

Increased variation in numbers of presacral vertebrae in suspensory mammals

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Restricted variation in numbers of presacral vertebrae in mammals is a classic example of evolutionary stasis. Cervical number is nearly invariable in most mammals, and numbers of thoracolumbar vertebrae are also highly conserved. A recent hypothesis posits that stasis in mammalian presacral count is due to stabilizing selection against the production of incomplete homeotic transformations at the lumbo-sacral border in fast-running mammals, while slower, ambulatory mammals more readily tolerate intermediate lumbar/sacral vertebrae. We test hypotheses of variation in presacral numbers of vertebrae based on running speed, positional behaviour and vertebral contribution to locomotion. We find support for the hypothesis that selection against changes in presacral vertebral number led to stasis in mammals that rely on dorsomobility of the spine during running and leaping, but our results are independent of running speed per se. Instead, we find that mammals adapted to dorsostability of the spine, such as those that engage in suspensory behaviour, demonstrate elevated variation in numbers of presacral vertebrae compared to dorsomobile mammals. We suggest that the evolution of dorsostability and reduced reliance on flexion and extension of the spine allowed for increased variation in numbers of presacral vertebrae, leading to departures from an otherwise stable evolutionary pattern.

Evolutionary stasis is one of the most commonly observed and least adequately explained phenomena in evolutionary biology^{1,2}. A striking example of stasis is the conserved variation in numbers of presacral vertebrae over more than 200 million years of mammal evolution^{3–6}. In mammals, cervical vertebrae are nearly constant, at seven, in all but three genera despite dramatic differences in measured neck length and function⁷. Cervical number is highly variable in sauropsids^{3,7–9} and, in fact, cervical number is more variable than other regions in birds, in stark contrast to mammals¹⁰. The thoracolumbar (combined thoracic and lumbar) region of mammals is also relatively conserved, with most mammals possessing 19 (or 20 in the case of carnivorans) thoracolumbar vertebrae^{8,9,11–15}. Together, cervical and thoracolumbar regions make up the presacral vertebral column and account for the overall low variation of vertebral counts found in mammals³.

The numerical composition of the vertebral column has generated newfound interest in recent years, due in part to the role of *Hox* genes in its evolution^{16,17} and, particularly, in light of our modern understanding of phylogenetic relationships among mammals^{3,8,9,12–15,18–20}. Phylogenetic inertia, developmental constraints, metabolic rates and stabilizing selection have variously been proposed to restrict variation in regional numbers of vertebrae in mammals^{1,3,7–9,11–15,21–23}. Explanations for cervical stasis in mammals invoke developmental constraints^{7,14,21–23}. Galis⁷ and Bots and colleagues²¹ proposed that deviations from seven cervical vertebrae are selected against due to negative pleiotropy, namely their

association with congenital abnormalities such as neonatal cancer. The few mammal species that are exceptions to the typical pattern of seven cervical vertebrae—manatees (*Trichechus*) and tree sloths (*Choloepus* and *Bradypus*)—are argued to have overcome these negative pleiotropic effects due to reduced oxidative DNA damage (and therefore reduced cancer susceptibility) associated with low metabolic rates^{7,23}. In humans, large percentages of perinates with congenital abnormalities, including early childhood cancers, also show evidence of homeotic change (for example, cervical ribs)^{21,24,25} (but see ref. ¹³, where this trend was not found in other mammals).

In addition, several mammalian innovations, namely atlas and axis specialization and the appearance of a muscular diaphragm, are developmentally and functionally linked to cervical stasis^{14,22,26}. Locomotor stamina, particularly during running, is achieved through dorsoventral movement of the vertebral column and continuous ventilation via the muscular diaphragm^{27–29}, a derived mammalian structure that acts to facilitate inhalation and compartmentalize viscera³⁰. The origin of the mammalian diaphragm has also been linked to the high metabolic rates generally found among mammals^{14,31}. The migratory muscle precursor cells that form the mammalian diaphragm and their innervation via the phrenic nerves are of mid-cervical somatic origin; therefore, potential disruption of the muscularization of the diaphragm, which is vitally important in mammalian respiration and locomotion, is identified as a source of constraint on cervical number change^{14,22,26}. It has been hypothesized (refs. ^{14,22}) that sloths and manatees have low ventilatory

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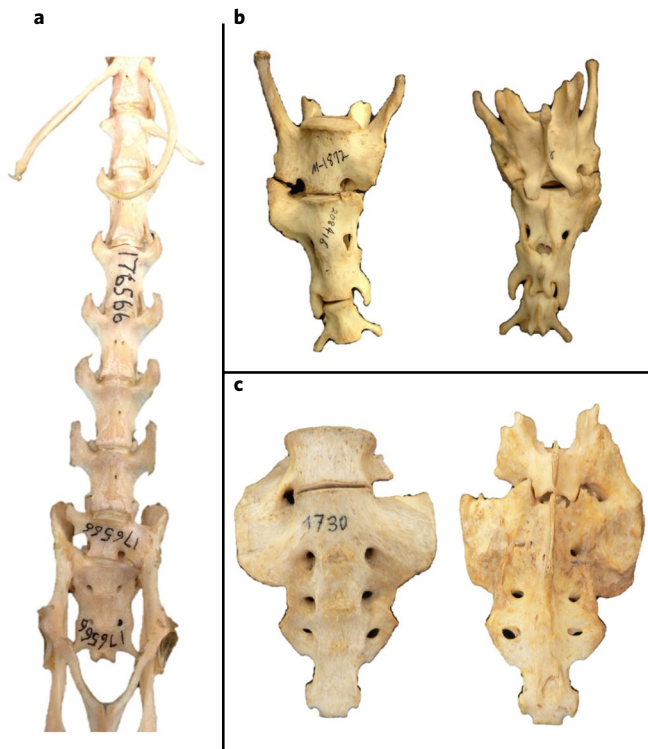


