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Running for life: Developmental and biomechanical constraints on homeotic transformations

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Running for life:
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The mammalian vertebral column is highly variable, reflecting adaptations to a wide range of lifestyles, from burrowing in moles to flying in bats. Yet, in many taxa the number of trunk vertebrae is surprisingly constant. We argue that the latter constancy results from strong selection against initial changes of these numbers in fast-running or agile mammals, while such selection is weak in slower-running, sturdier mammals. The rationale is that changes of the number of trunk vertebrae require homeotic transformations from trunk into sacral vertebrae, or vice versa, and mutations towards such transformations generally produce transitional lumbosacral vertebrae that are incompletely fused to the sacrum. We hypothesize that such incomplete homeotic transformations impair flexibility of the lumbosacral joint and, thereby threaten survival in species that depend on axial mobility for speed and agility. Such transformations will only marginally affect performance in slow sturdy species, so that sufficient individuals with transitional vertebrae survive to allow eventual evolutionary changes of trunk vertebral numbers. We present data on fast and slow carnivores and artiodactyls and on slow afrotherians and monotremes that strongly support this hypothesis. The conclusion

is that the selective constraints on the number of trunk vertebrae stem from a combination of developmental and biomechanical constraints.

Many mammalian taxa show a remarkable conservation of the number of presacral (cervical, thoracic plus lumbar) vertebrae. For instance, carnivores almost invariably have 27 and artiodactyls 26 presacral vertebrae. Yet, in some taxa, in particular afrotherians, there is considerable interspecific variation[1,2]. In this study we investigate the causal importance for this conservation of biomechanical problems associated with incipient homeotic transformations[3,4]. To this end, we compare the frequencies of abnormal (i.e., non-modal) presacral vertebral numbers in fast-running artiodactyls and carnivores versus slower-running species in the same taxa and slower-running afrotherians and monotremes. We predict that slower-running species harbour more abnormal presacral numbers and transitional lumbosacral vertebrae than fast ones, both within and between taxa. Furthermore, assuming that there are no other causes for variation, we predict that afrotherians are not more variable than similarly slow species of other taxa.

Fast versus slow

Variation in the number of presacral vertebrae in fast running artiodactyls and carnivores is almost absent in our dataset (Table 1, <2%), both in sprinters (felids) and endurance runners (canids and artiodactyls). We found only three abnormal numbers ($\neq$26) in 161 artiodactyl specimens (in *Saiga tartarica, Eudorcas rufifrons*, *Kobus vardoni*) and one ($\neq$27) in 269 carnivore specimens (in *Leptailurus serval*). In contrast, variation is common in slower running artiodactyls and carnivores ranging from ± 25% in badgers, muskoxen and bay duikers to >50% in water chevrotains and *Hippopotamus* (Tables S1,S2). Most abnormal presacral numbers are due to transitional lumbosacral vertebrae, i.e. to incomplete homeotic transformations (71.4%, Table 1). Within the Artiodactyla the differences between fast and slower runners are significant for transitional vertebrae and total abnormal presacral numbers (including transitional vertebrae, Table S2). This also holds at the family level for the Bovidae and for all non-bovid taxa together. Similarly, in the Carnivora, fast and slower runners differ significantly as, at the family level, do short-limbed mustelids.

Fast carnivores and fast artiodactyls do not differ significanty, and neither do slow carnivores and slow artiodactyls (Table 1). The slow carnivores, artiodactyls, monotremes and afrotherians differ significantly, but, posthoc pairwise comparisons show that only the afrotherians differ from slow carnivores and slow artiodactyls; the other differences are not significant (Table 1). The afrotherians do not differ significantly from the slowest artiodactyls, *Hyemoschus* and *Hippopotamus* (Tables S2,3). *Hippopotamus* has the highest frequency of abnormal presacral vertebrae, a striking 70%. However, the range of variation (25.5-26) is smaller than in other species, like *Hyemoschus* (24.5-26) and Elephas (28.5-31).

