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The evolution of mammalian morphology: a developmental perspective

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Abstract

Recent advances in developmental biology permit significant improvements to be made in the manner in which we interpret morphological evolution. Our knowledge of limb embryogenesis, innervation of the limb bud, and bone and cartilage biology all indicate that the inheritance of musculoskeletal morphology is best modeled as taking place via modifications of cellular relationships within developmental fields, and that details of adult limb structure should be viewed in this light. We here review some recent additions to our understanding of limb embryogenesis and discuss their use as a means for improving the interpretation of limb evolution at the species level. We provide examples from the Hominoidea, and suggest formal mechanisms for the classification of musculoskeletal traits.

INTRODUCTION

Recent advances in developmental biology have greatly improved our understanding of vertebrate morphological evolution, and molecular mechanisms for macroevolutionary events such as the origins of limbs and the fin/limb transition in gnathostomes have recently been proposed (Sordino *et al.*, 1995; Coates & Cohn, 1998). However, much of mammalian palaeontology deals with microevolutionary changes such as locomotor behaviour within orders. At this level the interpretation of musculoskeletal detail can be complicated by

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subjectivity and trait atomisation if clear genetic models for morphological evolution are lacking (Gould & Lewontin, 1979). This seriously compromises both functional and cladistic analyses of mammalian postcrania. Cladistic analysis can succeed only if the traits employed are truly independent, and problems in its application to mammalian postcrania are, therefore, exacerbated by the remarkable degree of bone plasticity in mammals, which can make an accurate interpretation of the genetic basis of adult morphology problematic. Therefore, it is fortunate that recent improvements in our understanding of skeletogenesis can provide new trait classification systems which can reduce redundancy error (Lovejoy *et al*, 1999).

EARLY PATTERNING OF THE LIMB SKELETON

Based on current knowledge, limb development is divisible into four broad phases (see Cohn & Tickle, 1996): (1) initiation (the limb bud emerges from lateral plate mesoderm); (2) pattern formation (positional information is assigned); (3) differentiation (positional addresses are interpreted leading to cell differentiation and the spatial organisation of tissues) and (4) growth of the miniature limb to adult size. Such a division is crude because there is substantial overlap among all four of these phases. However, it will be useful for the present discussion.

Our knowledge of phases 1 and 2 has recently burgeoned. Phase 1 involves the localised expression of fibroblast growth factors (FGFs) and has been extensively reviewed elsewhere (Cohn, *et al*., 1995; Vogel *et al*., 1995; Cohn & Tickle, 1996; Crossley *et al*., 1996; Cohn & Bright, 1999 Chapter 1). Phase 2 is orchestrated by specific transient specialised tissue regions, including the apical ectodermal ridge (AER), progress zone (PZ) and zone of polarising activity (ZPA). These signalling regions coordinate assignment of positional values along the primary axes of the limb bud (Saunders & Gasseling, 1968; Summerbell *et al*., 1973; MacCabe *et al*., 1974; Laufer, 1993). Recent work has identified gene products involved in such patterning, including FGFs, wnts and *sonic hedgehog* (Riddle *et al*., 1993, 1995; Laufer *et al*., 1994; Niswander *et al*., 1994; Vogel *et al*., 1995). Cells respond to these signalling molecules by expressing transcription factors such as HOX and LMX (Riddle *et al*., 1995; Vogel *et al*., 1995) which then coordinate assignment of their positional address. These are fundamental to skeletal development in vertebrates (Dolle *et al*., 1993; Small & Potter, 1996). For example, loss of function of *Hoxa11* alters the shape of the ulna/radius and tibia/fibula, and causes fused carpals, ectopic sesamoids and rib fusions (Small & Potter, 1996). When the paralogous *Hoxa11* and *Hoxd11* genes are both inactivated, the phenotype is even more severely changed (Davis *et al*., 1995). Overexpression of *Hoxa13* in the zeugopod results in the transformation of the radius and ulna into short bones by eliminating their presumptive proximal and distal growth plates (Yokouchi *et al*., 1995). Because such deviations result in extensive alterations of the body plan (Dolle *et al*., 1993; Zakany *et al*., 1997), they clearly cannot account for the limited morphological modifications that concern us here. Most of mammalian limb evolution (i.e. that below the ordinal level) must, therefore, be restricted to changes in interactions between *cis*-acting regulatory sequences and these Hox complexes and to their effects on a variety of downstream alleles (especially signalling proteins such as growth factors). The primary effects of the latter are typically delayed until phase 3 (or even phase 4), even though the positional information which guides that expression is probably acquired during phase 2.

