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Adaptation in the vertebral column: a comparative study of patterns of metameric variation in seven species of small mammals

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ABSTRACT

The pattern of variation of certain vertebral measurements along the vertebral column is known to differ in man and mouse. This paper investigates changes in this pattern in 7 species of small mammals and attempts to correlate them with locomotor adaptations and limb dimensions.

Key words: Osteology; spine; locomotion.

INTRODUCTION

In previous papers (Johnson & O'Higgins, 1994; O'Higgins et al. 1997) we have investigated variation in patterns of vertebral measurements between inbred strains of laboratory mice and the ontogenetic and adaptive significance of differences in pattern between the presacral vertebrae of man and mouse. In general we found that, within the small numbers of dimensions studied, those which varied most between individuals also varied most between inbred strains of mice and between man and mouse. We also suggested in the later paper that the differences that we found between man and mouse might reflect, at least in part, differences between bipedal and quadrupedal locomotion, and that the mouse patterns might represent, or at least approach, a primitive mammalian quadrupedal condition (Slijper, 1946). In order to follow up this point, and to see if the mouse is in any sense typical, it is necessary to investigate further the pattern found in small quadrupeds by replicating the measurements that we made on man and mouse on other mammalian species.

This paper looks at the shape differences between samples of presacral vertebrae of a number of species of small quadrupedal mammals and asks how the vertebral columns of these animals differ from each other in respect of 5 key measurements. We also consider whether any changes in pattern might be variations on a (primitive) quadrupedal arrangement and if variations reflect changes in the proportions of the fore and hindlimbs, which may be linked to locomotor mechanisms.

MATERIAL AND METHODS

The material used in this study comprised the presacral vertebrae from papain digested skeletons. Most of these form part of the Grüneberg collection, on loan to one of us (DRJ) from the British Museum of Natural History. We used samples of bandicoots, mice, rats, and squirrels. This was supplemented by such specially prepared material as was available to us (Table 1). Material was mostly from small rodents, but rabbit and shrew (Lagomorph, Insectivore) were also included.

The mice used in this study are from the 13th generation of Falconer's selection experiments (Falconer, 1973). We used 20 mice from each of 6 experimental categories QLA, QLF, QCA, QCF, QSA and QSF (QLA and QLF are replicates selected for large size, QSA and QSF for small size and QCA and QCF are unselected controls) in order to broaden our knowledge of factors possibly contributing to variation in patterns of skeletal dimensions.

Only adult individuals were included. Laboratory animals were of known age. For the bandicoots and rats Grüneberg recorded weights of animals captured: the same applies for squirrels. Bones from

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Table 1. *Materials used in this study*

Species	Number of specimens	Source
Shrew (*Microtus agrestis*)	20	Grüneberg collection (collected by R. J. Berry)
Mouse (*Mus musculus*)	120	20 examples each of QCA, QSA, QLA, QCF, QSF and QLF mice (Falconer, 1973)
Rat (*Rattus rattus*)	20	Grüneberg collection (collected in Kerala, South India; see Grüneberg et al. 1966)
Gerbil (*Gerbillus sp.*)	21	University of Leeds Animal House, 1993
Bandicoot (*Bandicos indica indica*)	21	Grüneberg collection. Collected in Kerala, South India; see Grüneberg et al. (1966)
Grey squirrel (*Sciurus carolinensis*)	14	Grüneberg collection
Rabbit (*Lagopus sp.*)	20	Wild (collected by D. R. J. Malham, N. Yorks, 1993)

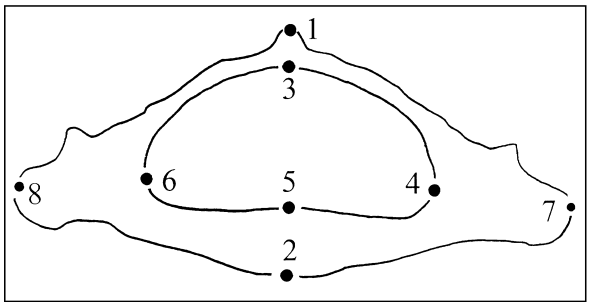


Fig. 1. Landmarks used in this study. See Table 1 for definition of measurements based on these.

