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Differential segmental growth of the vertebral column of the rat (*Rattus norvegicus*)

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Abstract

Despite the pervasive occurrence of segmental morphologies in the animal kingdom, the study of segmental growth is almost entirely lacking, but may have significant implications for understanding the development of these organisms. We investigate the segmental and regional growth of the entire vertebral column of the rat (*Rattus norvegicus*) by fitting a Gompertz curve to length and age data for each vertebra and each vertebral region. Regional lengths are calculated by summing constituent vertebral lengths and intervertebral space lengths for cervical, thoracic, lumbar, sacral, and caudal regions. Gompertz curves allow for the estimation of parameters representing neonatal and adult vertebral and regional lengths, as well as initial growth rate and the rate of exponential growth decay. Findings demonstrate differences between neonatal and adult rats in terms of relative vertebral lengths, and differential growth rates between sequential vertebrae and vertebral regions. Specifically, relative differences in the length of vertebrae indicate increasing differences caudad. Vertebral length in neonates increases from the atlas to the middle of the thoracic series and decreases in length caudad, while adult vertebral lengths tend to increase caudad. There is also a general trend of increasing vertebral and regional initial growth and rate of growth decay caudad. Anteroposterior patterns of growth are sexually dimorphic, with males having longer vertebrae than females at any given age. Differences are more pronounced (a) increasingly caudad along the body axis, and (b) in adulthood than in neonates. Elucidated patterns of growth are influenced by a combination of developmental, functional, and genetic factors.

Keywords: Segmental growth; Vertebrae; Morphometrics; Gompertz curves

Introduction

A segmental body plan is characteristic of many metazoan groups (Richardson et al., 1998; Brusca et al., 2002). Vertebrates are segmented throughout prenatal development and into adulthood (Morin-Kensicki et al., 2002). Prenatally, paraxial mesoderm gives rise to segmental somites, which are arranged along the anteroposterior (AP) axis (Hopper and Hart, 1985; Brickell, 1995). Somitic cells dissociate, giving rise to sclerotomes, which condense to form vertebrae (Hopper and Hart, 1985; Morin-Kensicki et al., 2002). During development, sclerotomes, and hence vertebrae, are shifted relative to the original somites, and adjacent somites contribute to more than one vertebra (Remak,

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1855; Brand-Saberi and Christ, 2000). This resegmentation results in vertebrae not corresponding directly to the original segmental boundaries (Morin-Kensicki et al., 2002). Ultimately, the vertebrae chondrify, and may subsequently ossify, and are retained as the axial skeleton throughout postnatal life.

Functionally, the vertebrae are assembled as a series of rigid, linked elements (Hukins and Meakin, 2000), which collectively determine the postcranial length of the animal, and integrate their growth to yield body regions that are represented as predictable portions of the axial length of the organism. Integrative segmental growth (in terms of vertebrae being surrogates of the primary pattern of segmentation), therefore, relates directly to the linear dimensions of the body, and to the linear disposition of other anatomical systems. Understanding how the vertebral column apportions growth throughout ontogeny, and between species, provides a window into the potential for regional differences in growth that influence overall body form.

The roles of the vertebral column are well established. It protects the spinal cord, allows for muscle attachment, and acts as a support structure (Slijper, 1946; Liem et al., 2000). In addition to being segmental, the mammalian vertebral column is regionalized (Flower, 1885). Segmentation and regionalization are distinct features of its development, with the former resulting from the establishment of repeated units along the AP axis, and the latter arising from the differentiation of morphologically distinctive areas along that axis (Morin-Kensicki et al., 2002) – cervical, thoracic, lumbar, sacral, and caudal (Flower, 1885; Pilbeam, 2004, and references therein). Adjacent vertebrae differ in morphology, even within a vertebral region (Liem et al., 2000), and their identities are determined by developmental genes such as *Hox* prenatally (Gaunt, 1994; Burke et al., 1995; Wellik and Capecchi, 2003).

Segmentation and regionalization lend themselves well to the study of integrative growth phenomena and the differentiation of segments. Vertebral morphology varies along the AP axis of the adult, and has been studied in a variety of mammalian species (Slijper, 1946) including humans, mice and a number of other rodent species (Johnson et al., 1988; O'Higgins et al., 1997; Kida et al., 1999). These differences in vertebral morphology along the AP axis raise the question of how the constituent regions grow. Segmental growth of the vertebral column has, however, only been examined for the caudal series of a few lizards (Bergmann and Russell, 2001; Bergmann et al., 2003, 2004), and has not heretofore been examined for the entire body axis of vertebrates. Likewise, no invertebrate segmental structural plans have been investigated in this way.

To elaborate on previous studies of segmental growth (Bergmann and Russell, 2001; Bergmann et al., 2003, 2004), we model vertebral and regional growth of the entire vertebral column of the rat, *Rattus norvegicus*. The rat is a well studied and established model organism, and being mammalian, has distinct vertebral regionalization. Furthermore, these qualities of the rat allow for the collection of specimens of known ages, something that is impossible when studying museum specimens of lizards, and that allows the use of direct age as an independent variable, rather than a proxy for age, such as snout-vent length (Bergmann and Russell, 2001). The null hypotheses posited here are that the lengths and growth rates of all vertebrae in the series and all vertebral regions are equal. However, comparative observations across mammalian taxa (Kida et al., 1999), and findings from the study of vertebral growth in the tail of lizards, lead to the expectation of differential segmental morphology and growth rate. By studying vertebral growth, we quantify the mechanism of the establishment of the adult segmental morphology from the neonatal morphology. We also address how studies of growth such as this one can be placed into an explicit experimental context by integrating them with developmental genetic studies, as many developmental genes (e.g. *Hox*) are expressed in a segmental manner and establish segmental identity (Gaunt, 1994; Haack and Kessel, 1994; Burke et al., 1995; Crawford, 2003; Pilbeam, 2004).