Fig. 1 | Examples of intermediate lumbar/sacral vertebrae. a, *Mustela vison* (AMNH 176566) lower thoracic, lumbar and sacral vertebrae, ventral view. **b,** *Lepus alleni* (AMNH M-1872) lumbar/sacral/caudal vertebrae, ventral (left) and dorsal (right) views. **c,** *Gorilla gorilla* (CMNH HTB-1730) lumbar/sacral vertebrae, ventral (left) and dorsal (right) views. Note that incomplete homeotic changes, resulting from perturbations in *Hox* gene expression boundaries that manifest in regional vertebral asymmetries, frequently appear across multiple vertebral borders (for example, at the thoracolumbar and lumbo-sacral borders in the *M. vison* specimen).

demands and are therefore at risk of compromised development of the diaphragm; both possess derived, atypical diaphragm morphologies^{32,33}. Therefore, the innovation of the muscular diaphragm provides an alternative explanation for stasis in cervical numbers of vertebrae in mammals.

Lineage-specific patterns of variation in thoracolumbar vertebral numbers are also apparent: the major clades Afrotheria and Xenarthra show increased departures from, and variation in, numbers of thoracolumbar vertebrae compared to both Marsupalia and Boreoeutheria (Laurasiatheria + Euarchontoglires), although certain primates are also divergent^{9,12,13,19}. Galis and colleagues³⁴ proposed that there exists a combination of biomechanical and developmental constraints on presacral vertebral numbers in mammals that rely on fast running and leaping for potential predator avoidance and prey capture. The authors argued that homeotic mutations that modify the numerical composition of the presacral column are often associated with incomplete homeotic shifts at the lumbo-sacral boundary, resulting in vertebrae that are partially or asymmetrically fused to the pelvis (Fig. 1). Because such incomplete shifts could potentially hinder spinal mobility, such mutations are expected to be selected against in fast-running and agile (cursorial) mammals, but tolerated in slower-moving (ambulatory or slow-climbing) mammals³⁴. The need to engage in fast running caused long-term stabilizing selection, possibly leading to developmental canalization of the number of presacral vertebrae and resulting in low variation in presacral counts in many clades of mammals³⁴.

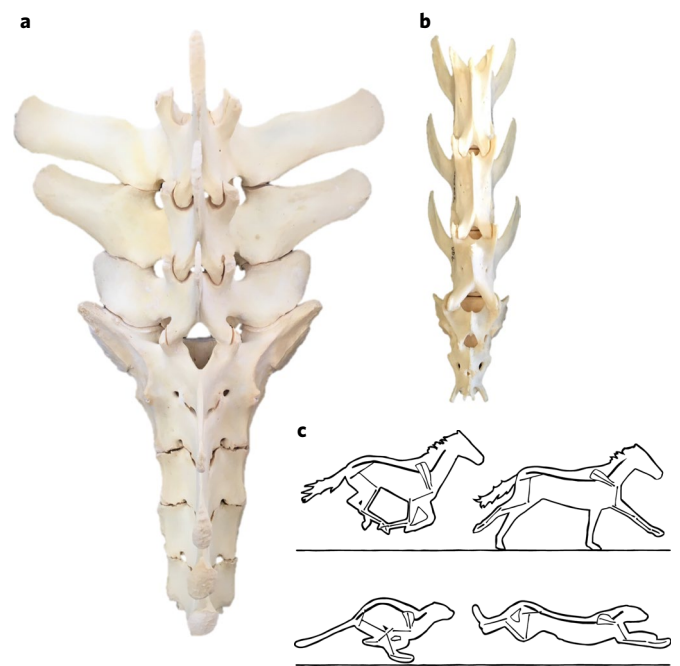


Fig. 2 | The last three lumbar vertebrae and sacrum in two fast-running mammals. a-c, Comparison of the dorsostable and dorsomobile vertebral column. The onager (*Equus hemionus*), a dorsostable runner (**a**) compared with the serval (*Leptailurus serval*), a dorsomobile runner (**b**). The line drawing (**c**, modified from ref. ⁴⁹) shows the flexibility of the vertebral column in a running dorsomobile mammal (lower), where stride length is increased through flexion and extension of the spine, in contrast to the relative stability of the vertebral column in a running dorsostable mammal (upper), where the spine is stiff and does not actively contribute to locomotion. Note that the onager is a sub-adult individual without a fully fused sacrum. Credit: Line drawing modified from Hildebrand, M. Motions of the running cheetah and horse. *J. Mammal.* **40**, 481–495, 1959 (ref. ⁴⁹), by permission of the American Society of Mammalogists/Oxford Academic.