PHASE 3: GENE EXPRESSION AND ANATOMICAL STRUCTURE

Current evidence makes it increasingly likely that the vertebrate limb is constructed by the sequential definition and construction of lineage-restricted cell domains roughly similar to the compartments of insects (Altabef *et al.*, 1997). There is now clear evidence that primary gene expression is by means of the sequential definition of spatially organised coordinate systems. These result in progressively more specific tissue boundaries. Although the molecular basis of this process is known only for the earliest phases of limb deployment, there is increasing evidence that the process is carried on for several additional steps until primary morphological structures are defined. This evidence comes from observations of limb bud behaviour following experimental manipulation of its skeleton, muscles and nerves conducted over the past two decades (Chevallier *et al.*, 1977; Landmesser, 1978a; Robson *et al.*, 1994). An excellent example is to be found in the process of muscle formation.

In early limb buds, myogenic precursor cells migrate from the somites and congregate into dorsal and ventral masses (Chevallier *et al.*, 1978). All limb muscles are formed by progressive subdivision of these masses in a sequence almost certainly orchestrated by their presumptive epimysia (the role of myogenic cells appears to be largely passive; see references in Thompson, 1988). Such an interpretation is made compelling by experimental manipulation of limbs designed to understand muscle patterning and motor innervation (Landmesser, 1978a,b). Using both retrograde horseradish peroxidase (HRP) labelling and EMG (Electromyography), Landmesser demonstrated a 'definite regionalization of . . . neuronal projection' within each primary muscle block prior to its cleavage into individual muscles. She observed that axons 'appeared to recognize and to respect pre-muscle boundaries' during the cleaving process (Landmesser, 1978a: 411–412). Each muscle block demonstrated a regular pattern of segmental innervation which could be mapped using HRP injection prior to its individuation into particular muscles. When the cell bodies of these axons were then mapped to the cord, it was demonstrated that the adult somatotypic pattern was present from the beginning of regionalisation, and that the segmental innervation of the adult musculature was traceable directly to the earliest penetration of axons into the dorsal and ventral blocks. In fact, some preliminary division of these blocks had certainly already occurred when the EMG/HRP mapping process was conducted. Detailed, ultrastructural studies of the muscle splitting process now confirm that it is under direct mesenchymal control and that 'an early role of positional information may be to instruct the pluripotent population of mesenchyme cells to form connective tissue along incipient cleavage zones or to assure that cells committed to this fate array themselves in proper places' (Schroeter & Tosney, 1991: 367). In fact, the probable fundamental basis of the earliest phases of such subdivision can now be linked to early HOX expression in the cells of the dorsal and ventral muscle blocks. Recent analyses demonstrate that some individual muscles which emanate from this progressive splitting process evince individually differentiated patterned histories of *Hoxa11* and *Hoxa13* expression. This suggests that specific combinations of gene expression are involved in the determination of individual muscle identity and that these identities are acquired within the early limb bud (Yamamoto *et al.*, 1998).

FUNCTIONAL INTEGRATION WITHIN THE LIMB BUD

The limb bud demonstrates an impressive degree of integration during the emergence of its tissues. If the skeleton is manipulated during Phase 2, for example (such as by a transplantation

of ZPA tissue), downstream changes in its investing musculature and nerves are later generated (Robson *et al.*, 1994; Yamamoto *et al.*, 1998). Essentially, any alteration of a cell signalling centre induces a cascade of events which simultaneously alters the bones, muscles and innervation of the limb (see below), but the novel derivatives are *fully recognisable* and are *functional structures* (reviewed in Hinchliffe, 1994).

This was demonstrated by Muller, who following Hampe's well-known earlier work (Hampe, 1959, 1960; Muller, 1989), inserted a foil barrier into the presumptive distal crura of the Y-shaped blastema of the emerging zeugopod in chick limb buds. Although interpretations differ on how the bony changes arise (Archer *et al.*, 1983), Muller found that *m. flexor perforans*, a muscle normally restricted in origin to the tibiotarsus and fibula, often showed a 'strong muscular head' from the lateral femoral condyle, a condition which is the normal state in the muscle's reptilian homologue. Similar changes were observed in *m. popliteus* and *m. fibularis brevis*. Muller concluded from his experiments that there is an extensive 'interdependence of muscle and bone formation, especially during the phase of muscle insertion and attachment' (Muller, 1989: 42).