Table 2. *Linear dimensions of vertebrae used in this study*

Spinous process length	1–3
Maximum anteroposterior diameter of neural canal	3–5
Anteroposterior diameter of vertebral body (or 'equivalent' for C1, C2)	2–5
Maximum transverse diameter of neural canal	4–6
Overall vertebral width	7–8

Numbers refer to landmarks in Fig. 1. Numbers 1–6 correspond to Johnson & O'Higgins (1994); 7, 8 have been added for this study.

small or immature rabbits were not included in the sample.

The presacral vertebrae from each animal were mounted in sequential order on black modelling clay under a dissection microscope and oriented with the neural canal at 90° to the plane of focus of the microscope. A video camera was attached to the dissecting microscope and a digitised image of each vertebra was saved, along with an image of a scale, at a suitable known magnification using a frame grabber card (Vidi-PC, Rombo Productions, Livingstone, Scotland) attached to a personal computer.

Vertebral images were displayed on a PC VGA display and measurements were taken using a purpose-designed and written program which enables the x, y coordinates of selected landmarks (see Fig. 1, Table 2) to be recorded and which calculates and writes to disk appropriate distances between landmarks. In all, 5 linear dimensions were calculated for each vertebra (Table 2: note that we use the terms anterior and posterior in preference to ventral and dorsal to maintain consistency with current studies in primates and in humans). Four of these dimensions (maximum anteroposterior diameter of the neural canal, maximum transverse diameter of the neural canal, maximum anteroposterior diameter of the vertebral body and spinous process height) were used in previous papers (Johnson & O'Higgins, 1994; O'Higgins et al. 1997). We added overall vertebral width (7–8) to this series.

Limb measurements

At the same time as vertebral measurements were taken the length of a number of limb bones was measured, using the same technology. We have selected from these the overall length of the femur, tibiofibula, humerus, radius and ulna. These were recorded singly, as bone lengths, as composites (forelimb length = humerus + radius, hindlimb length = femur + tibiofibula) or as ratios (forelimb : hindlimb, humerus : radius, femur : tibiofibula). Limb measurements were then correlated with vertebral dimensions.

All measurements (vertebral and limb) were performed by one of us (MYK) over a period of about 2 mo during a visit to the University of Leeds.

Analysis of data

The magnitude of each dimension in each individual at each vertebral level was used to calculate standard error to assess within species variation. Graphs of each species mean at each vertebral level were used to assess between species variation. A spline function was fitted to each curve in order to smooth it. Pearson

correlation coefficients were measured for each limb variable or derived ratio against each vertebral variable for each presacral vertebra.

RESULTS

Mice

We observed no significant differences in the pattern of measurements from replicate strains selected for large or small size or unselected for size (i.e. QLA versus QLF, etc). There were small but significant quantitative differences between large, control and small categories in unscaled data (Fig. 2): these differences disappeared when the data were scaled.

Other species

All other species showed a characteristic pattern of dimensions along the vertebral column (Fig. 3) which differed between species. These data are perhaps best