Because Afrotheria and Monotremata contain many slow-moving or ‘low-activity’ species^{23,34}, where biomechanical constraints on running would be weaker, it is argued that species in these clades possess greater variation in presacral numbers of vertebrae, as do slow-moving taxa in other clades³⁴.

Morpho-functional correlates between vertebral shape and ecology have been documented extensively^{35–47}. Most mammals, including many rodents, lagomorphs, marsupials, primates, artiodactyls and especially carnivorans, are dorsomobile runners whose spines flex and extend sharply during the gait cycle to augment speed during running^{36,48–52} (Fig. 2). Many arboreal species also possess a mobile spine that flexes and extends rapidly during leaping^{53,54}. In contrast, some mammals are dorsostable, possessing back-stiffening features that stabilize the vertebral column in various ways^{34,36,39,46,50,54–65}. Body size has been shown to negatively correlate with sagittal flexibility in bovids^{56,61}, such that large-bodied bovids (and other ungulates—for example, elephants and perissodactyls) have a relatively rigid vertebral column compared to smaller mammals (see ref. ⁵² regarding sagittal bending in small mammals), frequently achieved via cranio-caudally tall lumbar transverse processes that inter-articulate or closely approximate each other and the iliac blades of the pelvis^{34,36,50,60,62,66}.

Dorsostable features are also found in mammals adapted to suspensory and quadrumanous slow climbing, bridging and cantilevering, especially at large body sizes where lower back stability reduces buckling and damage to the intervertebral discs and allows

Table 1 | Phylogenetic ANOVA results

Analysis	Comparison	N	F value	P value ^a	Post hoc t value	HSB P
Analysis 1	Fast-slow	58-60	8.762	0.1048	2.960	0.1048
Analysis 2	Fast-slow	34-54	6.695	0.0798	3.579	0.1673
	Fast-int.	34-30			1.276	0.4097
	Slow-int.	54-30			2.037	0.2810
Analysis 3	Fast-slow	55-46	2.048	0.4890	1.431	0.4890
Analysis 4	Terr.-arb.	90-50	7.124	0.1428	4.183	0.5608
	Terr.-foss.	90-9			1.360	1
	Terr.-aqu.	90-5			2.451	0.1778
	Arb.-foss.	50-9			1.167	1
	Arb.-aqu.	50-5			0.828	1
	Foss.-aqu.	9-5			1.167	1
Analysis 5	Upright-non.	19-130	3.277	0.3999	1.810	0.3999
Analysis 6	Antipro.-not	19-131	36.269	0.0007	6.022	0.0007
Analysis 7	Quad.-susp.	91-29	13.832	0.0005	7.221	0.0021
	Quad.-bip.	91-6			0.509	1
	Quad.-dig.	91-21			2.293	0.9796
	Quad.-swim.	91-8			1.713	0.9796
	Bip.-susp.	6-29			3.916	0.0568
	Bip.-dig.	6-21			1.664	0.9796
	Bip.-swim.	6-8			1.543	0.9796
	Susp.-dig.	29-21			3.442	0.4549
	Susp.-swim.	29-8			2.472	0.9796
	Dig.-swim.	21-8			0.108	1
Analysis 8	Run.-climb.	85-28	22.27	<0.0001	7.737	<0.0001
	Run.-ambl.	85-26			3.143	0.2260
	Run.-other	85-16			3.942	0.0583
	Ambl.-climb.	26-28			3.604	0.2260
	Ambl.-other	26-16			1.164	0.5806
	Climb.-other	28-16			1.951	0.5806
Analysis 9	Mob.-stab.	90-65	45.075	<0.0001	6.714	<0.0001
Analysis 10	Mob.-DS climb.	73-26	49.308	<0.0001	9.759	<0.0001
	Mob.-DS quad.	73-18			4.073	0.0235
	DS climb.-quad.	26-18			3.633	0.1100

HSB P, Holm's sequential Bonferroni P value; int., intermediate speed; terr., terrestrial; arb., arboreal; foss., fossorial; aqu., aquatic; non., non-upright; antipro., antipronograde; not, not antipronograde; quad., quadrupedal; susp., suspensory; bip., bipedal; dig., digging; swim., swimming; run., running and leaping; climb., slow climbing and suspension; ambl., ambling; other, digging/swimming/flying; mob., dorsomobile adaptations; stab., dorso-stable adaptations; DS climb., dorso-stable slow climbing and suspension; DS quad., dorso-stable quadrupedal locomotion. ^aSignificant P values here and for HSB are in bold, and judged using alpha = 0.005.

rotation and lateral bending in the thoracic region during forelimb-dominated, antipronograde positional behaviours^{54,55,58-60,64,65,67-76}. In the large-bodied great apes, the lumbar column is relatively short and a major portion of it is 'entrapped' between the tall, wide iliac blades of the pelvis, ensuring its virtual immobility^{59,65,72}. Hominoids and sloths, in particular, are key examples of lineages that have experienced disruptions of vertebral borders and associated limb girdles (in hominoids, the lumbo-sacral border and lower limb^{55,64,77,78}, and in sloths, both the cervical-thoracic border and upper limb and the lumbo-sacral border and lower limb^{23,79}). Aquatic mammals, such as sirenians and cetaceans, that have experienced disruption of the pelvic girdle (that is, do not have sacra) also demonstrate highly variable departures in total presacral and regional numbers of vertebrae^{8,80,81}.