Materials and methods

Specimen and data collection

One hundred five Sprague–Dawley rats (*R. norvegicus*), spanning an age range from 0 to 84 days (neonate to adult), were raised under standard laboratory conditions and euthanized via carbon dioxide asphyxiation following University of Calgary Animal Care Protocol LBI2002-011. Specimens were stored frozen at −20 °C, and thawed at 4 °C to allow manipulation, at which time external measurements and radiographs were taken.

Five rats, representing both sexes, from each sampled post-natal age were included. Individuals were sampled every other day from birth until 22 days of age (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 days), and every seventh day thereafter (28, 35, 42, 49, 56, 63, 70, 77, and 84 days) (Bentley and Taylor, 1965). Six adult rats that were older than 84 days were also examined to confirm that 84-day-old rats had attained adult size. This procedure allowed the assembly of an ontogenetic series consisting of individuals of known age, equally sampled at all sizes.

Measurements of head-body length (HBL), and tail length (TL) were taken, using a straight edge ruler, to the nearest millimeter. Right hind foot length was measured using Mitutoyo digital calipers to the nearest

0.01 mm. Each measurement was taken three times, and the average used in all subsequent analyses. Radiographs were taken using a Hewlett-Packard Faxitron model 43805N radiology unit and Polaroid® Type 55 black and white positive/negative film. During radiography, rats were positioned on their right side and in direct contact with the film, yielding a 1:1 object to image size ratio (Myers, 1998; Bergmann and Russell, 2001).

Subsequent to radiography, all X-rays were scanned, saved as JPG images, and imported into Image J 1.29 (Rasband, 2002). A one cent coin, measured to the nearest 0.01 mm using Mitutoyo digital calipers, was used to calibrate Image J using the "set scale…" function. Image J was then used to measure skull, tibia, and femur lengths, as well as all vertebral lengths and the lengths of all intervertebral spaces to the nearest 0.01 mm (Fig. 1). Each measurement was made three times and all vertebral measurements were made by the same person (ADM), eliminating inter-observer error. The use of a digital version of each radiograph and measurement using Image J resulted in coefficients of variation for each three measurements not exceeding 0.01. Hence the procedure for measurement was highly precise. Skull length was measured from the occiput to the tip of the nasal bones, avoiding inclusion of incisors, which are absent in neonates. Measurement of limb bones included the diaphysis and both epiphyses. As rat vertebrae are amphiplatyan, being flat on both ends, measurement was not confounded by the overlapping of adjacent vertebral centra. Vertebral and intervertebral space lengths were measured from the same set of two landmarks per vertebra (as seen in two-dimensional images) – one defined as the dorsal anterior corner of the centrum of each vertebra and the other as the dorsal posterior corner of the centrum. These landmarks were not identifiable for the atlas (C1) because it lacks a centrum. Points of measurement for this vertebra were simply approximated, resulting in a measurement not directly homologous with those for other vertebrae and with a lower model R^2 value (see below). Vertebrae were assigned to type, with seven cervical, thirteen thoracic, six lumbar, four sacral, and a variable number of caudal vertebrae (following Greene, 1949; Hebel and Stromberg, 1976; but contra Wells, 1964, for the number of sacral vertebrae). All measurements were imported and compiled in Microsoft® Excel XP©. From these data, vertebral and intervertebral space lengths (Fig. 1) were summed for cervical, thoracic, lumbar, sacral, and caudal (for only the anterior 14 caudal vertebrae) regions to give total regional lengths.

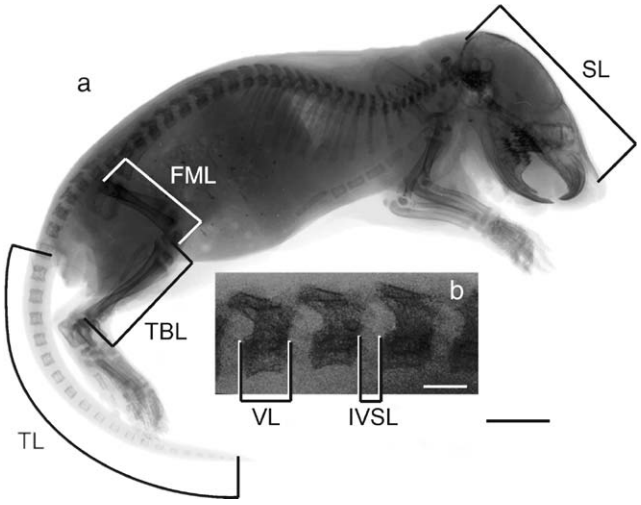

Fig. 1. Radiograph of an 18-day-old specimen of *Rattus norvegicus*, depicting morphometric variables collected for this study. (a) Whole body radiograph depicting major body dimensions: FML – femoral length, SL – skull length, TBL – tibia length, and TL – tail length, as the emergent portion. (b) Inset of the lumbar region depicting vertebral dimensions: IVSL – intervertebral space length and VL – vertebral length. HBL is not shown. Black scale bar represents 10 mm, white scale bar in inset represents 2 mm.

Statistical analysis

All data were log-transformed using Microsoft® Excel XP©, and all statistical analyses were conducted using SYSTAT© 10.2 (Wilkinson, 2002). Although the current study is similar in nature to those of Bergmann and Russell (2001) and Bergmann et al. (2003, 2004), the use of a model lab organism (*R. norvegicus*) allowed for collection of age data in days, which was unavailable for the previous studies, allowing for a more sensitive approach to studying vertebral growth.