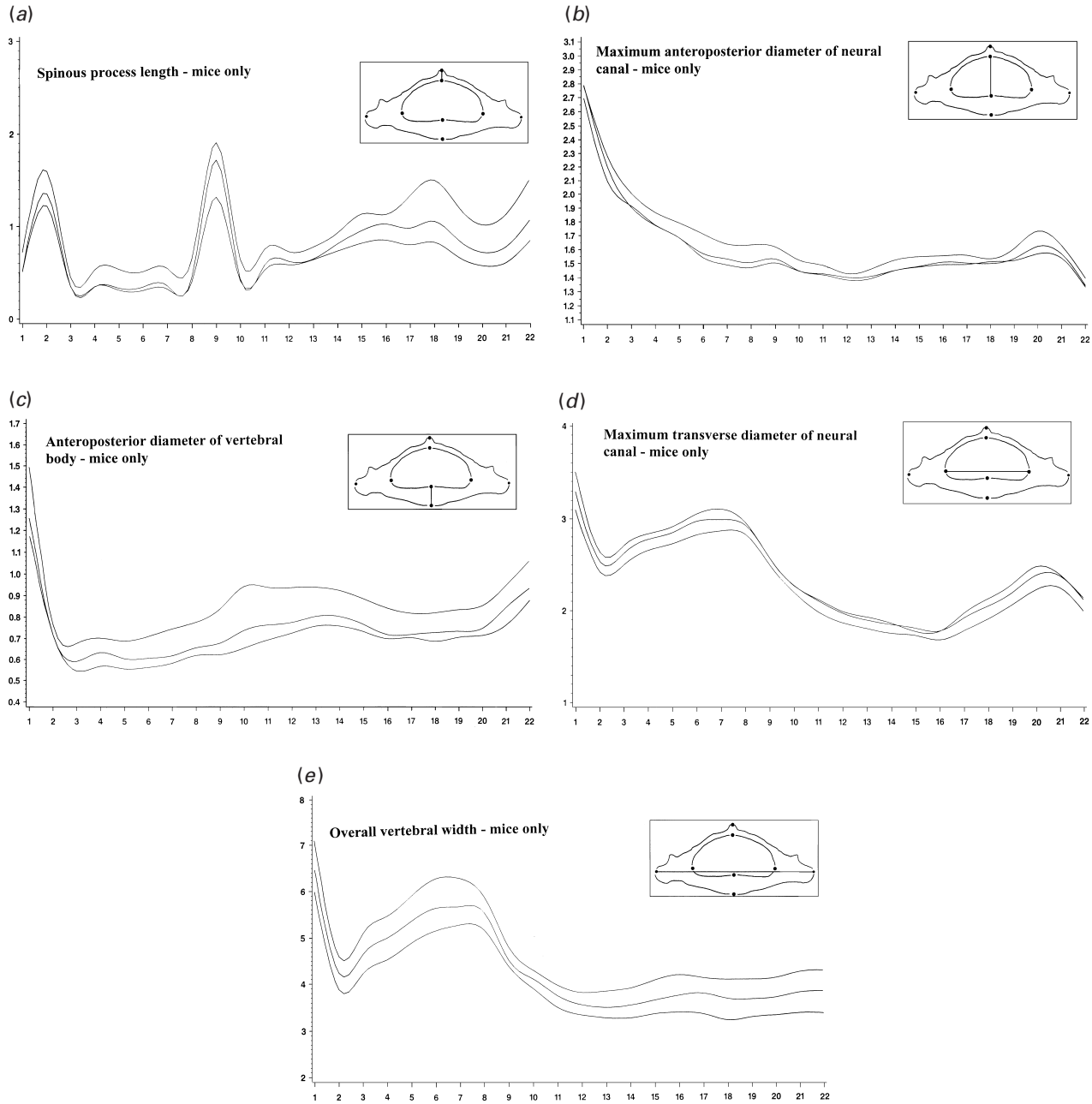


Fig. 2. Vertebral dimensions at each vertebral level (1–7 cervical, 8–18 thoracic, 19–22 lumbar) for large, control and small mice. The upper line in each graph represents mice selected for large size, the middle line unselected controls and the lower line mice selected for small size. Each group (large, control, small) is made up of individuals from 2 replicates, A and F, of Falconer's experiment (Falconer, 1973) which showed no significant size differences. Unscaled; vertical axis is in mm.