Results of sampling of terrestrial quadrupedal mammals from just four clades (Monotremata, Afrotheria, Carnivora and Cetartiodactyla) can be found in ref. ³⁴. Potentially informative orders including Rodentia, Primates, Perissodactyla and Marsupalia

have not been studied. Additionally, mammals live in various habitats (for example, arboreal, terrestrial, aquatic, fossorial) and possess adaptations of their locomotor skeletons, including mobility (or stability) of the vertebral column, to travel and feed in them (for example, via suspension, quadrupedalism, bipedalism, swimming, digging). Here, we analyse a phylogenetically and functionally broad comparative sample of mammals to test ten sets of hypotheses based on running speed (Analyses 1-3), habitat (Analysis 4), body posture (Analysis 5), positional behaviour (Analysis 6), limb and tail use (Analysis 7), broad locomotor modes (Analysis 8) and contribution of the spine to locomotion (Analyses 9-10) (see Methods and Supplementary Table 1).

Results

We do not find evidence for significantly elevated variation in numbers of presacral vertebrae in slow-running (versus fast-running) mammals in our phylogenetic analysis of variance (ANOVA)

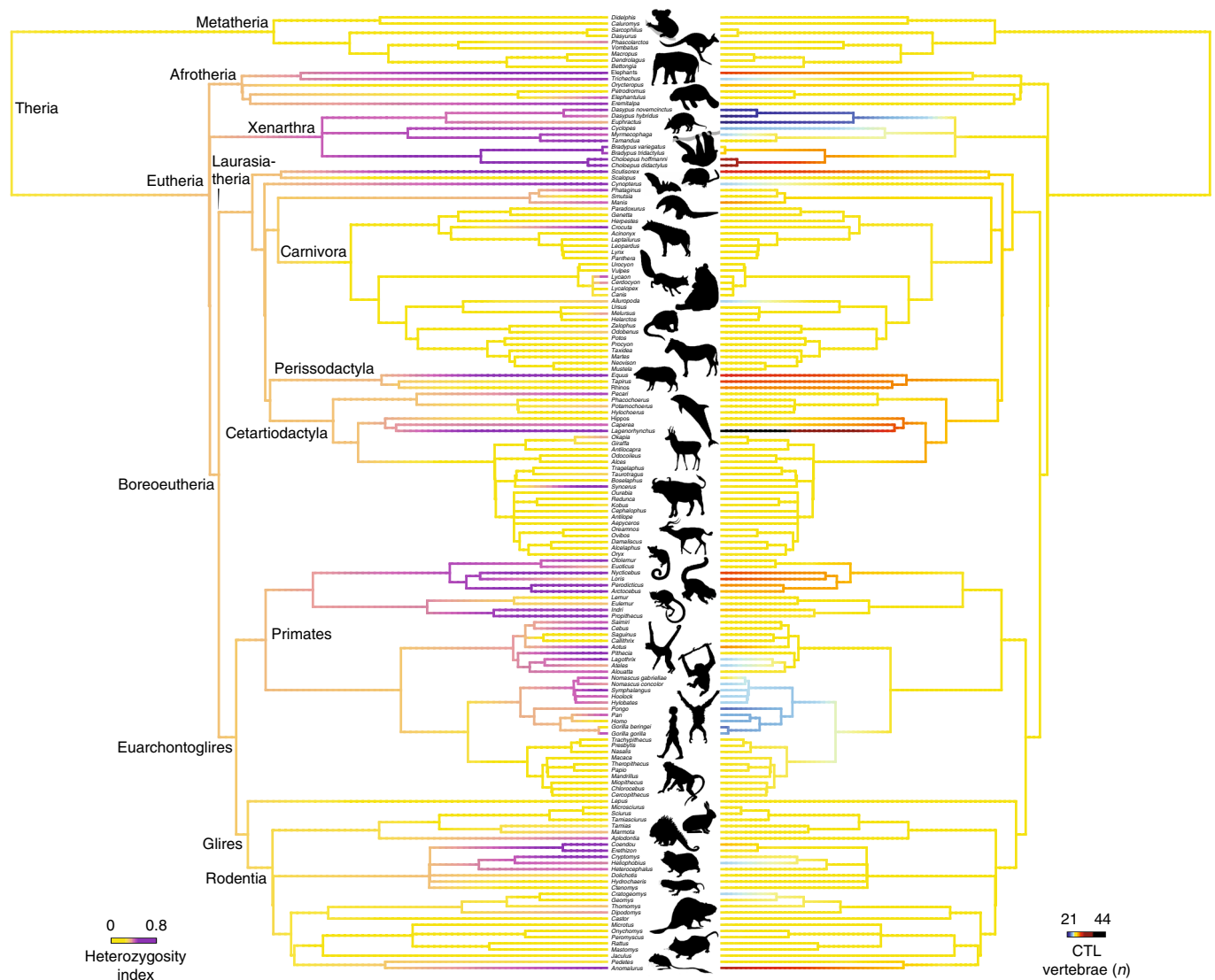


Fig. 3 | Ancestral state reconstruction of presacral vertebral numbers. The morphological heterogeneity index, a measure of intra-specific variation, is used in the ASR on the left; modal numbers of presacral vertebrae are used in the ASR on the right. Brownian motion is used to model the evolution of these variables. The left panel uses a heat map that reflects low (yellow) and high (purple) morphological heterogeneity indices. The right panel plots numbers of presacral vertebrae, with a heat map showing the presumed primitive number of presacral vertebrae (26–27, yellow), in contrast to marked decreases (blue) or increases in numbers of presacral vertebrae (red to black). Credit: Silhouettes for *Potos* and *Coendou* are from <https://www.123rf.com/>. All others are from <http://phylopic.org/> (Sarah Werning (*Phascolarctos* and *Bradypos*), T. Michael Keesey (*Elephas*), Jan A. Venter, Herbert H. T. Prins, David A. Balfour & Rob Slotow (vectorized by T. Michael Keesey; *Syncerus*), Gareth Monger (*Pongo*) and Maija Karala (*Jaculus*)) (<https://creativecommons.org/licenses/by/3.0/>).

analyses (Analysis 1: $P=0.1048$; Analysis 2: $P=0.0798$; Analysis 3: $P=0.489$; Table 1). Habitat also failed to explain variation in mammalian presacral numbers (Analysis 4: $P=0.1428$; Table 1). Body posture categories (upright or non-upright) were not significant (Analysis 5: $P=0.3999$), but antipronograde taxa (that is, those that are adapted to suspensory behaviour, cantilevering or quadrumanous slow climbing) are significantly more variable than bipeds and stereotypical above-branch or terrestrial quadrupeds (Analysis 6: $P=0.0007$; Table 1). Classification by limb and tail use (general quadruped, biped, suspensory, digging and swimming) produced a significant phylogenetic ANOVA result (Analysis 7: $P=0.0005$), and post hoc tests showed that only the suspensory versus quadrupedal comparison was significant ($P=0.0021$; Table 1). Broad locomotor modes are also significant (Analysis 8: $P<0.0001$): slow-climbing/suspensory species are more variable than runners/leapers ($P<0.0001$; Table 1). Mammals with dorsostable adaptations of the lower back are more variable in presacral numbers of vertebrae than

