Adaptation in the vertebral column: a comparative study of patterns of metameric variation in mice and men

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ABSTRACT

In this paper we examine metamerism in the vertebral column of certain mammals from the perspectives of development and adaptation. To this end we examine the patterns of metameric variation of dimensions of the neural (vertebral) canal, vertebral body and spinous process in man and inbred strains of mice. The data from inbred strains of mice indicate that variability in dimensions within a strain reflects the temporal ordering and nature of developmental influences on vertebral morphogenesis. Differences between strains parallel the within-strain findings. These findings are attributed to somatic and neural influences on morphogenesis. Comparisons between mice and man indicate that these same influences can be invoked to explain and interpret the mosaic nature of vertebral column evolution. These findings lead us to conclude that different vertebral elements and levels are subject to different interactions of evolutionary and morphogenetic influences. The study of these influences and their interactions should prove fruitful in developing an understanding of the relationship between adaptation, development, growth and function in the skeleton generally.

Key words: Vertebral column; metameric variation.

INTRODUCTION

The vertebral column consists of a series of repeated, serially homologous units each of which serves many functions: these include the provision of protection for the spinal cord, attachments for musculature, the bearing of weight, locomotor power transmission, shock absorption, provision of flexibility and conversely support. Functional demands together with morphological adaptations to these functional demands vary from vertebral level to vertebral level. In our terminology we recognise the importance of variation along the vertebral column within an animal and differentiate between cervical, thoracic, lumbar, sacral and caudal vertebrae on both functional and morphological grounds. The morphology of vertebrae shows metameric variation along the column, morphology grading from one level to the next (Lannier, 1939; Slijper, 1946). Between species, morphological adaptations to function are seen both as variations in the number, size and shape of vertebral elements and, importantly, as variations in the patterns of metameric variation of different vertebral elements such as the bodies or arches. This paper aims to investigate patterns of metameric variation of different vertebral elements in 2 mammalian genera (Mus and Homo) in order to consider the likely mechanisms underlying phylogenetic change in the vertebral column.

Evolution ultimately controls morphology through the selection and modification of morphogenetic pathways, mechanisms and interactions. In order to understand the basis of serial or metameric variations within the vertebral column of a single species and how these might be modified in different species we must address the extent to which different vertebral elements (e.g. the bodies, neural (vertebral) arches,
spinous processes) come under the influence of different developmental influences.

The paraxial mesoderm undergoes metameric segmentation forming somitomeres of which the more caudal members go on to form somites. The somites, in turn differentiate into sclerotome, myotome and dermatome and the sclerotomes migrate around the notochord and neural tube to give rise to the vertebral bodies and arches. The widely accepted view is that the segmental sclerotomes resegment but this has been challenged by Verbout (1976); yet there is good experimental evidence that there is a realignment of segmentation between the somite stage and the subsequent vertebral stage (Bagnall et al. 1988; Ewan & Everett, 1992). The vertebral primordia may vary in size along the column but, at this early stage there is little morphological differentiation between levels. Individual vertebrae develop from distinct cartilaginous elements which fuse to produce the vertebral arches and bodies. The distinctive morphology characterising different vertebrae within the column is a product of the subsequent differentiation and growth which follows segmentation.

Recent work (reviewed by Kessel & Gruss, 1991) has stressed the importance of a class of genes which are both highly conserved and expressed during early morphogenesis (homeobox or \textit{Hox} genes) in the segmented mesoderm which gives rise to the vertebral column. The expression of homeobox genes appears to be correlated with the differentiation of morphology in many developing skeletal systems, such as the limbs and the developing head as well as the vertebral axis. Different vertebral levels are characterised by differences in the subsets of homeobox genes which are expressed and these differences are graded along the vertebral columns such that adjacent levels may show differences in the expression of only one or two genes (the \textit{Hox} code, Kessel & Gruss, 1991). Likewise the developing vertebrae are graded in morphology from one level to the next. Treatment with retinoic acid alters the spatial pattern of \textit{Hox} gene expression and results in respecification of vertebral identities (Kessel, 1992). Additionally in comparing mouse and chick (which vary in the numbers of vertebrae in each region of the column) Burke et al. (1995) found that \textit{Hox} gene expression boundaries were transposed in concert with morphological boundaries. These studies underline the contribution of \textit{Hox} genes to the specification of vertebral identity. It may be that these genes regulate local growth timings and rates in developing vertebrae and so produce many of the morphological features which characterise individual vertebrae. The patterns of metameric variation of vertebral elements are likewise likely dependent to some degree on the way in which the \textit{Hox} code varies from level to level. One aim of this study is to examine the extent to which morphological metameric variation of different vertebral elements differs within and between species and so is likely to be related to the highly conserved \textit{Hox} code or other morphogenetic influences.

