Evolutionary developmental biology of the tetrapod limb

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SUMMARY

New insights into the origin of the tetrapod limb, and its early development and patterning, are emerging from a variety of fields. A wide diversity of approaches was reported at the BSDB Spring Symposium on 'The Evolution of Developmental Mechanisms' (Edinburgh, 1994); here I review the contributions these various approaches have made to understanding the evolutionary developmental biology of the tetrapod limb. The fields covered included palaeontology, descriptive embryology, experimental embryological analysis of interactions within developing limbs plus description and manipulation of homeobox gene expression in early limb buds. Concepts are equally varied, sometimes conflicting, sometimes overlap-ping. Some concern the limb 'archetype' (can the palaeon-tologists and morphologists still define this with precision? how far is there a limb developmental bauplan?); others are based on identification of epigenetic factors (eg secondary inductions), as generating pattern; while yet others assume a direct gene-morphology relationship. But all the contributors ask the same compelling question: can we explain both the similarity (homology) and variety of tetrapod limbs (and the fins of the Crossopterygians) in terms of developmental mechanisms?

Key words: limb development, palaeontogeny, Hox gene, tetrapod

INTRODUCTION

The developmental basis of macroevolution has long been ignored by the neo-Darwinian synthesis, with its emphasis on the population genetics approach to understanding evolutionary change. In recent years, however, the relationship between genes, development and macroevolution has aroused much greater interest (Gould, 1977; Hall, 1992; Duboule, 1992; Holland, 1992; Alberch, 1989). This represents a return to Charles Darwin's own view of embryology as second to none in importance, with his repeated citing of the embryological work of von Baer as the best evidence for the 'unity of structural plan' amongst vertebrates, and as a source of information about their phylogeny. In Darwin's time, of course, only descriptive embryological observations were available, but the most recent attempts to 'explain' phylogenetic changes integrate information from experimental embryology, and from cell and molecular biology. A recent and striking example is Carl Gans's citation (Gans and Northcutt, 1983) of the neural crest as a critical chordate invention, and a necessary precursor to the whole of skull and dermal axial skeleton evolution in vertebrates. In the field of limb development and evolution, current discussion has been enlivened by recent discoveries ranging from palaeontology to molecular biology. Palaeontologists investigating the Devonian period (360 Myr) have recently described (Coates and Clack, 1991) the polydactylous limbs of the first tetrapods - at a stroke undermining the classic pentadactyl archetype – as well as Panderichthyid lobe fin fish representing a sister group much closer to the first tetrapods (Vorobyeva and Schultze, 1991), than the longer known forms

(such as *Eusthenopteron*) traditionally allocated in textbooks of vertebrate evolution the role of the first conquerors of the land, and of tetrapod ancestors. In the earliest tetrapods, these first limbs with digit numbers ranging from 6-8 appear more experimental than later more strictly pentadactyl versions. Thus polydactylous tetrapods present novel patterns which developmental biologists need to take into account in their explanations. Recent attempts incorporate evidence from developmental processes and include models based on (i) generation processes elaborating the endoskeleton and attenuating the exoskeleton (e.g. the fin rays) (Thorogood, 1991), (ii) a 'developmental bauplan' of segmenting and asymmetrical branching of the skeletogenic mesenchyme of the limb bud (Shubin, 1991; Shubin and Alberch, 1986) and (iii) evidence that the developmental system in the limb bud is biased towards generating stability proximally and variability distally (Hinchliffe, 1991).

From the very different perspective of molecular biology, analysis of genetic regulation of limb development has provoked reassessment of the evolution of the vertebrate limb. In the chick and mouse limbs, homeobox expression domains along both proximodistal and anterior-posterior axes give the mesenchyme cells unique 'positional addresses' in terms of their patterns of Hox gene expression (Duboule, 1992). Evidence from mutants and from experimental manipulation of the domains (Morgan et al., 1992) suggests that the particular combinations of expression of homeobox genes may relate to the type of digit (or other structure) which forms, regulating its pattern and determining whether the structure is posterior or anterior, proximal or distal.