Gompertz curves were fitted to each vertebra (Laird et al., 1965, 1968), with age in days as the independent variable and vertebral length as the dependent variable, following the methods of Reichling and German (2000) and German (2004). Specifically, the NONLIN module of SYSTAT (Wilkinson, 2002) was used to fit a Gompertz curve, defined by the equation: $y = Ae^{-be^{-kt}}$, where y is the vertebral length, t is the age in days, and A, b, and k are parameters defining the shape of the curve (see below). The FUNPAR command in SYSTAT was used to calculate a further two parameters using the equations: $w = Ae^{-b}$ and $I = bk$ (Reichling and German, 2000). For each parameter, SYSTAT was used to calculate the asymptotic standard error (Wilkinson, 2002). Of the five parameters estimated, b is biologically unimportant (Reichling and German, 2000) and is not discussed further. A is the asymptote of the curve, or the maximum size of y, while w is the initial size at $t = 0$ (Reichling and German, 2000). I and k describe the actual growth of y, with the former being the instantaneous initial growth rate at $t = 0$, and the latter being the rate of exponential growth decay (Reichling

and German, 2000). Our approach has the caveat of being conducted on a cross-sectional data set (ontogenetic series) as opposed to a longitudinal one, decreasing its sensitivity (German, 2004). However, this approach is more sensitive and more fully characterizes growth than the use of a linear method, such as linear regression. It is also important that age data are available, allowing the implementation of this method, as opposed to a linear approach, where scaling is examined and age data are ignored or unavailable.

Sexual dimorphism was evaluated by fitting a Gompertz curve, as described above, to each vertebra, with the sexes pooled. Residuals were calculated using SYSTAT (Wilkinson, 2002), and compared using Mann–Whitney *U*-tests (see Pimentel, 1979; Ranta et al., 1994; Pan and Oxnard, 2001; Ji et al., 2002, for similar approaches). The Mann–Whitney *U*-test was used because for most vertebrae (24 out of 44 vertebrae) the assumptions of either normality or homoscedasticity of a two-sample *t*-test were violated (data not shown). Furthermore, the Mann–Whitney *U*-test is non-parametric, so is more conservative than a *t*-test. The Mann–Whitney *U*-tests were used to examine sexual differences for all vertebrae so that test statistics were comparable between individual tests. χ^2 approximations calculated by SYSTAT were plotted against vertebral position to examine the relative degrees of sexual dimorphism from a segmental perspective. This was deemed appropriate because sample sizes and type of test were held constant for all vertebrae. Gompertz curves were then fitted to each vertebra for each sex separately, allowing us to examine differences in parameters between the sexes.

In addition to a segmental analysis, a regional analysis was undertaken. Total lengths (all vertebrae and intervertebral spaces) of the cervical, thoracic, lumbar, sacral, and caudal regions, as well as externally measured HBL and TL, were considered. A Gompertz curve was fitted to each of these variables and the residuals were calculated. Sexual dimorphism was evaluated as described for the segmental analysis, and separate Gompertz curves were fitted to each variable for each sex. This allows for the comparison of segmental growth patterns to regional ones.

Results

Sexual dimorphism

Examining residuals from Gompertz curves that included all individuals for sexual differences allowed for a simple evaluation of sexual dimorphism, while considering all ages at once. From a segmental perspective, there were significant sexual differences in residual values for all vertebrae examined, except vertebra S4 (Fig. 2a). Likewise significant sexual dimorphism in Gompertz residuals was observed for HBL, TL, and each of the five vertebral regions (Fig. 2b). In both the segmental and the regional analyses, male residual values were greater than those of females (results not shown), indicating that males tend to be larger for all variables at the ages examined (0–84 days).

Our analysis of sexual dimorphism further allows a consideration of relative levels of dimorphism along the AP axis. From a vertebral perspective, dimorphism is relatively low in the anterior cervical vertebrae and increases considerably in the posterior cervicals (Fig. 2a). There is relatively low (but still significant) dimorphism in most of the thoracic vertebrae, and this again increases in the posterior four thoracics, continuing with the lumbar. Sexual dimorphism is least apparent for the sacrals, but increases tremendously in the caudal series. The anteriormost four caudal

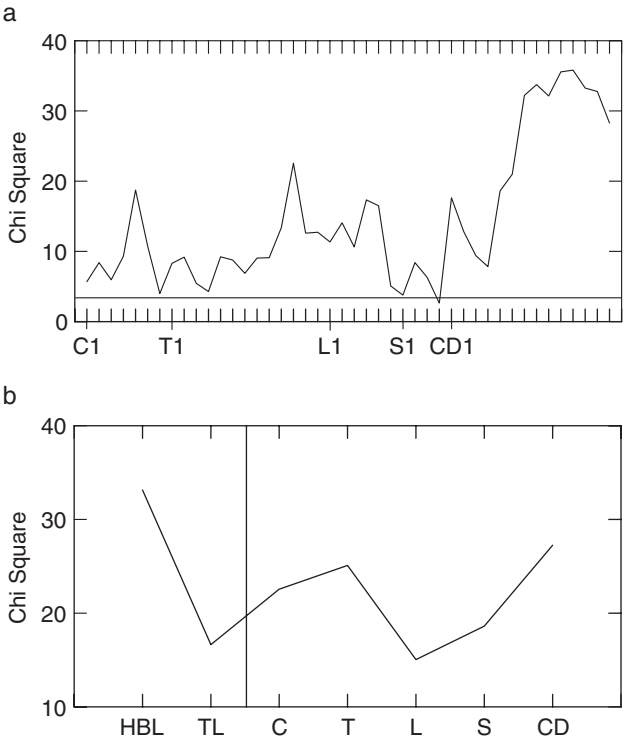

Fig. 2. Segmental (a) and regional (b) analysis of sexual dimorphism. χ^2 approximations from Mann–Whitney *U*-tests (df = 1) testing for significant differences between sexes in residuals from Gompertz curves calculated with sexes pooled for each vertebra. The higher the χ^2 value, the greater the sexual dimorphism. The solid horizontal line near the bottom of plot (a) indicates the critical χ^2 value for $\alpha = 0.05$. All regional values (b) are well above the critical value. C – cervical, T – thoracic, L – lumbar, S – sacral, and CD – caudal vertebrae. Vertical line in (b) segregates external variables (to the left) from vertebral regions (to the right). HBL – head-body length, TL – tail length.

vertebrae remain within the body wall (pers. obs.) and exhibit lower sexual dimorphism than those vertebrae contributing to the (emergent) tail proper (Fig. 2a). Regional sexual dimorphism (Fig. 2b) does not perfectly match the segmental situation (Fig. 2a). The caudal region remains the most dimorphic, but is followed by the thoracic region. The lumbar region exhibits the least sexual dimorphism. Oddly, when the tail is considered in its entirety (from external measurement), there is relatively low sexual dimorphism. Sexual dimorphism is high for HBL. Due to documented sexual dimorphism in all regions and for all vertebrae, all subsequent analyses were conducted separately for males and females.