dorsomobile mammals (Analysis 9: $P<0.0001$; Table 1). Among dorsostable mammals, those adapted to suspensory and other antipronograde behaviours are more variable than dorsomobile mammals (Analysis 10: $P<0.0001$), whereas those adapted to dorsostable quadrupedal locomotion are not ($P=0.0235$).

The ancestral state reconstruction (ASR) highlights the appearance of high variation in presacral numbers of vertebrae in certain lineages (Fig. 3). High morphological heterogeneity indices frequently accompany major departures from the presumed primitive number of presacral vertebrae in most mammal clades (26–27), whereas lower indices tend to correspond to mammals that retain the primitive number of presacral vertebrae (Fig. 3).

Discussion

An adaptive explanation for the stability of presacral numbers of vertebrae in mammals was proposed recently by Galis and colleagues³⁴: fast, agile running (cursoriality) and leaping, which

necessitate flexion and extension of the spine, would be compromised by intermediate morphologies that often accompany changes in numbers of vertebrae (for example, lumbarized sacral vertebra or sacralized lumbar vertebra; see Fig. 1), whereas slower-moving (ambulatory) mammals can tolerate intermediate morphologies because their locomotion would not be severely impaired. Slow-moving mammals (of clades Carnivora, Artiodactyla, Monotremata and Afrotheria) were found more commonly to possess non-modal numbers of presacral vertebrae compared to fast-running species (of Carnivora and Artiodactyla)³⁴. We expanded those comparisons to other mammalian clades and tested for the potential influence of habitat, limb and tail use, locomotor behaviour, posture and spinal contribution to locomotion, in addition to speed.

The results presented here do not explicitly support the hypothesis proposed in ref. ³⁴ that running speed per se acts as a constraint on presacral vertebral variation. Additionally, we did not find differences between terrestrial and arboreal taxa or between any other habitat comparisons, nor did we find upright (bipedal or otherwise orthograde) taxa more variable than quadrupedal, pronograde taxa (Analyses 4–5; Table 1). Dorsostability of the vertebral column is associated with high variation in, and departures from, modal numbers of presacral vertebrae (Analysis 9), particularly dorsostability as an adaptation to suspension and other antipronograde positional behaviours (Analysis 10; Table 1). The African buffalo (*Syncerus*) and elephants (*Elephas* and *Loxodonta*) are highly variable taxa that exemplify dorsostable runners such as other, large-bodied ungulates^{36,50,60,62,66} (Fig. 2). All xenarthrans possess back-stiffening morphologies, possibly as an ancestral digging adaptation in that lineage^{82,83}. The vertebral column of *Scutisorex somereni*, the hero or armored shrew, is highly derived in both morphology and numerical composition, with an extremely rigid, buttressed thoracolumbar vertebral column^{19,84–87}. Selection for spinal strength for leveraging heavy or compressed objects during foraging has been proposed to explain the rigidity of the armoured shrew spine⁸⁷.