PALAEONTOLOGICAL FINDINGS AND THE DECONSTRUCTION OF THE ARCHETYPE

To the developmental biologist, the palaeontological record of fins and limbs and, more specifically, the fin/limb transition, sets out the full range of adult fin/limb skeletal patterning that morphogenetic mechanisms must generate (or have generated). To rely on extant forms – lungfish, the coelacanth, the highly derived urodeles – would produce an impoverished set of fin and limb skeletons. Coates (1994 – this volume) sets out the rich diversity of vertebrate appendages (both fins and limbs) with forms very unfamiliar to developmental biologists who may just about remember the distinction between cartilaginous and bony fish. From his survey, Coates suggests that paired fins may have evolved twice and that pectoral fins preceded pelvic fins. Developmental biologists will be most interested in his account of the origin and diversification of the tetrapod limb. Classically, the origin has been sought among the Sarcopterygians, the fleshy or lobe-finned fish (forms in which a substantial endoskeleton enters the fin base and which include the present day lungfish (dipnoans) and the coelacanth, as well as extinct forms). The classification of sarcopterygians is controversial (Coates, 1991): Schultze (1991) identifies a 'rhipidistian crossopterygian' subgroup which includes most of the fossil forms which are divided into osteolepiforms, such as *Eusthenopteron*, (and their probably close relative, Sauripterus), the panderichthyids, and strictly also the tetrapods. The homology of the proximal part (eg humerus, radius, ulna of the forelimb) of the tetrapod limb and osteolepiform paired fin endoskeleton (see Fig. 1) has long been recognised (Jarvik, 1980; Gregory and Raven, 1941) and used as an argument to support the hypothesis of an osteolepiform origin of tetrapods. At a more distal level it has been difficult to detect homologies; for example, the digits do not correspond with elements of the simple branching patterns of the 'rhipidistian crossopterygian' paired fin skeleton. Thus the digits have generally been regarded as evolutionary novelties. The textbook scenario has been of osteolepiform fish dragging themselves with their fleshy lobed fins over the land from one drying out pool to a still-surviving water source. Later, a strengthened and elaborated fin skeleton could become permanently weight bearing on the land as envisaged in the traditional reconstruction of the early tetrapod, *Ichthyostega*, (eg Jarvik, 1980) as a thoroughly terrestrial form.

There are now many problems with this interpretation (Ahlberg and Milner, 1994). The osteolepid *Eusthenopteron* frequently selected for the role of terrestial conquest, appears to have been a fast free-swimming form unlikely to move at all convincingly in shallow water or on land. A more likely sister group for the first tetrapods is the order Panderichthyida (Vorobyeva and Schultze, 1991) named after *Panderichthys*, a species with several features shared with early tetrapods, and whose life-style was probably shore- and shallow water-based, which used the relatively massive proximal skeletal parts of the fore fin (humerus, radius, ulna) for hard substrate locomotion on the shore (Vorobyeva, 1991, 1992a,b; Vorobyeva and Kuznecov, 1992). *Panderichthys* is itself unlikely to be a tetrapod ancestor, on account of the early termination of the branching of the fin endoskeleton (only two branching events – see Fig. 1) which is composed of only five elements and is distally quite unlike that of the tetrapods.
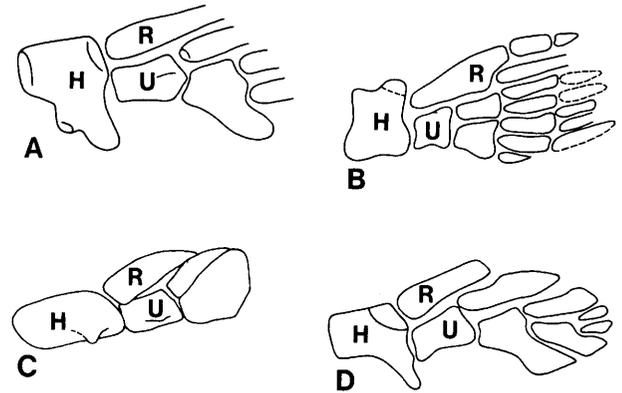
Fig. 1. Distal variation and proximal stability in the pectoral fin endoskeletons from the fossil 'rhipidistian crossopterygians', close relatives of the first tetrapods. ('Rhipidistian crossopterygians' form a subgroup of the sarcopterygians, Schultze, 1991). Proximally the structure is homologous with that of tetrapods (H, humerus; R, radius; U, ulna). Distally, the number of branchings shows variation. (A) *Sterropterygion* (an osteolepid); (B) *Sauripterus* (a rhizodont); (C) *Panderichthys*; (D) *Eusthenopteron* (an osteolepid).

