

The handedness and origin of supernumerary limb structures following 180° rotation of the chick wing bud on its stump

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SUMMARY

The pattern of differentiated wing structures formed following 180° rotation of the undifferentiated wing bud tip on its base was examined in detail. These analyses were performed to determine the handedness and origin of the supernumerary structures which arise. In contrast to the variable classes of symmetric and/or asymmetric limb anatomies observed following the same operation with amphibian regeneration blastemas, wings of predictable handedness were observed. Both the graft and stump contributed cells to the supernumerary structures. These results are discussed in the light of two current models describing the developing chick limb and analysed diagrammatically within the framework of one of these models, the polar coordinate model.

INTRODUCTION

It is well documented in the literature that supernumerary limbs and/or limb structures form after 180° rotation of an undifferentiated chick wing bud on its base; supernumerary limbs also form after 180° rotation of an amphibian regeneration blastema on its stump (see recent reviews of Javois, 1984; Muneoka & Bryant, 1984a). Only in the last few years have workers in the field begun to do detailed analyses of limb structures formed other than the easily visualized skeletal anatomy. These more detailed descriptions of resulting experimentally manipulated limbs have led to new insights and new questions regarding models for limb pattern formation.

180° rotation of an amphibian regeneration blastema on its stump opposes anterior/posterior and dorsal/ventral cells and gives rise to triplicated limb regenerates in a high percentage of cases (Bryant & Iten, 1976; Maden & Turner, 1978; Wallace & Watson, 1979; Stock, Krasner, Holder & Bryant, 1980; Turner, 1981). These investigators examined the skeletal pattern and the curvature of the digits in the supernumerary regenerates to determine the handedness of the

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supernumerary limbs. In limbs with supernumerary regenerates, the rotated blastema maintained its original (but upside-down) handedness, one supernumerary limb had the same handedness and orientation as the stump, and the other supernumerary was of opposite handedness and upside-down. Recent histological analyses of the muscle patterns of supernumerary limb regenerates, however, indicate that the handedness of the supernumerary regenerates is not as clear cut as was once thought (Maden, 1980, 1982; Maden & Mustafa, 1982). The work of Maden (1982) and Maden & Mustafa (1982, 1984) identified four classes of limb anatomy produced following 180° rotation of the axolotl blastema: normal symmetrical limbs; symmetrical double ventral or double dorsal limbs; partly symmetrical and partly asymmetrical limbs; and limbs of mixed handedness. Two additional studies, one using the axolotl (Tank, 1981) and the other using the newt (Papageorgiou & Holder, 1983) confirmed these findings, although the frequency of each type of limb anatomy varied depending on the study. The problems presented by these recent findings for models based on the principle of continuity are discussed by the authors of these works and will not be repeated here. However, the insights gleaned from these more detailed analyses of experimentally produced supernumerary regenerates now require investigators to examine more than the limb skeletal anatomy to determine a limb's asymmetry or symmetry. Also, these studies of limb regenerates suggest that limbs of variable symmetry or asymmetry may arise following 180° rotation of the developing chick wing bud on its base.

Early studies on the formation of supernumerary limb structures following 180° rotation of a right chick wing bud tip on its base indicate that when duplicate or triplicate hands result, there appears to be little ambiguity in the asymmetry (handedness) of the limb structures formed (Saunders, Gasseling & Gfeller, 1958). In wings with duplicate hands, the anterior hand is an upside-down right hand and the posterior is an upside-down left hand. Those wings with triplicate hands have an anterior right hand (right side up), a middle upside-down right hand, and a posterior upside-down left hand (Saunders & Gasseling, 1968). However, the determination of the handedness of these duplicate and triplicate hands was based only on the pattern of skeletal elements present and the overlying integumentary pattern.

The study reported here re-examines the formation of triplicate hands following 180° rotation of a right chick wing bud tip on its base. Particular emphasis is placed on the detailed analysis of the muscle/tendon patterns of resulting wings. Because of the characteristic dorsoventral and anteroposterior asymmetry of the muscle/tendon pattern surrounding the skeletal elements of the differentiated chick wing, we can unequivocally determine the handedness of resulting limb structures. This study shows that contrary to the variety of supernumerary limb anatomies resulting from 180° rotation of amphibian regeneration blastemas, the handedness of resulting chick limbs is quite straightforward. Additionally, the histological analysis of chimaeric chick/quail wings shows that graft and host cells contribute to the supernumerary hands formed. Possible reasons for the differences in the

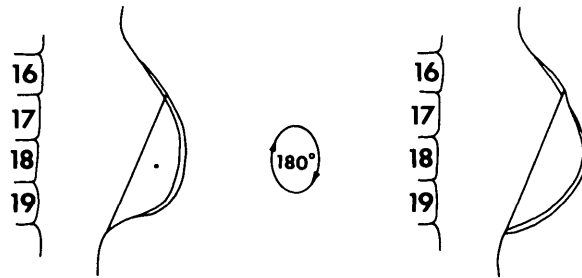


Fig. 1. A dorsal view diagram of a right stage-21 chick wing bud and adjacent somites (16–19) illustrating 180° rotation of the wing bud tip on its base. The wing bud is severed from the base by making a cut perpendicular to the future caudal direction of wing bud outgrowth. Following 180° rotation anterior/posterior and dorsal/ventral wing bud cells are opposed. (Note the dorsal carbon mark is no longer visible after rotation of the tip.)

handedness of supernumerary amphibian regenerates and developing chick wings will be discussed, as well as theoretical models that utilize supernumerary limb formation for their verification.

