elbow and the posture of the limb indicated whether it remained rotated through 180° or whether it had partly or fully returned to its normal position. Although this back rotation was noted for all the limbs its relevance to the outcome of the experiment is unclear because the supernumerary limbs took a long time to appear, and the exact time of back rotation for each limb could not be defined. In the cases where back rotation did occur therefore, the exact angle at which the supernumerary limb was induced was not known.

In this study, out of the 45 limbs which supported rotated blastemas, 26 appeared to be fully back rotated by the time the limbs were fixed, 10 appeared to be still rotated 180°, and 6 limbs had partly derotated. In three cases the degree of back rotation could not be determined. The important observation here is that no correlation was seen between the limbs which derotated partly or completely and the production of supernumerary limbs; for example, only 10 of the 26 completely derotated limbs produced no supernumerary outgrowths. Therefore,
although derotation limits the clarity of theoretical interpretation of these results, it does not prevent the induction of supernumerary limbs.

2. Frequency of supernumerary limb formation

Out of a total of 45 initially rotated limbs, 16 failed to produce extra structures (Table 1). The fraction of limbs producing extra structures was therefore 65%, which is higher than was reported following 180° rotations in the newt *Notophthalmus viridescens* (Stock et al. 1980), but lower than that seen in the axolotl, where frequencies as high as 95% occur (Turner, 1981).

3. Position of outgrowth of supernumerary limbs

The site of supernumerary limb outgrowth was judged retrospectively as the site of eventual position of the extra limb at the time of fixation. These positions are shown in Table 2. It can be seen from this table that supernumerary limbs appear almost all around the limb circumference.

A retrospective determination of position in supernumerary limb initiation has uncertainties. As was pointed out by Tank (1981), supernumerary limbs may change their circumferential position during growth and thus make their initial position of origin unclear. We accept this point and do not wish to put this data forward to contest the argument about the theoretical basis for unequal spacing of positional values around the limb circumference (see Maden & Turner, 1978; Wallace, 1978; Tank, 1981). Nonetheless we feel it unlikely that the initial sites of origin of the supernumerary limbs were solely at the anterior and posterior

### Table 1. Number of supernumerary limbs

<table>
<thead>
<tr>
<th>Total no. of expl. limbs.</th>
<th>No extra outgrowth</th>
<th>Single supers</th>
<th>Double supers (inc. 3 spikes)*</th>
<th>Structure of supernumeraries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spikes</td>
<td>Two digits</td>
</tr>
<tr>
<td>45</td>
<td>16</td>
<td>18</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

*A spike is a single-rayed structure which usually contains a series of jointed skeletal elements.

### Table 2. Final circumferential position of supernumerary limbs*

<table>
<thead>
<tr>
<th>A</th>
<th>AD</th>
<th>D</th>
<th>DP</th>
<th>P</th>
<th>PV</th>
<th>V</th>
<th>VA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

*The poles of the axes of the limb are Anterior (A), Posterior (P), Dorsal (D) and Ventral (V). Between each pole the limb was further subdivided. Each subdivision is marked by a combination of the extremes of the quadrant. For example, the quadrant from anterior to dorsal is bounded by designations A and D and includes the AD subdivision.
poles as Tank demonstrated in the axolotl (Tank, 1981). This is for two reasons. Firstly, the majority of extra limbs end up at the dorsal side of the limb (Table 2). Some of these dorsal or ventral supernumerary limbs appeared on limbs bearing non-derotated grafts. Secondly, in all of the cases where two clear supernumerary limbs with digits formed on a single host limb (eight cases – see Table 1), they were always fused. This means that the blastemal populations must have originated close together on the limb circumference. Such fused supernumerary limbs appeared in D, DP, AD, A and P regions of the circumference.

4. The skeletal structure of supernumerary limbs

In Victoria-blue whole mounts, the form of the supernumerary limbs varied greatly from single digit-like outgrowths, which were designated as spikes, to complete 4-digit limbs. Only a few supernumerary limbs were well separated from the host limb. By far the majority was fused at the radius and ulna level and showed various degrees of separation of the hands.