Flexible versus stiff trunk

The fast-running taxa with the lowest frequency of transitional vertebrae gallop at top speed and are generally long-limbed (Fig. 1a,b, Table S1,2). The spine is dorsoventrally and laterally flexible, the rigid ribcase rather short and narrow and the lumbar spine relatively long and slender[5-7]. The mobility of the trunk is largest at the lumbosacral transition[5,7-9]. The laterally projecting transverse processes are slender and point forward, clearly separated from the sacrum and ilium (Fig. 2h-j). The dorsal spinous processes of the thorax point backward up to the anticlinal vertebrae, which usually has a straight spinous process (Fig. 1a-c). Posterior to the anticlinal vertebra the spinous processes point forward. This anticlinality, particularly pronounced in fast carnivores, allows dorso-ventral flexion around the anticlinal vertebra. In fast artiodactyls, anticlinality is less pronounced (Fig. 1a), especially in larger species, with dorsoventral flexibility concentrated around the lumbosacral transition[5]. Dorsoventral flexibility significantly contributes to speed as it increases stride-length[5,9]. Additionally, many fast species are also agile, able to swerve and leap (e.g. servals, cheetahs and impalas), which requires not only dorsoventral, but also lateral mobility of the lumbosacral spine. Incomplete and asymmetric fusions of the lumbar spine to the sacrum necessarily reduce flexibility of the lumbosacral joint (Fig. 2k-n). In wolves, dogs and humans transitional lumbosacral vertebrae are furthermore associated with additional biomechanical problems in adjacent tissues, like pressure on blood vessels and nerves, intervertebral disc degeneration, iliolumbar ligament degeneration, scoliosis and hip dysplasia[10-12]. Hence, such transitional vertebrae dramatically reduce survival in species that depend on speed and agility to catch prey or to avoid predation.

The taxa with the highest frequency of transitional lumbosacral vertebrae and/or abnormal presacral numbers (> 47%, echidnas, afrotherians and slow artiodactyls) do not gallop and locomotion is cautious with usually three or four and minimally two feet on the ground, thus avoiding great transitory stresses on the joints[8,13-17]. The trunk has limited flexibility, due to a long, robust and stiff thoracic region, a stiff lumbar spine of variable length and little mobility at the lumbosacral joint (Fig. 1f and 2b-d). The stiffness of the lumbar spine can be realized in different ways. In elephants and echidnas stiffness is provided by sturdy dorsal spinous processes that all point backward (no anticlinality) (Fig. 1f). Additionally, the lumbar region is short and wedged between the rigid ribcage and sacrum (Fig. 1f,2a). In aardvarks, hippopotamuses and water chevrotains stiffness is provided by wide and long laterally projecting transverse processes. The most caudal ones often touch the ilium and sacrum, severely limiting mobility (Fig. 2b-d). In addition, ligaments and muscles interconnecting the transverse and spinous processes and connecting the lumbar vertebrae with the ilium and sacrum further stiffen the axial skeleton[9,18]. The restricted mobility of the lumbosacral transition and the usually slow movements make that structural abnormalities will only minimally affect performance so that indirect selection against change in vertebral numbers should be weak.

Species with an intermediate number of abnormal presacral numbers (24-33% in swine, badgers, musk oxen and bay divers, Table S1,2) are also intermediate in speed, agility and trunk stiffness (c.f. shape, size and position of transverse and spinous processes, relative lengths of thoracic and lumbar regions, Figs. 1d,e and 2e,f). These species gallop, but only infrequently. The variability in presacral numbers that we find in different taxa thus agrees well with the hypothesized strength of selection against homeotic transformations.