MATERIALS AND METHODS

Embryos used in these experiments were from a White Leghorn strain of chickens obtained from Commercial Chicks, Thorntown, Indiana, or from a flock of *Coturnix coturnix japonica* maintained at Purdue University, West Lafayette, Indiana. Eggs were incubated at 38°C , and at approximately $3\frac{1}{2}$ days incubation they were prepared for microsurgical manipulation. Albumen was withdrawn from chick eggs to lower the yolk and embryo before a window was sawn in the shell. Since quail embryos were used only for donor tissue, their eggs were not windowed in the same manner. Instead, quail embryos were exposed simply by opening the shell and shell membrane over the air cell. Extraembryonic membranes were opened using fine forceps, and tissue manipulations were performed on wing buds with sharpened tungsten needles. Stage-21 (length:width ratio of 2.3–2.9) right wing buds were severed from their bases with cuts made perpendicular to the future caudal direction of wing bud outgrowth (Fig. 1). Following 180° rotation about their proximodistal axes they were reattached to their stumps using tungsten microtacks. This operation has been demonstrated to result in a high frequency of limbs with ‘triplicated’ distal limb structures (Saunders *et al.* 1958; Iten, 1982). The same operation was also performed using donor quail wing buds to determine the contribution of stump and tip cells to the resulting structures. All surgical operations were recorded with camera-lucida drawings at the time they were performed as well as 1 and 2 days later. Tungsten microtacks were removed after approximately 2 h, at which time the eggs were sealed with Parafilm (American Can Co.) using a hot iron and returned to the incubator. The host embryos were sacrificed 7 days later. The chick and chimaeric chick/quail embryos were fixed and stained, and the wings were embedded and serially cross sectioned as described in Javois & Iten (1982). From the serial cross sections the muscle/tendon patterns, as well as the contribution of donor and host cells to the structures formed in the chimaeric wings, were recorded by making camera-lucida drawings of every 10th serial cross section. Longitudinal reconstructions of the wings were made from these drawings.

RESULTS

Severing a stage-21 wing bud tip from its base and rotating it 180° on its stump resulted in a wing bud with three distinct areas of outgrowth 1 to 2 days following

Table 1. *Limb structures formed after 180° rotation of a chick wing bud tip on its base*

Anterior-to-posterior sequence of skeletal elements						
Stylopodium		H*			H	
Zeugopodium		RUU†			RUUU‡	
Autopodium	2344334	234334	24334	34334	234334	?4334
Total number of wings	1	4	3	1	4	1

* Abbreviations for skeletal elements are: H, humerus; R, radius; U, ulna; 2, digit 2; 3, digit 3; 4, digit 4; ?, unidentifiable.

† Skeletal elements in italics were identified based on skeletal and integumentary patterns.

‡ Three of these forearms were split proximally into an anterior RU and posterior UU.

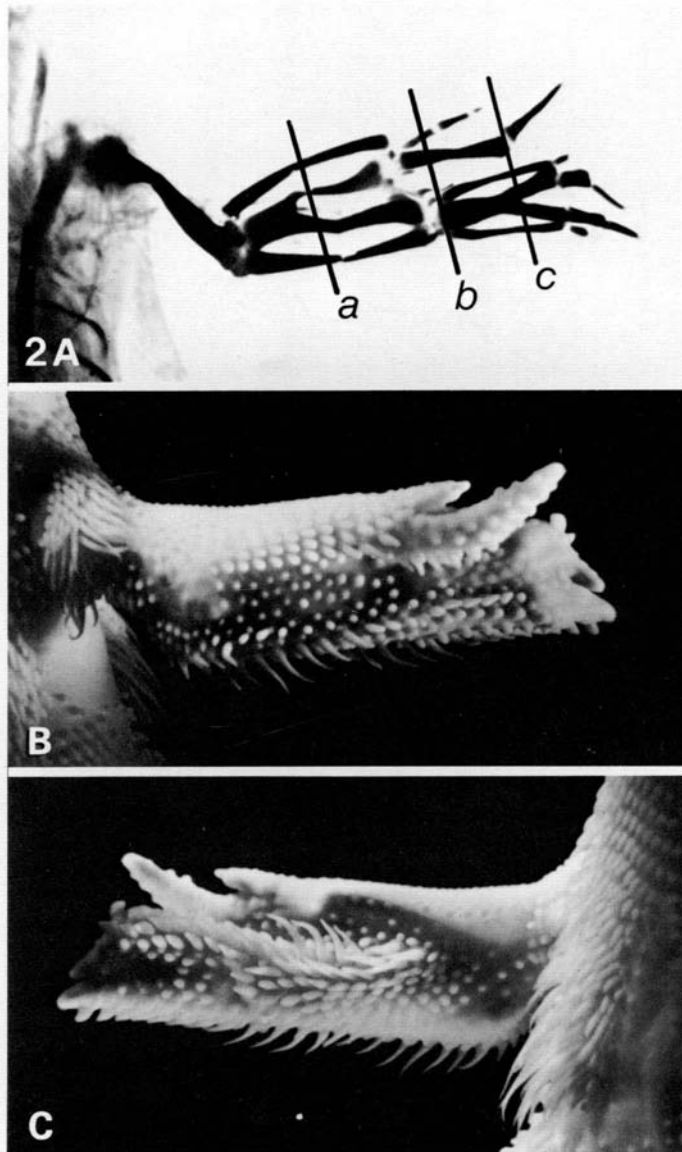
the operation. One outgrowth originated from the rotated tip and the other two appeared to be supernumerary outgrowths arising at the graft/stump junctions where anterior and posterior cells were juxtaposed.