Muller's experiments provide a direct window into potential mechanisms of local morphological evolution. Altering limb mesenchyme can clearly lead directly to systematic shifts in musculoskeletal morphology. Such shifts could be produced by slight changes in morphogen or cell communication gradients, by dosage effects (Zakany *et al.*, 1997) produced by changes in the cellular expression of signalling molecules, or simply by morphogenetic movements in which cells with slightly altered positional identities are diverted to new positions. Most importantly, Muller's experiments demonstrate that even when relatively crude (i.e. essentially non-directed) changes are introduced in such manipulative protocols, anabolic limb mechanisms are still fully capable of their integration into a new phenotype. There is no reason to suppose that the novel musculature which resulted from insertion of the foil would not have been capable of coordinated locomotor activity (entire limb segments, when transplanted to ectopic locations, become fully innervated (Lance-Jones & Landmesser, 1981; Lance-Jones & Dias, 1990; Weiss, 1990)).

Recent work on Hox genes can again shed light on how downstream genes might establish such morphological patterning. Ectopic expression of *Hoxa13* using a retroviral vector results in transformation of the tibia and fibula into shorter bones (resembling tarsals) by altering cell adhesive and histological properties (Yokouchi *et al.*, 1995). *In vitro* assays showed that cells expressing *Hoxa13* homophilically reassociate and sort-out from non-expressing cells. This suggests that Hox genes may up-regulate cell adhesion which would, in turn, determine the size (and shape) of initial cartilage condensations and their associated muscle blocks. Cell adhesion molecules (CAMs) contain homeoprotein-response elements in their promotor regions (reviewed in Edelman & Jones, 1995), and several Hox genes and NCAM are expressed in perichondria. Phase 3 morphogenesis certainly involves more than cell adhesion, but controlling the size and form of the initial anlagen is an important mechanism for guiding morphological pattern. Inasmuch as such events lie clearly downstream of the *initial* Hox expression in the limb bud, it is tempting to suggest that more fine scale patterning of the limb tissues is accomplished by additional phases of Hox expression within increasingly restricted lineage based territories. Such a means of tissue localisation would certainly be in keeping with the general pattern of *Hox* utilisation which is to sequentially redeploy previously expressed *Hox* systems during increasingly localised cycles of developmental events (Charite *et al.*, 1994).

In addition, anlage shape is almost certainly dependent on the local expression of growth factors, such as the secreted transforming growth factor-β-related proteins (TGFβ, BMP

(Bone Morphogenetic Protein), GDF (Growth and Development Factor); reviewed in Kingsley, 1994). BMP expression induces mesenchymal cell condensation and subsequent differentiation into cartilage and bone. Different BMPs are capable of dimerising to form complexes (Kingsley, 1994), and variation in BMP expression patterns, synthesis, diffusion kinetics and/or receptor activation could generate anatomical differences in the skeleton (Kingsley, 1994). For example, a mutation in the mouse BMP-5 gene causes the short ear mutation which results in multiple skeletal defects, including altered curvature and width of long bones, loss of ribs, decreased ability to repair fractures, and loss of ventral and lateral vertebral processes (Kingsley *et al.*, 1992). A mutation in the related GDF-5 gene is responsible for the brachypodism mutation which causes skeletal abnormalities specifically in limbs, such as shortened long bones and reduction in the size and number of bones in the paws (Storm *et al.*, 1994). Obviously, such mutations are not viable routes for localised, small-scale bone modification, but differential numbers and combinations of BMP expression during skeletogenesis would certainly seem a reasonable mechanism of generating variation in limb morphology.

