Variability and constraint in the mammalian vertebral column

R. J. ASHER*, K. H. LIN†, N. KARDJILOV‡ & L. HAUTIER*

*Department of Zoology, University of Cambridge, Cambridge, UK
†Department of Organismic and Evolutionary Biology, Harvard College, Cambridge, MA, USA
‡Helmholtz-Zentrum Berlin für Materialien und Energie GmbH, Berlin, Germany

Abstract

Patterns of vertebral variation across mammals have seldom been quantified, making it difficult to test hypotheses of covariation within the axial skeleton and mechanisms behind the high level of vertebral conservatism among mammals. We examined variation in vertebral counts within 42 species of mammals, representing monotremes, marsupials and major clades of placentals. These data show that xenarthrans and afrotherians have, on average, a high proportion of individuals with meristic deviations from species' median series counts. Monotremes, xenarthrans, afrotherians and primates show relatively high variation in thoracolumbar vertebral count. Among the clades sampled in our dataset, rodents are the least variable, with several species not showing any deviations from median vertebral counts, or vertebral anomalies such as asymmetric ribs or transitional vertebrae. Most mammals show significant correlations between sacral position and length of the rib cage; only a few show a correlation between sacral position and number of sternebrae. The former result is consistent with the hypothesis that adult axial skeletal structures patterned by distinct mesodermal tissues are modular and covary; the latter is not. Variable levels of correlation among these structures may indicate that the boundaries of prim/abaxial mesodermal precursors of the axial skeleton are not uniform across species. We do not find evidence for a higher frequency of vertebral anomalies in our sample of embryos or neonates than in post-natal individuals of any species, contrary to the hypothesis that stabilizing selection plays a major role in vertebral patterning.

Introduction

Awareness of mammalian vertebral diversity has a long history, dating to classic works of the 18th and 19th centuries (Buffon, 1770; Owen, 1853). A substantial body of evidence collected since then supports the interpretation that vertebral counts in mammals, plus their extinct synapsid relatives, show relatively little variation compared to other vertebrate groups (Hautier et al., 2010; Muller et al., 2010; Sánchez-Villagra, 2010). The mammalian cervical count of 7 shows a particularly high level of conservatism; to a lesser extent, so do more distal parts of the mammalian vertebral column. Based primarily on the data from Owen (1853), Narita & Kuratani (2005) observed that most mammalian species

Correspondence: Robert J. Asher, Department of Zoology,
University of Cambridge, Downing St, Cambridge CB2 3EJ, UK.
Tel.: +44 (0) 1223 336 680; fax: +44 (0) 1223 336 676;
e-mail: r.asher@zoo.cam.ac.uk

have a combined total of 19 thoracic and lumbar vertebrae. Sánchez-Villagra et al. (2007) extended this observation, but noted that a high thoracolumbar vertebral count was common among afrotherians. Most afrotherian species exhibit over 20 thoracolumbar vertebrae; this may exceed 30 in some hyraxes. Sánchez-Villagra et al. (2007) also observed frequent departures from 19 among xenarthrans, with armadillos showing as few as 14 and two-toed sloths up to 29.

Sánchez-Villagra et al. (2007) raised the important possibility that xenarthrans and/or afrotherians may differ in the extent to which they have been released from the high levels of vertebral constraint evident in other mammals. This was explored further by Asher & Lehmann (2008) and Asher et al. (2009), who supported previous conclusions (Narita & Kuratani, 2005; Sánchez-Villagra et al., 2007) that thoracolumbar vertebral count was highly variable across afrotherian and xenarthran species. In addition, Asher et al. (2009) suggested that

members of these clades exhibited more intraspecific variation in vertebral counts than members of Laurasiatheria and Euarchontoglires.

The extent to which the species listed in Owen (1853) and summarized by Narita & Kuratani (2005) depart from the vertebral counts observed in one or a few individuals has rarely been tested. Assessments of intraspecific vertebral variability are limited primarily to primates (Schultz, 1930; Bornstein & Peterson, 1966; Pilbeam, 2004; Oostra et al., 2005; Galis et al., 2006) and domesticated or lab animals (Sawin, 1937; Freeman, 1939; Stecher, 1962; McPherron et al., 1999). A few exceptions have focused on intraspecific variability in afrotherians and xenarthrans (Buchholtz et al., 2007; Asher et al., 2009; Buchholtz & Stepien, 2009; Galliari et al., 2010; Hautier et al., 2010).

Vertebral variation in mammals

Following the definition of Bateson (1894: 407), changes in vertebral counts are homeotic when 'one of the component parts of the axial skeleton assumes the morphological appearance and function of its neighbor either immediately preceding or immediately following it... in distinction from meristic variations characterized by changes in total number of component parts'. Thus, when a given individual shows variation in vertebral count, it may be because of either change of one series identity at the expense of another (homeotic) or to the addition of a segment (meristic). Asher et al. (2009) provided some documentation of the extent to which specific clades of mammals vary among these categories. They tentatively concluded that as a group, southern placental mammals (i.e. afrotherians and xenarthrans, or Atlantogenata) showed more frequent departures from median series counts, particularly meristic, compared with northern placental mammals (i.e. laurasiatheres and euarchontoglires, or Boreoeutheria). However, their samples of individuals across 20 placental species were relatively small.

Since the 19th century, biologists have noted intriguing correlations between components of the axial skeleton, such as locations of the ribcage, pelvis and sternum. For example, based on the work of Welcker (1878), Bateson (1894: 121) noted that in sloths

'...when the sacrum is far back, the ribs also begin further back...Backward homeosis of the lumbar segments is generally, though not quite always, correlated with backward homeosis of the cervicals, and *vice versa*.'

Similarly, Sawin (1937: 423) noted in his colony of domestic rabbits that

'the sacrum may begin to shift its point of attachment backward to include a part of the twenty-eighth vertebra in addition to the entire twenty-seventh. Where this tendency finds fuller expression, the extra (13th) pair of ribs is found more completely developed and the sacral shift toward the twenty-eighth vertebra is increased.'

These observations correspond to a mechanism behind variation in the sloth neck discussed by Buchholtz & Stepien (2009). They proposed that the abaxial mesoderm patterning environments of the limb girdles and part of the ribcage, distinct from the primaxial mesoderm patterning environments of vertebrae, result in covariance of the positions of the ribcage and pelvic girdle across individuals within a species. Consistent with the text quoted above, they observed that individuals of *Bradypus variegatus* and *Choloepus hoffmanni* (but not their samples of *Bradypus tridactylus* or *Choloepus didactylus*) with fewer neck vertebrae and a more proximally situated ribcage exhibited a more proximally situated sacrum, compared with conspecific individuals with more neck vertebrae and a more distally situated sacrum (Buchholtz & Stepien, 2009: Table 1). With the caveat that there is some ambiguity regarding the adult derivatives of abaxial patterning in mammals, and if the mouse is representative of other mammals (Burke & Nowicki, 2003; Buchholtz & Stepien, 2009), covariance in the positions of ribcage and sacrum along the vertebral column is consistent with the presence of abaxial and primaxial modules within the axial skeleton, as hypothesized for sloths (Buchholtz & Stepien, 2009) and across mammals generally (Burke & Nowicki, 2003; McIntyre et al., 2007; Durland et al., 2008). The existence of such modules in sloths was further supported by Hautier et al. (2010), who noted that sloths with 8–10 ribless neck vertebrae still exhibited seven cervicals using a developmental criterion (late ossification of centra) to identify vertebral homology.

