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REVIEW ARTICLE

Mechanoadaptation of developing limbs: shaking a leg

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Abstract

The proportion of total limb length taken up by the individual skeletal elements (limb proportionality), varies widely between species. These diverse skeletal forms have evolved to allow for a range of limb uses and they first emerge as the embryo develops, to achieve the characteristic skeletal architecture of each species. During this time, the developing skeleton experiences mechanical loading as a result of embryonic muscle contraction. The possibility that adaptation to such mechanical input may allow embryos to coordinate the appearance of skeletal design with their expanding range of movements has so far received little attention. This is surprising, given the critical role exerted by embryo movement in normal skeletal development; stage-specific *in ovo* immobilisation of embryonic chicks results in joint contractures and a reduction in longitudinal bone growth in the limbs. Epigenetic mechanisms allow for selective activation of genes in response to environmental signals, resulting in the production of phenotypic complexity in morphogenesis; mechanical loading of bone during movement appears to be one such signal. It may be that 'mechanosensitive' genes under regulation of mechanical input adjust proportionality along the bone's proximo-distal axis, introducing a level of phenotypic plasticity. If this hypothesis is upheld, species with more elongated distal limb elements will have a greater dependence on mechanical input for the differences in their growth, and mechanosensitive bone growth in the embryo may have evolved as an additional source of phenotypic diversity during skeletal development.

Key words: embryo movement; epigenetics; limb development; mechanical loading.

Introduction

Limb proportionality, or the proportion of the total limb length taken up by individual skeletal elements, can vary a lot between species. For example, the metatarsals of the jerboa, a hopping desert rodent, are hugely extended relative to those of the mouse. There is also a level of normal intra-species variation; the sprinter Usain Bolt has a larger proportion of lower to upper leg length compared to most humans (E. Otten, personal communication), and it has been suggested that his record-breaking running speed can be attributed to this variation in limb anatomy. Throughout evolution, vertebrates have adopted a vast range of different locomotor habits and limb uses, which their diversity in forms of musculoskeletal anatomy make possible.

These diverse skeletal forms emerge as embryos develop to achieve the characteristic skeletal architecture of each species and, to some extent, each individual. One important factor in development which may play a critical role in establishing these functional skeletal forms is mechanical input. Cellular mechanics play many roles in early limb morphogenesis. Thus, cell movement-generated forces influence condensation of cartilage elements in developing limbs. There is also evidence that fundamental processes, including growth, differentiation, death and directional motility of cells, are likely guided by forces exerted by the cell cytoskeleton. This conforms with 'tensegrity' principles described by Ingber (2006), with differential growth patterns producing local extracellular matrix distortion and the generation of tension in the cytoskeleton of associated cells. Microenvironmental cell-matrix mechanics have also been implicated in growth and transcription factor activation (Tenney & Discher, 2009). Tissue deformation has been shown to modulate *Twist* expression in Drosophila embryos, which is necessary for midgut differentiation (Farge, 2011). However, in this review, we will focus on the role of mechanics in later stages of vertebrate limb development, when the developing skeletal elements experience mechanical loading forces as a result of embryonic muscle contraction.

In all species, the developing skeleton experiences mechanical loading from embryonic movements *in utero* and *in ovo*. These movements begin early in development; as early as day 5 of incubation in the chick (Hamburger &

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Balaban, 1963; Bekoff, 1981), day 12.5 of gestation in the mouse (Carry et al. 1983; Hanson & Landmesser, 2003) and in humans the first movements have been detected at 7.5–8 weeks' gestation (deVries et al. 1982). These embryonic muscle contractions exert loads of increasing magnitude and coordination with growth (Hamburger & Balaban, 1963; Hamburger & Oppenheim, 1967; Sharir et al. 2011). These movements and loads can be influenced by a range of environmental factors and also altered both genetically and pharmacologically (Pitsillides, 2006).