The newly discovered (or reinterpreted) polydactylous tetrapods (Coates and Clack, 1990; Coates, 1994 – this volume) also challenge the traditional interpretation. There are 3 species of these from the Devonian period whose limbs have been preserved: from Greenland, *Ichthyostega* (Jarvik, 1980) and *Acanthostega* (see Coates, Fig. 4) at the base of the amphibian radiation, and *Tulerpeton* (Lebedev, 1985), a reptiliomorph, from Russia. All have lost the fin rays of their rhipidistian crossopterygian relatives, and all have digits, being polydactylous with digit numbers ranging from 8 (*Acanthostega*, fore and hindlimbs), 7 (*Ichthyostega*, hindlimb), to 6 (*Tulerpeton*, fore and possibly hindlimb). Also the low number of carpus or tarsus elements, and their position seems quite different from that typical for the tetrapod limb. These limbs appear likely to have been used horizontally as props and/or aquatic paddles exploiting a shore and shallow water niche, rather than as terrestrial weight-bearing structures. Thus the tetrapod limb may well have evolved initially as a shallow water rather than a terrestrial adaptation (Coates and Clack, 1991; Vorobyeva, 1992b). Later forms of the tetrapod limb are more terrestrially adapted, with the digit number reduced to 5 as a maximum (later amphibians have a maximum of 4 in the forelimb) and with carpus and tarsus elements closer in both number and spatial arrangement to the traditional tetrapod archetype. It appears that pentadactyly – having 5 digits – has been independently evolved along both the reptile and amphibian lines of evolution (Coates, 1991).

One casualty of this discovery of digit variation in early tetrapod limbs is the pentadactyl archetype (see Fig. 2B). This represents the attempt to define a canonical formula or a primitive, numerically fixed pattern of skeletal elements. But with variation in both digit number, and in tarsal pattern, no archetype can be defined. In fact in the interpretation of this subject, the archetype has been very persistent, generating attempts based on recapitulation (ontogeny repeating phylogeny) to read into development at the prechondrogenic stage an ancestral pattern based on the adult limb skeleton of the first tetrapods. This pattern itself supposedly corresponds

with that of the hypothesised dipnoan or osteolepiform tetrapod ancestors (see Hinchliffe, 1977, 1989 for criticism of archetype-based analyses by Holmgren, 1933; Montagna, 1945; Jarvik, 1980). The absence of a precisely defined archetype must affect the credibility of such ancestral archetype 'explanations' of the development of tetrapod limb structure, which attempt to account for both the general common features of fin/limbs (eg the proximal parts) as well as the specialised and variable features. Absence of an archetype gives added weight to process-based explanations.