Analysis of segmental axial growth

Fitting of a Gompertz curve to each vertebra considered allowed for examination of four biologically interesting parameters from a segmental perspective. Corrected R^2 values were consistently greater than 0.85, except for the atlas (C1), where $R^2 = 0.79$ for males, and the curve could not be estimated for females (parameter values missing in Fig. 3). The parameter w is an estimate of neonatal size at $t = 0$ (Fig. 3a). At birth, there is a general trend of vertebrae increasing slightly in length through the cervical and thoracic series, peaking at approximately vertebra T6, and then decreasing steadily in length caudad. Both sexes follow the same trend, but males have marginally shorter vertebrae between C2 and L1. The pattern of vertebral length at adulthood, parameter A (Fig. 3b) is quite different. Parameter estimates for A have far less standard error than those for w and males tend to have longer vertebrae than females caudal to L1. Values of A correlate positively and very closely with actual vertebral lengths for the largest individuals (Pearson correlation: males $R = 0.946$, females $R = 0.964$), indicating a close match between model-estimated and observed values. At adulthood, cervical vertebrae are the shortest (also see Fig. 1, where this pattern is already becoming established), and thoracics are only slightly longer. Lumbar vertebrae increase substantially in length relative to more anterior vertebrae and are approximately the same length as the sacrals. The first four caudal vertebrae are the shortest in that series, but more posterior ones (measured up to CD14) are the longest vertebrae of all.

The final two parameters characterizing growth are I and k, and both show the same patterns for both sexes. The instantaneous initial growth rate, I, is comparable and low between C1 and the end of the thoracic series (Fig. 3c), but then increases steadily through the lumbar, sacral, and caudal vertebrae. The rate of exponential growth decay, k, increases from the atlas to the last caudal vertebra considered – CD14 (Fig. 3d). However,

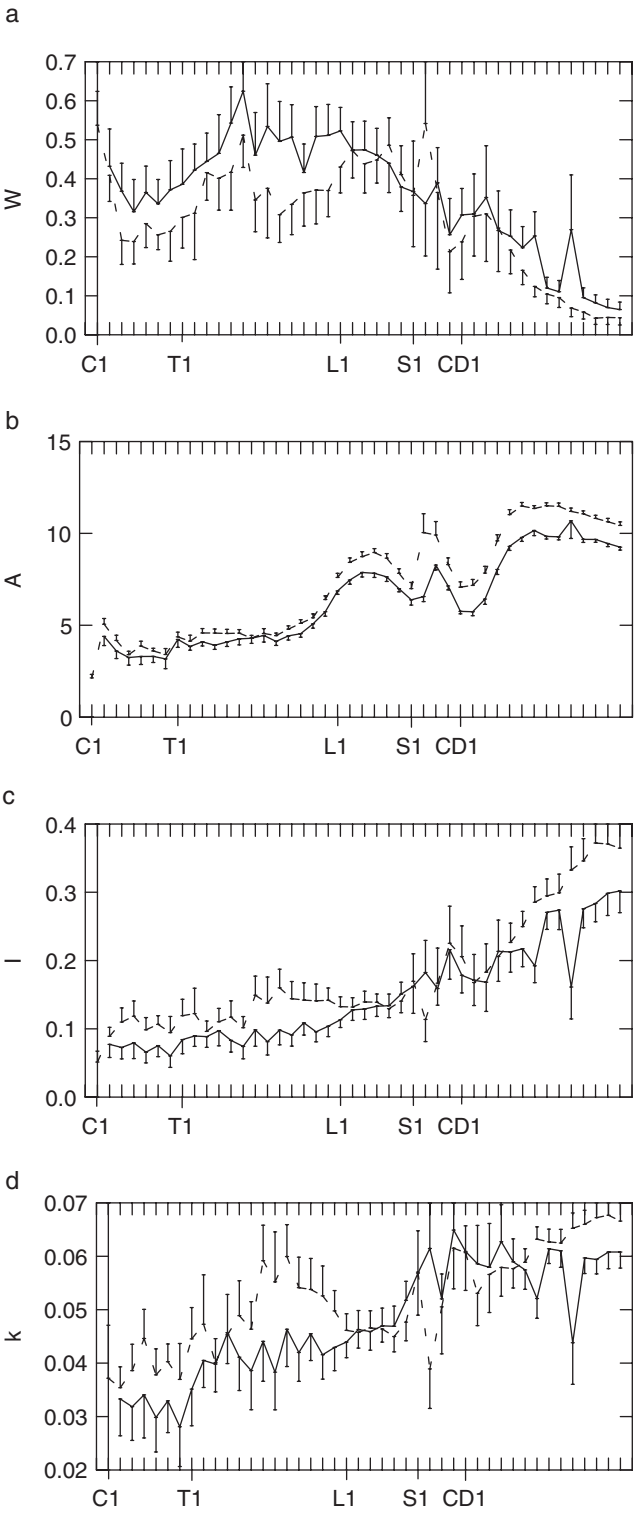

Fig. 3. Gompertz parameters for the segmental analysis of growth of vertebrae for *Rattus norvegicus*. Parameters w (a), A (b), I (c), and k (d) are plotted segmentally, with vertebral position represented on the x-axis. C – cervical, T – thoracic, L – lumbar, S – sacral, and CD – caudal vertebrae. Dashed line represents parameters for the male sample, solid line for the female sample. Error bars represent asymptotic standard error. See text for a detailed explanation of parameters.

this increase is less substantial than that for I (Fig. 3c). There is also a slight leveling-off in the AP increase at the lumbar series.