Within a vertebra, individual elements such as the bodies or the arches follow different patterns of growth throughout ontogeny. Early in development these growth patterns are largely influenced by genetically mediated mechanisms. As they ossify (a process which extends well into postnatal life) the growth and remodelling of vertebral elements comes increasingly under the influence of various systemic factors and of its biomechanical milieu including the activity of muscles (Oxnard, 1991; Erlebacher et al. 1995). We might expect, then, that the duration and rate of growth of particular elements within each vertebra will, to some extent, also reflect the pattern of growth of the adjacent tissues. Thus the neural (vertebral) arch, being adjacent to and functionally concerned with nervous tissue, might to some degree follow a neural pattern of growth in which growth ceases relatively early, modifications of its morphology come about through remodelling after fusion of epiphyses (O’Higgins et al. 1989). The vertebral body and vertebral processes might likewise be expected to follow a somatic pattern of growth (Harrison et al. 1988) through the influence of applied forces and the musculature arising from them. An earlier study (Johnson & O’Higgins, 1994) has demonstrated that these growth patterns are, in turn, reflected in the patterns of metameric variation and the variability of these patterns in different parts of the vertebral column.

In this paper we are concerned with the ontogenetic and adaptive significance of differences in the patterns of metameric variation displayed by different vertebral elements such as the body, the arches and the spinous processes. In order to allow an interpretation of the developmental significance of these patterns in \textit{Homo sapiens} we compare them with the patterns observed in inbred strains of mice. Inbred strains are brother/sister mated over many generations: any differences between animals within a strain can therefore be attributed to the effects of the environment. Differences between samples from different inbred strains will have a large genetic component.

Comparisons between these species of the patterns of metameric variation of different elements of the vertebral column may contribute to a better under-
standing of the interaction of the evolutionary, ontogenetic and environmental factors influencing vertebral column metamerism.

MATERIALS AND METHODS

Materials

The vertebral columns of 2 species, Mus musculus and Homo sapiens were examined. The mouse sample consists of 6 vertebral columns from males from each of 2 inbred strains, BALB/c and CBA (there is no sexual dimorphism in the mouse vertebral column). They form part of the Grüneberg collection of the British Museum (NH), London. The human sample consists of 9 male and 8 female (Milne, 1991) skeletons from India held in the Department of Anatomy, University of Western Australia. All vertebral columns come from adult individuals. Data were only available for the first 20 presacral vertebrae in the mice.

Methods

Data collection. The mouse vertebrae were placed on a piece of black plasticine so that their cranial aspect was parallel to the microscope stage. They were measured in incident light using a low-power microscope (×6 objective) fitted with a video camera by means of a standard C mount. This gave a working distance of approximately 20 cm. Images of the cranial aspect (rostral) of each vertebra were displayed on the digital display of a computer (PC) and a specially written program was used to locate the coordinates of selected landmarks and to calculate the projected distances between them (Fig. 1). The human vertebrae were measured in a similar way except that black and white negative photographic images of standardised cranial views were placed under a low power microscope with video camera and data were recorded using the same system.

The following measurements were recorded (Fig. 1): (1) neural (vertebral) canal depth: the distance between the dorsal and ventral extremities of the neural (vertebral) canal, measured in the median plane; (2) neural (vertebral) canal width: the distance between the left and right extremities of the neural (vertebral) canal as seen in the cranial vertebral outline; (3) vertebral body depth: the dorsoventral diameter of the cranial vertebral endplate, measured in the median plane; (4) spinous process length: the projected length of the spinous process as seen from the cranial aspect; this measurement takes no account of the angulation of the spinous process, but reflects the leverage afforded by the spinous process for longitudinally running spinal musculature.