Gallop versus half-bound

The fast short-limbed mustelids have a somewhat higher incidence of abnormal presacral numbers than fast long-limbed carnivores and artiodactyls (~5% vs ~1%), notwithstanding the flexibility of their lumbosacral spine (Figs. 1c and 2g). These mustelids do not gallop, but employ a half-bounding gait with the left and right hind-limb simultaneously striking the ground. The increased tolerance of abnormal lumbosacral transitions probably has to do with this symmetric strike. Asymmetric striking of the hind-limbs should lead to greater torsional strains on an asymmetric lumbosacral boundary, with longer limb lengths increasing the effect (except for fully parasagittal strides); longer limb lengths also lead to higher parasagittal shear stresses, further increasing the biomechanical adversity of abnormal lumbosacral joints.

Body size

Body size appears to matter less than stiffness of the lumbosacral spine, as we find highly variable presacral numbers in large (elephants and hippopotamuses) and small species (tragulids,bay divers, echidnas, Table S2-S4). Naturally, weight plays a role in that extremely heavy mammals always have stiff lumbar spines, to prevent structural damage and minimize muscular stabilization costs[5,8,9].

Domestication and inbreeding

Domesticated species usually harbour high numbers of transitional lumbosacral vertebrae, also those that originate from fast and agile wild counterparts (e.g. cats, dogs, horses)[11,19,20] Human care relaxes selection by increasing the survival of less adapted individuals. Inbreeding probably also plays a role, as inbred wild wolves have higher numbers of transitional lumbosacral vertebrae than outbred ones[12,21]. The *Saiga tatarica* with a transitional vertebra may well be the product of the strong inbreeding in this endangered species[22,23].

Developmental buffering and canalization

The incidence of abnormal lumbosacral transitions in slower-running species was higher than we expected, with a quarter or more affected individuals. One possible cause is low developmental robustness. That is, during the embryonic stage when the identities of the lumbar and sacral vertebrae are determined as part of the A-P patterning of the embryonic axis, buffering mechanisms are rather ineffective at neutralizing environmental and mutational disturbances that cause some degree of homeotic transformation. The high frequency of transitional lumbosacral vertebrae in inbred mammals supports this hypothesis as inbreeding appears to weaken developmental stability[24-26]. In contrast, in fast running species the transition at the lumbosacral boundary is sharp and vertebral shape is regular (Fig. 2g-j), suggesting strong selection for robust and stable vertebral development. Any weakening of this selection in slow and domesticated species, due to the mitigated fitness effects of lumbosacral abnormalities, probably leads to a sharp decrease in robustness. This can in part be explained by the high interactivity and low modularity of the vulnerable early organogenesis stage, when lumbosacral vertebral identities are determined[27,28]. Moreover, the early irreversibility of the determination of vertebral identity further limits the buffering potential[3].

Fast and inbred cheetahs

Unexpectedly, we did not find any abnormal lumbosacral transitions in cheetahs (Table S1), despite their dramatically low genetic diversity[29] and our (exceptional) inclusion of captive-born specimens (9 of 38 specimens). Apparently, the extreme demands for high speed in this fastest of all terrestrial species have resulted in the selective maintenance of a highly canalized vertebral development, despite severe inbreeding. It will be of interest to study more cheetahs in zoos, to see whether and after how many generations the canalized lumbosacral development breaks down.

Developmental and biomechanical constraints

Our results indicate that the selective constraints limiting the evolution of mammalian presacral vertebral numbers are due to a combination of developmental and biomechanical constraints. Many genes (including *Hox*) are involved in determining vertebral identity, with initial mutations for shifts of the lumbosacral boundary typically leading to incomplete homeotic transformations (a developmental constraint), associated with later acting biomechanical problems hampering locomotory performance (biomechanical constraints). The biomechanical problems come from (i) incomplete and often asymmetric fusions of transitional lumbosacral vertebrae with the sacrum and, (ii) correlated biomechanical problems, because many genes that pattern the vertebrae also influence patterning of adjacent nerves and muscles (developmental constraints). Fast and agile mammals, thus, provide a powerful example of the potential importance of the interplay of developmental and biomechanical constraints in evolution.