Analysis of regional axial growth

Although examination of differential growth of all individual vertebrae is perhaps the most holistic approach, it is possible that such a detailed treatment obscures regional patterns of growth. This justifies the consideration of the cervical, thoracic, lumbar, sacral, and caudal regions as individual units. The same set of parameters were examined regionally as well as segmentally. The lowest corrected R^2 value for the regional analysis was 0.91, reflecting excellent characterization of the data by the Gompertz curves. At birth, relative regional lengths are similar between the sexes, with females having slightly longer cervical, thoracic, and caudal regions (Fig. 4a). At birth, the thoracic region is longest, followed by the caudal, lumbar, cervical, and sacral. Care must be exercised when considering the caudal region, however, because we considered only the first 14 caudal vertebrae (and intervertebral spaces) when calculating regional lengths. However, when caudal region length is compared to externally measured TL at birth (Fig. 4a), they are comparable, indicating that the first 14 vertebrae represent almost the complete length of the tail at birth. At adulthood, the regional length patterns exhibited by the sexes are again very similar, with males having a slightly longer tail and HBL (Fig. 4b). The tail and caudal region is much longer, relatively, than at birth and less of the externally measured TL is accounted for by the first 14 caudal vertebrae. The caudal region is the longest, followed by thoracic, lumbar, sacral, and finally cervical (Fig. 4b). TL is almost as long as HBL.

Regionally, the instantaneous initial growth rate (Fig. 4c) and the exponential rate of growth decay (Fig. 4d) exhibited very similar patterns. Males had higher values for cervical, thoracic, and caudal regions, as well as for HBL and TL. For lumbar and sacral regions the sexes had similar growth patterns. The instantaneous initial growth rate increased steadily caudad (Fig. 4c). Also, the rate of growth for HBL was quite low, while that for TL was very high, higher than for any vertebral region. The exponential rate of growth decay followed a similar, but less well defined pattern, especially for males, who had a comparable value for this parameter for the thoracic, lumbar, and sacral regions (Fig. 4d).

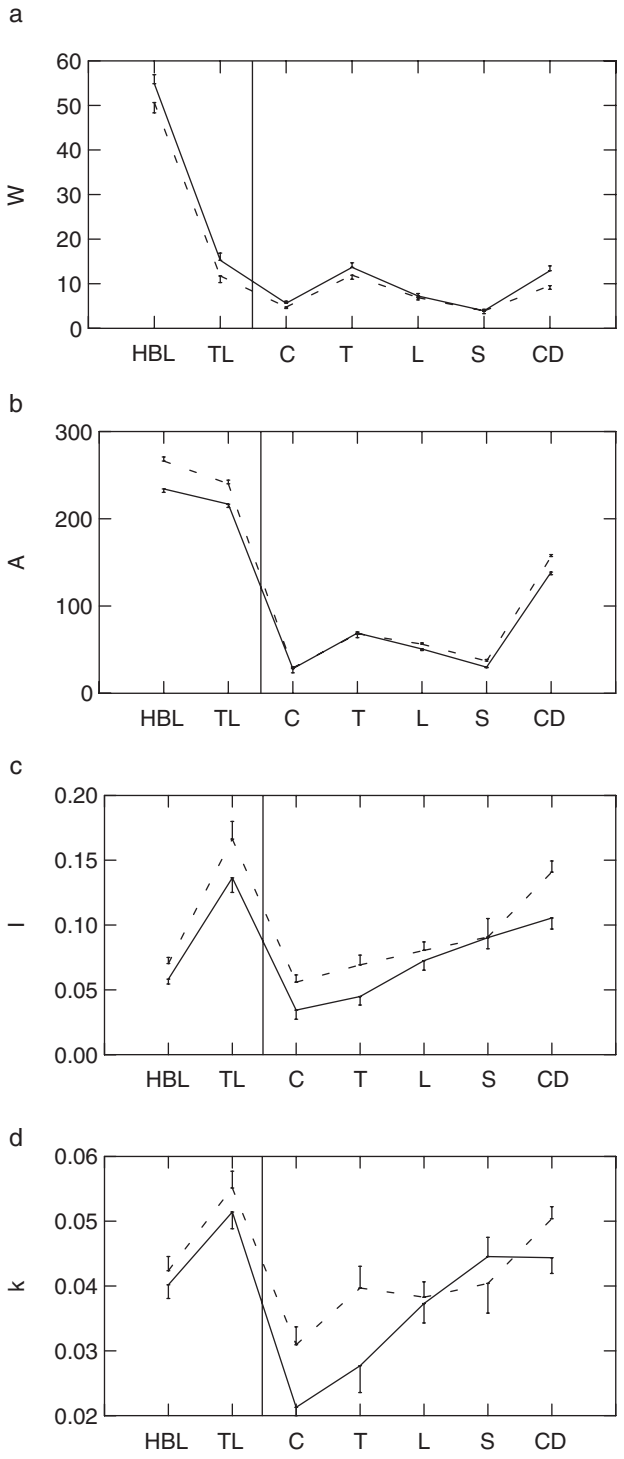

Fig. 4. Gompertz parameters for the regional analysis of growth for *Rattus norvegicus*. Parameters w (a), A (b), I (c), and k (d) are plotted for each vertebral region and for head-body length (HBL) and tail length (TL). C – cervical, T – thoracic, L – lumbar, S – sacral, and CD – caudal vertebrae. Dashed line represents parameters for the male sample, solid line for the female sample. Error bars represent asymptotic standard error. Vertical line separates vertebral regions from external variables. See text for a detailed explanation of parameters.