PHASE 4: GROWTH AND MODELLING

A key innovation in tetrapod evolution was the capacity of connective tissue cells to respond differentially to local mechanical stimuli. Much of mammalian bony morphology emerges by this mechanism during growth and is maintained during adulthood as a consequence of highly conserved response protocols resident within osteocytes and related cells. Modelling involves spatially coordinated bone formation. In addition to bone strain (Hall, 1992a,b), factors produced both systemically by the immune system and locally within bone matrix are thought to be involved, including interferon, interleukin-1 (IL-1), tumour necrosis factor, insulin-like growth factors and TGFβ (Mundy, 1989). The latter is believed to play a role in modulating the switch between resorption by osteoclasts and deposition by osteoblasts. This could operate by a feedback loop in which active TGFβ released from bone matrix during resorption by osteoclasts would, perhaps at threshold levels, inhibit osteoclast activity (Mundy, 1989). Factors released during resorption are chemotactic and mitotic for osteoblasts, which are recruited to generate bone matrix. This plasticity is likely to be the result of genetically determined (and highly conserved) cellular response protocols resident within each cell.

These same processes are very likely pivotal to normal development and the emergence of the adult bone from its initial anlagen. In fact, it has recently been demonstrated that even the cells of undifferentiated mesenchyme respond differentially to imposed local compression by up-regulating and down-regulating (respectively) the *Sox9* and IL-1 genes, which result in both cellular positional changes and the production of type II collagen (Takahashi *et al.*, 1998). Carter and colleagues have used finite element modelling to demonstrate that using relatively simple cellular response 'rules', excellent representations of adult bone form can be generated from simple anlage and that primary and secondary ossification patterns can be accurately predicted (Carter, 1987; Carter & Wong, 1988; Carter *et al.*, 1991; Carter & Orr, 1992). The combination of these two kinds of studies raises the strong possibility that bone rudiment growth, initial and progressive ossification, and joint cavitation may be partially autonomous responses, epigenetically 'canalised' by local mechanical forces, and regulated by the interaction of each original anlage with its investing soft tissue fields (Murray, 1935; Moss, 1978; Wolpert, 1981). Carter has

suggested that much of the process of ossification (*sensu stricto*) of the anlage may be epigenetic: 'Although it appears that positional information is involved in early skeletal chondrogenesis, . . . our studies raise the possibility that . . . further skeletal development is directed primarily by the influence of stress history on gene expression. (Carter, 1987: 1096; see also Wong & Carter, 1988: 56–58). We take a more 'conservative' view at this time, however, and continue to regard at least the earliest phases of ossification as most probably involving both mechanical reactivity on the part of connective tissue cells and downstream expression of cell programmes assigned earlier as a consequence of positional address.

The morphological patterning that is generated during Phases 3 and 4 could take place by various combinations of these two very different anabolic mechanisms. It might be the consequence of specific strain regimens imposed on cells (which would be position dependent and thereby a product of primary anlagen form (along with its soft tissue envelope), or it could be the direct effects of positional information on subsequent transcriptional regulation. In either case, however, adult limb morphology represents (either directly or indirectly) an expression of Phase 2 positional information, followed by regimented and highly conserved cell response mechanisms operating downstream. The primary locus of local morphological evolution must therefore lie in the establishment of positional fields during Phase 2 of limb development.

SOME IMPLICATIONS FOR THE INTERPRETATION OF MAMMALIAN SKELETAL EVOLUTION

These recent advances in our understanding of development carry important implications with respect to the evolution of mammalian limb morphology. One of the most striking is that local morphological changes are almost certainly generated by slight modulations of Phase 2 patterning. These then lead to fully coordinated changes in adult structures because of the highly conserved anabolic machinery of the limb. This implies that many morphological changes which lack direct mechanical significance must uniformly accompany novel postcranial adaptations, and that the genetic basis of limb evolution lies in polymorphic systems and not isolated structures. If most target phenotypes (those favoured by selection) are acquired by slight shifts in limb tissue fields, then many of the individual adult 'traits' currently used in broad-scale morphometric and cladistic analyses are both artificial and potentially misleading. Such considerations are especially crucial in the analysis of completely novel locomotor and phylogenetic characters which may appear in unique fossil taxa such as the new hominid *Ardipithecus ramidus* (White *et al.*, 1994, 1995).