Adaptation to altered load bearing is known to modify adult skeletal tissue but the possibility that this highly dynamic and exquisitely sensitive system may also therefore furnish the embryo with the capacity to coordinate the appearance of its skeletal design with its expanding range of movement during development has been largely ignored. Current paradigms dictate that dynamic loading in adult bones produces extracellular fluid flow within the bone's lacunar-cannalicular system, which is detected by osteocytes (Kim et al. 2006). This is associated with the production of secreted factors such as nitric oxide and prostanoids (Pitsillides et al. 1995; Klein-Nulend et al. 1998; Westbroek et al. 2000), and with decreased levels of sclerostin (Robling et al. 2008), which act to regulate bone resorption and formation. These responses allow for matching of the dynamic loading associated with exercise to resultant increases in bone mass and mineral density *in vivo*, and also underpin the reduction in bone mass, mineral density and matrix protein formation associated with extended periods of disuse. However, less is known about the role of mechanical input during the creation of skeletal form and morphology during development, and this lack of knowledge regarding bone architecture and mass extends equally to both the forming cartilage and the growth plate.

There is substantial evidence that embryo movement is necessary, however, for normal skeletal development. Congenital neuromuscular disease and other conditions which cause decreased fetal movement can result in fetal akinesia deformation sequence. Anatomical abnormalities associated with this syndrome include joint contractures and bone changes such as decreased cortical thickness, among other clinical signs (Rodríguez & Palacios, 1989; Fanconi et al. 1995), indicating that this sensitivity to the embryonic movement extends beyond bone to other components of the musculoskeletal system. Embryonic muscle contraction also appears to be necessary for the formation of bone ridges, which act as anchoring points for muscle attachment and are therefore important in the transduction of muscle-induced loading via tendons to the skeleton. (Blitz et al. 2009). Observations from animal models of *in ovo* and *in utero* immobilisation have also demonstrated that the skeletal proportionality along the bone's proximo-distal axis, essential for differing skeletal designs (Fig. 1), is influenced by embryonic movement. This suggests that the development of skeletal morphology may be subject to epigenetic mechanical regulation. Whether species with different limb proportionalities exhibit differential sensitivity to the effects of embryo movement is not currently known.

The establishment of skeletal form in limb development is a complex problem. Limb buds arise from the lateral plate mesoderm and the limb bud mesenchyme condenses into centres of chondrification to form cartilage models of the limb bones. These go through the stages of proliferation