Morphometric analysis. Graphs were plotted of vertebral level vs dimension magnitude for each dimension in each individual in the data set. These metameric curves were used to assess visually the variability of each dimension. Similarly, plots of the mean of each dimension at each vertebral level for each strain of mice and each sex of humans were used to assess differences between strains and sexes in their patterns of metameric variation and to compare visually the patterns of metameric variation between variables.

Clearly mice are much smaller than humans and within each species vertebral dimensions differ in relative scale. Quantitative comparisons between curves were therefore made, taking account of isometric scale differences between curves. The absolute difference between the metameric curves of mouse strains or hominoid sexes was quantified by summing the differences between pairs (sexes or strains) of curves (e.g. of spinous process lengths) at each vertebral level and dividing this total by the number of vertebral levels. The relative differences between sexes or strains were calculated by dividing the absolute differences by the average magnitude of each variable (i.e. spinous process length) in each species.

RESULTS

The plots of magnitude versus vertebral level for each of the 4 linear dimensions in each of the 6 inbred BALB/c mice are given in Figure 2. From these plots it can be seen that, relative to variation along the vertebral column, there is little variation between the
6 individuals in the curves describing the pattern of metameric variation of the neural (vertebral) canal diameters. Spinous process lengths and vertebral body depths appear more variable between individuals. Thus variability is proportionately small in dimensions of the neural (vertebral) canal, and large in the vertebral body depth and in the length of the spinous process. Four BALB/c mice had a large spinous process at T2, while the other 2 did not; this large process develops in all mice, its occasional absence in some individuals is due to epigenetic factors late in ontogeny (Johnson & Kida, 1995). The data from CBA behaved in an identical manner to those from BALB/c; plots are omitted for brevity.

The mean curves for each inbred strain of mice are plotted in Figure 3. The relative differences between inbred strains appear greatest in spinous process length, less in vertebral body depth, and least in the neural (vertebral) canal dimensions. These appearances are substantiated by the values for relative

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Fig. 2. Variability within BALB/c mice. The 4 vertebral dimensions are plotted for each BALB/c individual.
Vertebral metamerism

Fig. 3. Differences between BALB/c and CBA mice. The means for the BALB/c and CBA inbred mice are plotted for each of the 4 vertebral dimensions.

differences (average differences corrected for isometric scale differences) presented in Table 1. Thus, between-strain differences parallel within-strain differences.

The mean curves for both sexes of humans are plotted in Figure 4. The relative differences between the sexes appear to follow a similar pattern to that found in the mice; the greatest differences are seen in the spinous process length and vertebral body depth, and the smallest differences are seen in the neural (vertebral) canal dimensions. Quantitative comparison of differences (Table 2) confirms these appearances.

The differences between species in the curves for the neural (vertebral) canal depth (Figs 3, 4) are not pronounced. In both species there is a progressive (if slight) decrease in diameter from the cervical to the lumbar regions. The curves are very simple. The transverse diameter of the neural (vertebral) canal (Figs 3, 4) follows a more complex, but similar curve in both species.
Table 1. Average and relative differences between inbred strains of mice

<table>
<thead>
<tr>
<th></th>
<th>Average (mm)</th>
<th>Relative</th>
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<tbody>
<tr>
<td>Neural (vertebral) canal depth</td>
<td>0.063</td>
<td>3.90</td>
</tr>
<tr>
<td>Neural (vertebral) canal width</td>
<td>0.134</td>
<td>5.24</td>
</tr>
<tr>
<td>Vertebral body depth</td>
<td>0.055</td>
<td>7.08</td>
</tr>
<tr>
<td>Spinous process length</td>
<td>0.670</td>
<td>9.75</td>
</tr>
</tbody>
</table>

For definition of relative and average differences, see text.

Table 2. Relative differences between strains of mice and sexes of humans in the metameric variation of certain vertebral dimensions

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural (vertebral) canal depth</td>
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<td>5.41</td>
</tr>
<tr>
<td>Neural (vertebral) canal width</td>
<td>5.24</td>
<td>5.40</td>
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<tr>
<td>Vertebral body depth</td>
<td>7.08</td>
<td>8.54</td>
</tr>
<tr>
<td>Spinous process length</td>
<td>9.75</td>
<td>11.45</td>
</tr>
</tbody>
</table>

Fig. 4. Differences between male and female humans. The means for males and females are plotted for each of the 4 vertebral dimensions.
Greater differences between the species are apparent in the curves of metameric variation of the vertebral body depth (Figs 3, 4). In mice the curve has 2 troughs, in the lower cervical and the lower thoracic regions. In humans, it is simpler and shows a gradual increase in variable magnitude from the cranial to the lumbar segments. The curves for spinous process lengths (Figs 3, 4) are very different between species.