Consider, for example, some 'traits' which can be enumerated in separating the hips of bipedal humans from those of quadrupedal apes. Humans bear uniquely broad sacra, lumbar columns with distally expanding (i.e. L1 to L5) zygopophyseal joints and centra, marked retroflexion and anteroposterior broadening of the ilium, and a host of additional features which can each be individually defined and enumerated (Lovejoy *et al.*, 1973; Lovejoy & Latimer, 1997). Each of these could be isolated and treated independently during either a taxonomic (cladistic) or functional analysis. However, not even a minority of these traits are likely to have been individually fixed in the human genome by the action of natural selection, simply because virtually none is an isolated product of simple gene expression upon which selection could act. Most are almost certainly the consequence of field shifts in presumptive limb tissue fields, and the mechanism by which any change in

pelvic form has been achieved is therefore by alteration of their antecedent positional address. Such changes are almost certain to yield many downstream effects, only some of which represent target (selected) adaptations. The highly modified pelvis of hominids can therefore be expected to demonstrate morphological consequences of field shifts which have altered patterns of periosteal investment and its effects on the underlying bone's surface topography. However, many such phenotypic adjustments probably have no other direct mechanical significance.

This leads to an equally important corollary with respect to the interpretation of musculoskeletal function. Structures which can easily be shown to be the epigenetic effects of others can still be readily shown to demonstrate 'function'. In humans the *m. plantaris* is obviously a plantarflexor and in apes the *m. dorsi-epitrochlearis*, if tested by EMG, can probably significantly affect forelimb mechanics. Are these therefore positively selected structures which have been fixed because they improved actual Darwinian fitness? Almost certainly they are not, and instead almost surely represent collateral consequences of field shifts whose primary morphological effects were far more functionally significant.

In summary, adult bone form is a product of (1) the precise construction of its primordium (and its accompanying soft tissue envelope) by assignment of positional information during limb bud deployment, and (2) the highly conserved anabolic behaviour of the cells within that primordium and their descendants. Although the former is unique to a species, or group of closely related species, the latter is not. It is instead shared among large numbers of related taxa, and is the primary source of the remarkable phenotypic plasticity (often collectively termed modelling and remodelling) characteristic of mammals. By definition, any differences between individuals which derive from the latter are not subject to selection, since they are not individually heritable (though they clearly play an important role in such phenomena as genetic assimilation (reviewed in Hall, 1992c)). Conversely, any change in Phase 2 pattern formation is completely heritable, because the precise structure of condensations and anlagen is directly generated by positional information. Therefore we propose that adult traits be formally classified, whenever possible, into two broad categories which reflect these two very different components of their development.

We suggest that traits which differ in two or more taxa because they differ in their respective positional fields be called archigenetic (G. archai, origin, beginning + G. genos, birth). Their adult manifestation is a direct consequence of positional information deployed during Phase 2. If a trait differs in two taxa because a change in a developmental field has occurred, it is archigenetic.

Such traits can be contrasted with ones which we shall call actogenetic (L. actio, to do + G. genos). These owe their expression to cell response regimens which are common to most mammals and which are shared by them, i.e. the generalised anabolic machinery of mesenchyme and its derivatives. Actogenesis is that process defined above as anabolism during Phases 3 and 4. The importance of making a clear distinction between these two types of morphogenesis justifies their specific definition. As an example, we have elsewhere (Lovejoy *et al.*, 1999) defined five specific trait categories which then can be formally applied to fossils in a systematic way. These are included in Table 1 together with an example of each for the hominid postcranium. Types 1, 2 and 3 are archigenetic and Types 4 and 5 are actogenetic.

Although Type 2 traits are archigenetic, they are reproductively neutral and therefore non-Darwinian. In the analysis of fossils they may be used as evidence that a field shift has occurred even though they themselves were not the target of selection. For example, hominid pelves exhibit dramatically shortened pubic symphyses compared to those of apes. Although mechanical interpretations can be invoked for this difference, it is most likely a

Table 1 Proposed analytical trait types

Type 1 A trait which differs in two taxa because its presence and/or expression are downstream consequences of significant differences in the positional information of its cells and their resultant effects on pattern formation. Type 1 traits are fixed by directional and/or stabilising selection because their primary functional features have a real effect on fitness, and result largely from a direct interaction between genes expressed during tertiary field deployment and the functional biology of their adult product. Example: the superoinferior shortening of the ilium in hominids.

Type 2 A trait which is a collateral consequence of changes in positional fields which are naturally selected (Type 1), i.e. they are byproducts of field changes whose principal morphological consequences do provide significant functional benefits to their phenotype. Type 2 traits differ in two taxa because of differences in pattern formation (as in Type 1), but their functional effect is so minimal as to have had no probable real interaction with natural selection. Their principal difference from Types 4 and 5 is that they represent true field derived pleiotropy. Example: the superoinferiorly shortened pubic symphyseal joint of hominids (for discussion see text).