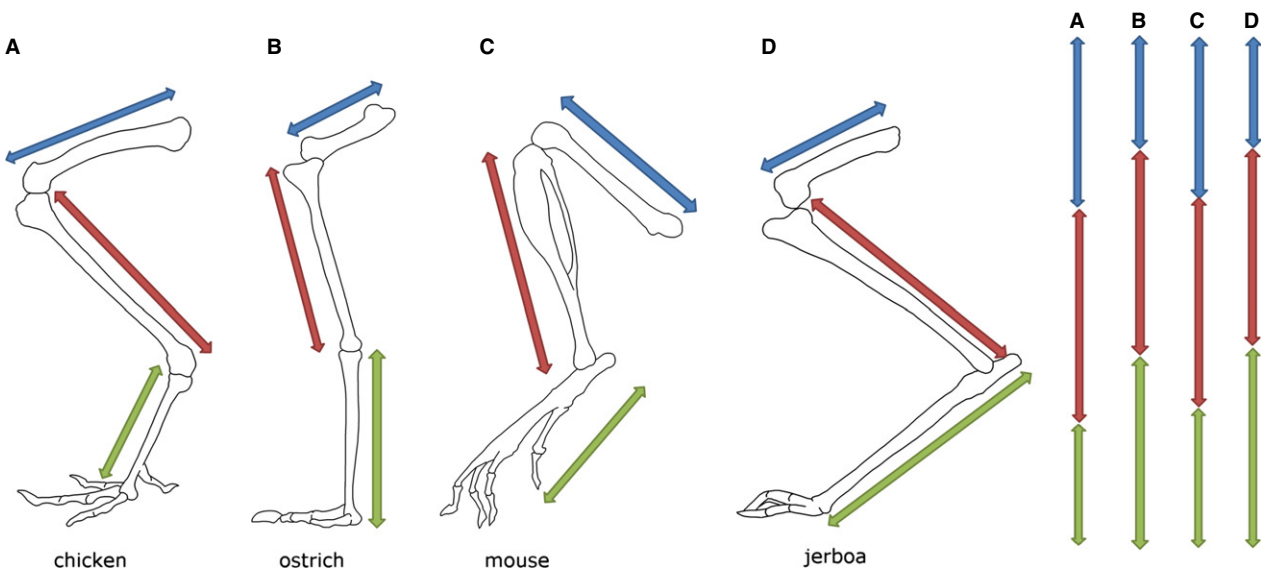

Fig. 1 Variations in limb element proportionality – the proportion of total limb length taken up by the individual limb elements – between species. In species adapted for rapid terrestrial locomotion such as the ostrich (B) and jerboa (D) there is a tendency towards relative elongation of the distal and reduction of the proximal limb elements.

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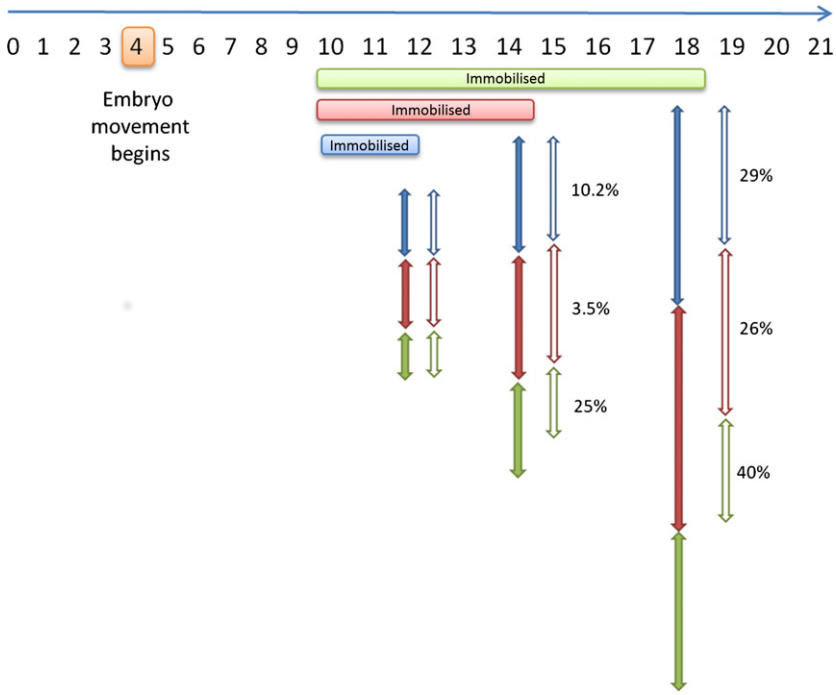

Fig. 2 Reduced longitudinal growth of chicken embryo limb elements following *in ovo* immobilisation between E10–11, 10–14 and 10–18. Normal limb elements are represented by solid arrows and immobilised elements by outlined arrows. Percentage reductions in element length are given, except for E10–11 limbs where no significant reduction was reported. Data from Lamb et al. (2003) and Pitsillides (2006). Immobilisation prior to E14 appears to have little effect on growth.

and hypertrophy and subsequently form individual skeletal elements, which develop sequentially in a proximo-distal direction (reviewed, Towers & Tickle, 2009 and Oster et al. 1988). These cartilage models are eventually replaced by bone in the process of endochondral ossification (Staines et al. 2013). These endochondral ossification processes, involving chondrocyte proliferation, hypertrophy and mineralisation, may each be influenced by different levels of embryo movement to coordinate longitundinal expansion of the skeletal elements. The mechanisms by which these processes are regulated by mechanical input are not, however, well understood; in this review we hope to identify avenues for further investigation.