**Discussion**

This study has examined patterns of metameric variation in the vertebral columns of mice and humans. The aim has been to examine the interplay of ontogeny, phylogeny and function in this metameric structure. The results can be considered in 3 parts; patterns of variability within groups, patterns of similarity and difference in the metameric curves of different dimensions of the vertebral column within species, and differences between species in the patterns of metameric variation manifest by different vertebral dimensions.

**Variability within groups**

Variability within BALB/c mice is least in the dimensions of the neural (vertebral) canal which, we infer, follow a neural pattern of growth (Sinclair, 1969) and greatest in the spinous processes which follow a somatic growth pattern. Environmental influences will have a greater effect in structures which grow over a longer period. Such influences might include hormones, diet, activity and disease. They need not act directly: differences in diet might produce differences in body weight and therefore in the forces acting on the bones during activity. Differences in diet or disease might also affect the secretion of hormones which might in turn affect the growth of bone. This finding is particularly relevant to our understanding of the mechanisms by which sexual differences are manifest in different vertebral elements. Sexual differences in skeletal morphology in mammals are less pronounced early in ontogeny and become more apparent in later stages of growth (Ford & Corruccini, 1985). Shea (1985) has considered the mechanisms of sexual differentiation in hominoids where adult males are generally larger than females. Briefly a considerable proportion of the sexual differences in craniofacial morphology may be explained in terms of peramorphic growth, that is, males extend a growth pattern which is common to both sexes. This extension may come about either through time or rate hypermorphosis (growth for a longer period or at a faster rate).

Sexual differences in hormonal milieu are important mediators of the differences in the growth patterns of human males and females. These hormonal differences may exert their effects by affecting bone growth directly and/or indirectly through effects on body weight, muscle mass and tone and possibly vascularity and connective tissue elasticity. Hormonal differences are most pronounced in humans during puberty, which occurs towards the end of the growth period. We might expect, therefore, that sexual differences would be most pronounced in those dimensions which complete their growth later rather than earlier in ontogeny. Our findings indicate that this is the case. Thus sexual differences between humans parallel variability within BALB/c mice in that they are most pronounced in the vertebral bodies and spinous processes. The reasons for this parallel seem, in turn, to be related to the patterns of growth and the functional and epigenetic influences acting on different elements of the vertebral column.

Our findings indicate that differences between mouse strains also parallel variability within BALB/c. Thus the greatest differences are apparent between the mean curves of metameric variation of the spinous processes and vertebral body and the least between the dimensions of the neural (vertebral) canal. Since we are comparing 2 inbred strains we can conclude that these differences are in some way genetically mediated (Fig. 3). This genetic effect may be relatively direct, i.e. to relate to specific genetic control of vertebral body and spinous process morphology. Alternatively, it may operate through a less direct route, i.e. genetically determined differences in the hormonal or behavioural milieu. That the differences between strains parallel those within strains supports the latter hypothesis. In any case this finding underlines the need for further ontogenetic studies of the vertebral column.

**Patterns of covariance between vertebral dimensions**

The neural (vertebral) canal depth follows a simple curve which parallels the dorsoventral diameter of the spinal cord. Casual inspection of Figures 2 or 3 suggests that in the mouse neural (vertebral) canal width follows a dissimilar pattern to that of vertebral body depth and spinous process length. If, however, the curve for neural (vertebral) canal width is inverted a basic similarity with vertebral body depth and spinous process length becomes apparent. Neural (vertebral) canal width shows a peak in the lower
cervical region and a trough in the midthoracic region, whilst the others show the inverse pattern (discounting the variable peak in the T2 spinous processes).