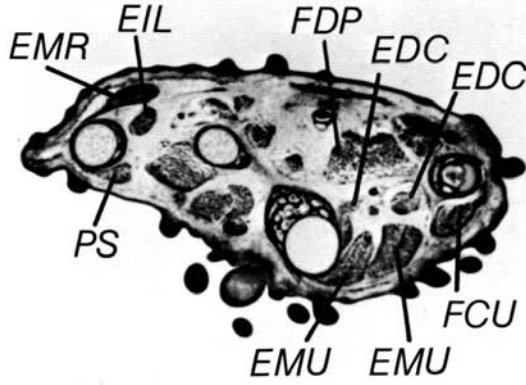
The normal chick wing has an upper arm comprising a humerus, a forearm with an anterior radius and a posterior ulna, and a hand with an anterior to posterior digital sequence of 2, 3, and 4. Of the 14 experimental limbs analysed in this study, all formed supernumerary forearm elements and extra digits (Table 1). Eight limbs had digits comprising three 'hands': an anterior hand of digits 2 and 3; a middle hand of digits 4 and 3; and a posterior hand of digits 3 and 4. Four limbs had an anterior hand composed of just a digit 2 or 3. One limb had an anterior hand with digits 2, 3, and 4, and the remaining limb had an anterior hand with a single cartilagenous spur. An analysis of the integuments suggested that of the elements composing the resulting forearms, the anterior-most was a radius and the posterior-most was an ulna. The middle element was an ulna or two partially fused ulnas as suggested by the presence of the long secondary feather coverts associated with the posterior edge of the normal wing (Fig. 2).

Histological analysis of all 14 wings confirmed the identity of the anterior-most and posterior-most forearm elements as radius and ulna respectively. Muscles characteristically associated with the radius and ulna were identified according to

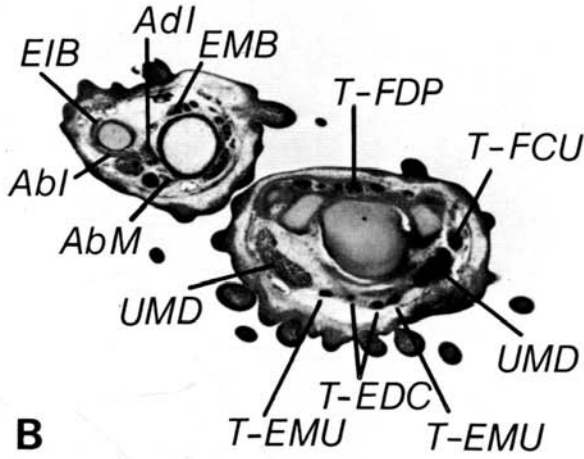
Fig. 2. A typical wing resulting from the manipulation illustrated in Fig. 1 at 7 days incubation. (A) A dorsal view of the resulting wing's skeleton illustrating supernumerary skeletal elements in the forearm and autopodium. Based on histological reconstruction of the muscles and tendons of this limb, the forearm consists of an anterior radius, a posterior ulna, and partially fused unidentifiable extra elements in between. The digits comprise three 'hands': an anterior hand of digits 2 and 3; a middle hand of digits 4 and 3, and a posterior hand of digits 3 and 4. (B,C) Dorsal and ventral integumentary views of the wing illustrated in (A). In a normal wing there are more feather germs present on the dorsal as opposed to ventral wing surface. There is also an anteroposterior asymmetry of the feather germs across the dorsal surface of the wing. Long posterior secondary coverts begin just proximal to the elbow and extend to the wrist; primary posterior coverts extend from the wrist to the distal tip of digit 3 (see fig. 2, Javois & Iten, 1981). A comparison of the integuments illustrated in (B,C) suggests the anterior radius and hand have the normal orientation. The middle and posterior forearm elements and hands have reversed dorsal/ventral asymmetry beginning at the midproximal forearm (from Javois, 1984).

their origins and insertions on the wings' skeletal elements. These muscles, e.g. the anterior dorsal extensor metacarpi radialis (EMR) or the posterior ventral flexor carpi ulnaris (FCU), are seen in cross section in Fig. 3. The middle forearm elements, however, demonstrated a reproducible pattern of numerous super-numerary muscles. Since muscles are traditionally named according to their origins and insertions, many of these were classified 'unidentifiable' as their origins and/or insertions, if present, were not characteristic of any known wing muscles. They are left unlabelled in Fig. 3. Hence, this middle forearm element was identified as an ulna (or ulnas) on the basis of the overlying integumentary pattern.

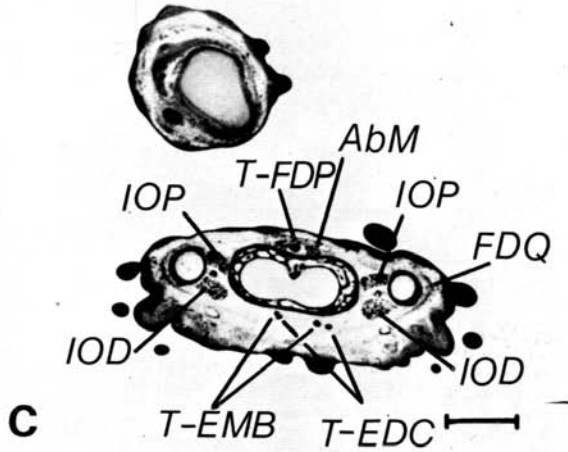




3A



B



C

Distally, the pattern of hand muscles and tendons was considerably more organized and clearly indicated the handedness of the structures. The dorsoventral asymmetry of the autopodium pictured in Fig. 2 can be determined from the