Embryonic motility is necessary for normal joint formation

Skeletogenesis is an area of developmental biology for which the importance of mechanical input has been established since the 1920s (Murray, 1926; Murray & Selby, 1930 and Fell & Canti, 1934). These early experiments showed that the initial stages of limb development in embryonic chicks can occur independently of the limb's normally contiguous elements, including skeletal muscle attachment but that, in contrast, muscle contraction is absolutely necessary for joint cavitation to occur. Drachman & Sokoloff (1966) demonstrated that the induction of paralysis in embryonic chicks *in ovo* with the neuromuscular blocking agents decamethonium bromide (DMB) and type A botulinum toxin resulted in absent or minimal knee, ankle and toe joint cavities (other joints were not examined), leading to cartilaginous fusion of opposing limb elements. This demonstrates that muscle contraction, and presumably the load that it generates, is essential for the formation of all of these joints in this species. The extent to which this dependence on mechanical input extends to all species and all joints is only now beginning to surface.

Since these early experiments, further studies have confirmed the importance of skeletal muscle contraction in cavitation of the hip, knee, ankle and metatarsophalangeal (MTP) joints (Ruano-Gil et al. 1978; Mikic et al. 2000; Pitsillides, 2006). Roddy et al. (2011a,b) showed that *in ovo* immobilisation of embryonic chicks alters cellular organisation of the interzone and results in changes in shape of the distal femur and proximal epiphysis of the tibiotarsus and fibula. After cavitation occurs, maintenance of joint cavities is also dependent on mechanical input. Post-cavitation induction of flaccid paralysis with pancuronium bromide, a non-depolarising neuromuscular blocker, also leads to loss of the joint cavities. Rigid paralysis induced with DMB, a depolarising neuromuscular blocking agent, causes muscle contraction and has been shown to partially maintain joint cavities (Osborne et al. 2002). This indicates that some degree of static loading, resulting from DMB-induced muscle contraction, on embryonic bone prevents total loss of the joint cavity. However, dynamic loading is necessary for normal development. In the elbow joints of mice, in which there is a genetically engineered absence or failure in contraction of skeletal muscle, joint progenitor cells do not maintain their designated fate; they fail to express normal joint markers and instead become chondrocytes (Kahn et al. 2009). It should be noted that, at least in the currently

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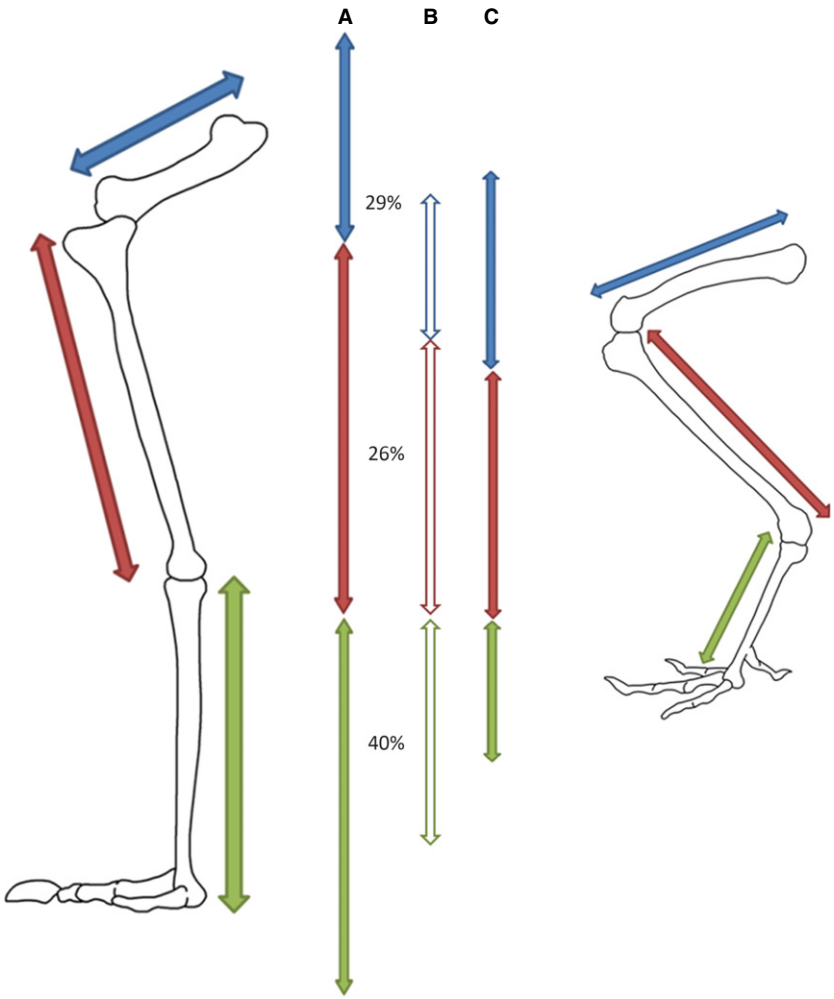