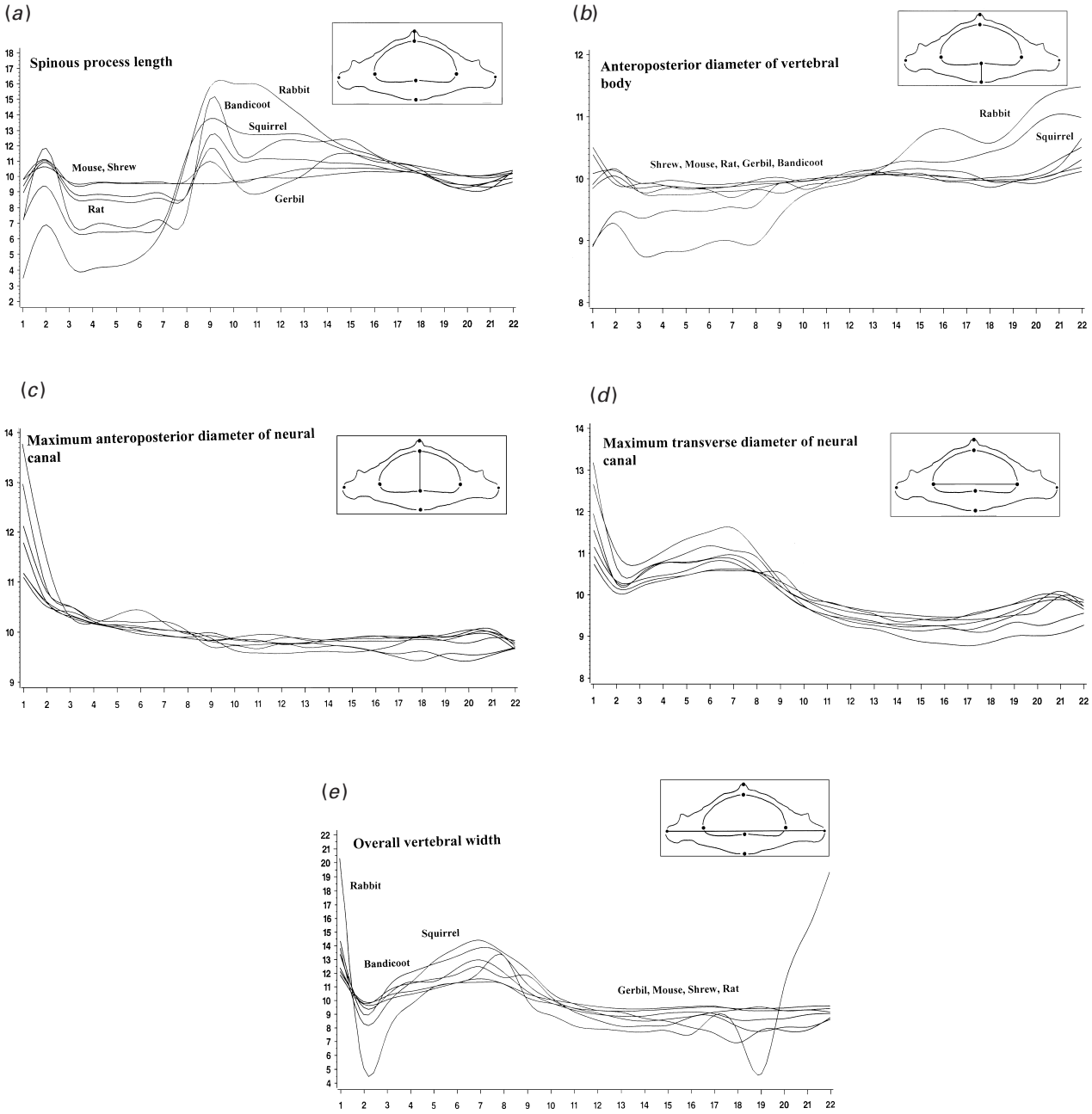


Fig. 3. Vertebral dimensions at each vertebral level for all species in the study. Scaled; the vertical axis is in arbitrary units.

appreciated if scaled (because of the range of body sizes in our sample), and considered variable by variable.

Spinous process length (Fig. 3*a*) was the most variable dimension in the sample. It showed a localised increase in the region of vertebra 2 (C2) in all species. Apart from this the pattern for shrew was almost a horizontal line: mouse was similar except for a further localised increase at vertebra 9 (T2). Rat, gerbil and bandicoot also showed this localised peak, followed by a drop and a small increase in the lower thoracic region. Squirrel, and especially rabbit had large thoracic spinous processes over the upper thorax.