Methods (supplementary online info)

Specimens

We analysed skeletons of 753 wild-born and 9 captive-born individuals of 89 species of 14 different mammal families of 8 European natural history museums: Naturalis Biodiversity Center, Leiden (Naturalis), The Natural History Museum, London (NNM), the Royal Museum for Central Africa, Tervuren (RMCA), the Royal Belgian Institute of Natural Sciences, Brussels (RBINS), the Natural History Museum of Denmark, Copenhagen (ZMUC), Naturhistorisches Museum Wien, Vienna (NHMW), the Swedish Museum of Natural History, Stockholm (NRM) and Museum für Naturkunde, Berlin (ZMB). To avoid the potentially confounding effects of inbreeding, we excluded mammals that were born in zoos, except for 9 cheetahs (*Acinonyx jubatus*) that were included for additional information on this extremely fast species.

Carnivora. We analysed 419 skeletons of Carnivora including 84 Canidae, 183 Felidae, 134 Mustelidae and 18 Procyonidae (Table S1).

Artiodactyla. We analysed 266 skeletons of Artiodactyla including 3 Antilocapridae, 165 Bovidae, 21 Cervidae, 10 Hippopotamidae, 33 Suidae and 34 Tragulidae (Table S2).

Afrotheria. We analysed 48 skeletons of Afrotheria including 21 Tubulidentata and 27 Elephantidae (Table S3).

Monotremata. We analysed skeletons of 30 Tachyglossidae of the Order Monotremata (Table S4).

Vertebral formula

We have determined the vertebral formula of the skeletons by determining the number of cervical, thoracic, lumbar, sacral and coccygeal vertebrae. Transitional vertebrae at boundaries were counted as half for each of the neighboring regions, e.g. half thoracic and half lumbar. The thoracolumbar boundary is sometimes difficult to establish with precision, because transitional thoracolumbar vertebrae have one or two rudimentary ribs and these are often lost and the detection of their small articulations on the vertebra is often difficult, especially when the vertebrae are worn or damaged by strong maceration during preparation. Therefore, the sum of the thoracic and lumbar vertebrae is more precise than the separate numbers, but this does not affect the precision of the presacral number. We considered the most frequent whole presacral number (mode) as normal. For carnivore species the normal number is 27 and for artiodactyl species 26 (tables S1,S2). For Afrotherians, the normal number is 28 for *Orycteropus*, 30 for *Elephas* and 31 for *Loxodonta* (table S3). For the monotremes it is 26 for *Tachyglossus* and 27 for *Zaglossus* (table S4). Abnormal numbers were divided into two groups: a) with a transitional lumbosacral vertebra (abnormal transitional) and b) without one (abnormal non-transitional).

Classification of fast-running versus slower-running

Predictions regarding running speed and gait were based on references found in the literature [13-17,30-39] and surmised from the anatomy and observations. To avoid classification mistakes, we selected as far as available the fastest and most agile of galloping, long-limbed species versus the slowest and sturdiest species within the taxon. The slower-running species consist of those that never gallop on land (the afrotherian *Elephas*, *Loxodonta*, *Orycteropus* and the artiodactyl *Hyemoschus* and *Hippopotamus*, of which the latter only gallops under water) and those that infrequently gallop (the artiodactyl *Ovibos*, *Cephalophus* and the suid species and the carnivore *Meles* and *Procyon*), Classifications can be found in tables S1-S4 and were made before the analyses of the vertebral columns. In total we classified 252 specimens of carnivore as fast running and 51 as slower running. An exception was made for the family Mustelidae (Carnivora), for which we included as fast species, exclusively for the analysis at the family level, the fast, but short-limbed *Martes foina*, *Mustela erminea* and *Mustela nivalis*. The rationale was that in this set of mustelid species there are no long-limbed and galloping fast species, but only fast and agile elongate species that use a half-bound gait and have short limbs.

Statistical tests

We analyzed overall contingency tables of the different slow and fast running taxa and their presacral numbers (normal, abnormal non-transitional or abnormal transitional) using Fisher's exact tests. Posthoc comparisons were performed by Fisher's exact tests and p-values were Holm-Bonferroni adjusted.