Fig. 3 Schematic showing the likely impact of *in ovo* immobilisation on the proportions of an ostrich limb, assuming the same pattern of reduced longitudinal growth reported in embryonic chickens immobilised from E10–18. (A) Normal ostrich limb. (B) Predicted effect of immobilisation on an ostrich limb. (C) Normal chicken limb. Immobilisation may alter ostrich limb proportions, resulting in a limb which appears more 'chicken-like'.

available mouse models of *in utero* immobilisation, not all joints are affected equally. In 'muscle-less' mouse models of *in utero* paralysis, the knee joint is conserved whereas other hind- and forelimb joints are lost, indicating that some joints may develop irrespective of muscle contraction. This joint-specific distinction in the requirement for muscle-induced loads in the embryonic mouse skeleton clearly requires deeper examination and the extent of joint flexion that emerges during initial limb outgrowth may be a crucial factor in these considerations (Pitsillides & Ashhurst, 2008).

Embryo movement and skeletal proportionality

There is substantial evidence that mechanical input has a role in defining limb element proportionality. *In ovo* immobilisation of embryonic chicks leads to significant reductions in limb bone length. This was first reported by Drachman & Sokoloff (1966) in the lower limbs of chicks immobilised with DMB. Hall & Herring (1990) used single injections of decamethonium iodide to show similar effects, and intriguingly found that the induction of paralysis at Hamburger and Hamilton stages 34 and 36 (Hamburger & Hamilton, 1992) (equivalent to E8 and E10) produces greater reductions in limb bone length than when paralysis is induced at HH31 (E7). Osborne et al. (2002) built on this by demonstrating that induction of paralysis at E8 results in no greater effect on limb growth than induction of paralysis at E14, to suggest that later stages of limb development exhibit greater sensitivity to the impact of movement-induced loading than earlier stages of embryogenesis. It should be noted that the current literature focuses on immobilisation effects on the chick hind limb; whether forelimb skeletal proportionality is also affected in the same way has yet to be reported.

More detailed 'targeting' of specific temporal windows during development indicates that the effects of *in ovo* paralysis on bone length become significant at approximately E13 of development (Osborne et al. 2002; Lamb et al. 2003). This indicates that embryo bone growth is initially not sensitive to mechanical stimulus, but that mechanosensitivity is acquired later during development. This suggests that intrinsically regulated initial limb growth 'switches' later to regulation dominated by extrinsic factors

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such as mechanical signals. It remains to be determined whether this immobilisation-related skeletal growth retardation is due to deficient chondrocyte proliferation, differentiation, matrix synthesis or hypertrophy or due to insufficient replacement of calcified cartilage by bone during the endochondral ossification process. It has been suggested that mechanical loading regulates the elongation of chondrocyte columns during zebrafish craniofacial development (Shwartz et al. 2012) and our data suggest that such growth deficiencies are at least partly due to an immobilisation-induced diminution in cartilage-to-bone transition (Lamb et al. 2003). Recent data indicates that any reduction in skeletal element elongation is most likely to rely ultimately on the behaviour of the chondrocytes during the terminal phase of their hypertrophy (Cooper et al. 2013).

Our data indicate that more distal limb elements appear to have a greater sensitivity to immobilisation effects compared with proximal elements, with greater percentage decreases in length reported in the tibiotarsus and tarsometatarsus than in the femur in immobilised chick embryos (Osborne et al. 2002; Lamb et al. 2003) (Fig. 2). The general proximo-distal pattern of limb bone development means that distal elements are, in a sense, 'newer' than those positioned proximally in the later stages of development, when the effects of immobilisation are most apparent. Proximal segments might therefore be expected to show greater sensitivity to immobilisation earlier in development, but this does not appear to be the case. This merits further investigation; it may be that plasticity in the form of the distal skeleton in response to mechanical input confers some sort of evolutionary advantage and this also remains to be examined. Formal comparison of temporal maps of gene expression in respective limbs of different mammalian species with varying proportions have not been undertaken, but if these were found to match with proximal-distal elements and also to *in utero* muscle activity, it would strengthen this notion.