In eight cases the supernumerary limbs possessed greater than four digits. Six of these structures were duplicated across the anterior to posterior axis and
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possessed a clear line of symmetry. These were taken as being double supernumerary limbs. However, it was not possible to assess whether these symmetrical multiple structures were derived from a single blastema which diverged its anterior to posterior pattern during distal outgrowth or whether the structures formed as the result of fusion of two initially separate blastemas. One such fused symmetrical supernumerary limb is shown in Fig. 1. This 7-digit limb has a digital pattern of 4321234 and is separated from the main limb axis about halfway down the forearm region. Four of these double supernumerary limbs were of this type with seven digits fused around a central digit 1, one fused supernumerary outgrowth had eight digits with the digital formula 43211234, and one had six digits, some of which did not have a normal complement of phalanges. The remaining two supernumerary limbs had five digits which appeared to be in groups of two and three. Although these structures were fused proximally they appeared to be two separate incomplete patterns distally. No clear line of symmetry would be determined in these two cases.

Of the eighteen single supernumerary limbs, four were spikes, three had two digits, six had three digits, and five had four digits.

5. The muscle structure, symmetry about the dorsal ventral axis

A total of 33 supernumerary limbs were sectioned, all of which had two or more digits. In addition to these two normal limbs were sectioned so that the normal limb muscle pattern of Triturus could be ascertained. In all major respects the muscle pattern of the forelimb of Triturus is the same as that of the axolotl (see Fig. 2 and Maden, 1980, 1982). Briefly, the proximodistal level where the dorsal and ventral muscle patterns are most clearly distinguished is that of the metacarpals. Here the dorsal muscles are a set of four, the extensores breves digitorum (Fig. 2A–B), which arise from the carpals (Francis, 1934; Grim & Carlson, 1974). They appear as semilunar-shaped caps over each of the metacarpals and distally they taper into long tendons which extend to the phalanges. Ventrally the muscles are more complex. The pattern that is seen depends on the exact proximodistal level of the phalanges and metacarpals that is examined. The most conspicuous muscles are the flexores breves profundi, which flex the metacarpals; the contrahentes digitorum and the short triangular intermetacarpales which lie between the metacarpals on the ventral side of the palm (Fig. 2A–B).

Four different classes of symmetry were found in the 33 sectioned limbs (Table 3).

Table 3. Structure and handedness of supernumerary limbs

<table>
<thead>
<tr>
<th>Total supers (exc. spikes)</th>
<th>Normal</th>
<th>Partly symmetrical, partly normal</th>
<th>Unclear</th>
<th>Mixed handedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>
Fig. 2. The normal muscle pattern of the newt forelimb. (A). Camera-lucida drawing of a transverse section of a normal hand at the mid metacarpal level. The dorsal muscles (extensor breves digitorum, ebd) are only seen on the central, longest, metacarpals. The lateral digits are covered dorsally by the distal tendon (t) of the same muscles. Ventrally the extensive flexor breves profundi (fbp) are clearly seen as are the deeper intermetacarpales (imc). The small ventral contrahentes digitorum muscle is difficult to identify. ×32. (B). A photograph taken from a normal 4-digit asymmetrical supernumerary which had been serially transverse sectioned through the metacarpals. The clear dorsal and ventral asymmetry can be seen. The abbreviations are as for Fig. 2A. ×65.
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Fig. 3. A symmetrical double ventral 4-digit supernumerary limb shown in a low-power camera-lucida drawing (A) and a high-power photograph (B) of the central region of the same section drawn in A. Abbreviations as in Fig. 2. The drawing is at a magnification of ×32, the photograph at ×72.
Twelve cases were normal, showing dorsal–ventral asymmetry (Fig. 2A–B). Complete symmetry was seen in 12 cases, seven of which were double ventral and five of which were double dorsal (Fig. 3A–B). Three supernumerary limbs showed a combination of digits which were partly symmetrical and partly normal (Fig. 4A–B). No cases of mixed-handed limbs were seen. The anatomical structure of six cases remained unclear because they contained too little muscle to allow identification of dorsal and ventral symmetry.

6. Control transplants

A total of 12 control transplants, where the blastema was removed and replaced with no axial misalignment, were performed. In no case did any supernumerary outgrowth of any kind appear.