Legends

Figure 1. **Skeletons of fast and slower running mammals, lateral views**. (**a-c**) Fast-running and agile species with slender vertebral columns with a relatively short thoracic region (ribs provide rigidity), a relatively long lumbar region and a highly flexible lumbosacral transition: long-limbed gallopers, *Gazella dorcas* (**a**), coyote (**b**, *Canis latrans*) and short-limbed and half-bounding marten (**c**, *Martes martes*). (**d-f**) Slower running species with more sturdy skeletons, longer thoracic and shorter lumbar regions and stiffer lumbosacral transitions: badger (**d**, *Meles meles*) and Babirusa swine (**e**, *Babyrousa babyrussa*) and Asian elephant (**f**, *Elephas maximus*, juvenile specimen). Swine and badgers occasionally run fast, whereas elephants never uses the

gallop and have a particularly stiff lumbosacral transition. The stiffness of the elephant spine comes from the dorsal spinous processes which are all backward pointing (no anticlinality**)** and a particularly short lumbar region that is wedged between the long and sturdy ribcage and rigid sacrum. For a comparison of fast-running and slower-running species within one family, cf. the slender and flexible marten (**c)** and the sturdier and stockier badger (**d)**. The spinous and transverse processes are more robust in the badger, which provides rigidity in combination with the attached ligaments. Additionally, in martens the thoracic region has one less vertebra and the lumbar region one more vertebra, adding to the flexibility (see Fig. **2f-g** for dorsal views of the lumbosacral spines). Anticlinality is particularly pronounced in fast carnivores (**b**,**c)**, allowing dorsoventral flexibility at the end of the thoracic region. However, in fast artiodactyls (**a**) this is less the case, with flexibility of the lumbosacral transition being especially important.

Figure 2. **Lumbosacral spines of fast and slower running mammals**, **dorsal views.** (**a-d**) Relatively slow and cautiously moving species with a stiff lumbosacral transition. In Asian elephants (**a**, *Elephas maximus*), stiffness is due to a short lumbar region that is wedged in between a rigid ribcase and sacrum, in combination with a backward orientation of all spinous processes of the trunk (see Fig. **1f**). In aardvarks (**b**, *Orycteropus afer),* hippopotamuses (**c**, *Hippopotomus amphibius*) and water chevrotains (**d**, *Hyemoschus aquaticus*), stiff lumbosacral transitions are due to wide and long laterally projecting transverse processes of the lumbar vertebrae, that are close to, or touch each other, or the sacrum and ilium. (**e**,**f**,) Babirusa swine (**e**, *Babyrousa babyrussa*) and badgers **(f**, *Meles meles*) are species that occasionally run and that have intermediately stiff lumbosacral transitions. The transverse processes of the lumbar vertebrae are clearly separated from each other and less robust compared to those in (**a-d**), but more robust than those of the fast species in (**g-j**). The most caudal transverse processes generally do not touch the sacrum or ilium **(e**), but occasionally do so slightly **(f**). (**g-j**)

Lumbosacral spines of fast running species with flexible lumbosacral transitions: the short-limbed half-bounding pine marten (**g**, *Martes martes*) and the long limbed gallopers, cheetah (**h**, *Acinonyx jubatus*), coyote, (**i**, *Canis latrans*) and *Gazella dorcas* (**j**). These fast species have flexible and slender lumbar spines with a sharp lumbosacral transition. The lateral transverse processes are slender and forward pointing, clearly separated from each other and from the sacrum and ilium. Asymmetrical transitional lumbosacral vertebrae in a badger (**k**, *Meles meles*) and a *Saiga tatarica* (**l**) and symmetrical transitional lumbosacral vertebrae in an aardvark (**m**, *Orycteropus afer*) and a water chevrotain (**n**, *Hyemoschus aquaticus*. The partial fusions with the sacrum drastically limit the flexibility of the lumbosacral joint, which is especially problematic in fast and agile mammals.