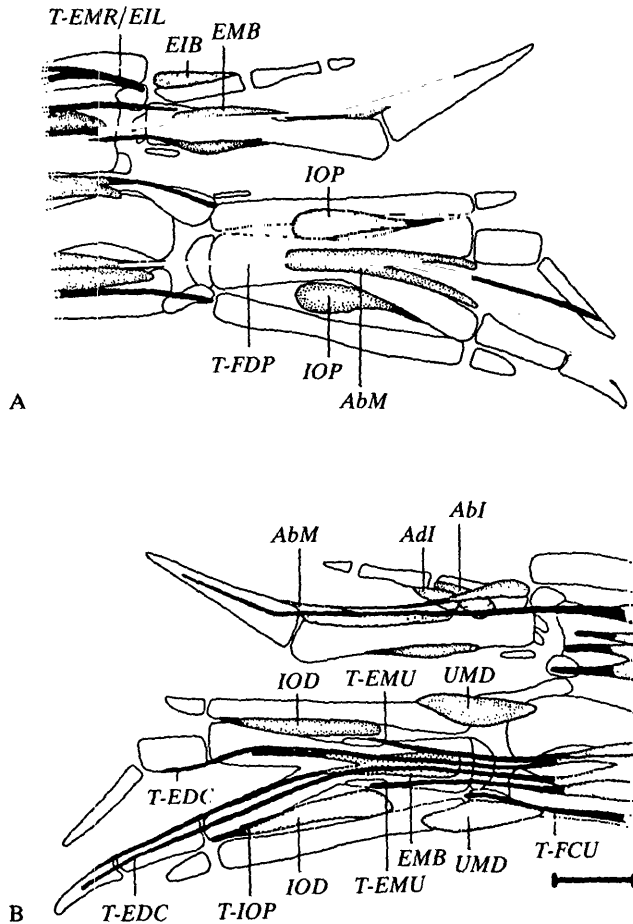


Fig. 4. Longitudinal reconstruction of the autopodium muscle/tendon pattern of the wing illustrated in Fig. 2. (A) Dorsal view; (B) ventral view. Muscles are stippled and tendons are blackened. Only those muscles and tendons with origins and insertions of known wing muscles and tendons are labelled. Abbreviations as in the text or Table 2. Scale bar equals 3330 μm .

Fig. 3. Histological cross sections from levels (a), (b), and (c) of the wing in Fig. 2. Those muscles with an origin and insertion of a known wing muscle have been identified. The unlabelled supernumerary muscles surrounding the middle forearm elements could not be unequivocally identified because their origins and/or insertions did not correspond to those of any known wing muscle. The abbreviations for wing muscles are those used by Javois & Iten (1981, 1982) and Sullivan (1962): *EMR*, extensor metacarpi radialis; *EIL*, extensor indicis longus; *PS*, pronator superficialis; *FDP*, flexor digitorum profundus; *EDC*, extensor digitorum communis; *EMU*, extensor metacarpi ulnaris; *FCU*, flexor carpi ulnaris; other abbreviations as in the text or Table 2. Dorsal, top; anterior, left. Scale bar equals 420 μm .

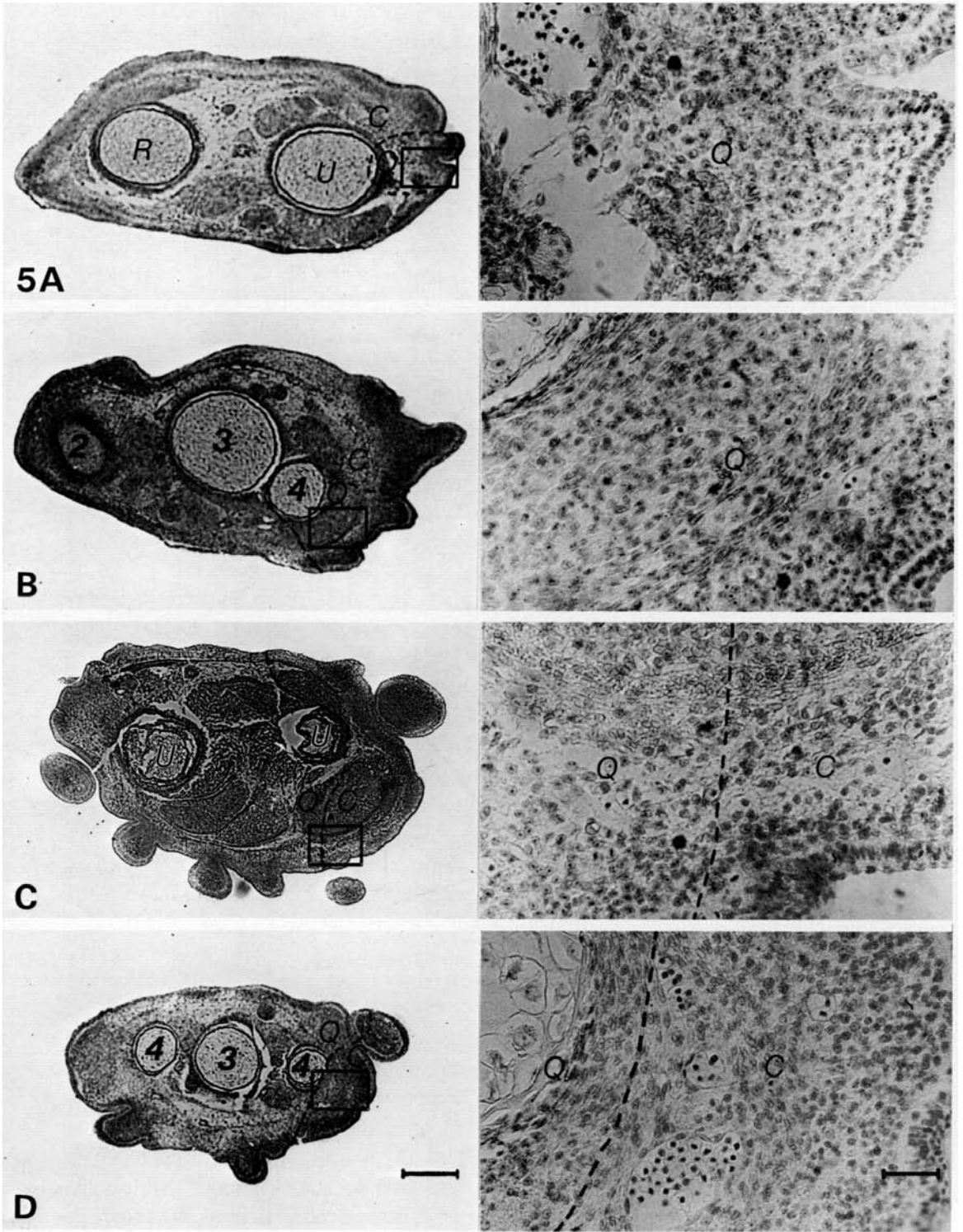
muscle/tendon pattern, seen in cross section in Fig. 3B,C and in the longitudinal reconstruction in Fig. 4. Table 2 lists the dorsal and ventral muscles of the normal autopodium. When the resulting limb is examined from the dorsal aspect, the anterior hand, composed of digits 2 and 3, is seen to have the same handedness as the stump: it is a right hand with normal dorsal/ventral asymmetry. The dorsal extensor indicis brevis (EIB) and extensor medius brevis (EMB) are present. From the ventral aspect, the ventral abductor medius (AbM), adductor indicis (AdI), and abductor indicis (AbI) are present. The middle hand comprising digits 4 and 3 is fused with the posterior hand which is composed of digits 3 and 4. Both are upside-down in relation to the stump and the anterior hand. From the dorsal aspect the ventral interosseus palmaris (IOP) and AbM are present, while from the ventral aspect the dorsal interosseus dorsalis (IOD), ulni metacarpalis dorsalis (UMD), and EMB are present. Accordingly, the middle hand is an upside-down right hand while the posterior hand is a mirror-symmetric upside-down left hand.