Another mechanism behind variation in the mammalian axial skeleton, one that does not exclude that of primaxial–abaxial modularity, was proposed by Galis et al. (2006). Based on a sample of pre- and post-natal humans, they noted a role for stabilizing selection in the highly conserved vertebral count of the mammalian neck. Among adult humans, the proportion of adults with cervical ribs is low, around 1%. In contrast, among cases of foetal or infant mortality recorded in hospital radiographs, this frequency climbs to 30–60% and is higher in individuals with multiple pathologies (Galis et al., 2006: Fig. 3). They suggested that pleiotropic effects of *Hox* mutations lead to both cervical ribs and other abnormalities (e.g. cancer), resulting in a much lower proportion of individuals with cervical ribs that survive to reach sexual maturity.

Galis & Metz (2007) have further proposed that mammals deviating from typical vertebral counts may have succeeded in doing so because of a lower metabolic rate. However, they have also noted that endothermic diapsids (represented today by Aves) do not have this association between high metabolism and vertebral conservatism. In addition, synapsids acquired their high level of vertebral conservatism long before the appearance of what we now regard as a characteristic mammalian metabolic rate (Muller et al., 2010). Clearly,

therefore, factors beyond metabolic rate have contributed to vertebral constraint in mammals.

Goals of present study

Here, we present and interpret data concerning intra-specific variation in vertebral counts across 42 mammalian species, focusing on placentals. Such data are necessary to critically evaluate the above issues regarding variation and constraint in the mammalian axial skeleton. In particular, we seek to address the following questions:

1 Do certain clades exhibit more intraspecific vertebral variation than others?

2 Are meristic and homeotic categories of vertebral change distributed evenly throughout mammals?

3 Does length of the ribcage and/or number of sternebrae correlate with position of the sacrum?

4 Do embryonic, foetal and infant mammals show more vertebral anomalies than post-natal specimens?

Answers to the first two questions will help address the phylogenetic hypothesis articulated by Sánchez-Villagra *et al.* (2007) and Asher *et al.* (2009) that afrotherians and xenarthrans show less axial constraint than other mammals. An answer to the third will help test the mechanism behind vertebral variation implied by Buchholtz & Stepien (2009). Although they did not directly make predictions regarding the relationship between number of sternebrae and the position of the sacrum, and while we do not deny the potential role of other factors in development influencing this relationship, such a correlation would follow from the modularity implicit in prim/abaxial patterning. If at least part of the ribcage and sternum is modular along with the pelvic girdle (abaxial), they should positionally covary relative to the vertebral column (primaxial).

An answer to the fourth question will help test the mechanism behind vertebral variation articulated by Galis *et al.* (2006). Implicit in their discussion is that a higher proportion of vertebral anomalies should be found among embryonic and neonatal mammals relative to post-natal mammals, because deleterious pleiotropic effects of *Hox* patterning resulting in vertebral anomalies do not necessarily prevent conception or birth, but show a high rate of mortality during early development. In other words, the effects of stabilizing selection should be evident in a population as individuals age.

Methods

We assembled counts of presacral vertebrae from museum skeletal preparations, cleared-and-stained specimens and X-ray computerized tomography (CT) scans of alcohol-preserved mammals, representing two species of monotremes, four marsupials and 24 placentals, adding substantially to the sample discussed by Asher *et al.* (2009). For three of these species (*Amblysomus hottentotus*,

Eremitalpa granti, *Sorex araneus*), radiographs also contributed to tabulations of vertebral counts. Specimens housed at the following institutions contributed to our sample: London, UK (BMNH); King Williams Town, South Africa (KM); Madrid, Spain (MNCN); Paris, France (MNHN); Stockholm, Sweden (NRM); Oxford, UK (OUM); Zürich, Switzerland (PIMUZ); Tervuren, Belgium (RMCA), Pretoria, South Africa (TM); Cambridge, UK (UMZC); Cape Town, South Africa (ZM); and Berlin, Germany (ZMB).

The median sample size for data on each species we collected (not including literature sources), including skeletons, X-ray CT scans, cleared-and-stained specimens and radiographs, was 21 individuals. The smallest samples were *Peromyscus* and *Phascolarctos* (9) and the largest *Amblysomus* (121). Data from an additional 12 species were added from previous studies of vertebral diversity (Table 1): five primates and one rodent from Pilbeam (2004); two xenarthrans from Buchholtz & Stepien (2009); and domesticated horses, pigs, mice and rabbits from (respectively) Stecher (1962), Freeman (1939), McPherron *et al.* (1999) and Sawin (1937).

For the 30 species represented by data we collected, specimens were selected that unambiguously preserved articulations between all vertebrae. In most cases, this meant retained, natural intervertebral articulations still bound by dried connective tissue. Where breaks in the vertebral column occurred, fits between vertebrae were checked by carefully placing the adjacent elements together to ensure a natural fit. Specimens that consisted of ambiguously associated parts of the skeleton, without clear evidence of vertebrae belonging to a single individual, were excluded.

Skeletons of alcohol-preserved embryos and foetuses were analysed using high-resolution X-ray microtomography (μCT) at the engineering department of the University of Cambridge (Cambridge, UK), at the Helmholtz Zentrum (Berlin, Germany), at the Natural History Museum (London, UK) and at VISCOM SARL (Saint Ouen l'Aumône, France). 3D rendering and visualization were performed using the open source software Drishti v.1.0 (Drishti Paint and Render, Limaye, 2006). Threshold values between ossified elements and soft tissues were substantial and easily allowed osteological reconstructions. Following methods described in Wilson *et al.* (2010), we also examined cleared-and-stained mammals at the Paläontologisches Institut und Museum, Zürich.

Cervical vertebrae were defined as those between the skull and the first vertebra bearing large, bilateral ribs. A developmental criterion for recognizing cervical vertebrae based on the late ossification of centra has recently been discovered (Hautier *et al.*, 2010), but is generally unavailable for most museum specimens. Among mammals, the first rib-bearing vertebra was defined as showing independent articulations via the first costal cartilage to the manubrium sterni. Hence, a neck vertebra with asymmetric, free-floating riblets, unilateral ribs

Table 1 Correlations between number of thoracolumbar vertebrae, sternebrae and rib-bearing vertebrae.