Type 3 A trait which differs in two taxa because of modification of a systemic growth factor which affects multiple elements, such as an anabolic steroid. Example: body size and its allometric effects.

Type 4 A trait which differs between taxa and/or members of the same taxon because its presence/absence and/or 'grade' are attributable exclusively to phenotypic effects of the interaction of connective tissue 'assembly rules' and mechanical stimuli. Such traits have no antecedent differences in pattern formation, and therefore have no value in phyletic analysis. They are epigenetic and not pleiotropic. However, they provide significant behavioural information, and are of expository or evidentiary value in interpreting fossils. They often result from habitual behaviours during development and/or adulthood. Example: the cortical bone patterning of the hominid femoral neck.

Type 5 Traits arising by the same process as Type 4 but which have no reliable diagnostic value with respect to behaviour (even though they may have been previously so regarded, e.g. development of the intertrochanteric line in human femora). Such traits are not consistently expressed within species and often show marked variation of expression within individuals and local populations. Example: femoral anteversion.

simple consequence of reduction in the superoinferior height of the entire pelvic field in hominids, the primary effects of which are to reposition the ilia for effective abduction and to approximate the sacroiliac and hip joints; i.e. the novel (dramatically shortened) pubic symphyseal face of hominids has no immediate mechanical significance and is a byproduct of changes induced elsewhere by natural selection: it is a Type 2 character.

Clearly if this is the case, a mechanical explanation of pubic symphyseal shortening and its treatment as a Type 1 character would seriously compromise its inclusion in a cladistic analysis. Such inappropriate reliance on adaptationist interpretations of structures greatly reduces cladistic power because it excessively weights what are in reality single characters. A number of additional examples may be noted, but another relating to the primate postcranium would seem most appropriate here.

A number of primates have greatly reduced first metacarpal rays, some of which are vestigial. Selective explanations of such structural reductions have been offered (e.g. a long first ray 'interferes' with use of the lateral four digits during active suspension). If accepted, such attributions of first ray reduction to the action of natural selection could justify their classification as Type 1 traits, and thereby their inclusion in a cladistic analysis. However,

given the autopod's response to experimental manipulation of the ZPA, there is an obvious probability that marked differences in presence/absence of rays and their relative development is directly regulated by genes expressed in the earliest phases of autopod definition including those responsible for long- and short-range secreted signalling molecules, modulation of homeobox-containing genes, etc. This provides a more probable explanation of first ray reduction than natural selection to reduce its size. For example, change in one or more enhancer elements, their timing of expression, or other mechanisms that have potential dosage effects could have been involved. Such changes could readily result in elongation of the posterior four digits and accompanying reduction of the first. This is certainly in accord with the distribution of thumb reduction in primates which is usually greatest in those species that rely heavily on forelimb suspension and exhibit elongated digits 2–5. In fact there now appears to be some substantial basis for such a change as early as first Hox expression in the autopod. Various combinations of loss of function mutations for *Hoxd13* and *Hoxa13* have very substantial effects on the first ray, and both the dose and distribution of their protein products may specifically underlie first ray reductions in primates (Fromental-Ramain *et al.*, 1996).

Presuming an inverse relationship between selection intensity and phenotypic variability, Tague (1997) tested a form of this hypothesis on a large sample of anthropoid primates. A strong correlation was found between reduction of the first ray and relative variance in several simple dimensions (if selection was the cause of first ray reduction, it should not have led to increased variability). On the other hand, great care must be taken in accepting this particular explanation for increasing or decreasing variation in any particular skeletal character. Given that much of the process by which early positional information is 'translated' into final morphology involves the expression of highly conserved cell response protocols as described above, much of the downstream variation arising in such structures may therefore be largely epigenetic and a consequence of the subtleties of the interplay between early tissue fields and the entire genomic background and connective tissue configuration which constitute the 'canvas' on which they are expressed. Thus much of adult variation may not be individually heritable because it results from the cascade of events originating from graded cellular expression as 'interpreted' by largely immutable response rules (i.e. changing any of such rules would have a systemic effect on the entire skeleton: see below). Such an interpretation has a profound impact on the way we have traditionally viewed variations in skeletal morphology, i.e. that such variation can serve as the 'raw material' for alterations of musculoskeletal form. Such may very well not be the case.