Table 2. *Muscles of the autopodium*

		Origin	Insertion
<i>Dorsal autopodium</i>			
EIB	Extensor indicis brevis	Anterior metacarpal digit 2	Dorsal first phalanx metacarpal digit 2
EMB	Extensor medius brevis	Dorsal metacarpal digit 3 (no tendon)	Muscle applied to tendon of extensor medius longus
IOD	Interosseus dorsalis	Proximal metacarpals digits 3 and 4	First phalanx digit 3 common tendon with extensor digitorum communis
UMD	Ulnimetacarpalis dorsalis	Distal dorsal posterior ulna	Proximal posterior metacarpal digit 4
<i>Ventral autopodium</i>			
AbI	Abductor indicis	Anterior ventral proximal metacarpal digit 3	Anterior ventral distal metacarpal digit 2
FI	Flexor indicis	Ventral metacarpal digit 2	Midventral first phalanx digit 2
AdI	Adductor indicis	Anterior proximal metacarpal digit 3	Posterior first phalanx digit 2
AbM	Abductor medius	Ventral proximal metacarpal digit 3	Ventral proximal first phalanx digit 3
IOP	Interosseus palmaris	Ventral proximal metacarpals digits 3 and 4	Tendon to dorsal posterior first phalanx digit 3
FDQ	Flexor digiti quarti	Ventral posterior metacarpal digit 4 just distal to insertion of UMD	Phalanx digit 4

There are four tendons illustrated in Fig. 4 which originate from identifiable forearm muscles and insert distally on the digits. In a normal wing, T-EMR/extensor indicis longus (EIL), T-extensor digitorum communis (EDC), and T-extensor metacarpi ulnaris (EMU) have dorsal insertions, while T-flexor digitorum profundis (FDP) has a ventral insertion. The dorsoventral orientation of these tendons agrees with the handedness of the digits as determined by the muscles of the hands. There is one ventral hand muscle which is not present, the flexor digiti quarti (FDQ), and one which has an abnormal insertion and is therefore unnamed ('flexor indicis'). There are several tendons in the hand which originate from unidentifiable supernumerary forearm muscles and are unlabelled. The limb illustrated in Figs 2, 3 and 4 is representative of the other seven limbs with this digital sequence although some of these limbs have FDQs and FIs. Those wings with fewer digits in the anterior hand than illustrated here have fewer hand muscles present. For a complete description of the muscles and tendons of a normal wing see Javois & Iten (1982) or Sullivan (1962).

In an additional five cases, rotated right quail wing bud tips were grafted onto right chick wing bud stumps. Resulting chimaeric wings had extra forearm elements and digits representing three hands as above. In four cases the forearms were split proximally such that the anterior consisted of a radius and ulna while the posterior consisted of a duplicated ulna. Based on analysis of the skeletal, integumentary, and muscle/tendon patterns the anterior to posterior digital sequences were determined to be **2344334**, **234434**, **?4334**, and **23443?**. The italicized digits comprised the fused middle and posterior hands while the question marks represent unidentifiable cartilagenous spurs. The fifth limb had an anterior forearm with a radius and ulna and digits **2, 3**, while the posterior forearm had an ulna and digits **3, 4**. Histological analysis of these chimaeric wings allowed us to determine where and in what structures the progeny of donor quail cells and host chick cells were found, as determined by the nucleolar difference between interphase chick and quail cells (Le Douarin, 1973). Of the anterior radius/ulna forearms all (one out of five) or a portion (four out of five) of the ulna, as well as the posterior connective tissue was of quail origin. Of the three anterior hands with digits **234**, digit **4** and the posterior connective tissue were of quail origin. The anterior hand with digits **2** and **3** had quail connective tissue extending the posterior length of the hand. The one anterior hand with a cartilagenous spur had no quail tissue. Of the posterior ulna/ulna forearms, the anterior-most ulna, as well as the middle hands comprising digits **4** and **3** were entirely of quail origin. Of the posterior-most ulnas, two out of five were entirely of quail origin while three out of five were partially of chick origin. The posterior hands were entirely quail (two out of five) or had chick cells contributing to digit **4** and the posterior-most connective tissue (three out of five). One of these limbs is illustrated in cross section in Fig. 5. In summary, both donor quail and host chick cells contributed to the anterior and posterior supernumerary structures which arose as the result of surgically juxtaposing normally non-adjacent wing bud cells.