Clade	Taxon	Ribs-sacrum			Sternebrae-sacrum		
		n	r	P	n	r	P
Monotremata	*Ornithorhynchus anatinus*	**16**	**0.68**	**<0.01**	8	nsv	nsv
	Tachyglossus aculeatus	18	0.33	>0.05	6	−0.42	>0.05
Marsupialia	*Didelphis marsupialis*	**13**	**0.74**	**<0.01**	11	nsv	nsv
	Didelphis virginiana	**14**	**0.81**	**<0.01**	13	0	>0.05
	Phascolarctos cinereus	9	0.57	>0.05	5	−0.25	>0.05
	Trichosurus vulpecula	21	nsv	nsv	16	nsv	nsv
Xenarthra	*Bradypus tridactylus*[1]*	**36**	**0.35**	**<0.05**	6	0.17	>0.05
	Bradypus variegatus[1]*	**25**	**0.43**	**<0.05**	–	–	–
	Choloepus hoffmanni[1]*	**48**	**0.56**	**<0.01**	–	–	–
	Dasypus novemcinctus	**25**	**0.80**	**<0.01**	11	0.36	>0.05
Afrotheria	*Amblysomus hottentotus*	**121**	**0.65**	**<0.01**	25	−0.12	>0.05
	Eremitalpa granti	**42**	**0.66**	**<0.01**	–	–	–
	Dendrohyrax arboreus	**21**	**0.77**	**<0.01**	**9**	**0.75**	**<0.05**
	Dendrohyrax dorsalis	**15**	**0.80**	**<0.01**	7	0.38	>0.05
	Procavia capensis	74	0.12	>0.05	23	0.14	>0.05
	Setifer setosus	**21**	**0.62**	**<0.01**	**15**	**0.84**	**<0.01**
	Tenrec ecaudatus	**37**	**0.40**	**<0.05**	**22**	**0.51**	**<0.05**
	Macroscelides proboscideus	12	0.41	>0.05	12	nsv	nsv
Euarchontoglires	*Eulemur fulvus*	**13**	**0.74**	**<0.01**	11	0.42	>0.05
	Lepilemur mustelinus	15	−0.03	>0.05	15	0.23	>0.05
	Hylobates lar[2]	**105**	**0.44**	**<0.01**	–	–	–
	Pongo pygmaeus[2]	**153**	**0.58**	**<0.01**	–	–	–
	Gorilla gorilla[2]	**86**	**0.48**	**<0.01**	–	–	–
	Pan troglodytes[2]	**179**	**0.34**	**<0.01**	—	–	–
	Homo sapiens[2]	**181**	**0.44**	**<0.01**	–	–	–
	Mus musculus[3]	41	nsv	nsv	–	–	–
	Ctenomys talarum	12	nsv	nsv	7	nsv	nsv
	Microtus oeconomus	25	nsv	nsv	25	nsv	nsv
	Pedetes capensis	11	nsv	nsv	10	nsv	nsv
	Peromyscus melanophrys	9	nsv	nsv	8	0.02	>0.1
	Rhabdomys pumilio	**56**	**1**	**<0.01**	28	nsv	nsv
	Onychomys leucogaster[2]	71	−0.02	>0.05	–	–	–
	Sciurus vulgaris	12	nsv	nsv	11	nsv	nsv
	Lepus europaeus	10	0.38	>0.05	–	–	–
	Oryctolagus cuniculus[6]	**489**	**0.48**	**<0.01**	–	–	–
Laurasiatheria	*Cryptotis parva*	**62**	**0.69**	**<0.01**	58	0	>0.05
	Sorex araneus	34	nsv	nsv	34	nsv	nsv
	Talpa europaea	34	nsv	nsv	33	nsv	nsv
	Erinaceus europaeus	**35**	**0.5**	**<0.01**	24	0.12	>0.05
	Sus scrofa[4]	**182**	**0.83**	**<0.01**	–	–	–
	Equus caballus[5]	**55**	**0.56**	**<0.01**	–	–	–
	Vulpes vulpes	24	nsv	nsv	–	–	–

'Ribs-sacrum' indicates relation between number of thoracic vertebrae and location of sacrum. 'Sternebrae-sacrum' indicates relation between number of sternebrae and location of sacrum. 'n' = sample size; 'r' = Pearson correlation coefficient; 'nsv' = not sufficient variation to calculate r; P = two-tailed significance level (values at or below 0.05 are shown in bold). Dashes indicate insufficient sample to carry out comparison. *Rhabdomys* shows an r of 1 because of a single individual with fewer thoracic and thoracolumbar counts, interrupting an otherwise invariant sample. Superscripts indicate data taken from Buchholtz & Stepien (2009)[1], Pilbeam (2004)[2], McPherron *et al.* (1999)[3], Freeman (1939)[4], Stecher (1962)[5] or Sawin (1937)[6]. For *Bradypus tridactylus,* our sample was supplemented by data from Buchholtz & Stepien (2009). Data for other taxa were collected during the course of this study and that of Asher *et al.* (2009); all data are shown in Appendix S1 in electronic spreadsheet format. Asterisks denote use of presacral count, rather than thoracolumbar count, to infer sacral position in sloths (as discussed in Methods).

or ones that join with T1 ribs to articulate with the manubrium would still be 'cervical'.

Thoracic vertebrae were defined as those elements bearing large, bilateral ribs longer than the centrum is wide, with corresponding rib facets on or near each vertebral neural arch.

Lumbar vertebrae were defined as those elements cranial to the sacro-iliac articulation that lack conspicuous

rib facets and show transverse processes. Vertebrae that show riblets similar in size to the facets that bear them, or miniscule or unilateral rib facets, were defined as 'lumbar'. Posterior lumbar vertebrae may show some articulations with the sacrum, but not more than the first sacral vertebra.

The proximal-most sacral vertebra was defined as the first vertebral element to show complete, bilateral fusion of its transverse processes to articulate with the ilium on each side.

Sternebrae were counted as all distinct, ossified elements articulating with costal cartilages between (and including) the manubrium sterni and the xiphisternum. Elements cranial to the manubrium (e.g. the monotreme interclavicle) were excluded.

Outside of sloths, we observed no variation in cervical vertebral count. In addition, caudal vertebral counts are not reliably obtainable from museum preparations (Pilbeam, 2004). Hence, we relied primarily on thoracolumbar counts to quantify vertebral variation across mammals except for sloths. For all individuals in a given species, we took the median values for cervical, thoracic and lumbar categories. The sum of median thoracic and median lumbar values for a given species was defined as its median thoracolumbar count. This is usually, but not necessarily, equal to the median of summed individual thoracolumbar values. We recognized a 'homeotic' change when an individual conformed to its species' median thoracolumbar count, but differed from its species' lumbar and thoracic medians. We recognized a 'meristic' change when an individual deviated from its species' median thoracolumbar count.