Discussion

Growth, a general change in size over time, occurs in concert with changes in shape, structure, and function

(Kirkwood and Mace, 1997). Differences in these factors, whether inter- or intraspecifically, generally arise through variation in the timing of the onset and duration of development, and variation in growth rates (Kirkwood and Mace, 1997; German, 2004). All of these aspects of ontogeny are interrelated and pertinent to the consideration of vertebral column growth in *R. norvegicus*, and are addressed herein. Patterns of segmental and regional growth in the rat can differ between the sexes and be influenced by developmental, functional, and genetic factors. This necessitates consideration of embryology (prenatal growth influencing postnatal growth patterns), functional morphology (the functional differentiation of vertebrae in different positions along the column), and *Hox* gene expression in our assessment of overall patterns of growth.

Segmental and regional sexual dimorphism

We found sexual dimorphism to be present in virtually all variables considered (Fig. 2). Only in the fourth sacral vertebra was dimorphism not significant. A general finding of sexual dimorphism in *R. norvegicus*, with males being larger, has been documented previously (Reichling and German, 2000). However, this study is the first to document it from a segmental perspective. Two of our findings in this regard are particularly surprising. First, sexual dimorphism is lowest for the sacral vertebrae, whether three or four vertebrae are included. One might expect it to be greatest in this region, given that the sacrum is the site of articulation between the pelvis and spine (Hebel and Stromberg, 1976). Second, sexual dimorphism was by far the greatest in the caudal vertebrae, especially those furthest caudad (out of the 14 considered). The tail is expected to play the same roles in both sexes and is not associated with limbs and internal organs. One potential explanation for this finding is that the larger sex (males) requires heavier tails to act as adequate counterbalances during locomotion. From a regional perspective, the sacrum as a whole mirrors segmental patterns (Fig. 2b). The caudal region also reflects high degrees of sexual dimorphism. However, this dimorphism is greater than that exhibited by the externally measured tail (TL), suggesting that much of the documented sexual dimorphism is contained in the first 14 caudal vertebrae, as opposed to those further caudad, or the intervertebral spaces. Examination of segmental asymptotic (final) length of vertebrae (Fig. 3b) further documents relative levels of sexual dimorphism observed from comparison of Gompertz residuals. Specifically, dimorphism is relatively low along much of the anterior vertebral column and becomes greater in the lumbar region. The male and female lines are most widely separated (males with greater asymptotic length) for the caudal vertebrae (Fig. 3b).

Despite consistently high sexual dimorphism when Gompertz residuals are compared between the sexes (Fig. 2), dimorphism is far less apparent when Gompertz parameters are considered, and this differs from the findings of Reichling and German (2000). For example, initial vertebral length (Fig. 3a) is virtually indistinguishable between sexes for different vertebrae. This is also the case regionally (Fig. 4a). A similar situation exists for segmental initial growth rates (Fig. 3c) and rates of growth decay (Fig. 3d). For both parameters, error bars associated with the estimates prohibit differentiation between the sexes in most cases. One exception to this is higher male initial growth rates between vertebrae T7 and T12, and again between CD7 and CD 14 (Fig. 3c). From a regional perspective, males tend to have faster growth and a higher rate of growth decay (Figs. 4c and d) in most situations. However, even in these situations, male and female parameter estimates tend to be rank-ordered similarly between regions. Hence, despite consistent male-biased sexual dimorphism in size of vertebrae and regions, the manner in which the two sexes grow is very similar.

Neonatal and adult morphology, and patterns of growth

Despite highly significant sexual differences in vertebral and regional length, the primary focus of this study is the contrast between neonatal and adult morphologies, and how the latter is attained from the former through growth.

The observation that vertebrae within an animal differ in length (Figs. 3a and b) is symptomatic of numerous morphological differences between these vertebrae (Slijper, 1946; Kida et al., 1999). Although all vertebrae act to protect the spinal cord and serve as points of attachment for muscles involved in locomotion and body support (Slijper, 1946; Hukins and Meakin, 2000; Liem et al., 2000), their morphology also differs based on functional differences associated with differing stresses applied to them by various muscles (German, 1982; Currey, 1984; Bergmann and Russell, 2001). This is reflected in differences in the size and shape of adnexae of the vertebral centra, such as the transverse processes and zygapophyses (Flower, 1885). In addition to minor differences in morphology between adjacent vertebrae, there are more substantial contrasts between vertebrae of different regions of the column (Flower, 1885). For example, neural spines are very pronounced in thoracic vertebrae, while transverse processes are relatively enlarged in lumbar vertebrae. In addition to differences in muscle attachment and forces acting on vertebrae, there is also a differential requirement for

rigidity or flexibility of different regions of the column (Slijper, 1946). For example, the thoracic region is held much more rigidly than the caudal or cervical regions, and this is reflected in more robust anterior and posterior zygapophyses in thoracic vertebrae (Flower, 1885; Liem et al., 2000). The short length of cervical vertebrae (Fig. 3b) contributes to the relatively high flexibility of that region. Also, functional demands associated with the bounding run exhibited by many small mammals require a flexible lumbar region, allowing the arching of the back (Pough et al., 2002).

If there is a direct connection between morphology and function (Wainwright et al., 1976), then differential morphology between neonates and adults has far reaching functional implications. It can be argued that functional demands on the vertebral column of *R. norvegicus* differ prenatally (see below), neonatally, and in adulthood. For example, a peak in lengths of sacral vertebrae in adults (Fig. 3b), which is absent in neonates (Fig. 3a), is suggestive of a more robust and extensive articulation with the anteriorly projected ilium in adults (Flower, 1885). However, these patterns may also be influenced by developmental constraints, with the posterior vertebrae increasingly strongly lagging in their relative development. Faster growth of vertebrae further caudad (Fig. 3c) has the implication that sacral and caudal vertebrae are short at birth, but grow to be among the longest vertebrae in adult rats.