In addition to immobilisation studies, further evidence for mechanosensitivity in skeletal development is provided by observations of increased limb bone length when the level of embryo motility is increased in chicks. Incubation temperature increases embryo movement, with a 1 °C increase in incubation temperature producing a significant increase in embryo motility. This is associated with an increase in the number of myonuclei in embryo limb muscles and increased limb element lengths (Hammond et al. 2007). This increase in limb length with temperature did not become significant until E12.5, providing further evidence that mechanosensitivity in skeletal element growth is acquired at a relatively late stage of development. Treatment with 4-aminopyridine (4-AP), a drug which stimulates the release of acetylcholine, thereby increasing its availability at the synaptic cleft and resulting in skeletal muscle hyperactivity, also stimulates embryo movement. Increases in tibia and femur lengths have been reported in chick embryos treated with 4-AP at E15 and E16, but not E14 (Heywood et al. 2005). This stage-specific distinction in the input of muscle-induced load in longitudinal skeleton element expansion along the proximo-distal axis also clearly requires more thorough study.

Investigation into the cellular basis of these changes in bone growth, indicates that the level of embryo motility may act to influence skeletal growth by several mechanisms. There is evidence that mechanical stimuli modulate chondrocyte proliferation in the growth plate (Germiller & Goldstein, 1997). In immobilised zebrafish, which show a significant reduction in the size of all pharyngeal cartilage elements, and in muscle-deficient mouse embryos, chondrocyte number does not appear to be targeted by the absence of muscle contraction, but chondrocyte intercalation is, however, abnormal (Shwartz et al. 2012). It has not yet been determined whether these mechanical effects on chondrocyte behaviour are the product of changes in asymmetric cell division, growth, differentiation or cellular morphology.

A recent paper by Cooper et al. (2013) has provided a new insight into the mechanisms responsible for differential longitudinal growth. They demonstrated significant differences between the width of the hypertrophic chondrocyte zone in the metatarsal growth plates of mice and in the lesser Egyptian gerboa, a rodent which exhibits significant elongation of the metatarsus compared with mice. This elegant study provides evidence to suggest that the rate of growth plate elongation is dependent on the third and terminal phase of hypertrophic chondrocyte enlargement, which is modulated by insulin-like growth factor-1 signalling. The effect of mechanical input on this process, and in particular its various stages, is yet to be investigated, but altered hypertrophic chondrocyte enlargement may also underlie the differential long bone elongation seen in immobilised embryos and hence underpin the skeletal form attained by movement during normal development.

Mammalian models of *in utero* immobilisation

Lack of movement in cases of fetal akinesia deformation sequence in human neonates is fairly well characterised and is associated with multiple joint contractures and bone changes such as decreased cortical area, cortical thickness and external diameters of the ribs and long bones, among other clinical signs (Rodríguez et al. 1988a,b; Rodríguez & Palacios, 1989). Multiple pregnancy is another natural cause of decreased fetal movement in humans and it is therefore intriguing that there are reports of an increased incidence of arthrogryposis in twins than in single human babies, and that its severity is proportional to the extent of the restriction upon movement (Gordon, 1998).

Although there are many muscle-less mouse mutants, their usefulness as models of *in utero* paralysis is questionable, as

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the impact of muscle deficiency on skeletal development may be independent of the effects of embryo movement. Thus, Myf5$^{nlacZ/nlacZ}$:MyoD$^{-/-}$ mice and Pax3$^{Sp/Sp}$, or 'Splotch-delayed', mice are 'muscle-less' mutants which exhibit a range of skeletal abnormalities (Vogan et al. 1993; Yang et al. 1996; Rot-Nikcevic et al. 2006), but a potentially better model is the muscular dysgenesis (mdg) mouse. This is a spontaneously occurring mutant strain which exhibits skeletal muscle paralysis *in utero*. Homozygotes suffer from a failure in excitation-contraction coupling caused by a frame-shift mutation in the alpha 1 subunit of the skeletal muscle calcium channel (Chaudhari, 1992), so the bones of these mice develop in the presence of muscle but in the absence of muscle contraction-induced mechanical loading.