Anomalous vertebrae were those that mixed features of the above vertebral definitions, e.g. by combining small riblets and transverse processes, or that showed asymmetrical ribs, transverse or ventral processes. We tabulated occurrences of such anomalies for each species and noted if they occurred in embryonic or neonatal (before or near birth) vs. post-natal (showing ossification throughout the skeleton and at least some epiphyseal fusion) individuals. For our samples of *Cryptotis* and *Rhabdomys* with at least partial data on age, we arbitrarily recognized 10 days as the cut-off between the categories 'neonatal' and 'post-natal'. Because vertebral anomalies can be difficult to detect in radiographs, we used only specimens represented by X-ray CT scans, cleared and stained preparations or macerated skeletons to calculate their frequency. We could not be certain about previous investigators' criteria for identifying vertebral anomalies and therefore did not use our literature sources to infer their frequency. Based on these criteria, seven species (*Dasypus novemcinctus*, *B. tridactylus*, *Procavia capensis*, *Tenrec ecaudatus*, *Erinaceus europaeus*, *Rhabdomys pumilio*, *Cryptotis parva*) in this study had the best samples of pre/neonatal vs. post-natal individuals.

Pearson's *r* was calculated to evaluate the significance of correlations between two pairs of intraspecific variables: position of the sacrum with number of rib-bearing vertebrae, and position of the sacrum with the number of sternebrae (Table 1). For all mammals but sloths, we used thoracolumbar count to infer the position of the sacrum, because their cervical count is invariant in our sample. For sloths, we used presacral count to infer position of the sacrum. Coefficients of variation (CV) represent the standard deviation of a data series expressed as a percentage of the mean and were calculated for thoracolumbar counts at intraspecific and selected suprageneric levels (Table 2), using the correction for small sample sizes ($CV(1 + (1/4n))$) described in Sokal & Rohlf (1995). Our original data and those derived from literature sources (Appendix S1) and summary statistics from both (Appendix S2) are available in spreadsheet format, available upon request from the corresponding author and/or journal website.

Results

Do certain clades exhibit more intraspecific vertebral variation than others?

Our data support the interpretation that vertebral variation differs substantially across mammalian clades (Fig. 1). Most conspicuously, the rodents *Ctenomys*, *Microtus*, *Pedetes* and *Sciurus*, the caniform *Vulpes*, the lipotyphlan *Talpa*, and the marsupial *Trichosurus* exhibited no deviations from their median thoracolumbar vertebral counts. Other rodents show a coefficient of variation (CV) of thoracolumbar count between 0.7 and 1.8, slightly below the clade mean for lagomorphs (1.99) and well below those of xenarthrans (3.34), primates (2.78) and afrotheres (2.6; see Fig. 1 and Table 2). The most variable species for thoracolumbar count are *D. novemcinctus* (CV = 4.65) and *Procavia capensis* (CV = 4.59). Outside of southern placentals, the most variable taxa are *Pongo pygmaeus* (3.88) and a sample of domesticated *Sus scrofa* (3.58).

Averaging across high-level clades, the interpretation of Asher *et al.* (2009) that southern placental mammals (Atlantogenata) show more vertebral variation than other high-level groups is partly true, but underestimates the variability exhibited in clades such as primates (Fig. 1; see Pilbeam, 2004). Although the vertebrally most variable species (*Dasypus*, *Procavia*) are atlantogenatan, thoracolumbar CVs of the remaining afrotheres and xenarthrans fall within the range of those reported for primates and monotremes (Fig. 1). The average thoracolumbar CV for laurasiatheres is low because of the almost nonexistent variation in *Sorex*, *Talpa* and *Vulpes*.

Data on frequency of vertebral anomalies (e.g. non-thoracic or asymmetric ribs, transitional features at vertebral series boundaries) were available only for species we sampled from museum collections, either by direct observation or X-ray CT imaging. Although the species with the highest thoracolumbar CVs in our

Table 2 Summary statistics for vertebral counts across clades of mammals. Nomenclature follows Asher & Helgen (2010). Data for constituent individual species are given in the Appendix S2. 'TL CV*' refers to the corrected thoracolumbar coefficient of variation, or coefficients of variation (CV) = stdev(100)/average, using the correction for small sample size [CV(1 + (1/4n))] as described in Sokal & Rohlf (1995).

	TL CV*	% homeotic	% meristic	% normal	% anomalies	n individ	n species	Notes
Monotremata	2.557	14.2	17	68.8	9	34	2	
Marsupialia	1.333	7.7	11.1	81.3	23	57	4	
Atlantogenata	2.844	10.6	43.9	45.5	25.7	462	12	E,F
Boreoeutheria	1.673	6.5	15.3	78.1	10	1894	24	A,B,C,D
Xenarthra	3.340	11.1	44.5	44.4	34.2	119	4	F
Afrotheria	2.596	10.3	43.6	46.1	23.1	343	8	E
Laurasiatheria	1.575	2.7	16.1	81.2	13.8	426	7	B
Euarchontoglires	1.713	8.1	15.0	76.8	8.4	1469	17	A
Lipotyphlan	1.435	2.9	10.9	86.2	18.4	165	4	G
Primates	2.780	7.2	25.3	66.9	28.7	732	7	A
Rodentia	0.710	7.0	5.8	87.5	3	237	8	C
Lagomorpha	1.989	15.8	15.8	68.3	0	499	2	D

A: data on anomalies in Primates available only for *Eulemur* and *Lepilemur* ($n = 28$).
B: data on anomalies in Carnivora+Euungulata available only for *Vulpes* ($n = 24$).
C: data on anomalies in Rodentia available for all ($n = 125$) except *Onychomys* and *Mus.*
D: data on anomalies in Lagomorpha available for *Lepus* ($n = 10$), not *Oryctolagus.*
E: data on anomalies in Afrotheria available for all ($n = 209$) except *Eremitalpa* and radiographs of *Amblysomus.*
F: data on anomalies in Xenarthra available only for subset of *Bradypus tridactylus* ($n = 21$) and *Dasypus novemcinctus* ($n = 46$).
G: data on anomalies in Lipotyphlan available for all ($n = 131$) except *Sorex.*

sample (*Dasypus* and *Procavia*) are not among the species with the highest frequency of anomalies, overall, the rate of vertebral anomalies is positively correlated with the thoracolumbar CV ($r = 0.451$, $n = 28$, $P < 0.02$). Thus, high frequency of vertebral anomalies (e.g. the xenarthran *B. tridactylus*, afrotherian *Setifer setosus*, lipotyphlan *Erinaceus europaeus* and primate *Lepilemur mustelinus*) co-occurs with a high thoracolumbar CV (Fig. 1). A few species comprise exceptions, with a high thoracolumbar CV and relatively low rate of vertebral anomalies (xenarthran *D. novemcinctus*, afrotherian *Procavia capensis*, lagomorph *Lepus europaeus*).

Outside of southern placentals, the highest rates of vertebral anomalies are exhibited by the marsupial *Phascolarctos cinereus* (44%, $n = 9$) and the laurasiathere *Erinaceus europaeus* (43%, $n = 35$). Rodents again appear to be among the most vertebrally constrained mammals, with only one of the six examined species (*Microtus*) exhibiting a modest frequency of vertebral anomalies (16%).

Are meristic and homeotic categories of vertebral change distributed evenly throughout mammals?

