Testing the clockface model of amphibian limb regeneration

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Summary. Contrary to the predictions of the clockface model, rotating a regenerate by 90° produces duplications identical to those resulting from 180° axial reversals. Exchanging regenerates between arms indicates the presence of 2 determined transverse axes.

French et al.[1] have devised a two-dimensional model involving polar co-ordinates of positional information to describe pattern regulation in epimorphic fields, including the field postulated for amphibian limb regeneration. They claim this 'clockface model' (figure 1) accounts for the number, position and orientation of supernumerary arms which arise during regeneration when the transverse axes of some limb tissue have been reversed. Transplanting a young regenerate from one arm to the other, so that either is anteroposterior (AP) or dorsoventral (DV) axis has a reversed polarity to that of the supporting arm, or replacing it after 180° rotation to reverse both axes (APDV) regularly elicits the formation of supernumerary arms or analogous duplications[2-5]. An axial reversal of arm skin or muscle prior to amputation yields identical duplications[6-8]. I have repeated the former type of experiment and also executed the corresponding 4 90°-rotations to effect a discrepancy rather than reversal between graft and host axes. The occurrence of duplications after the latter operations is so different from that predicted by the clockface model as to refute its validity.

The operations reported here were performed on palette stage regenerates produced by amputation through the mid-forearm of 60–65-mm-long larval iberian newts, *Pleurodeles waltl.* 10 reliable examples for each of the 8 rotational classes of graft were obtained, ignoring cases where the graft was lost or grossly resorbed. These arms were scored 6 weeks later as possessing either a simple hand with only 4 digits or a complex hand with 5–9 digits (figure 2). Complex hands which usually carried 2 or 3 digits maintaining the graft orientation flanked by several accessory digits conforming to the host arm orientation, correspond to earlier descriptions of fan-hands[2], expanded and double hands[4]. They are clearly expressions of duplication for merely operating higher up the arm converts complex hands into duplicate or triplicate complete hands or forearms.

The table shows that control grafts replaced without rotation formed 4-digit hands, possibly with a host contribution to some digits. The vast majority of grafts which were replaced after various rotations or transferred to the contralateral arm yielded complex hands. Detailed inspection reveals that rotated grafts often reverted to a more normal posture and usually formed defective hands (sometimes only a single digit) which were invariably supplemented by

Numbers of arms and digits

Graft rotation	Ipsilateral grafts				Contralateral grafts			
	0° Normal	90°	180° APDV	270°	0° AP	90°	180° DV	270°
Simple hands	10	3*	1*	2*	0	2*	0	0
Complex hands	0	7	9	8	10	8	10	10
Mean number of digits	4.0	5.1	6.1	4.8	6.7	5.9	6.3	6.2
With graft orientation	–	2.3	2.4	1.7	2.3	2.8	2.9	2.6
With host orientation	–	2.8	3.7	3.1	4.4	3.1	3.4	3.6

* Anatomically composite.

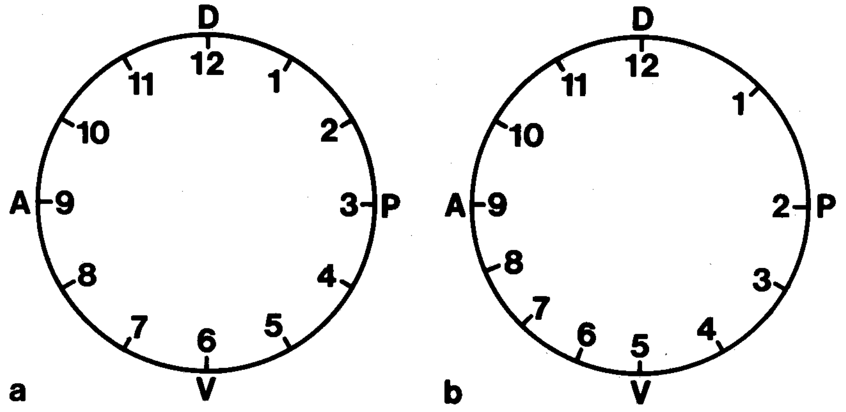

Fig. 1. Clockface model versions of this experiment. The circles represent an end-on view of a left limb-stump with AP and DV axes shown by letters. The numbers indicate assigned positional values, regularly spaced in the basic model *a* or displaced *b* to accommodate previous results[5]. The ipsilateral grafts reported here can be visualised as identical circles places over *a* or *b* and then rotated clockwise through successive 90°-intervals. Contralateral grafts would appear as a mirror image of itself superimposed on *a* or *b* and similarly rotated clockwise. In either case the graft orientation is palm down at 0°, forward at 90° up at 180° and back at 270° rotation. - The model stipulates that a discrepancy between superimposed numbers results in intercalation of the shortest sequence of intermediate numbers. Maximum discrepancies (equivalent to a complete axial reversal) allow 2 sequences of intercalation, e.g. 2, 3, 4, 5, 6 and 8, 9, 10, 11, 12 between position 1 and 7, and thus generate subsidiary complete circles. Only complete circles are capable of outgrowth: stump and graft combine as the main regenerate, duplicated by supernumerary or accessory structures from any subsidiary circle. Ipsilateral grafts should yield triplicate arms when rotated 180° according to model *b* but not at 90° or 270° when only minor discrepancies occur on either model or at the control 0°. Contralateral grafts should result in triplicate arms at any of the rotations tested.