DEVELOPMENTAL PROCESSES

One common feature of recent attempts to explain limb pattern generation in developmental terms is that they are all process based. Developmental biology has revealed a hierarchy of such processes ranging, for example, from apical ridge induction of limb mesenchyme outgrowth, through prechondrogenic condensation, to polarising zone role in control of the anteroposterior axis (Fig. 2A). According to one scheme, the mesenchyme will generate branching precartilaginous condensations whose number relates to the area of available mesenchyme (Pautou, 1973). Positional identity is then imprinted on the condensations (the 'prepattern') by the polarising zone. Variants of this scheme are given by Wolpert, 1989; Ede, 1982, 1991; Hinchliffe, 1991. The most detailed of such schemes (Fig. 2B) emphasising generation of the limb skeleton through branching has been put forward by Alberch and his co-workers (both at the conference and in Shubin and Alberch, 1986; Shubin, 1991), and aims to explain both general and specific features of developing limbs. Condensing mesenchyme is considered to branch asymmetrically (eg the ulna branches distally but not the radius) in generating precartilaginous pattern in a proximodistal direction. This pattern is common to anuran amphibians and amniotes (though urodeles may do it rather differently). The branches segment thus forming individual condensations. Specific variation may be produced by differences in the position of segmentation divisions. From this model, Alberch has evolved the concept of 'developmental constraints' – in particular the last-forming elements being the most easily lost in conditions of mesenchymal cell shortage – which fits evidence provided by digit reduction examples occurring either naturally or experimentally. The model also fits rather well digit reduction and other pattern modifications in birds as compared with chelonians (turtles), and archosaurs (eg crocodiles) in Muller's scheme (1991) of heterochronic modification through paedomorphic truncation of the skeletogenic branching and segmenting patttern.

One characteristic of the evolution of tetrapod limbs and sarcopterygian fins (especially osteolepiforms, *Sauripterus* and *Panderichthys*) (Fig. 1) is proximal stability and distal variability in structure. Unlike the proximal elements, digits are frequently lost in tetrapod limb evolution. This probably relates to the developmental properties of the limb bud, since in experimental manipulation such as ZPA grafting, and in mutants, it is relatively easy to add supernumerary digits, but difficult to affect zeugopod and stylopod. Hinchliffe (1991) suggests this structural outcome is related to relatively minor changes in limb bud boundaries arising through alteration in mesenchyme
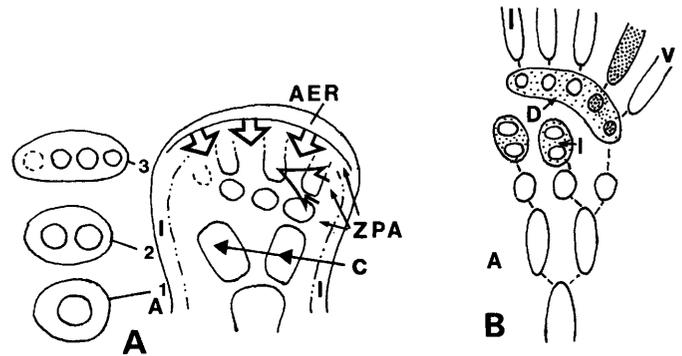
Fig. 2. (A) Schematic diagram of main processes in chick limb development. These processes may represent the common developmental basis to the homology of the tetrapod limb (possibly urodeles are an exception). Cross-sectional areas represented (left) at limb levels 1, 2, 3. Abbreviations. A, anterior; AER, apical ectodermal ridge, mesoderm outgrowth inducer (and site of activity of FGF, fibroblast growth factor); C, branching and segmenting condensations; I, ectoderm-induced zone of inhibition of chondrogenesis; ZPA, zone of polarising activity controlling posterior to anterior axis (also site of *HoxD13* (stage 22) and '*hedgehog*' gene expression). (B) Patterns of branching and segmentation of pre-cartilaginous condensations in Shubin's (1991) scheme for amniotes. Dotted lines represent embryonic connections between condensations. While this partly resembles Holmgren's eutetrapod archetype (1933), this pattern is not a fixed archetype since the number of elements within each stippled area may vary in a species-specific way. Heavy shading represents earliest forming elements in digital arch or among digits. Abbreviations. A, anterior; D, digital arch; I, intermedium/centrale series; I–V, digit ray condensations.

cell number or in the period of AER activity. Quantitative changes in cell number would readily result in distal qualitative changes eg in digit number. Such distal channelling of structural change may represent a developmental constraint very similar to that proposed by Alberch.