With the exception of sloths, the most variable species show changes in their thoracolumbar count without changes in their cervical count. Relative to their species' median values, this equates to the frequent addition or subtraction of a thoracolumbar vertebra, synonymous with a meristic change as defined here. Thus, we observe a positive correlation between species' thoracolumbar CV and the frequency of meristic deviations ($r = 0.75$, $n = 42$, $P < 0.01$). The correlation between thoracolumbar CV and frequency of homeotic deviations is not significant ($r = 0.12$, $n = 42$, $P > 0.05$). Although not undertaken in this study, further scrutiny of sacral and caudal variation would be valuable to distinguish between globally meristic changes and homeotic changes between the lumbar and sacral series.

All but four of the 30 sampled laurasiatheres, euarchontoglires, monotremes and marsupials exhibit median vertebral counts in well over 50% of the sampled individuals for each species (Fig. 1; Table 2). Domestic *Sus scrofa*, the short-nosed echidna (*Tachyglossus*), plus *Pan* and *Gorilla* are the only exceptions, with 44%, 50%, 50% and 52% (respectively) of individuals showing median counts. In contrast, of the 12 afrotherians and xenarthrans we sampled, only three (*Amblysomus*, *Eremitalpa* and *Macroscelides*) substantially exceed 50% of individuals with median thoracolumbar counts; the armadillo (*Dasypus*) is close at 54%. Monotremes, marsupials and boreoeutherians show an average of 69, 81 and 78% of individuals with median vertebral counts, whereas xenarthrans and afrotherians average 44% and 46%, respectively. Both southern placental mammal groups show 44% of individuals with meristic deviations. In contrast, monotremes show 17%, marsupials 11% and boreoeutherians 15% (Table 2). Of any high-level clade, rodents are again the least variable, with 88% exhibiting median vertebral counts, 6% meristic and 7% homeotic. Lagomorphs show the highest average percentage of homeotic changes (16%), followed by xenarthrans (11%) and afrotherians (10%). Other clades averaged below 10% homeotic changes (Table 2).
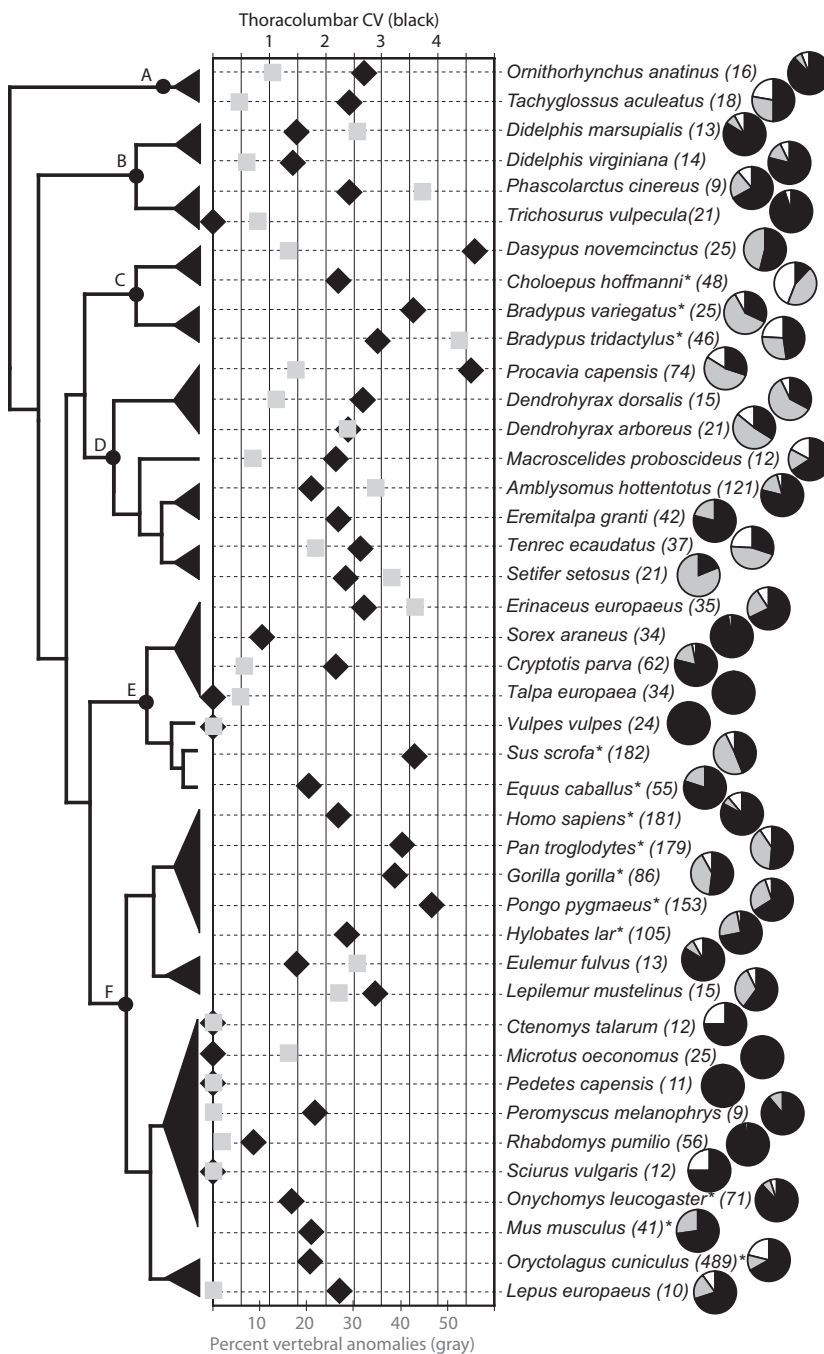
Fig. 1 Phylogenetic tree of mammals derived from Murphy *et al.* (2007) and Prasad *et al.* (2008). Lettered nodes A–F represent monotremes (A), marsupials (B), xenarthrans (C), afrotherians (D), laurasiatheres (E) and euarchontoglires (F). Pie-charts represent proportion of individuals in sample (given in parentheses adjacent to taxon names) with median thoracolumbar count (black), meristic deviations (grey) and homeotic deviations (white). The graph represents thoracolumbar coefficient of variation (CV, black diamonds, top scale) and percentage of specimens with vertebral anomalies (grey squares, bottom scale), also summarized in Table 2. Note that sample sizes for per cent anomalies are in some cases less than those for CV and proportion median thoracolumbar count (see Table 2). Asterisks represent species for which data have been added from the literature.

Does length of the ribcage correspond to the position of the sacrum?

Of the 32 mammals in our sample that exhibited variation in number of rib-bearing vertebrae and position of the sacrum, 24 showed a positive correlation between the two (Table 1). This is consistent with the comment of Bateson (1894) that 'when the sacrum is far back, the ribs also begin further back', reflected also in the observations of Sawin (1937). However, there are seven taxa with sufficient presacral variation in which this correlation is not significant: the monotreme *Tachyglossus aculeatus*, the marsupial *Phascolarctos cinereus*, two afrotherians (*Procavia capensis*, *Macroscelides proboscideus*) and three euarchontoglires (*Lepilemur mustelinus*, *Onychomys leucogaster* and *Lepus europaeus*).