This similarity might be explained on functional grounds. The limbs require both increased outflow from the spinal cord (influencing neural (vertebral) canal width) and modifications for the attachment of musculature (spinous processes) and load bearing (vertebral bodies). Clearly the relationship between spinal cord enlargements and vertebral level changes with age owing to differential growth; it may be however that the relationship between vertebra and spinal cord is more important early rather than later in development. Similar arguments apply to muscle development. These could be examined in future studies. Alternatively it may be that the similarities between these vertebral dimensions in their patterns of metameric variation are the result of (developmental) factors influencing the overall shape of vertebrae. In this regard it may be significant that neural (vertebral) canal width is a transverse measurement, whilst vertebral body depth and spinous process length are both dorsoventral measurements. These alternative explanations provide a stimulus for further ontogenetic and biomechanical studies of the vertebral column.

**Interspecific differences in patterns of metameric variation of vertebral dimensions**

The patterns of metameric variation of each of the 4 variables in mice and humans can be compared between Figures 3 and 4.

Similarities between species are greatest in the dimensions of the neural (vertebral) canal. The vertebral body depth follows a pattern which differs between humans and mice. The pattern in humans, one of increasing diameter caudally, is likely to be consequent upon the adoption of predominantly upright posture (Slijper, 1946), where natural selection has resulted in a pattern of metameric variation which is well adapted to bearing the weight of a more vertically disposed trunk and head. The pattern in mice (which is repeated in other small quadrupeds, Slijper, 1946; and work in progress) possibly reflects the primitive quadrupedal mammalian condition.

The spinous processes (Figs 3, 4) show patterns of metameric variation which differ markedly between the species included in this study. Again we may cite genetic and environmental interaction in the morphogenesis of these differences. Thus locomotor adaptations lie at the basis of these differences; during ontogeny genetic and epigenetic mechanisms coordinate the development of the spinous processes which in turn provide important muscle attachments and lever-arms for these attachments. During growth, locomotor and postural activity may well further modulate and stimulate further growth and differentiation of these bony elements.

**Conclusions**

The principal findings of this study are that measurements associated with the neural (vertebral) canal show less variation within species, between inbred strains, and between species than do vertebral body depth and spinous process length. Within-strain variability in mice is related to the growth pattern (neural or somatic) of adjacent structures and the biomechanical milieu. Sexual differences in humans again reflect adjacent growth patterns and possibly the biomechanical milieu but hormonal influences also come into play. Between-species differences in part reflect adaptive genetic differences but also are most clearly seen in those dimensions which follow a somatic growth pattern.

These findings underline the mosaic nature of adaptation within the vertebral column and lead us to suggest an evolutionary and possibly developmental ordering or hierarchy of patterns of metameric variation of different elements of vertebrae. Thus the most simple patterns are displayed by the dimensions of the neural (vertebral) canal and the most complex by vertebral body depth and spinous process length. This ordering reflects the conservative nature of nervous system organisation relative to that of the vertebral bodies and spinous processes given the specialisations of locomotor function in the hominoids. Knowledge of such ordering of patterns of metameric variation may have significance in phylogenetic analysis. Thus adaptations of closely related species are likely to be strongly reflected in the morphology of spinous processes, less so in the vertebral bodies and least in the neural (vertebral) canal.

One aim of this study was to examine the extent to which morphological metameric variation of different vertebral elements differs within and between species and so is either likely to be related to the highly conserved Hox code and its immediate downstream effects, or to other morphogenetic influences. Our findings indicate that some features (especially neural (vertebral) canal dimensions) of the vertebral column show little within and between-species differences in their patterns of metameric variation. As such it is
possible that their morphology is regulated primarily by the highly conserved Hox code. Other features show more marked variation within and between species and so their morphology is also influenced by other genetic and epigenetic factors. Thus knowledge of variability of features and of their curves of metameric variation may be important in unravelling the complex interactions between genes and the internal and external environment in studies of vertebral column adaptation.

The findings of this study indicate many interesting avenues for the further investigation of the interaction of ontogeny and phylogeny in relation to form and function in the mammalian vertebral column. Studies of metameric variation in other species and in other dimensions are required, together with investigations of the patterns of growth and of influences on these patterns.

In attempting to understand the links between morphology and locomotion in mammals we must recognise that mosaicism characterises vertebral column evolution. Different vertebral elements and levels are subject to different interactions of evolutionary and morphogenetic influences. The study of these influences and their interactions should prove fruitful in developing an understanding of the relationship between adaptation, development, growth and function in the skeleton generally.

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