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References

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

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

3 ten Broek, C. M. et al. Evo-Devo of the Human Vertebral Column: On Homeotic Transformations, Pathologies and Prenatal Selection. Evol. Biol. 39, 456-471 (2012).

4 Varela-Lasheras, I., Bakker, A. J., van der Mije, S., van Alphen, J. & Galis, F. Breaking evolutionary and pleiotropic constraints in mammals: On sloths, manatees and homeotic mutations. EvoDevo 2, 11 (2011).

5 Halpert, A. P., Jenkins, F. A. & Franks, H. Structure and Scaling of the Lumbar Vertebrae in African Bovids (Mammalia, Artiodactyla). J. Zool. 211, 239-258 (1987).

6 Spoor, C. F. & Badoux, D. M. Descriptive and Functional Myology of the Back and Hindlimb of the Striped Hyena (Hyaena-Hyaena, L 1758). Anat. Anzeiger 167, 313-321 (1988).

7 Boszczyk, B. M., Boszczyk, A. A. & Putz, R. Comparative and functional anatomy of the mammalian lumbar spine. Anat. Rec. 264, 157-168, doi:Doi 10.1002/Ar.1156 (2001).

8 Slijper, E. J. Comparative biologic–anatomical investigations on the vertebral column and spinal musculature of mammals. Verhand. Kon. Ned. Akad. Wet. Amst. II, 1-128 (1946).

9 Smeathers, J. E. A Mechanical Analysis of the Mammalian Lumbar Spine Ph.D. thesis, University of Reading, (1981).

10 Aihara, T. et al. Intervertebral disc degeneration associated with lumbosacral transitional vertebrae: a clinical and anatomical study. J. Bone Joint Surg. Br. 87, 687-691 (2005).

11 Damur-Djuric, N., Steffen, F., Hässig, M., Morgan, J. P. & Flückiger, M. A. Lumbosacral transitional vertebrae in dogs: classification, prevalence, and association with sacroiliac morphology. Vet. Radiol. Ultrasound 47, 32-38, doi:10.1111/j.1740-8261.2005.00102.x (2006).

12 Räikkönen, J., Bignert, A., Mortensen, P. & Fernholm, B. Congenital defects in a highly inbred wild wolf population (Canis lupus). Z. Saugetierkd. 71, 65-73 (2006).

13 Genin, J., Willems, P., Cavagna, G., Lair, R. & Heglund, N. Biomechanics of locomotion in Asian elephants. J. Exp. Biol. 213, 694-706 (2010).

14 Dubost, G. Account on Biology of African Chevrotain, Hyemoschus-Aquaticus--Ogilby, Artiodactyl Tragulid. Mammalia 42, 1-62, doi:Doi 10.1515/Mamm.1978.42.1.1 (1978).

15 Shoshani, J., Goldman, C. A. & Thewissen, J. G. M. Orycteropus afer. Mamm. Species, 1-6 (1988).

16 Coughlin, B. L. & Fish, F. E. Hippopotamus Underwater Locomotion: Reduced-Gravity Movements for a Massive Mammal. J. Mammal. 90, 675-679, doi:Doi 10.1644/08-Mamm-a-279r.1 (2009).

17 Hildebrand, M. & Hurley, J. P. Energy of the oscillating legs of a fast-moving cheetah, pronghorn, jackrabbit, and elephant. J. Morphol. 184, 23-31 (1985).

18 Gal, J. M. Mammalian Spinal Biomechanics .1. Static and Dynamic Mechanical-Properties of Intact Intervertebral Joints. J. Exp. Biol. 174, 247-280 (1993).

19 Haussler, K. K., Stover, S. M. & Willits, N. H. Developmental variation in lumbosacropelvic anatomy of Thoroughbred racehorses. Am. J. Vet. Res. 58, 1083-1091 (1997).