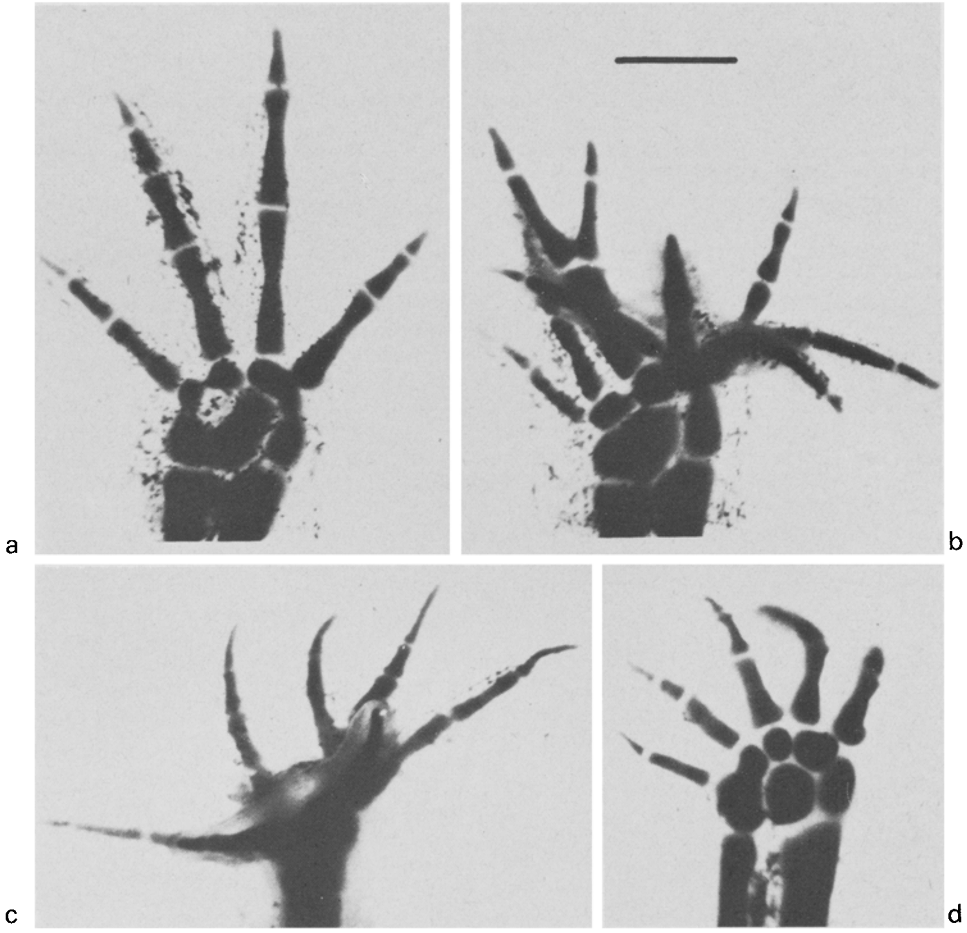

Fig. 2. Skeletal preparations of left hands after ipsilateral grafts rotated 0 ° (a), 180 ° (b), 90 ° (c), 270 ° (d). Note the flexed digit in *d* conforms to the graft orientation. Scale bar is 1 mm.

accessory digits, even where that only made up a total of 4. The table thus understates the yield of duplications, which appears to be 100% at all rotations tested.

Considering first the ipsilateral grafts, the basic clockface model (figure 1, a) specifically predicts that only a rotation of 180 ° should generate duplicate or triplicate structures. A revised model (figure 1, b) permits duplications after a wider range of rotations, estimated as 135–225 °. In practice 90 °, 180 ° and 270 ° rotations yield an identical spectrum of duplications at the same high frequency. Either the shortest intercalation rule or the complete circle rule of the model (see legend to figure 1) is thus shown to be false. It is impossible to locate the fault more precisely, because the only meaningful attribute of the shortest intercalation rule here is that of specifying complete circles. The model's complete circle rule is probably contravened by the outgrowth of 1 or 2 partial limbs represented by a variable number of accessory digits, and always lacked credibility because a supernumerary arm can grow from a small lateral wound in the host arm (examples, are cited by Lheureux[8]).

Eliminating the clockface model leaves a variety of more conventional ones which employ cartesian coordinates. The present results essentially contradict the classical view underlying most of these models that a complete reversal of some transverse axis is required to produce duplications, but substantiate the rule formulated by Abeloos and Lecamp[3]: duplications occur when graft and host axes do not coincide. Assuming duplications only occur after a substantial rotation approaching 20 ° (Maden[9]) the series of contralateral grafts may indicate the number and position of axes involved. Any single orthogonal or diagonal axis of the host arm should be reasonable well matched by that of the graft in one of the contralateral rotations reported here, for instance, and be revealed by a markedly reduced frequency of duplications. The table does not show the predicted effect and hence suggests the limb must contain at least 2 polarised transverse axes. These may well be the conventional AP and DV axes established early in development which seem to be permanently encoded in the limb's mesodermal cells[2,6]. I trust this analysis will clear the way for a unified theory of limb morphogenesis embracing both development and regeneration, if only as a means of restricting any further proliferation of models.

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