A second, similar example, can be used to demonstrate that functional analyses can be improved by a developmental approach as well. Pedal phalangeal length in *Australopithecus afarensis* is intermediate between modern humans and the great apes. Two very different interpretations have been proffered. One is that their length reflects active use in arboreal substrates. A second is that it reflects ongoing reduction of an anatomical structure whose primary function (grasping) is no longer employed because the animal is an habitual terrestrial biped.

Neither explanation is entirely satisfactory from the point of view of evolutionary theory. The first suffers simply from the fact that the pedal digits of *A. afarensis* exhibit any reduction at all. If still employed in arboreal grasping (and therefore under the purview of natural selection), why should any reduction have occurred? The second suffers from a similar dilemma when viewed from the perspective of classical evolutionary theory: why have toes undergone significant reduction instead of simply becoming more variable following a relaxation of stabilising selection?

Attempts to posit directional selection for toe reduction are notably weak – suggestions of a greater likelihood of injury or greater energetic cost during locomotion being wholly inadequate when examined from the perspective of their potential impact on actual reproductive success. This is especially true since equally good 'functional' arguments can be proffered for retention of long toes by a metatarsifulcrimating biped known to have frequented swampy lake margins.

Reference to the limb's developmental cascade may again provide a more probable accounting of pedal digital reduction in these early hominids. Substantial evidence now indicates that a significant number of pattern formation alleles are shared by the autopods of both fore and hind limbs. In fact, whereas chick zeugopods differ substantially in their overall Hox expression patterns, their autopods exhibit very similar patterns, despite obvious strong adult morphological differences (Nelson *et al.*, 1996). One very real possibility, therefore, is that the enhanced power and precision grip of later hominids (e.g. *Homo habilis*) made possible by an increased length of the pollical phalanx and a simultaneous reduction in the phalanges of the posterior four digits, was at least partially effected by changes among alleles contributing to autopod pattern formation in both limbs. Absent selection for retention of long pedal digits in a fully terrestrial biped, pedal digital proportions would have no stabilising effect on any genomic shifts affecting digital proportions in the metacarpus and its phalanges, which would serve as the primary focus for genetic change. Directional selection could therefore readily cause simultaneous reduction of the phalanges of the posterior four rays in both hands and feet, even though the primary target of selection was restricted to the digital proportions of the hand. This same type of (Type 2) mechanism is the most likely explanation of the enlarged thumb-like sesamoid bones in the hind feet of the giant panda, which 'appear to be without function, but which match a functional set on the forelimbs' (Roth, 1984: 21; see also Endo *et al.*, 1996).

Such a hypothesis is testable. If correct, there should be a significant correlation between the individual digital elements of the hands and feet, independent of any covariation with body size. We measured the length of the proximal phalanx of the third digit in 30 modern human hands and feet (data not shown), and then removed the effects of size by calculating a Pearson partial correlation, controlling for femoral and humeral lengths. The phalanges exhibited partial correlations of 0.56 (P=0.001). As expected, the coefficient of variation (CV) was higher for the pedal phalanges (11.5) than for those of the hand (8.7).

As with the earlier case, however, there remains an additional and more probable explanation of toe reduction in hominids. In the absence of any need for grasping, the phalanges of the toes become largely superfluous structures, essentially secondary to the mechanical and anatomical relationships among the five metatarsals, whose heads become the primary point of fulcrimation during locomotion and whose form and structure therefore determine the benefits of any change in their anatomical structure. Any changes in the tissue fields of the foot which altered the anlagen of the metatarsals (and their relationships with the more proximal tarsals) could very well have downstream effects which greatly altered the epigenetic metamorphosis of any more distal structures (i.e. the phalanges) during development. Absent any selection to prevent such increased epigenetic 'entropy', reduction and dysgenesis become increasingly likely, and contra the suggestion made earlier on the basis of classical evolutionary theory, increased variation is therefore not necessarily the expected outcome of relaxed selection on specific aspects of morphogenesis. Inasmuch as final morphological form is dependent on both gene expression and the mechanical environment in which musculoskeletal structures develop, additional changes specific to the foot which affected the distribution of its digital musculature, in combination with length reduction of its phalanges

brought about by coincident reduction of those in the hand, become the most likely pathway by which the highly reduced phalanges of the lateral four digits of the hominid foot evolved.