DISCUSSION

The purpose of this study was to examine thoroughly the skeletal, integumentary, and muscle/tendon patterns of limbs resulting from 180° rotation of the chick wing bud tip on its base in order to determine the handedness and origin of the supernumerary structures formed. This study provides new data in two areas: (1) the *absence* of limbs with variable symmetry or asymmetry as seen in similar studies performed with amphibian regeneration blastemas; (2) the contribution of donor and host cells to the extra structures formed.

Handedness or asymmetry of limb structures

The consistent handedness of resulting wing structures in this study is in marked contrast to the variable classes of symmetric and/or asymmetric anatomies of supernumerary limbs resulting from the same manipulation done with amphibian regeneration blastemas. By examining the skeletal and muscle/tendon patterns of the chick wings, we found that the anterior supernumerary digits 2, 3 (and 4) and carpals comprise a normally oriented right hand derived from both stump and rotated tip cells. The middle digits 4 and 3 and carpals were an upside-down right hand arising entirely from the rotated right limb bud tip. The posterior supernumerary digits 3 and 4 and carpals comprised an upside-down left hand of both stump and tip origin. Examination of the overlying integument's asymmetry also supported these conclusions.

Anatomical differences between regenerating amphibian limbs and developing chick limbs during outgrowth may account for the dramatic differences in limb asymmetry observed following the same experimental manipulation. In amphibians, supernumerary limbs can arise at variable locations around the circumference of the limb following 180° rotation of the regeneration blastema. During distal outgrowth, fusion of supernumerary outgrowths could give rise to limbs with mixed dorsoventral asymmetry (Maden & Mustafa, 1982). In contrast, rotation of the chick limb bud tip results in outgrowths only along the anteroposterior plane of the limb. A possible explanation for why chick limb buds do not form supernumerary limb *outgrowths* from areas where dorsal and ventral cells are juxtaposed is that the ectodermal specialization necessary for limb bud outgrowth, the apical ectodermal ridge (AER), does not form at this wound site. It should be

Fig. 5. Histological cross sections from a chimaeric wing resulting from the 180° rotation of a quail wing bud tip on a chick wing bud base. On the left are sections from the midanterior forearm (A), midanterior digits (B), midposterior forearm (C), and midposterior digits (D). Scale bar equals 170 µm. On the right are enlargements of the boxed areas indicated on the cross sections illustrating the nucleolar difference between the host chick cells and the donor quail cells. Scale bar equals 14 µm. Unlike the wing illustrated in Fig. 2 this chimaeric wing has two separate forearms, an anterior radius (R) and ulna (U) and a posterior duplicated ulna. The anterior hand consists of digits 2, 3, and 4. The middle and posterior hands are fused and consist of a 4, a 3 duplicated distal to the level illustrated, and a 4. Note donor quail cells and host chick cells both contribute to the extra structures formed. Dorsal, top; anterior, left.

noted, however, that Javois & Iten (1982) have demonstrated that supernumerary structures, extra muscles, do arise when dorsal and ventral wing bud cells are juxtaposed. In the study reported here, an AER is present at the sites where anterior and posterior cells are opposed, and this is where the supernumerary outgrowths are seen to originate. Fusion of outgrowths like that proposed to occur in amphibian regeneration blastemas is not observed with the chick system, and supernumeraries of predictable asymmetry arise following 180° rotation of the wing bud tip on its stump.

Origin of limb structures

We have previously reported the contribution of both donor and host cells to the formation of supernumerary structures following a variety of grafting operations in the developing chick limb (Iten, 1982; Iten & Murphy, 1980; Iten, Murphy & Muneoka, 1983; Javois & Iten, 1982). The present results are similar: both donor rotated tip and host stump cells contribute to the extra structures formed (Fig. 5). Examination of the anterior and posterior supernumeraries in this study indicates the progeny of anterior cells (whether they be donor quail or host chick) tend to contribute more to the extra structures formed. However, posterior cells are capable of contributing to the formation of extra digits as well as extra muscles and connective tissue. This is in agreement with Carlson (1984) who reports contribution of both donor and host cells to the supernumerary structures resulting from grafts of anterior tissue to a posterior host site. He, too, states that anterior tissue contributes more to the extra structures formed.

Does the ZPA model adequately describe these results?

Many investigators have attempted to explain how supernumerary limb structures arise in a variety of experimental circumstances. As a result of this body of work, two models predominate in the literature on limb development and regeneration. Historically, the idea that a special region of the developing limb bud played a key role in determining the anterior-to-posterior polarization of the limb parts was suggested by the work of Saunders & Gasseling (1968). MacCabe, Gasseling & Saunders (1973) coined the term 'zone of polarizing activity' (ZPA) with reference to the posterior edge of the developing limb. Based on Wolpert's concept of positional information (1969), Tickle, Summerbell & Wolpert (1975) proposed a model which mechanistically describes how the anterior-to-posterior sequence of digits arises using a gradient of polarizing morphogen. Assuming the ZPA to be the source of this polarizing morphogen, much subsequent work examining the formation of supernumerary limb structures has been analysed from this perspective (for reviews see Summerbell & Tickle, 1977; Tickle, 1980). While this model describes the anterior-to-posterior sequence of resulting digits in relation to the position of the ZPA or additional grafted ZPAs ('posterior' being dictated by the higher morphogen concentration nearer the ZPA), it *does not* address the question of dorsal/ventral asymmetry nor the handedness of the

structures. Only the anterior-to-posterior sequence of structures can be said to be dictated by the location of the ZPA(s) in the developing limb.