As documented by Buchholtz & Stepien (2009), we found a positive correlation between number of neck vertebrae and position of the sacrum in sloths. Buchholtz & Stepien (2009) showed this correlation in *B. variegatus* and *C. hoffmanni*, but not in their sample of *B. tridactylus* ($n = 15$). In contrast, we do find such a correlation in our independent sample of *B. tridactylus* ($n = 21$, $r = 0.72$, $P < 0.01$), and in pooling our data ($n = 35$, $r = 0.36$, $P < 0.05$). We also find a positive correlation between sacral position and number of rib-bearing vertebrae in all of our sloth species (Table 1).

Three species with a nonsignificant correlation between position of the sacrum and number of thoracic vertebrae are among the least well sampled: *Phascolarctos cinereus* ($n = 9$), *Lepus europaeus* ($n = 10$) and *Macroscelides proboscideus* ($n = 12$); another two have (for this study) relatively large sample sizes: *Procavia* ($n = 74$) and *Onychomys* ($n = 71$). In addition, the only rodent to show a significant correlation, *Rhabdomys pumilio*, does so because of a single outlier with an extra rib-bearing vertebra and a caudally displaced sacrum. Of 56 individuals, all but this one individual show 12 thoracics and seven lumbars.

One specimen of *Talpa* (NRM A58-0799) that showed riblets on its first lumbar vertebra (the 21st vertebra in the column, or V21) also exhibited a slightly more posterior extent of its pelvis, although its sacrum was still on V27. Compared with all other specimens (which lacked riblets), the anterior margins of the ilia in NRM A58-0799 are situated slightly more posteriorly in this individual, not reaching the V26 anterior zygopophyses. This underscores the possibility that a more subtle means of measuring the posterior extent of ribcage and the precise position of the pelvic articulation, for example using continuous length measurements rather than counts of discrete vertebrae, may yield a correlation

between the two that the discrete quantification methods of this study have failed to distinguish.

In sum, the high-level clades containing species that do show a significant correlation between pelvic position and rib-count appear to be evenly distributed throughout mammals, including at least some monotremes, marsupials, xenarthrans, afrotherians, euarchontoglires and laurasiatheres. An important qualification to this is that most rodents show exceedingly little intraspecific variation, not enough to potentially correlate position of the sacrum with length of the ribcage.

Does number of sternebrae correspond to position of the sacrum?

In contrast to the widespread correlation between number of rib-bearing vertebrae and position of the sacrum, only three species show a significant correlation between the latter and number of sternebrae: the tenrecids *Tenrec* and *Setifer* and the hyracoid *Dendrohyrax arboreus*. For example, most specimens of *T. ecaudatus* show a pelvic articulation at V31, after seven cervical, 18 thoracic and five lumbar vertebrae. In one individual (NRM A59-3739) that shows a posterior shift of the main vertebral–pelvic articulation to V33, costal ribs seven and eight articulate with the sternum at separate points (Fig. 2a), leading to an additional element between the two for an overall count of eight sternebrae. An individual with seven sternebrae (NRM A59-2425) shows the main vertebral–pelvic articulation at V32, and costal ribs seven and eight articulating at the same point (Fig. 2b), just proximal to the xiphisternum.

In *Setifer*, the pelvis typically articulates at V32, after seven cervical, 17 thoracic and seven lumbar vertebrae. In such individuals (e.g. UMZC 2011.2.5, NRM A59-0500), costal ribs seven and eight articulate with the
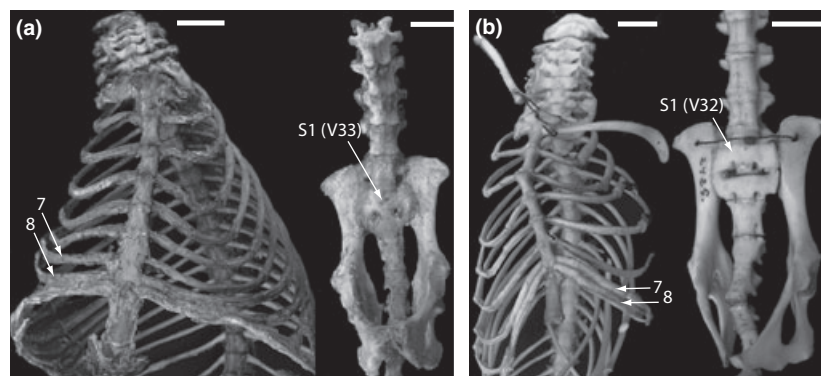

Fig. 2 Specimens of *Tenrec ecaudatus* demonstrating correlation between number of sternebrae and position of sacrum. (a) In a specimen with the sacrum at V33 (NRM A59-3739 with 19 thoracics and six lumbars), costal ribs seven and eight articulate separately at the sternum, leading to the presence of eight sternebrae. (b) An individual with the sacrum at V32 (NRM A59-2425 with 19 thoracics and five lumbars) shows costal ribs seven and eight articulating with the sternum at the same point, yielding a total of seven sternebrae. The correlation between position of sacrum and number of sternebrae is significant in *Tenrec*, *Setifer* and *Dendrohyrax arboreus*, but not in other species sampled in this study. Scale bars = 10 mm.

sternum separately, defining seven distinct sternebrae. In contrast, and as described above for *Tenrec*, individuals with a sacral articulation at V30 (e.g. UMZC 2011.2.1, NRM A58-5803) tend to show costal ribs seven and eight joined at a common sternal articulation, leading to five distinct sternebrae. In *Setifer*, cranial displacement of the sacrum is significantly correlated with a decrease in the number of sternebrae (Table 1).

The correlation between sternebrae and pelvic position is insignificant in other species, such as *Amblysomus* and *Procavia*. In the former, sacral position varies between V30 and V31, and the sternum shows between eight and nine sternebrae, with no apparent link between the two. In *Procavia*, the number of presacral vertebrae ranges widely from 34 to 39, with individuals showing 5–6 sternebrae regardless of sacral position.

Although the number of sternebrae in *Lepilemur* varies only between 5 and 6, without a correlation with pelvic position, there may be an association of the latter with extent of xiphisternum ossification (included here as part of sternebral count). Specimens with 22 thoracolumbar vertebrae and a sacrum at v30 have an ossified xiphisternum, even a very young individual (NRM A61-5895) that has not yet completely fused its innominate bones. An older individual (NRM A61-5904) with 20 thoracolumbar vertebrae and a sacrum at v28 shows only five sternebrae, without an ossified xiphisternum. In *Lepilemur*, the association is not consistent, however, as other specimens (e.g. NRM A61-5899 and A61-5212) show five sternebrae, but have a sacrum at V30.