The mdg mutation is lethal in homozygous animals, with mice dying perinatally. However, heterozygotes exhibit 'clasped' limbs due to a failure of development in multiple joints. Other skeletal abnormalities observed in these mice include shortening of the mandible and clavicle and fusion of cervical and thoracic vertebrae (Pai, 1965). Although it is considered that this is caused by the complete arrest of chondrocyte proliferation, a thorough analysis of limb bone morphology, architecture and bone mineral density from these animals is not available in the current literature, but might reveal insights into the role of movement in the development of mammalian limb bones.

The significant differences in length of all limb elements observed in immobilised chicks do not appear to be repeated in mdg mice; there are no data available on whether the limb proportionality of mdg mice is altered, but no visible differences in limb length have been reported. In the Splotch-delayed 'muscle-less' mice, some bones are reported to be shorter than in wild-types, such as the humerus and tibia but not the femur or ulna. This may reflect the growth rate of each element at the stage at which measurements were recorded (E18.5), or the possibility of a more complex system for regulation of long bone growth during development in mammals than in oviparous avians, where passive movement of the limbs as a result of movements of the mother or normal siblings may ameliorate some effects of the *in utero* immobilisation. It might also highlight the existence of a 'modular-type' of evolution in which particular components of the musculoskeletal system may have developed a specific range of sensitivities to mechanical input in order to secure the development of a functional locomotor system.

Species differences

Chicken embryos are a widely used and well characterised model of vertebrate development (Tickle, 2004). Their use in the investigation of immobilisation effects on skeletal growth have been outlined in this review and include their contribution to skeletal proportionality. It is possible that the extent to which movement contributes to the skeletal proportions in avian species – where these proximal-distal ratios produce markedly diverse anatomies – may differ. An interesting question to pose is whether the longer distal metatarsal elements in ostrich limbs, which are adapted for efficient running, show more marked sensitivity to the input of embryonic movement compared with chickens (Fig. 3).

This notion has circumstantial support in the reported limb growth rate differences observed in ostriches and chickens during development (Gefen & Ar, 2001). It is possible that the stages of development at which mechanosensitivity is acquired in these two species are not equivalent. Thorough investigation of the developmental period during which these chick-ostrich differences in bone elongation are established, and whether this coincides with the embryo phase at which immobilisation exerts a critical role on proximo-distal proportionality, would reveal more about how embryo movement contributes to establishing skeletal form in different species.

There are also numerous examples of divergent limb skeletal proportionality among mammalian species. For example, the metararsal elements of the horse and many other prey species are extremely elongated relative to those of humans, and some primates exhibit short metacarpals and long proximal phalanges relative to other arboreal mammals for grasping and climbing in thin branches (Hamrick, 2001). The growth of forelimb elements of mice and bats is another valuable comparison. However, the effect of immobilisation on digit growth and the existence of species differences in sensitivity to the input of embryonic movement have yet to be investigated in any species. As a small rodent with relatively elongated metatarsal elements compared with mice, the lesser Egyptian jerboa, mentioned earlier in this review and described by Cooper (2011) and Cooper et al. (2013), may be a very appropriate model in which to investigate potential species differences in embryonic skeletal mechanosensitivity in mammals.

Embryo movement as an epigenetic regulator of skeletal growth

Epigenetic mechanisms allow for selective gene regulation in response to environmental signals in order to produce phenotypic complexity in morphogenesis. We propose that mechanical loading of the developing skeletal system, resulting from embryo movement, is one such environmental signal. It is well established that other environmental factors can influence phenotypes in humans, evident in the risk factors for diabetes and cardiovascular disease (Kaati et al. 2002; Wong et al. 2005). The evidence outlined in this review suggests that bone growth during development is also subject to mechano-epigenetic regulation.