Patterns of vertebral length tend to be different from other vertebral dimensions, as shown by Kida et al. (1999, Fig. 3). The patterns of morphological variation presented for mice (*Mus musculus*) are very similar to those for various other rodents, including *R. rattus* (Johnson and O'Higgins, 1994; Kida et al., 1999). Those authors examined only adult individuals, and their findings, in concert with ours, suggest that adult vertebral length is not a good indicator of other dimensions. Instead, the dorsoventral diameter of the neural canal (Fig. 8b in Johnson and O'Higgins, 1994) matches neonatal vertebral length quite closely (Fig. 3a in this study). This is suggestive of a constraint on minimal vertebral size at birth, associated with the requirements for housing the spinal cord. Long vertebral lengths (this study) and large neural canal diameters (Johnson and O'Higgins, 1994), particularly around the posterior cervical and anterior thoracic region, coincide well with widening of the spinal cord (the cervical enlargement) and the positioning of the brachial plexus (Crouch, 1969). In addition, calculations of moments of resistance to dorsal flexion for a variety of terrestrial mammals exhibit an increasing trend caudad, with a second peak in magnitude in the posterior cervical region (Slijper, 1946). Although Slijper's (1946) examination did not include caudal vertebrae, there is also a correlation between adult vertebral length and vertebral resistance to dorsal flexion.

Differential morphology between neonatal and adult *R. norvegicus* along the length of the column indicates that growth of vertebrae is also differential. In other words, allometry occurs and an adult rat's vertebral column is not simply that of a scaled-up neonate. This is confirmed when segment length is examined for neonatal (Fig. 3a) and adult individuals (Fig. 3b). The lines indicating vertebral length on these two graphs are neither straight, indicating morphological differences in the AP axis, nor parallel, indicating divergent morphology between neonates and adults.

Observed segmental growth patterns (Fig. 3c) are consistent with the general pattern of anterior to posterior prenatal development in amniote embryos (Hopper and Hart, 1985). Directly resultant from this is the observation that, at birth, the head and trunk of a neonate are larger and more developed than are the posterior parts of the animal. Growth from birth to adulthood is, then, allometric, yielding a differently proportioned adult when compared to the neonate (Medawar, 1945).

Growth patterns of individual vertebrae (Fig. 3c) reveal a distinct increase in initial growth rate caudad. This is most evident when vertebral growth rates are examined: there is a relatively smooth increase in growth rate posteriorly along the column, with cervical vertebrae growing the slowest, and vertebrae positioned increasingly posteriorly growing more quickly (Fig. 3c). Such a pattern is reaffirmed when growth is examined regionally. The cervical region grows slowest, with the thoracic, lumbar, sacral, and caudal regions growing at successively increasing rates (Fig. 4c). This is further supported by the observation that externally measured TL grows much faster than HBL (Fig. 4c).

These patterns are consistent with an AP prenatal progression of development, yielding a neonate that is well developed cranially, and less so caudally. Specifically, fast postnatal growth observed in the posterior regions of the animal is complemented by slow prenatal growth of those same regions. Conversely, fast prenatal growth of cervical and thoracic regions results in slower anterior postnatal growth. A similar situation is seen in equids, where the cervical vertebrae grow rapidly prenatally, lengthening the neck (Bard, 1977). Further potential developmental impacts on the growth of vertebrae may result from the size of somites prenatally. In mice, caudal somites tend to be small and numerous, resulting in slow growth of the tail (Tam, 1981). In contrast, the somites in the lumbar region are larger and allow faster absolute axial elongation (Tam, 1981). This provides a mechanism for establishing prenatal regional growth rates, and, as argued here, in turn determines postnatal growth rates. Such a hypothesis requires further testing in numerous species, including the rat.

In addition to an AP pattern of increasing initial growth rate (discussed above), a similar pattern exists

for the rate of exponential growth decay, both segmentally (Fig. 3d) and regionally (Fig. 4d). A high rate of exponential growth decay indicates that growth rate decreases quickly through time, and that growth occurs over a shorter period of time (Reichling and German, 2000; German, 2004). Since this rate correlates positively with initial growth rate, posterior segments and regions grow more quickly, but for a shorter period of time (Reichling and German, 2000). The caudally increasing trend is less well defined for the rate of growth decay (approximately doubling from cervical to caudal vertebrae and regions – Figs. 3d and 4d) than for the initial rate of growth (approximately tripling – Figs. 3c and 4c). This suggests that increasing rate of growth decay does not completely negate the effects of increases in initial growth rate.

Differential growth of vertebrae (Fig. 3c) may also be influenced by constraints imposed by neighboring, articulating elements. The slowest growing vertebra is the atlas, which articulates with the occipital condyles of the skull (Liem et al., 2000), and this matches negative allometry of the skull, relative to HBL (Melin et al., 2005). The growth rate of the atlas appears to be constrained by its articulation with the skull, and because it, unlike other vertebrae, lacks a centrum (Wake, 1979). Measurement of its length is, therefore, not directly homologous to the length of subsequent vertebrae, its centrum being incorporated into the odontoid process of the axis (Wake, 1979). These factors also lead to operational problems with measuring the atlas and resulting in either a decreased R^2 value (0.79 in males), or else an inability to calculate the Gompertz curve for that vertebra (females).

All of the anteriormost fourteen caudal vertebrae grow very quickly, relative to vertebrae further craniad or even other vertebral regions (compare parameter I values – Figs. 3c and 4c). However, the anterior four caudals, although growing quickly, remain the shortest units of the tail (Fig. 3b) in adulthood. These first four caudals are not part of the emergent tail, but instead remain within the body wall (Fig. 1). Those vertebrae further caudad belong to the narrowed, emergent section of the tail proper. Non-emergent and emergent caudal vertebrae are expected to be functionally different because a greater array of muscles is likely to attach to the former, and greater flexibility is expected of the latter. Furthermore, the anteriormost caudals are morphologically more complex than those further caudad (Flower, 1885). For example, the anterior four caudal vertebrae have the most pronounced transverse processes (pers. obs.).