As demonstrated by the example just cited, the definition of 'total morphological pattern' (Le Gros Clark, 1978) of any musculoskeletal structure is clearly a complex interaction of traits potentially belonging to all five of the operational classes designated in Table 1. In fact, many traits may be the product of more than one aetiological class. One obvious area of difficulty is traits which could be allocated to either Type 2 or Type 4. As noted above, hominids evince sweeping changes in the structure of their pelvis, with dramatic supero-inferior reduction in iliac height and an equally significant increase in anteroposterior breadth. It is possible, therefore, that reorganisation of the pattern formation field(s) which generate the ilium may have so altered its anteroinferior region to have caused isolation of a portion during growth. If so, then their unique anterior inferior iliac spine (AIIS; arising from a separate apophysis) represents a Type 2 trait. However, the alteration of iliac position, with the adoption of complete bipedality, causes this site of attachment of the iliofemoral ligament and long head of *m. rectus femoris* to undergo greater shear stress than it would if the hip were predominantly more flexed as in quadrupedal progression, and its separate apophysis may simply be a modelling phenomenon. If so the trait is of Type 4. In either case, however, the AIIS is a byproduct of a Type 1 change and is not itself, therefore, a product of selection. If the latter hypothesis is correct, however, it does serve as a significant functional marker of an habitual behaviour (erect posture), and is therefore of functional, but not taxonomic, significance.

As always, detailed study of both comparative anatomy and the fossil record can supply important data in carrying out trait classifications. An obvious example involves one of the specialized morphological features of the hominid lumbar spine. Chimpanzees must normally walk with a flexed hip and knee while bipedal because of their virtually immobile lower back. Early hominids, in order to either maintain or reintroduce spinal mobility [see Lovejoy and Latimer, 1997 for discussion], appear to have increased the number of lumbars to six (or they may have maintained the long lumbar column of a less derived common ancestor) and also to have evolved a progressive (craniocauded) increase in the interfacet distances of their lumbar zygopophyseal joints (Latimer and Ward, 1997). These two features permit substantial lordosis and allow the center of mass to be positioned over the foot, eliminating any need for a flexed hip and knee gait.

A simple developmental mechanism by which such a progressive increase in interfacet distance could be introduced would be a sequential enlargement of the lumbar anlagen. However, Latimer and Ward have shown that humans exhibit the same pattern of centrum size increase as do other hominoids, concluding that "no regional increase in areal dimensions has occurred in hominid evolution" (p.289). Moreover, a review of the data presented by these same authors also demonstrates that the progressive increase in interfacet distance seen in human lumbars may be a simple natural continuation of the same pattern seen in the thoracics of all hominoids or even primates (see their Figures 12.3 and 12.4). It would be of interest to investigate whether or not this pattern, in fact, is a simple primitive primate pattern, rather than a hominid specialization as currently held. If this is the case, then the reduced interfacet distance of chimpanzees should instead be viewed as a specialized (Type 1) adaptation of their lower spine to reduce mobility, with the capacity of imbrication being simply either a retained 'primitive' character in hominids or one which has bee 'reintroduced' if the last common ancestor of humans and chimpanzees exhibited these major adaptations to restrict lumbar mobility. In short, the loss of the ability to imbricate may be a Type1 apomorphy in chimpanzees and a retained primitive character in hominids.

CONCLUSIONS

We have provided some guidelines for the interpretation of fossils based on an emerging understanding of mammalian limb development. Our approach emphasises overall transitions in morphology rather than minor shifts of individual structural detail. Our purpose has been to systematically differentiate functional traits which have been directly fixed by selection from collateral, largely pleiotropic, ones. Furthermore, just what morphological variants become available for review by the selective process is highly circumscribed by a rigorously preserved developmental cascade, and the nature of that cascade negates many contemporary hypotheses about musculoskeletal functions which invoke meticulous detailing of anatomical structures in mammals. Selection cannot act on individual characters unless they are independently heritable, and our current knowledge of musculoskeletal development proscribes a majority of such 'functional' explanations. Therefore, interpretations of novel mammalian morphology should incorporate, wherever possible, proposed accounts of adult structural change which are congruent with known underlying mechanisms of pattern formation, and in the absence of such pathways, morphological analyses should be regarded as incomplete statements of hypothesis.

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