A further process-based scheme has been put forward by Thorogood (1991) in relation to one of the most striking features of the fin/limb evolutionary transition: the loss of the distal fin ray skeleton. Most analyses of the transition focus on the endoskeleton, but limb evolution can be seen as a process of elaboration of this, and elimination of the exoskeleton, paralleling a similar loss or reduction of fin rays from the unpaired fins of early tetrapods and their sister groups. Separate mechanisms govern endoskeletal and exoskeletal (fin ray) formation in teleosts. The endoskeleton forms from mesoderm generated by an apical ectodermal ridge, but this is succeeded by a fin fold within which the actinotrich precursors of the fin rays develop. Different cell lineages are likely to be involved: the endoskeleton being mesoderm derived and the exoskeleton probably neural crest derived. Neural crest participation in the exoskeleton is likely by analogy with unpaired fins where there is now good evidence in a teleost, the zebrafish, that the dermal skeleton depends for its formation on a neural-crest-derived mesenchyme (Smith et al., 1994). Thorogood (1991) proposes a heterochronic shift that would explain the distinction between ray-finned and lobe-finned fish: in the latter, the endoskeleton generation process would be extended, and the exoskeletal generation process abbreviated. In the transition to tetrapods,

the latter process would be entirely eliminated. The model cannot be tested directly, but its predictions can be tested by examining fin development in extant fishes. Preliminary findings (Thorogood, personal communication) suggest that, in the dipnoan *Neoceratodus,* there is such a late transition from apical ridge to fin fold in the development of their paired fins.

Generic properties of developmental processes were shown by Muller in his paper (see also Muller, 1991; Muller and Wagner, 1991) to play an active role in the generation of evolutionary novelty, in contrast to the concept of developmental constraint emphasised by Alberch. Muller has attempted to analyse some of the secondary interactions that generate adult structure. In general, these have been more difficult to analyse than the more familiar overall limb field interactions (for example, of ridge induced outgrowth and ZPA patterning control in early limb buds). This is partly due to the difficulty of clean surgical intervention and grafting in the post-limb-bud stages.

An example of a morphological novelty resulting from this property of vertebrate tissues is the sesamoid osseous fibular crest on the tibia, which links the reduced fibula to the tibia in birds. Sesamoid bones appear in tendons, usually initiated by pressure. The fibular crest is a neomorphic structure appearing first in the theropod dinosaurs, the presumed ancestors of birds. Developmentally the crest forms initially as a separate cartilaginous sesamoid which ossifies and becomes incorporated first into the tibia. As Hall (1986) has demonstrated, embryonic movement acts as an inducer and is necessary for the formation of sesamoids and secondary cartilages. The dependence of fibular crest on embryonic movement has been demonstrated in paralyzed embryos, which lose their crest. The necessity for the presence of the fibula for the developmental interactions has been shown also by deficiency experiments on chick leg buds which eliminate the fibula: in consequence the crest is also missing (Muller and Streicher, 1989). In the evolution of dinosaurs, reduction of the archosaur fibula could have increased mechanical instability during embryonic movement, thus creating pressure stress on the connective tissue and eliciting formation of the sesamoid cartilage precursor of the crest. Muller argues that the appearance of this novelty is based on specific conditions: skeletal proportions, biomechanical changes and the potential of connective tissues to react to mechanical stimulation. The initial trigger may well have been quantitative – an alteration in mesenchyme cell number in the zeugopod region of the limb bud generating a qualitative change through its effects on a critical threshold.

GENE-MORPHOLOGY RELATIONSHIP

Interesting as is the evidence from palaeontology and from analysis of developmental processes, the dynamic for the current 'buzz' of animated discussion is new molecular evidence for genetic regulation of limb pattern. This falls into a number of categories. One is the comparison of Hox gene complexes in different chordates (and invertebrates) enabling phylogenetic relations to be analysed on the basis of molecular homology, but without necessarily an understanding of the precise developmental role of the genes. Thus amphioxus has a single cluster of Hox genes, contrasting with mammal (mouse and man) which have four Hox clusters apparently

resulting from duplication of the initial cluster (Holland, 1994 – this volume). Holland (1992) has suggested that Hox gene duplication is necessary to support the increasing complexity of vertebrate development in evolution: duplication of the complexes permitting related genes to diversify their function. A second category is the expression pattern of the Hox genes, not only along the main body axis of the embryo, but also in the limb buds of mouse and chick embryos. A third category is the experimental modification of the expression domains of the Hox genes, following grafting experiments (e.g. preaxial grafting of ZPAs or of retinoic-acid-containing beads) or transfection experiments. The aim here is investigation of the relation between the domains and the structures they regulate.