Patterns of caudal growth contrast with those documented in lizards – the only other example of modeling of the growth of a subset of the vertebral column. Four species of iguanian lizards all exhibit the reverse pattern of growth evident in the rat tail: a quickly growing anterior region, with decreases in growth rate caudad (Bergmann and Russell, 2001; Bergmann et al., 2003, 2004). The only exception to this pattern is the iguanian *Phrynosoma coronatum*, where, as in *R. norvegicus*, the base of the tail grows the slowest, with growth rates increasing caudad (unpublished data). The initial phase of postnatal life of a rat is spent as an individual totally dependent upon parental care and incapable of independent locomotion. Neonatal lizards, in contrast, must be freely mobile and able to actively forage from the moment of hatching/birth. Thus, in the neonatal rat the tail is able to "catch up" with the remainder of the body after birth and to become a functional appendage by the time that free mobility is achieved at weaning.

Despite the current study being the only detailed examination of the growth patterns of an entire vertebral column, it provides only a partial picture. We have robustly characterized the growth and morphology of the vertebrae of the rat spine, as well as that of the five vertebral regions exhibited by mammals. However, although intervertebral space lengths were included in vertebral region lengths, we have omitted an analysis of the morphology and changes in the lengths of the individual spaces because huge variation in the data made the fitting of Gompertz or regression curves to the data meaningless ($R^2 < 0.1$). Furthermore, the Gompertz curve is designed to model growth (an increase in size), and, from examination of simple plots of intervertebral space lengths against time, they shrink slightly and then remain unchanged. This makes the use of the Gompertz curve inappropriate for this purpose (Laird et al., 1965; German, pers. comm). Despite these methodological problems, intervertebral spaces play an important role in the proper functioning of the vertebral column. The tissues occupying them act as spacers, resist compression, and give elastic resistance to bending (Slijper, 1946; Hukins and Meakin, 2000). Furthermore, these tissues undergo considerable ontogenetic change over the life of the organism (Urban et al., 2000). Intervertebral space lengths, however, were included in calculation of regional lengths, and their omission does not invalidate our study of vertebral growth.

Genetic-developmental considerations for segmental growth

In recent years, considerable attention has been focused on the genetic basis of segmental development and various developmental genes may influence segmental growth as well. The relevant processes to consider are the development of segmentation and segment identity, which are decoupled and controlled by different genes (Richardson et al., 1998). Although the genetic basis of body segmentation is poorly

understood (but see McPherron et al., 1999; Dubrulle et al., 2001), a number of genes have been identified that specify segment identity, including *Hox*, *Notch*, and *Cdx* (Lewis, 1978; Krumlauf, 1994; Burke et al., 1995; Van den Akker et al., 2002; Crawford, 2003; Hombria and Lovegrove, 2003; Cordes et al., 2004).

Since growth is differential between vertebrae, it is also a component of segment identity and may be influenced by the genes mentioned above. The anterior expression boundaries of numerous *Hox* genes along the AP axis have been mapped (Burke et al., 1995). For example, *Hox*c-6 is expressed at the cervical–thoracic boundary in mice, chickens, geese, and clawed frogs (Burke et al., 1995), and in the zebrafish, *Danio rerio* (Morin-Kensicki et al., 2002). Since all of these species have different numbers of cervical vertebrae, it has been suggested that this gene gives thoracic vertebrae their identity (Gaunt, 1994; Burke et al., 1995). This is further supported by *Hox* knock-out experiments, where the disabling of given *Hox* genes shifts or alters identities of some, but not all, vertebrae (Wellik and Capecchi, 2003). When all *Hox*-10 paralogues are knocked out, ribs are expressed on lumbar, sacral, and anterior caudal vertebrae, suggesting that *Hox*-10 genes act to suppress the expression of ribs (Wellik and Capecchi, 2003). Furthermore, *Hox* genes act in a layered manner, in turn influencing the effects of other *Hox* genes (Wellik and Capecchi, 2003).

Further knock-out experiments may also have a bearing on growth, and future studies, employing methods used by us in conjunction with gene knock-outs would constitute a rigorous approach to determining whether various developmental factors influence or control segmental growth. Such effects on caudal vertebral growth could be studied with *Hoxb-13* knock-outs (Economides et al., 2003). When this gene is knocked out, overgrowth of all major structures derived from the tail bud occurs. Specifically, mice with lost *Hoxb-13* function have two extra and longer posterior caudal vertebrae than wild type mice (Economides et al., 2003). When copies of *Cdx1* and *Cdx2* genes are knocked out, mutant mice show a posterior shift in all vertebral regional transitions (Van den Akker et al., 2002). These genes are upstream regulators of *Hox* genes (Gaunt et al., 2004), and may influence global segmental growth patterns.

Much emphasis has been placed on expression of *Hox*c-6 at the cervical–thoracic transition (Gaunt, 1994; Burke et al., 1995; Morin-Kensicki et al., 2002). The expression of this gene in the mouse does not extend all the way into the tail, but trails off after about seven vertebrae (Burke et al., 1995). From a correlatory perspective, this region coincides very closely with the depressed lengths of anterior thoracic vertebrae (Fig. 3b), which are associated with the true ribs. It is, therefore, possible that the expression of this gene is associated with the limitation of growth of these vertebrae.

Clearly, developmental gene expression patterns and their modification may provide insight into origins of differential growth patterns along the vertebral column. However, no such integrative study has yet been conducted. Further *Hox* paralogue knock-out experiments will be instrumental in going beyond a simple correlatory analysis of *Hox* expression, morphology, and growth. Indeed, it would be interesting to discover how gene knock-out influences growth. Furthermore, regionalization of vertebrae in the tail of fishes cannot be explained by the *Hox* code, which ceases to be differential at the proximal end of the tail (Morin-Kensicki et al., 2002). It is probable that a different set of developmental genes are responsible for differential morphology and growth at the posterior end of the AP axis. Finally, the paired box gene *Pax-1* is expressed in intervertebral disks (Kessel and Gruss, 1990). Further study of these and related genes may shed light on the ontogeny of intervertebral spaces and disks, which exhibit growth patterns not concordant with vertebral growth.

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