In sum, only the tenrecid *Setifer* shows a strong correlation between number of sternebrae and location of sacrum along the vertebral column; in *Tenrec* (Fig. 2) and *D. arboreus*, the correlation is significant but weaker (Table 1). In no other species of our sample does the sacrum consistently appear to be shifted posteriorly on the vertebral column in specimens with increased sternebral number.

Do embryonic, foetal and infant mammals show more vertebral anomalies than post-natal specimens?

Seven species in our sample contain at least eight individuals of both pre- and post-natal stages: *D. novemcinctus*, *B. tridactylus*, *Procavia capensis*, *T. ecaudatus*, *Erinaceus europaeus*, *Cryptotis parva* and *Rhabdomys pumilio*. Compared to previous studies of humans, these samples

are still relatively small. Nevertheless, if stabilizing selection is a key factor in mammalian vertebral conservatism, one would expect the frequency of vertebral atavisms (e.g. nonthoracic ribs, asymmetries) to be higher among embryos and neonates than among individuals who have survived longer towards or past sexual maturity. This expectation is not met in any of the taxa with our largest pre- and post-natal samples. On the contrary, there is a higher frequency of *Dasypus*, *Bradypus*, *Tenrec*, *Erinaceus* and *Rhabdomys* individuals near or past sexual maturity with vertebral anomalies than among those at embryonic or foetal stages; in *Procavia* and *Cryptotis*, the proportions of anomalies in distinct age-classes are nearly equal (Fig. 3).

Summing across all mammalian species for which we have vertebral data for pre/neonatal ($n = 148$) and post-natal ($n = 588$) individuals (and not just those species for which both age categories are represented), 9.5% of the former and 16.5% of the latter exhibit anomalies such as asymmetries and nonthoracic ribs. Focusing only on anomalies near the cervical–thoracic border, there is a single embryonic specimen of *D. novemcinctus* that exhibits such ribs or asymmetries, compared with 15 adult specimens of *Bradypus*, *Dasypus*, *Procavia*, *Setifer*, *Didelphis* and *Trichosurus* (Appendix S1). Again, the hypothesis that stabilizing selection has acted upon mammals to prevent those with vertebral anomalies from reaching adulthood is not supported, as it predicts the opposite distribution, with more anomalies in early ontogeny than late.

Discussion

Constraint in vertebral patterning shows marked differences among clades of mammals. In this dataset, the sampled monotremes, afrotherians, xenarthrans and primates show higher thoracolumbar CVs than other clades (Fig. 1). The rodent species in our analysis are the most conservative in the makeup of their axial skeleton, with low CVs for thoracolumbar count and a low frequency of vertebral anomalies (Fig. 1, Table 2). Afrotherians and xenarthrans depart more frequently than other clades in the proportion of individuals departing from median presacral vertebral counts, often showing more individuals with meristic variations than normal counts (Fig. 1). Both of these southern groups show fewer individuals with species-median vertebral counts
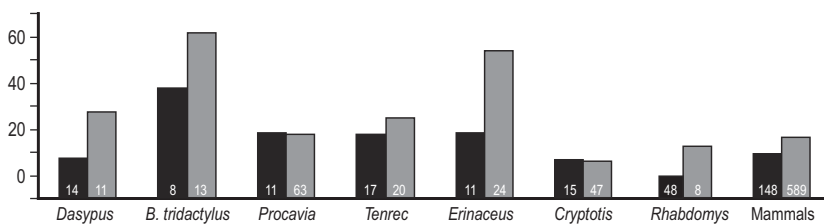

Fig. 3 Percentage of embryos and neonates (black) and individuals near or past sexual maturity (grey) showing vertebral anomalies (asymmetries, transitional vertebrae). The number of individuals in each category is given in white at base of each column.

than primates, even though all three groups exhibit a similarly high CV for thoracolumbar variation. Exceptions to low species-median counts include the two sampled golden moles (*Amblysomus* and *Eremitalpa*) and the sengi (*Macroscelides*), in which 67–79% of individuals exhibit species-median counts. Another exception may be the dasypodid *Chaetophractus* as discussed by Galliari *et al.* (2010), but not included in this study. Our sample of the closely related *D. novemcinctus* showed slightly over half of individuals with median vertebral counts (Fig. 1).

Interestingly, pairs of ecological analogues between afrotherians and other mammals, such as burrowing chrysochlorids (Afrotheria) vs. talpids (Lipotyphla), and insectivoran-grade *Setifer* (Afrotheria) vs. *Erinaceus* (Lipotyphla), differ substantially in level of vertebral variation. In each case, the afrotherians show fewer individuals with species-median counts, and chrysochlorids (average CV 1.98) show a much higher thoracolumbar CV than *Talpa* (CV 0). Although the thoracolumbar CV of *Erinaceus* falls within the Afrotherian range (slightly higher than that of *Setifer*), and although it also shows a high proportion of individuals with vertebral anomalies (43% vs. 38% in *Setifer*), *Erinaceus* shows a substantially greater proportion of individuals with a median vertebral count (69%) than *Setifer* (19%).

If all mammals follow the tissue-patterns exhibited in mice, in which the sternebrae, sternal ribs and limb girdles are patterned by abaxial mesoderm and vertebrae and proximal ribs by primaxial mesoderm (Burke & Nowicki, 2003), then the mechanism of prim/abaxial modularity would lead to the expectation that number of sternebrae should covary with position of the sacrum, because both are abaxially patterned. For most mammals in our sample, this is not the case. Only three (*S. setosus*, *T. ecaudatus*, *D. arboreus*) exhibited a significant correlation between number of sternebrae and sacral position (Fig. 2).

The more consistent (but still not universal) correlation we do recover is between number of rib-bearing vertebrae and position of the sacrum. If the model suggested by Buchholtz & Stepien (2009) for sloths is applicable across mammals, this might indicate that abaxial mesoderm-patterned structures vary across mammalian species, and that more of the ribcage in those species that exhibit this correlation is abaxially patterned, not primaxially as inferred for mice. This observation provides a framework for testing the mesodermal patterning of the axial skeleton in mammals. Species that lack a correlation between sacral position and length of ribcage, such as *Procavia*, may differ in the nature of the lateral somitic frontier (the structure that separates primaxial and abaxial domains following Burke & Nowicki, 2003) relative to those that exhibit this correlation, such as the shrew *Cryptotis*.

In our sample, vertebral anomalies among pre/neonatal individuals were less frequent than among older specimens. This contrasts with the pattern among humans discussed by Galis *et al.* (2006), in which the proportion of foetuses and neonates with anomalies greatly outnumbered that of adults. The foetal and neonatal humans used by Galis *et al.* (2006) were drawn from a hospital sample which, relative to the population at large, may overrepresent pathologies. It therefore remains to be demonstrated that the sample of Galis *et al.* (2006) is representative of pre/neonatal humans in general. Whatever the frequency of vertebral anomalies is among natural age-cohorts of *Homo sapiens*, a higher rate of such anomalies would be expected among younger individuals than older ones if the hypothesis of stabilizing selection is correct for mammals generally. This expectation is not borne out by our data (Fig. 3).