One hypothesis is that in the evolution of the tetrapod limb, the Hox genes originally expressed co-linearly along the main body axis of the embryo, and presumed to act as positional information markers in regulating its patterning, have later been co-opted into regulation of limb patterning (Duboule, 1992). Two Hox gene complexes have been shown to be expressed in mouse and chick limb buds. HoxD component genes are expressed as a series of nested domains along an axis from posterior distal to anterior proximal in mouse limb buds. In chick limb buds, HoxA domains are arranged more or less proximally to distally. Both fore and hind mouse and chick limb buds have rather similar HoxA and HoxD domains. All have the same *HoxD13* domain found at the posterior end of the main embryonic axis and present in limb buds along the posterior distal margin. Since this corresponds in chick limb buds with the ZPA position (and with 'hedgehog' gene expression in both chick and mouse as reported by Riddle et al., 1993; Laufer et al., 1994, Fietz et al., 1994 – this volume), this domain may represent the molecular basis of posterior positional value.

In chick limb buds, experimental embryology may be combined with domain mappings. In the classic experiment on pattern control, grafting the ZPA into a preaxial position provokes the formation of supernumerary digits in tissue from the adjacent host limb bud. The most posterior digit is always the closest to the graft. Normally this anterior limb bud mesenchyme expresses 'anterior' HoxD domains, but following the graft it is switched to a posterior mode of HoxD expression (Izpisua-Belmonte et al., 1991). Retinoic-acid-saturated beads provoke the same response: 'posterior' HoxD expression in an anterior region preceding supernumerary posterior digit formation. Manipulation of the domains was also achieved by transfection experiments in which HoxD genes appropriate to a more posterior position are expressed in the region prospective for the anterior leg digit. In some 30% of cases, a more posterior than normal digit ('2' rather than '1') formed from this region (Morgan et al., 1992). The hypothesis of correlation of HoxD gene expression with specific digits is supported by evidence from a mutant chick embryo, talpid, whose polydactylous digits lack anteroposterior specificity, and in whose limb buds HoxD genes are expressed throughout, rather than demonstrating the normal posterior to anterior sequence of domains (Izpisua-Belmonte et al., 1992).

These experiments and observations are interpreted as evidence that the HoxD code specifies the anterior posterior axis, allocating an identity to individual digits. According to the theory, proximodistal specification is through HoxA activity: mesenchyme cells' unique combination of HoxA and

HoxD expression will determine their differentiation appropriate to their position along the two axes. A more specific variation of this identity allocation hypothesis is given by Tabin (1992) in response to the question "Why do we have 5 digits?". His answer is that the 5 nested HoxD domains of the distal part of the mouse limb bud (Duboule, 1992) code for the 5 digits of the pentadactyl limb. In the chick wing and limb buds, Morgan et al. (1992), describe 5 corresponding HoxD domains (the 'loss' of the supposed ancestral number 1 bird digit is attributed to the intervention of cell death anteriorly in the limb bud). Tabin attempts to homologise digits of both fossil and extant forms on the basis of conserved genes and expression domains, applying the 5 domains to the 8 digit limb of *Acanthostega* in which 5 different digit types are supposedly specified. Perhaps because of its simplicity, such a direct explanation for the (later) pentadactyl nature of tetrapod limbs has proved immediately attractive, with accounts in recent textbooks (Alberts et al., 1994; Gilbert, 1994).