Maximum anteroposterior diameter of the vertebral body (Fig. 3*b*) was almost constant over the whole column in bandicoot, gerbil, mouse, rat and shrew, but depressed in the neck and increased in the lower thorax (beyond T6–T7, vertebra 15–16) in rabbit and squirrel.

Maximum anteroposterior diameter of the neural canal (Fig. 3*c*) was large in all species for C1, proportionately more so in larger animals, then constant throughout. Maximum transverse diameter of the neural canal (Fig. 3*d*) followed a similar curve in all species, being widest in the neck region of rabbit and squirrel.

Table 3. *Mean limb bone lengths (in mm ± standard error) in different species*

Bone	Species						
	Shrew (*Microtus agrestis*)	Mouse (*Mus musculus*)	Gerbil (*Gerbillus sp.*)	Rat (*Rattus rattus*)	Bandicoot (*Bandicos indica indica*)	Grey squirrel (*Sciurus carolinensis*)	Rabbit (*Lagopus sp.*)
Humerus	13.21 ± 0.82	12.64 ± 0.37	16.45 ± 0.31	22.24 ± 3.45	33.34 ± 3.62	44.32 ± 1.49	60.28 ± 4.42
Radius	12.08 ± 0.72	11.71 ± 0.37	18.03 ± 0.32	20.56 ± 0.73	29.63 ± 1.90	41.92 ± 1.98	54.35 ± 5.95
Ulna	14.49 ± 0.76	14.10 ± 0.40	21.59 ± 0.54	24.83 ± 1.12	36.66 ± 2.84	49.81 ± 1.30	64.57 ± 7.25
Femur	14.99 ± 1.18	15.73 ± 0.43	25.44 ± 0.51	30.56 ± 1.26	45.03 ± 5.28	57.41 ± 1.55	79.17 ± 6.04
Tibiofibula	18.91 ± 1.14	18.14 ± 0.45	29.62 ± 0.62	34.34 ± 1.48	49.14 ± 4.12	62.8 ± 1.63	87.12 ± 6.33

Overall vertebral width (Fig. 3*e*) showed a rather complex and variable set of patterns but was essentially similar in most species with a little narrowing around C2 (vertebra 2) and widening in the lower neck region. Rabbit diverged widely at each end of the column.

Limb dimensions

The lengths of the major bones of the fore and hindlimb are presented in Table 3. All differences were significant at the 0.05% level (SAS GLM) except comparisons between mouse and shrew, where no significant differences were seen. Ratios of forelimb: hindlimb length measured in various ways (Table 4) also showed interspecific differences.

DISCUSSION

A structure like a bony vertebra or a longbone inevitably responds to a number of constraints governing its dimensions: some of these will be genetic, some due to other causes. We can begin to analyse these differences by looking at animals which differ in some aspect of nature or nurture: those with different genetic backgrounds or different upbringings. If we choose to look at a longbone we have essentially only one data point per animal (left and right femora from the same individual, for instance, tend to be similar in length): vertebrae differ, in that a mammal which has in effect only one femur has many vertebrae. We may therefore measure corresponding dimensions anywhere or everywhere within this metameric vertebral series and extract a pattern, a picture of the way a particular vertebral dimension varies along the column.

We know that in most animals vertebrae are not all the same size, neither are they all the same shape: yet they are similar. Corresponding vertebrae from different species also resemble one another. This paper asks what happens to a small suite of measurements as we move along the column and from species to species.

We have already demonstrated (Johnson & O'Higgins, 1994) that inbred strains of mice, genetically separated over many generations, show minor variations in the pattern of skeletal measurements. The first part of the current study demonstrates that this pattern is robust. The mice used in this study have been highly selected for body size, with large mice weighing an average of 145% and small lines 62% of control weights (Falconer, 1973). We (Johnson et al. 1988) had previously been able to distinguish the shapes of T1 and T2 vertebrae both from large versus unselected versus small mice and from different replicates of Falconer's experiment using Fourier analysis. The measurements we took for the present study were unable to distinguish between similar mice from 2 separate replicates of the experiment (e.g. between QLA and QLF) but could detect selection for size (e.g. between QLA and QCA). The overall pattern of each variable as we progress along the vertebral column is, however, characteristic of mice, and the differences between mice selected for size disappear when the data are scaled. It seems reasonable to assume that the patterns of data from other species in this study (which may have come from a genetically mixed wild population of varying age and hence size) are equally robust.