The dorsal/ventral asymmetry of the overlying integumentary pattern was the original basis used by Saunders to describe the handedness of supernumerary structures resulting from 180° rotation of the limb bud tip on its base. For the ZPA model to be applied to these results requires the assumption that the dorsal/ventral polarity of cells is already established at the time of the rotation. Responding cells are 'repolarized' with respect to the anterior-posterior axis alone because of exposure to the reoriented ZPA. Hence, the anterior supernumerary limb was assumed to be a normally oriented right hand entirely of host origin with its posterior edge nearest the rotated ZPA. The middle and posterior hands were mirror-symmetric with their posterior-most digits near either the rotated ZPA or the stump ZPA. These latter two limbs were assumed to have arisen from the rotated tip in response to the signals from the ZPAs and therefore were upside-down in relation to the stump with right and left handedness respectively.

The results of this study conflict with this interpretation. The histological analyses of the skeletal and muscle/tendon patterns of resulting wings substantiate the handedness inferred from the original integumentary analyses (Saunders *et al.* 1958), but the contribution of both donor and host cells to the extra structures observed here is not easily reconciled with the above interpretation.

Given that the progeny of quail cells from the rotated tip contribute to the anterior supernumerary hand, and the progeny of chick cells from the stump contribute to the posterior supernumerary hand, one may question the concept of the ZPA as a 'signalling' region (Honig, 1983). These results are not consistent with the idea that supernumerary structures should be formed all, or almost entirely, from responding tissue whether it be graft (at the posterior location) or host (at the anterior location). Additionally, one may question the nature of stable dorsal/ventral polarity inferred by the ZPA model. According to the ZPA model, one would have to assume these cells changed their dorsal/ventral polarity since they contributed to structures of opposite handedness. Interpretations of this nature are tenuous at best, and the ZPA model, as presently formulated in the literature, does not specifically address the issue of dorsal/ventral polarity or handedness.

How the polar coordinate model can describe the results obtained

More recently, analysing limb development from a different perspective, we have used the polar coordinate model to describe limb development (for reviews see Iten, 1982; Javois, 1981, 1984). This model is formulated from a synthesis of experimental findings from work done with regenerating amphibian and insect limbs and *Drosophila* imaginal discs (Bryant, French & Bryant, 1981; French, Bryant & Bryant, 1976). It is also based on the idea that cells possess positional information (Wolpert, 1969), specifically about their location along the proximo-distal axis as well as around the circumference. In addition, it is proposed that tissues have the general property of intercalation: localized growth in response to

positional disparity. The polar coordinate model makes specific predictions regarding the handedness of supernumerary structures arising during pattern specification following 180° rotation of the limb bud tip on its stump.

The first of these predictions deals with the handedness and orientation of the supernumerary structures. Two sets of supernumerary digits should arise, one of stump handedness and orientation, the other of opposite handedness and orientation. It is proposed that these structures arise as the result of intercalation which eliminates the positional disparity created by the grafting operation. The dorsal/ventral asymmetry of the structures would be dictated by the circumferential sequence of positional values present following intercalation. Javois (1984) presented a detailed description of chick limb development using the polar coordinate model framework that will not be repeated here.

In the experiments reported here the interactions between stump and rotated tip cells following 180° rotation of the wing bud tip on its stump are illustrated in Fig. 6. While this grafting operation opposes cells of non-adjacent circumferential positional values around the entire limb, it is postulated that intercalation giving rise to supernumerary *outgrowths* occurs only at the anterior and posterior edges of the wing bud where the graft/host junction has an overlying AER. The intercalation that occurs at these two sites eliminates the circumferential positional disparity, but in doing so creates a region of positional symmetry around the circumference (Fig. 6B). As discussed by Javois (1981, fig. 9; 1984, fig. 11), when cells with the same circumferential positional values interact during distal outgrowth in regions of symmetry they recognize no positional disparity and therefore produce no progeny. As a result, cells with these circumferential positional values are not present at more distal radial levels, and in effect there is a 'convergence' of circumferential positional values at this area of symmetry. Examination of the sequence of circumferential positional values present at the end of the process of intercalation and distalization allows one to predict both what structures will be present and what their handedness will be. As can be seen in Fig. 6C, the anterior supernumerary structures are of normal orientation and right-handed; the structures arising from the rotated graft are upside-down and right-handed; the posterior supernumerary structures are also upside-down but left-handed.

The phenomena of convergence may in part explain the variability of digital sequences seen in the resulting limbs. Depending on the degree of convergence which occurs during distalization, more or fewer structures may be present. The fully intercalated digital pattern of **234-432*234** is never observed, suggesting there is always some convergence. All 14 of the resulting limbs lack digit 2s in the region of symmetry (asterisk in the above sequence) (Table 1). One limb has the fully intercalated pattern present anterior to the dash in the above expanded sequence. The remaining 13 have a reduced pattern. In three of these there is a digit 2 next to a digit 4, representing a clear discontinuity. We have seen digital sequences with this discontinuity in previous studies (Iten, Murphy & Javois, 1981; Javois & Iten, 1981). These discontinuities as well as the reduced anterior patterns could result from the lack of time necessary for complete intercalation or be due to

a lack of interactions which result in intercalation. By far, the majority of resulting limbs do not have discontinuities in their digital sequences, suggesting the former possibility.