DISCUSSION

This study demonstrates that newts are capable of making supernumerary limbs which show a range of anatomical classes following blastemal rotation. These results are confirmatory in the sense that they are similar in principle to those demonstrated in the axolotl (Maden, 1980, 1982; Maden & Mustafa, 1982; Tank, 1981) but are novel in that they require a reassessment of all previous data.
relating to the generation in general and handedness in particular of supernumerary limbs following ipsilateral blastema rotation in the newt. This is a significant point because much of the crucial work which initiated the present interest in pattern regulation in amphibian limbs involved the newt Notophthalmus viridescens (see, for example, Bryant & Iten, 1976, 1977). In addition, results presented in this paper further demonstrate the variation in frequency of particular limb anatomy that is evident from recent results from experiments performed on the axolotl.
Maden (1982) and Maden & Mustafa (1982) have now clearly identified four major classes of limb anatomy which are produced following blastema rotation. These are normal asymmetrical limbs, symmetrical double ventral and double dorsal limbs, partly symmetrical and partly asymmetrical limbs and limbs of mixed handedness. A variation in frequency of each of these types is apparent from each of the three laboratories which have examined the problem. Maden & Mustafa (1982) have now examined the muscle and cartilage structure of about 100 supernumeraries and find each type of major class of anatomy in roughly equal frequency. Tank (1981), on the other hand, has demonstrated predominantly normal and mixed-handed limbs but found no symmetrical structures. Our study demonstrates a spectrum which is different again. Of the 33 sectioned limbs no limbs of mixed handedness occurred, rather the majority were normal or symmetrical, and some partly symmetrical partly asymmetrical limbs were seen (Table 3 and Figs 2–3). The reasons for these discrepancies in frequency are unclear. It is almost certainly the case that the differences between the results of Tank and Maden are due to trivial reasons in terms of patterning mechanisms, such as operative technique, because these authors were using the same species, the axolotl. Furthermore, Tank demonstrated no new type of limb anatomy. The difference in frequency of each type of anatomy in our study may also be due to trivial reasons, but other differences between newts and axolotls, such as the frequency of supernumerary limb induction after 180° rotation, have been noted in the past (cf. Turner, 1981; Stock et al. 1980).

There are other features of the supernumerary limbs induced in this study which deserve comment. The first and most striking of these is the production of six fused supernumerary structures bearing six to eight digits. The interactions leading to the formation of these structures is unclear. For example, it could not be ascertained whether they appeared as initially separate blastemas which later moved together, or whether the supernumerary had a single base which then diverged as it grew distally. In nearly all the cases the proximodistal level at which separation of the fused supernumerary occurred did not allow this point to be clarified by examining the skeletal anatomy of the finished product. However, the occurrence of these fused outgrowths does strongly suggest that the blastemas which made them appeared close together around the circumference. This observation, taken together with the variation in eventual position of the other supernumerary limbs, does suggest that there is no clearly localised position or positions around the circumferences where supernumerary limbs arise following 180° rotations. However, because of the problems of derotation, unexplained variation between laboratories, and growth distortions of supernumerary outgrowth during regeneration, any statements concerning the spacing of positional values around the limb circumference which are drawn from ipsilateral rotation experiments must be treated with caution. In addition, it must be remembered that an unequal spacing of values around the limb circumference
in the newt was initially put forward to explain the apparent handedness and location of origin of supernumerary limbs formed following ipsilateral blastema rotation (Bryant & Iten, 1976). It is now clear that the assessment of handedness was based on insufficient criteria because the muscle patterns of these supernumerary limbs were never analysed. Furthermore, the clustering of circumferential values onto the anterior half of the limb can be reversed and the same predictions for the location of initial supernumerary limbs found in Bryant & Iten's experiments will be achieved. In fact, a spacing which has greater than half of the circumferential values on the posterior limb region has been suggested following experiments concerned with regeneration of double anterior and double posterior upper arms in the newt (Wise, Fox, Holder & Bryant, 1983). In these experiments, as is the case in comparable experiments in the axolotl (Holder, Tank & Bryant, 1980; Tank & Holder, 1978), double posterior stumps regenerate more than double anterior stumps.

It is also interesting to note that in all of the reports where partly asymmetrical, partly symmetrical and mixed handed supernumerary limbs have been found (Maden, 1982; Maden & Mustafa, 1982; Tank, 1982; and see Table 3) the line of apparent anatomical discontinuity can occur at any position across the a-p axis. Thus in a 4-digit supernumerary, one digit may be symmetrical and three normal (Fig. 4), or vice versa (see also Maden & Mustafa, 1982). However, it does seem to be the case that only a single apparent line of discontinuity exists in any one limb with this type of hybrid structure.