When we compare measurements between species differences in pattern begin to appear. These are most marked in spinous process length and vertebral body a-p diameter, and least evident in dimensions associated with the neural canal, confirming both our comparison of man and mouse and our studies on inbred mouse strains where these dimensions showed

Table 4. *Limb proportions in 7 small mammals*

	Shrew	Mouse	Gerbil	Rat	Bandi-coot	Squirrel	Rabbit
Humerus : femur	0.88	0.80	0.65	0.73	0.75	0.77	0.76
Humerus : radius	1.09	1.08	0.91	1.08	1.13	1.06	1.11
Femur : tibiofibula	0.79	0.87	0.86	0.89	0.92	0.91	0.91
Front limb : hind limb (humerus + radius : femur + tibiofibula)	0.75	0.72	0.63	0.65	0.67	0.72	0.69

GLM gave significant differences (0.05 %) for humerus : femur all except rabbit-squirrel, bandicoot-rat; front limb : hind limb all except mouse-squirrel, bandicoot-rat.

the greatest and least difference respectively between individuals within strain, between strains and between species. Measures which vary show a specific pattern, which may well be a variation of a more general mammalian pattern.

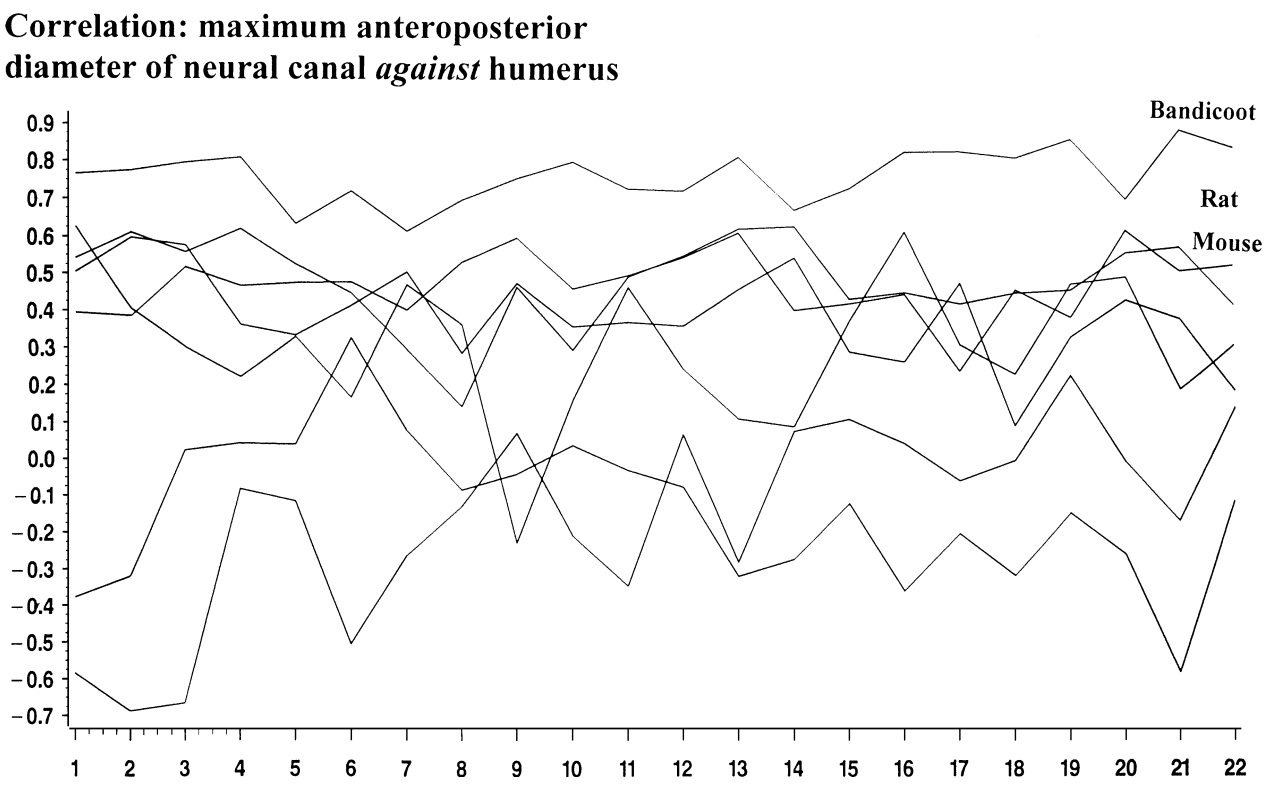
The greatest variation is in the pattern of the spinous process. Although we have taken a crude measure, the vertical height of the process, which ignores its angulation, a clear picture emerges. Much of the interspecific difference centres on T2, an important pivotal point to which are attached many muscles running to the occipital region (Searle, 1954). The spinous process of T2 in mouse is formed as a separate ossification which becomes incorporated as a bony extension of the vertebral arch late in development, in fact after birth (Johnson & Kida, 1995). In some mice this union fails to occur or is incomplete and bony preparations of T2 show spurs of bone of different sizes representing the spinous process (Grüneberg, 1963). We suspect that the dorsal spine of shrew (which was not present in any of our sample, nor in any other shrews in the Grüneberg collection) consistently fails to make this bony union and is not preserved in papain digested preparations. In rat, gerbil, squirrel, bandicoot and rabbit it is almost always present. In these species, however, the pattern of spinous process differs from that in shrew and mouse. Bandicoot, rat and gerbil show a decline beyond T2 followed by a second increase in the lower thorax whilst rabbit and squirrel have large processes throughout the thoracic region.