Furthermore, we found suspensory behaviour and other antipronograde positional behaviours to be associated with increased variation in numbers of presacral vertebrae compared to non-suspensory, quadrupedal taxa (Analyses 6–8,10; Table 1). Suspensory and quadrumanous slow-climbing, bridging and cantilevering mammals are amongst those with a dorsostable spine^{54,55,58–60,64,65,67–76} and represent many of the highly variable taxa in our study. Given the convergence on high variation in presacral numbers of vertebrae and divergence from the primitive number of presacral vertebrae in extant apes, tree sloths, lorises and other taxa (Fig. 3), we propose that adaptation to antipronograde positional behaviours reduced biomechanical and developmental constraints on presacral numbers of vertebrae in these lineages: mammals in these clades do not increase stride length during running and leaping by flexing and extending the spine as do dorsomobile runners^{36,48,50}, resulting in populations that can evolve atypical vertebral formulae through subsequent selection or genetic drift. High morphological heterogeneity indices tend to be found in species that deviate from the common mammalian pattern of 26 or 27 presacral vertebrae (Fig. 3).

A rigid lumbar column that contributes minimally to flexion and extension of the spine is released from stabilizing selection for dorsomobility of the vertebral column in typical mammalian locomotion, allowing for greater variation in regional and total presacral numbers of vertebrae. While adaptation to antipronograde is identified here as one source of dorsostability that resulted in increased variation in presacral numbers of vertebrae in mammals, other adaptive and potentially neutral factors should be considered. Dorsostability has evolved in other contexts (discussed above) and is frequently associated with increased variation in presacral numbers of vertebrae. Additionally, although our habitat analysis did not find major differences in aquatic, volant or fossorial taxa, this might

be attributed to limited sample sizes and therefore limited statistical power in this analysis. Cetaceans and chiropterans are both highly variable in numbers of presacral vertebrae^{88,89} and, like many antipronograde species, have clearly evolved derived body plans distinct from typical cursorial and scansorial mammals. Population-related evolutionary dynamics that might affect variation in vertebral numbers (for example, ref. ⁹⁰) also need to be considered. Work on manatees and tree sloths, species that possess atypical cervical counts, has advanced our understanding of cervical stasis^{7,23,79,91,92}. Focus on species that vary highly in thoracolumbar and other regional vertebral counts (see Fig. 1) will shed light on the evolutionary processes that have produced mammalian vertebral patterning and driven large-scale stasis in presacral numbers of vertebrae.

Methods

Data on vertebral numbers were sourced from US and European museum collections (see Acknowledgements) using criteria established by Schultz²⁷: cervical vertebrae lack ribs that articulate with the sternum; thoracic vertebrae possess ribs and bear rib facets bilaterally; lumbar vertebrae lack ribs and rib facets, including ankylosed (fused) ribs; and sacral vertebrae are defined as those contributing to sacral foraminae. ‘Intermediate’ vertebrae are recorded as half-counts, so that, for example, a vertebra at the thoracolumbar border bearing a rib on one side and not on the other is recorded as 0.5 thoracic, 0.5 lumbar. These criteria are used to obtain a total presacral count for each specimen (Supplementary Table 1). An additional ‘functional definition’ of thoracic and lumbar vertebrae (in contrast to the ‘costal definition’ just described), which is based on the orientation of the zygapophyses^{38,93}, was also employed. The vertebra with coronally oriented (thoracic-like) superior zygapophyseal articular facets and sagittally oriented (lumbar-like) inferior zygapophyseal articular facets was identified as the transitional or diaphragmatic vertebra, and demarcates pre- and post-transitional (or pre- and post-diaphragmatic) vertebrae^{36,40,60,94,95}. Along with other information, the number of pre- and post-transitional vertebrae was used to categorize taxa into dorsomobile and dorsostable categories (see below). However, these alternative definitions do not affect the total presacral count, which is the focus of this study.

In addition to the data collected in this study, two additional data sources were used. Data compiled and collected by Pilbeam¹⁸ follow the criteria outlined above³⁷ and were combined with our data, with repeated specimens removed (see ref. ⁹⁶). Data on three additional taxa, the manatee (*Trichechus*) and two cetaceans (*Caperea* and *Lagenorhynchus*), were included from Buchholtz^{91,97}. As in other fully aquatic taxa the sacrum has been lost, rendering the criteria discussed above ineffective in differentiating lumbar and caudal vertebrae. Instead, these regions were differentiated by Buchholtz and colleagues^{80,91,97} using the presence of chevron bones on the caudal vertebrae.