In sum, we have found that southern placental mammals share a high level of thoracolumbar vertebral variability with certain northern placental mammals, particularly primates. However, as a group, they differ from other mammals in showing frequent departures from median vertebral counts. Rodents in our sample are the most vertebrally conservative mammals quantified thus far. Covariation between the length of the ribcage and sacral position may indicate a level of modularity within prim- and abaxially patterned elements of the axial skeleton. Although the less frequent correlation between number of sternebrae and sacral position is not consistent with such modularity, it may reflect variation in the identity of prim- and abaxially patterned adult structures across mammals.

Acknowledgments

References

Asher, R.J. & Helgen, K.M. 2010. Nomenclature and placental mammal phylogeny. *BMC Evol. Biol.* **10**: 102.

Asher, R.J. & Lehmann, T. 2008. Dental eruption in afrotherian mammals. *BMC Biol.* **6**: 14.

Asher, R.J., Bennett, N. & Lehmann, T. 2009. The new framework for understanding placental mammal evolution. *Bioessays* **31**: 853–864.

Bateson, W. 1894. *Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species.* Macmillan and Co., London, New York.

Bornstein, P.E. & Peterson, R.R. 1966. Numerical variation of the presacral vertebral column in three population groups in North America. *Am. J. Phys. Anthropol.* **25**: 139–146.

Buchholtz, E.A. & Stepien, C.C. 2009. Anatomical transformation in mammals: developmental origin of aberrant cervical anatomy in tree sloths. *Evol. Dev.* **11**: 69–79.

Buchholtz, E.A., Booth, A.C. & Webbink, K.E. 2007. Vertebral anatomy in the Florida manatee, Trichechus manatus latirostris: a developmental and evolutionary analysis. *Anat. Rec. (Hoboken)* **290**: 624–637.

Buffon, G.L.L. 1770. *Histoire Naturelle, Générale et Particulière*, Nouv. éd. t.12 [Animaux quadrumanes]. ed. Imprimerie royale, Paris.

Burke, A.C. & Nowicki, J.L. 2003. A new view of patterning domains in the vertebrate mesoderm. *Dev. Cell* **4**: 159–165.

Durland, J.L., Sferlazzo, M., Logan, M. & Burke, A.C. 2008. Visualizing the lateral somitic frontier in the Prx1Cre transgenic mouse. *J. Anat.* **212**: 590–602.

Freeman, V.A. 1939. Variations in the number of vertebrae in swine. *J. Hered.* **30**: 61–64.

Galis, F. & Metz, J.A.J. 2007. Evolutionary novelties: the making and breaking of pleiotropic constraints. *Integr. Comp. Biol.* **47**: 409–419.

Galis, F., Van Dooren, T.J., Feuth, J.D., Metz, J.A., Witkam, A., Ruinard, S. *et al.* 2006. Extreme selection in humans against homeotic transformations of cervical vertebrae. *Evolution* **60**: 2643–2654.

Galliari, F.C., Carlini, A.A. & Sanchez-Villagra, M.R. 2010. Evolution of the axial skeleton in armadillos (Mammalia, Dasypodidae). *Mamm. Biol.* **75**: 326–333.

Hautier, L., Weisbecker, V., Sanchez-Villagra, M.R., Goswami, A. & Asher, R.J. 2010. Skeletal development in sloths and the evolution of mammalian vertebral patterning. *Proc. Natl. Acad. Sci. USA* **107**: 18903–18908.

Limaye, A. (2006) *Drishti – Volume Exploration and Presentation Tool.* Poster presentation. *Vis.* Baltimore.

McIntyre, D.C., Rakshit, S., Yallowitz, A.R., Loken, L., Jeannotte, L., Capecchi, M.R. & Wellik, D.M. 2007. Hox patterning of the vertebrate rib cage. *Development* **134**: 2981–2989.

McPherron, A.C., Lawler, A.M. & Lee, S.J. 1999. Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. *Nat. Genet.* **22**: 260–264.

Muller, J., Scheyer, T.M., Head, J.J., Barrett, P.M., Werneburg, I., Ericson, P.G. *et al.* 2010. Homeotic effects, somitogenesis and the evolution of vertebral numbers in recent and fossil amniotes. *Proc. Natl. Acad. Sci. USA* **107**: 2118–2123.

Murphy, W.J., Pringle, T.H., Crider, T.A., Springer, M.S. & Miller, W. 2007. Using genomic data to unravel the root of the placental mammal phylogeny. *Genome Res.* **17**: 413–421.

Narita, Y. & Kuratani, S. 2005. Evolution of the vertebral formulae in mammals: a perspective on developmental constraints. *J. Exp. Zool. B Mol. Dev. Evol.* **304**: 91–106.

Oostra, R.J., van der Wolk, S., Maas, M. & Hennekam, R.C.M. 2005. Malformations of the axial skeleton in the Museum Vrolik: II: Craniosynostoses and suture-related conditions. *Am. J. Med. Genet. A* **136A**: 327–342.

Owen, R. 1853. *Descriptive Catalogue of the Osteological Series Contained in the Museum of the Royal College of Surgeons of England.* Royal College of Surgeons, London.

Pilbeam, D. 2004. The anthropoid postcranial axial skeleton: comments on development, variation, and evolution. *J. Exp. Zool. B Mol. Dev. Evol.* **302**: 241–267.

Prasad, A.B., Allard, M.W. & Green, E.D. 2008. Confirming the phylogeny of mammals by use of large comparative sequence data sets. *Mol. Biol. Evol.* **25**: 1795–1808.

Sánchez-Villagra, M.R. 2010. Developmental palaeontology in synapsids: the fossil record of ontogeny in mammals and their closest relatives. *Proc. Biol. Sci.* **277**: 1139–1147.

Sánchez-Villagra, M.R., Narita, Y. & Kuratani, S. 2007. Thoracolumbar vertebral number: the first skeletal synapomorphy for afrotherian mammals. *System. Biodivers.* **5**: 1–7.

Sawin, P.B. 1937. Preliminary studies of hereditary variation in the axial skeleton of the rabbit. *Anat. Rec.* **69**: 407–428.

Schultz, A. 1930. The skeleton of the trunk and limbs of higher primates. *Hum. Biol.* **2**: 303–438.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman, New York.

Stecher, R.M. 1962. Anatomical variations of the spine in the horse. *J. Mammal.* **43**: 205–219.

Welcker, H. 1878. Zur Lehre von Bau und Entwickelung der Wirbelsäule. *Zool. Anz.* **1**: 311–314.

Wilson, L.A.B., Schradin, C., Mitgutsch, C., Galliari, F.C., Mess, A. & Sanchez-Villagra, M.R. 2010. Skeletogenesis and sequence heterochrony in rodent evolution, with particular emphasis on the African striped mouse, *Rhabdomys pumilio* (Mammalia). *Org. Divers. Evol.* **10**: 243–258.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Original data across specimens examined directly by the authors and collated from the literature.

Appendix S2 Summary statistics for data presented in Appendix S1.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.