Other measurements show more or less marked deviation from the pattern seen in mouse. The anteroposterior height of the vertebral body groups closely for bandicoot, rat, mouse, shrew and gerbil but squirrel and rabbit differ with smaller values in the upper part of the column, down to T5–T6 and larger ones in the lower part of the column. Patterns for the height and width of the spinal canal are remarkably consistent and follow the mouse pattern rather closely. The validity of the final dimension considered (overall vertebral width) is open to criticism as the maximum width of the bone does not necessarily represent homologous structures in different parts of the column. However, it is likely that the same structure was represented in all columns at any particular level. Again there is good correspondence between most species, with rabbit showing a characteristic narrowing between C1 and C2 and further deviations in the presacral region. Overall we may summarise: there are many commonalities in the 7 species of small quadrupeds which we have examined and some discrepancies.

This pattern is not, however, universal to all mammals. In a previous paper (O'Higgins et al. 1997) we showed that the patterns in mice and men were very different. The obvious difference seems to be one of gait: man is a biped and mouse a quadruped. Are we distinguishing locomotor pattern? If this is true in man vs mouse (where locomotion is very different) might we not see smaller differences in the pattern amongst different quadrupeds? If this were so it would be reasonable to expect some correlation between variation in patterns of vertebral dimensions and some aspect of limb dimensions (crudely an animal with long hind limbs, like a rabbit might be expected to have a different gait from a shrew, and an animal which runs to be different from one which hops).