We pooled taxa at the genus level when species in the same genus were found not to differ in modal numbers of presacral vertebrae. In some cases, differences in modes did not allow for pooling at the genus level, and those species were treated separately (for example, *G. gorilla* and *G. beringei*). In three cases (hippopotamuses, rhinoceroses and elephants), we combined closely related genera that did not differ in modal presacral count to adequately sample and include Hippopotamidae, Rhinocerotidae and Elephantidae in our analyses. Our total sample consisted of 7,162 specimens representing 155 taxa (Supplementary Table 1). Domestic mammals were not included in our analyses due to potential effects of artificial selection (for example, work in ref. ⁹⁸) on presacral metric variation.

The morphological heterogeneity index^{18,20,96}, analogous to measures of gene diversity, was used to quantify intra-specific variation: $\sum_{i=1}^n f_i^2 \left[\frac{n}{n-1} \right]$ where f_i is the frequency of finding a single presacral count in a population and n is sample size. The morphological heterogeneity index is calculated per species and can range from 0 (an invariant count across specimens sampled) to 1 (a different count observed for each specimen). It is a better measure of variation in meristic data than the coefficient of variation because it is designed for qualitative data, it is not influenced by the size or scale of data (that is, low or high numbers of vertebrae) and a sample size correction⁹⁹ renders it more effective than simple frequencies. To adequately sample intra-specific variation, only species represented by at least ten individuals were included in our analyses although the mean (46) and median (20) sample sizes were substantially greater than ten.

For analyses of speed, maximum running speed records and average body mass data were taken from the published literature^{100–102}. Phylogenetic least squares (PGLS) regressions were carried out on running speed and average body mass per species using a mammalian supertree¹⁰³. The relationship between running speed and body mass has been shown to shift between a positive relationship and a negative one, with the slope transitioning at a body mass ~30 kg (ref. ¹⁰¹). Therefore, two separate PGLS regressions were conducted, one on mammalian species with an average body mass ≤ 30 kg ($n=96$) and another on mammalian species with an average body mass > 30 kg or greater ($n=71$) (Supplementary Table 3). Standardized residuals were calculated (PGLS residual/residual standard error) for

each analysis, and their median was used to demarcate fast and slow taxa. A third, intermediate category was introduced using the 1st and 3rd quartiles as guides (see Supplementary Table 3).

Taxa in the vertebral number dataset were assigned categories based on these results, and additional taxa not represented in the PGLS dataset were added based on two criteria: (1) if they have close relatives of similar size and presumed speed represented in the PGLS dataset (for example, two-toed sloths were considered slow because three-toed sloths were categorized as slow); and/or (2) if they were included and categorized in a speed category in Galis et al.³⁴ (Supplementary Table 1). A series of phylogenetic ANOVA tests¹⁰⁴ based on speed were then performed (Table 1): fast and slow (Analysis 1); fast, intermediate and slow (Analysis 2); and fast and slow ambler and runners (not including antipronograde taxa) (Analysis 3). In Analysis 3, only terrestrial and arboreal quadrupedal taxa were included (that is, antipronograde and aquatic taxa were excluded), which is more directly comparable to the dataset in ref.³⁴ (Table 1).

Subsequent phylogenetic ANOVAs were conducted to test the hypotheses that the following factors explain variation in numbers of presacral vertebrae: habitat (arboreal, terrestrial, fossorial, aquatic; Analysis 4); body posture (upright versus non-upright; Analysis 5); suspensory or otherwise antipronograde behaviour (Analysis 6); limb and tail use (general quadrupedalism, bipedalism, suspensory behaviour, digging, swimming; Analysis 7); broad locomotor modes (run-leap, amble, slow climb-suspend, other; Analysis 8); vertebral contribution to locomotion (dorsomobile versus dorsostable spines; Analysis 9); and dorsomobile versus dorsostable activities (dorsomobile locomotion, dorsostable running, antipronograde; Analysis 10). The categories used in Analyses 4–7 to classify mammals are based on descriptions and accounts in various published sources^{16,49,50,105–109}.

For Analysis 5, we classified bipeds and mammals that habitually hold their body in orthograde posture as upright (and those that do not as non-upright) (Supplementary Table 1). For Analysis 6, we define antipronograde positional behaviours as those in which the limbs are held in tension during suspension, cantilevering and quadrumanous slow climbing, including clambering and bridging¹¹⁰. Mammals that do not typically engage in antipronograde activities are classified as 'not antipronograde' (as opposed to pronograde, to avoid confusion). We do not fully adhere to the original definitions of the terms orthograde and pronograde, because these were applied only to arboreal mammals (and primates specifically) and used to emphasize the orientation of the body relative to the plane of movement (that is, the branch)^{77,111}. Applying that definition to sloths, for example, would result in their classification as both antipronograde and pronograde (since their bodies are held parallel with the plane of movement; that is, they are inverted quadrupeds^{112,113}). For Analysis 7, we include in our suspensory category mammals that engage in occasional suspension (for example, using the hind limbs and/or a prehensile tail). Some of these taxa (for example, howler monkeys, tamanduas, kinkajous) are not classified as antipronograde in other analyses because they habitually carry their bodies prone to the ground while terrestrial and as above-branch quadrupeds when arboreal.