It may be that differential skeletal morphology relies upon specific 'mechanosensitive' genes, and particular molecular and mechanical cues to regulate bone/skeletal

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mechano-adaptation during development. The expression of *IHH* and hypertrophic markers such as *MMP13* have been shown to be regulated in chondrocytes *in vitro* by cyclic mechanical stress (Wu et al. 2001; Wong et al. 2003; Rubin et al. 2006) but these experiments do not tell us much about how mechanical stimuli are interpreted *in vivo* in the developing limb.

In ovo immobilisation has been shown to alter expression patterns of *COL X* and *IHH* in embryonic limbs, suggesting that these genes are involved in linking mechanical stimuli from embryonic muscle contraction with regulation of bone formation in the limbs (Nowlan et al. 2008). This *in vivo* study investigated the effect of immobilisation on gene expression in relatively early stages of development: E6, 7 and 8. Considering the data from Osborne et al. (2002), Lamb et al. (2003) and Pitsillides (2006), it seems likely that the critical period for acquisition of mechanosensitivity is E13–18 in chick embryos. Further investigation into the effect of immobilisation of gene expression patterns during these developmental time points is likely to reveal more about the mechanisms involved in establishing limb bone proportionality.

Potential evolutionary roles of mechanosensitive bone growth in development

The potential role of mechanosensitive limb growth in embryos as a source of phenotypic plasticity during development has been discussed in an elegant review by Müller (2003). The general premise proposed by this review is that environmental change may induce variations in size and shape of skeletal elements, upon which natural selection can subsequently act. This is reminiscent of Lamarckist theories in that it suggests that the emergence of phenotypic variation is not completely independent of selective pressures. Thus, rather than arising only from random mutation followed by selection, the differences in limb bone morphology that we see between species may also have a basis in the epigenetic effect of embryo movement.

The greater reductions in more distal limb element lengths than in proximal segments observed in immobilised chicks is particularly interesting in this context; distal limb proportionality varies more widely between species and is intimately linked with divergent types of locomotion. For instance, it is known that cursorial species tend to have relatively long limbs with proportionally longer distal elements compared with proximal elements, which allows for a greater stride length and greater running speeds (Hall, 2007). Our review of the literature leads us to speculate that it is possible that species with more elongated distal limb elements are more reliant upon embryo movement for the differences in their limb bone growth rate.

How the emergence of species-specific skeletal form and the influence of movement-induced mechanical inputs interlock has yet to be investigated. Nevertheless, Richardson (1999, review) raises the idea of developmental penetrance, i.e. changes in developmental mechanisms driven by selection for adult traits. For example, the differences in digit proportions between primates and other mammals including rats and possums are observable relatively early in development, during joint interzone formation (Hamrick, 2001). Differences in the spatial and temporal expression of conserved genes such as those involved in the regulation of limb skeletal development may account for these differences. It is therefore tempting to speculate that the genes which define the joint interzones (Pitsillides & Ashhurst, 2008; Purcell et al. 2009; Kan & Tabin, 2013), which are crucial in defining the relative proportions of individual elements as they emerge during outgrowth, will play a central role in providing the 'blueprint' upon which these mechano-epigenetic influences will act.

Conclusions

Our review of the literature leads us to suggest that limb development has evolved such that skeletal growth in the later stages of development is not only intrinsically controlled but is also subject to regulation by environmental factors. This may be advantageous as it would provide another mechanism for the generation of phenotypic variation. Most previous studies into the effect of environmental factors have only considered this in the context of disease risk, but there is some evidence which points to morphological changes with environment. Female Great Tits artificially exposed to a predation risk produce offspring with accelerated wing growth and longer wings at maturity, indicating that maternal factors or altered incubation conditions can lead to adaptive responses in avian offspring (Coslovsky & Richner, 2011). Alterations in embryo movement induced by environmental factors may be a mechanism by which similar responses in limb proportionality occur. Until recently, work on limb development has not considered the mechanical status of the limbs, i.e. whether there is a normal level of embryo motility, but the evidence discussed in this review suggests that this is something which requires further investigation. Our review also highlights that differences in the emergence of such skeletal mechanosensitivity may exist in different embryonic environments, such as in viviparous species compared to avians.

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