In practice all measures of limb bone length differ from each other (Table 3) except in mouse and shrew where there was no significant difference in any bone measured: this simply demonstrates that mouse and shrew are similar in size and the other animals larger. More information might be expected from the ratios of bones in fore and hindlimb. If we compute some of these (Table 4) we find that the shrew has the ratio of humerus : femur and front : hind limb closest to unity and the gerbil the smallest. This tells us that the gerbil has relatively long back legs, and we can assemble a list of decreasing disproportion based on these ratios which runs gerbil, rat, bandicoot, squirrel/rabbit,

(*a*)



(*b*)

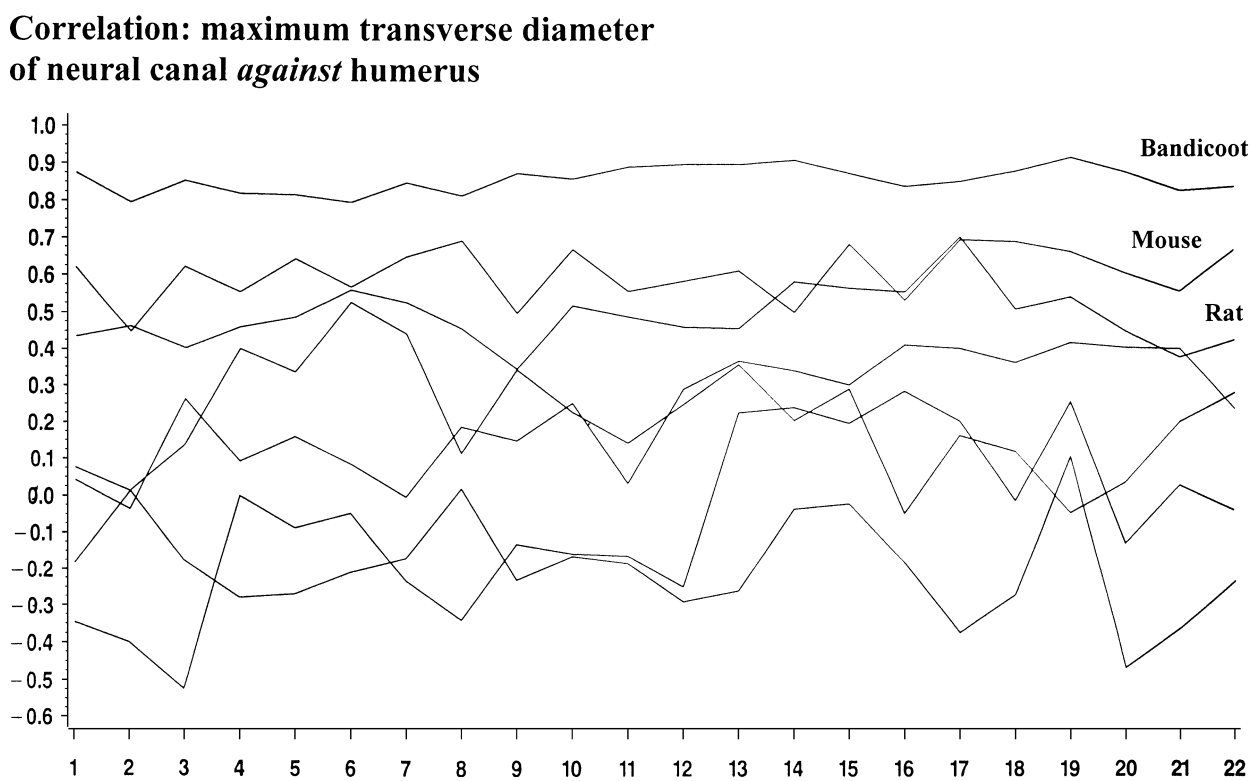


Fig. 4. Correlations of height and width of spinal canal with length of humerus.

mouse and shrew. Unfortunately this is not simply related to the pattern of vertebral dimensions. Rabbit and squirrel do share rather similar curves for vertebral body height and spinous process height, but gerbil and shrew do not form extremes: in fact they are fairly similar to each other. Neither can we see any clear distinction between animals that hop (rabbit, gerbil) and those that run (the remainder).

Correlations between vertebral dimensions and long bone lengths are variable, usually lying between $+0.9$ and -0.8. The only trend we can isolate is a tendency for bandicoot, mouse and rat correlations to cluster together as high, positive values (Fig. 4). This seems not to relate to body size and may be gait related. If the changes in specific pattern are related to gait, locomotor pattern and relative limb length, as was suggested by the differences between man and mouse, we have failed to demonstrate it here.

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