Categories for Analysis 8 used our speed results in addition to the published accounts of positional behaviour and other activities (that is, slow-speed mammals were classified as amblers whereas fast-speed mammals were classified as runners/leapers). The antipronograde category from Analysis 6 and the suspensory category from Analysis 7 were combined, along with upright arboreal taxa (for example, koalas) in Analysis 8 (as slow climb-suspend). Volant taxa were excluded from the slow climb-suspend category and combined with aquatic and fossorial taxa and together are classified as 'other' in Analysis 8. In Analysis 9, mammals were classified as dorsostable (versus dorsomobile) if they met one of two criteria: (1) the numerical composition of the lumbar and/or post-transitional column is short compared to total thoracolumbar count (<30% for lumbar vertebrae, <40% for post-transitional vertebrae; see Supplementary Table 1); or (2) taxa are discussed in the literature as having musculoskeletal adaptations to dorsostability^{19,34,54,55,58–60,63–65,67–75,82–87,113–116}. In Analysis 10, taxa were classified into the categories dorsomobile quadrupedal, dorsostable slow climbing-suspensory and dorsostable terrestrial quadrupedal (excluding bipeds and taxa in the 'other' category from Analysis 8) (Supplementary Table 1).

We based *P* values for the phylogenetic ANOVAs on a comparison of the *F*-statistic of the observed data and a distribution of *F*-statistics for simulated data under Brownian motion¹⁰⁴. One million iterations were conducted to build the distribution of simulated *F*-statistics for each analysis using the phytools package in R (ref.¹¹⁷). Reported *P* values were post hoc adjusted for multiple comparisons using Holm's sequential Bonferroni. We further adjusted our alpha level for statistical significance by correcting for the number of analyses we ran (0.05/10 = 0.005).

For visualization purposes, we carried out ASR of numbers of presacral vertebrae and their calculated morphological heterogeneity indices using a Brownian motion model. We did not interpret the ASR results as accurate estimations of ancestral states for deep nodes and stems, as we did not sample the key taxa that would be necessary for such a study. Rather, we carried out ASR to highlight clear cases of deviation from modal numbers of presacral vertebrae and high variation in extant taxa. For both the ASR and phylogenetic ANOVAs, we used the mammalian supertree from Bininda-Emonds et al.¹⁰³.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data analyzed in this study and related data are included in Supplementary Tables 1–3.

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Author contributions

S.A.W., J.K.S. and M.R.S. conceived and designed the study. S.A.W., J.K.S., A.B.L. and M.R.S. analysed the data. S.A.W., J.K.S., L.P. and M.R.S. wrote the manuscript. All authors collected data, edited the manuscript and gave final approval for publication.

Competing interests

The authors declare no competing interests.

Additional information

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- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

Provide a description of all commercial, open source and custom code used to analyse the data in this study, specifying the version used OR state that no software was used.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data analyzed in this study are included in Supplementary Tables 1-3.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	We quantify variation in numbers of vertebrae in mammals using an index of qualitative variation with a sample size correction and analyze the data in a phylogenetic Analysis of Variance framework. We test for differences in speed and postural and locomotor categories. Our total taxon sample size is 155, with each taxon represented by at least 10 individuals (7158 specimens total). Individual phylogenetic ANOVAs include subsets of the total of 155 species, depending on applicability (e.g., in some analyses, only species with known running speed are included), such that sample sizes range from 101-155 taxa.
Research sample	Data are numerical, consisting of presacral (cervical + thoracic + lumbar) vertebral counts. Data were mostly collected by the authors on skeletal material at natural history museums and other institutions. Some species were supplemented with or comprised of published data.
Sampling strategy	155 mammal taxa were sampled at >9 specimens per species. The minimum sample size of 10 was determined at first based on the first author's experience sampling vertebral counts across mammal species; with the current data set, sample size is not correlated with our measure of intraspecific variability ($r^2=0.00005$, $p=0.9305$). For phylogenetic ANOVAs, our sample sizes of taxa range from 101-155, with two to seven groups per comparison. Degrees of freedom range from 99 to 149.
Data collection	Vertebral count data were recorded in excel. The first author collected the majority of the data. The other authors were trained by the first author to collect the data, then collected data on a subset of specimens to compare to the first author's data. Data were highly reproducible, and any discrepancies were identified and resolved.
Timing and spatial scale	The first author began collecting data related to this project in 2009 as part of his PhD dissertation. Data collection and recruitment and training of other authors occurred from 2012-2018. Data collection protocol (counting vertebrae) has not changed over this time period as criteria published in 1930 and 1961 (Schultz, 1930, 1961) were used.
Data exclusions	Species represented by <10 specimens were not included in the study (see Sampling strategy statement above).
Reproducibility	Reproducibility is not a significant concern in this study since the data consist of counts of vertebrae (see Data collection above).
Randomization	Factors in the phylogenetic ANOVA were determined based on phylogenetic least squares regression on published running speed and body mass data from the literature, or by published descriptions of mammals' substrate use and postural and locomotor behavior.
Blinding	We did not blind data, but the second author, who is relatively new to the project, ran the phylogenetic ANOVA analyses in R and reported those results to the first author, who interpreted them.
Did the study involve field work?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Clinical data

Methods

n/a	Involved in the study	
<input checked="" type="checkbox"/>	<input type="checkbox"/>	ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/>	Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/>	MRI-based neuroimaging