Such a reductionist 'one domain, one digit type' hypothesis has had to be modified however, (Morgan and Tabin, 1994 – this volume), since it now appears that, in the chick limb bud, there are not 5 distinct regional nested HoxD domains and that these expression areas in the distal part shift with time in development. For example, *HoxD13* begins its expression posteriorly at stage 22, but then extends its area anteriorly until it is expressed in most of the distal mesenchyme by stages 23 and 25 (Morgan and Tabin; Fig. 2). In these later stages, almost all the distal mesenchyme is expressing all the *HoxD9-HoxD13* genes. As development continues Hox D expression fades proximally with the exception of the perichondrial tissue, surrounding the cartilage skeletal elements. Morgan and Tabin suggest now that early Hox expression is concerned in growth regulation (rather than pattern specification) of the limb bud while later expression may be regulating bone growth.

FUTURE PROSPECTS

A note of caution should be sounded about the ability of the new homeobox expression data, even when combined with experimental techniques, to solve the problem of the evolutionary developmental biology of the limb. We have information from two species, chick and mouse, and probably we can expect data soon from Zebrafish (a teleost) and from *Xenopus*. Developmental biologists sometimes give the appearance of believing evolution is from Zebrafish via axolotl and *Xenopus* to either chick or mouse and man (Hanken, 1993) and of forgetting that teleosts and urodeles should not be regarded as 'primitive' ancestors, for example of the amniotes. Coates account is a useful antidote to this belief. But in principle at least we can obtain data about gene expression in fins and limbs additionally from lungfish, coelacanth and urodeles. This at least will provide data relevant to Holland's hypothesis of gene duplication as a necessary basis for vertebrate complexity and add molecular to structural data on homology, potentially, for example, clarifying the controversial relation between urodele and non-urodele (anuran, amniote) limbs.

Implicit in much of the recent emphasis on homeobox expression domains encoding positional address is the belief in a simple relation between genes and morphology, exemplified by the 5 domains-5 digit hypothesis. But, in my view, the development of structure cannot be fragmented in this way. Parts of the skeleton are formed by co-related cellular processes and inductions organised in both time and space. A consequence of the domination of analysis of limb development by single factor one-step mechanisms (whether domains or ZPA) is the diversion of attention from experiments demonstrating sequential local interactions, including epithelial/mesenchymal ones (Hinchliffe and Horder, 1993). The extradigit phenomenon, in which an incision in interdigital mesenchyme in the developing chick leg triggers the formation of an additional digit, with joints and associated tendons, is difficult to explain in terms of ZPA or gene domain control, emphasising instead the importance of multiple local interactions (Hinchliffe and Horder, 1993). For example, one way of looking at generation of differences between the chick leg digits is to see them in terms of apical ridge stimulating the supply of mesenchyme distally to a forming digit condensation ray which is being divided into phalangeal elements by a segmentation process. Early, or late, termination of ridge activity will then control the number of phalangeal elements, respectively by reducing or increasing their number. Looked at in this way, differences in digit structure can be attributed to modification of interacting hierarchical processes (rather than to a single factor causation) and considered in Waddington's terminology (1962) as 'condition generated': a purely genetic theory of the development and evolution of structure is deficient because, as Alberch (1989) argues, genes and developmental processes cannot be dissociated as different levels of interaction.

On the positive side in our struggle to explain pattern generation within individual species, and in evolution as a whole, important evidence is provided by the new homeobox expression analysis. It is clear that at many different levels developmental mechanisms are conservative; at the molecular level, for example, in Hox D gene duplication and diversification in vertebrate evolution, and in the similarities in anteroposterior expression domains of Hox D and '*hedgehog*' genes in the tetrapod limb buds of the two species (mouse and chick) thus far examined. But mechanisms are conservative also at other levels analysed; in the iterative asymmetric branching of prechondrogenic condensations, and in ectoderm/mesoderm inductive mechanisms shown to be common to reptile, bird and mammal limb buds (since the tissue of one class understands instructive messages issued by the tissue of another class – Hinchliffe, 1991). We can surely hope to assemble in the next few years a model of limb development, with both regulatory gene and developmental process components, which, given some heterochronic variation and some novelty generation, will go far to provide a developmental basis to the 'variation on a theme' which Richard Owen identified (1849) in his pentadactyl limb paradigm model of homology. If so, this would bring development back to its true – though neglected – role as a major actor in our interpretation of the drama of vertebrate evolution.

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