The question remains, how are supernumerary limbs with such a range of anatomies created? The normal asymmetrical and totally symmetrical patterns do not contain discontinuities and can be explained in straightforward terms by, for example, the polar coordinate model. In this case shortest route intercalation between points of maximum disparity can lead to either an asymmetrical complete circle or two sets of the same newly intercalated circumferential values resulting in the formation of a symmetrical set of values. These two possible outcomes can occur because there are two shortest routes between points of maximum circumferential disparity. However, these principles cannot simply be applied to those limbs which bear clear anatomical discontinuities such as those with part symmetrical and part asymmetrical patterns and limbs of mixed handedness. Any model based purely on principles of continuity (Lewis, 1981) fail to explain the formation of these structures. Virtually all of the popular models which are being tested at the present time, whether they be based on long-range diffusion or short-range cell contact, predict the structural ironing out of such discrepancies (see Holder & Tank, 1981).

There seem to be two possible approaches to the problem. The first involves simply disregarding the theoretical frameworks based on principles of continuity. This radical step seems wholly unnecessary at the present time because we still remain very much in the dark about the cellular details of pattern regulation and the interactions which bring it about. The second approach is to attempt to
understand what the constraints on cell behaviour are during pattern regulation. Several approaches to this question have recently been suggested.

Maden & Mustafa (1982) have proposed that all of the classes of supernumerary limbs seen after ipsilateral rotation can be explained by the fusion of initially discrete supernumerary blastema populations during outgrowth. This attractive possibility is supported by the observation that all paired supernumerary outgrowths with normal anatomy have opposite handedness. This suggestion requires an analysis of cellular contribution to supernumerary outgrowth at the graft–host junction. However, blastemal fusion will only explain so much because it still leaves open the question of interaction, or the lack of it, when the initial populations of cells first fuse together.

This vexing question may be resolved in a different way. Although blastemal fusion may occur at the outset of supernumerary limb formation it is clear that limbs bearing anatomical discontinuities can regenerate following their amputation. This occurs following both the amputation of limbs derived from blastemal rotation (Maden, 1982; Maden & Mustafa, 1982), or the surgical construction of forearms which have mixed handedness (Holder, unpublished results). The answer to the problem may lie in the initial interactions of cells lying close to the amputation plane during the initial stages of regeneration. To date all of these limbs bearing discontinuities have been examined after amputations at or distal to the elbow region and have involved analysis of muscle patterns in the digits and forearm. We have proposed previously (Holder et al. 1980; Bryant, Holder & Tank, 1982; Holder, 1981; Holder & Reynolds, 1982) that the forearm blastema shows a strongly preferred healing mode because of its elliptical shape whereby cells from the dorsal and ventral sides of the blastema interact frequently whereas cells from the anterior and posterior blastema poles fail to interact. It is possible therefore that preferential healing and the restrictions of cell contacts which it imposes on blastema cells may allow discrete regions of blastema cells to interact as essentially autonomous populations. In this sense, therefore, whole regions of cells will interact and distally transform independently of the blastema population as a whole. Autonomous outgrowth of adjacent populations of cells within one blastema may result at the end of regeneration in one complete limb pattern, whereas, at least during the early stages of outgrowth, they were essentially separate. This kind of autonomous regeneration of a partial blastema population has already been identified in both axolotls and newts following the amputation of symmetrical limb stumps (Holder et al. 1980; Wise, Fox, Holder & Bryant, 1983). In these situations, half of the stump can regenerate when the grafted tissue fails to do so and either half limbs or half limbs showing distal expansion in the anterior–posterior axis result. If such autonomous regeneration of localised blastema populations occurs then it may explain how in the eventual regenerated limb, discrete, and apparently anatomically discontinuous limb regions can lie side by side.

In addition to these approaches is the problem of derotation and the time of
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initiation and population size of supernumerary limb blastemas. A further understanding of these phenomena coupled with an examination of blastemal fusion, cell contribution and healing restrictions should give a more complete understanding of the cellular interactions governing pattern regulation in urodele limbs. It may also allow us to stay faithful to models based on the principles of continuity.

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REFERENCES


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