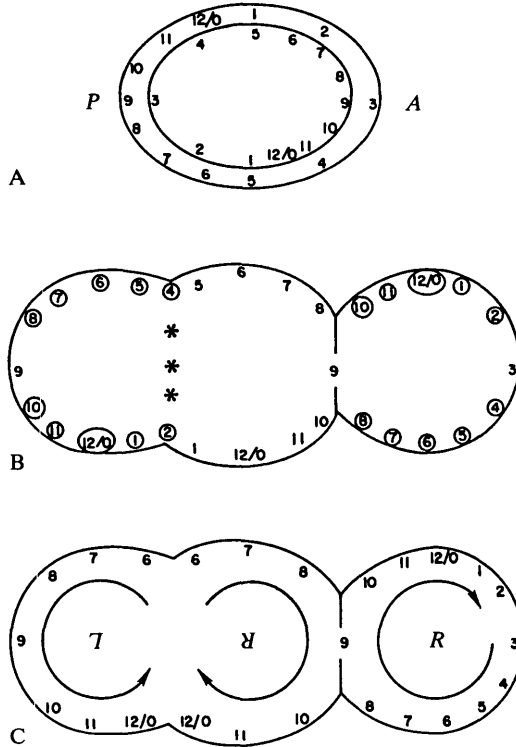


Fig. 6. Diagrammatic polar coordinate description of limb outgrowth following 180° rotation of a right wing bud tip on its stump. Diagram (A) illustrates the positional disparity created by the grafting operation. The outer ellipse represents the wing bud stump (A, anterior; P, posterior) while the inner ellipse represents the rotated tip in an end-on view. The sizes of the two ellipses are dissimilar for clarity only. Diagram (B) illustrates the intercalation of cells with intervening positional values (circled). The model postulates that intercalation occurs via the shorter circumferential route, but when cells with positional value 9 confront cells with positional value 3, as illustrated in the diagram, there is no shorter route. Since there is no variability in the handedness of the resulting structures, the data indicate intercalation occurs in the direction illustrated. This intercalation eliminates the positional disparity and gives rise to two supernumerary outgrowths. It is postulated that intercalation and distal outgrowth occur only at the anterior and posterior edges of the wing bud where the graft/stump junction has an overlying apical ectodermal ridge. The area of symmetry which results following intercalation is indicated by asterisks. Convergence occurs in this symmetric area during distalization (Javois, 1981, 1984). The final distribution of circumferential positional values is shown in (C). A clockwise sequence of circumferential positional values specifies a right (R) outgrowth and the mirror-image counter-clockwise sequence specifies a left (L) outgrowth. The anterior supernumerary outgrowth is of right handedness and normal orientation while the rotated right tip develops into an upside-down right outgrowth. The posterior supernumerary outgrowth is upside-down with left handedness.

The disorganization of the muscle pattern at the level of the forearm may be due to intercalation and continued distal outgrowth during the wound-healing process. While muscle patterns in the forearm indicate the anterior-most element is a radius and the posterior-most element is an ulna, there are many reproducible but unidentifiable muscles surrounding the middle element(s). The overlying integumentary pattern suggests this element is an ulna or two partially fused ulnas (Fig. 2). While supernumerary outgrowths do not originate from areas where dorsal and ventral cells are opposed, it is known that this confrontation of cells with disparate positional values does give rise to supernumerary muscles (Javois & Iten, 1982). This may also contribute to the pattern of muscles observed in the forearm.

It is interesting to note that the cross-sectional morphology of one of the posterior forearm supernumerary muscles repeatedly suggests it may be a duplicated flexor carpi ulnaris, although it does not have the origin of a normal FCU. This pattern is indicative of an area of symmetric double ventrality along the distal posterior forearm (Fig. 3A). However, it may be postulated that once circumferential intercalation has eliminated the circumferential discontinuities, distal outgrowth displays a more organized pattern of structures. Indeed, both the normal FCU and the supernumerary muscle insert on the ulnari at the wrist, and the pattern of muscles and tendons in the autopodium distal to this point of insertion has normal dorsal/ventral asymmetry. There is no indication of any region of double ventral or double dorsal muscles in the autopodium.

The second prediction made by the polar coordinate model states that both stump and rotated tip cells may contribute to the extra structures formed. The polar coordinate model proposes that these extra structures arise as the result of intercalation between juxtaposed cells. This is in contrast to the ZPA model where, as a signalling region, ZPA tissue is not expected to contribute to the extra structures formed (Honig, 1983). The polar coordinate model does not specify how much the donor and host cells will each contribute to the structures formed. However, Bryant *et al.* (1981) suggest that during an intercalary division, one or both daughter cells may be able to change positional values. In terms of the polar coordinate model, unequal contribution by anterior and posterior tissue such as that seen in this study could result. Following intercalary division of an anterior cell, both daughter cells could adopt new intermediate positional values, while one daughter of a posterior cell maintained the original positional value and the other adopted a new intermediate value. Muneoka & Bryant (1984b) suggest that because the capacity to intercalate involves cell division, it may also be related to the degree of cellular differentiation. Differentiation occurs from posterior to anterior in the chick limb bud, hence the greater intercalary capacity of anterior cells may be related to a less-differentiated state. This is in agreement with a recent study of Iten *et al.* (1983) in which older limb bud tips were grafted onto younger stumps. Older (more-differentiated) limbs buds did not form supernumerary structures following 180° rotation on their stumps. Yet grafting these older limb bud tips onto younger stumps did result in the formation of extra limb structures

which were of stump origin. This interpretation could also account for the one-sided contribution of anterior (less-differentiated) tissue to supernumerary structures reported by Honig (1983).

Thus, the framework of the polar coordinate model allows for another interpretation of the formation of supernumerary structures following 180° rotation of the chick wing bud tip on its stump. This model predicts both the anterior/posterior and dorsal/ventral asymmetry or handedness of the extra structures formed. It accounts for the likely contribution of both graft and host cells to these extra structures. And finally, it suggests possible explanations for the pattern of supernumerary muscles seen in the forearm and the array of digital sequences observed in the hands.

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DEDICATION

This paper is dedicated to Professor John W. Saunders, Jr, in celebration of his retirement. He is a leader in the field of developmental biology and a founding father in the area of chick limb development. His contributions to the field are immense and everlasting. His helpful comments, criticisms and support are a constant inspiration to us and we look forward to further interactions with him.

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