20 Newitt, A. L. M., German, A. J. & Barr, F. J. Lumbosacral transitional vertebrae in cats and their effects on morphology of adjacent joints. J. Feline Med. Surg. 11, 941-947, doi:Doi 10.1016/J.Jfms.2009.01.013 (2009).

21 Räikkönen, J., Vucetich, J. A., Peterson, R. O. & Nelson, M. P. Congenital bone deformities and the inbred wolves (Canis lupus) of Isle Royale. Biol. Conserv. 142, 1025-1031 (2009).

22 Taylor, V. J. & Dunstone, N. The exploitation of mammal populations. 435 (Chapman & Hall, 1996).

23 Sokolov, V. E. Saiga tatarica. Mamm. Species 38, 1-4 (1974).

24 Leary, R. F., Allendorf, F. W. & Knudsen, K. L. Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. Am. Nat., 540-551 (1984).

25 Markow, T. A. & Martin, J. F. Inbreeding and Developmental Stability in a Small Human-Population. Ann. Hum. Biol. 20, 389-394, doi:Doi 10.1080/03014469300002792 (1993).

26 Lens, L. et al. Developmental instability and inbreeding in natural bird populations exposed to different levels of habitat disturbance. J. Evol. Biol. 13, 889-896, doi:Doi 10.1046/J.1420-9101.2000.00232.X (2000).

27 Galis, F. & Metz, J. A. J. Testing the vulnerability of the phylotypic stage: On modularity and evolutionary conservation. J. Exp. Zool. 291, 195-204, doi:Doi 10.1002/Jez.1069 (2001).

28 Galis, F., van Dooren, T. J. M. & Metz, J. A. J. Conservation of the segmented germband stage: robustness or pleiotropy? Trends Genet. 18, 504-509, doi:Pii S0168-9525(02)02739-7

29 Menotti-Raymond, M. & O'Brien, S. J. Dating the genetic bottleneck of the African cheetah. Proc. Natl. Acad. Sci. U. S. A. 90, 3172-3176 (1993).

Additional references for the methods section (online only)

30 Christiansen, P. Locomotion in terrestrial mammals: the influence of body mass, limb length and bone proportions on speed. Zool. J. Linn. Soc. 136, 685-714 (2002).

31 Garland, T. The relation between maximal running speed and body mass in terrestrial mammals. J. Zool. 199, 157-170 (1983).

32 Garland, T. & Janis, C. M. Does metatarsal/femur ratio predict maximal running speed in cursorial mammals? J. Zool. 229, 133-151 (2009).

33 Geertsema, A. Impressions and observations on serval behaviour in Tanzania, East Africa. Mammalia 40, 13-20 (1976).

34 Hildebrand, M. The quadrupedal gaits of vertebrates. Bioscience 39 (1989).

35 Howell, A. B. Speed in animals, their specialization for running and leaping. 270 (Chicago University Press, 1944).

36 Hutchinson, J. R., Famini, D., Lair, R. & Kram, R. Biomechanics: Are fast-moving elephants really running? Nature 422, 493-494 (2003).

37 Iriarte-Díaz, J. Differential scaling of locomotor performance in small and large terrestrial mammals. J. Exp. Biol. 205, 2897-2908 (2002).

38 Ren, L. & Hutchinson, J. R. The three-dimensional locomotor dynamics of African (Loxodonta africana) and Asian (Elephas maximus) elephants reveal a smooth gait transition at moderate speed. J. Royal Soc. Interface 5, 195-211 (2008).

39 Seidensticker, J. & Lumpkin, S. Great Cats: Majestic Creatures of the Wild. (Rodale Press, 1991).

Author contributions

FG and CTB analysed the skeletal patterns. CTB and FG analysed the data. FG, DC and CTB formulated the predictions. FG, CTB and JAJM wrote the manuscript with contributions from JVA, DC and SJVM. JVA and CTB made the Figures. All authors read and approved the final manuscript.

Author information

The authors declare no competing interests.

Fig. 1

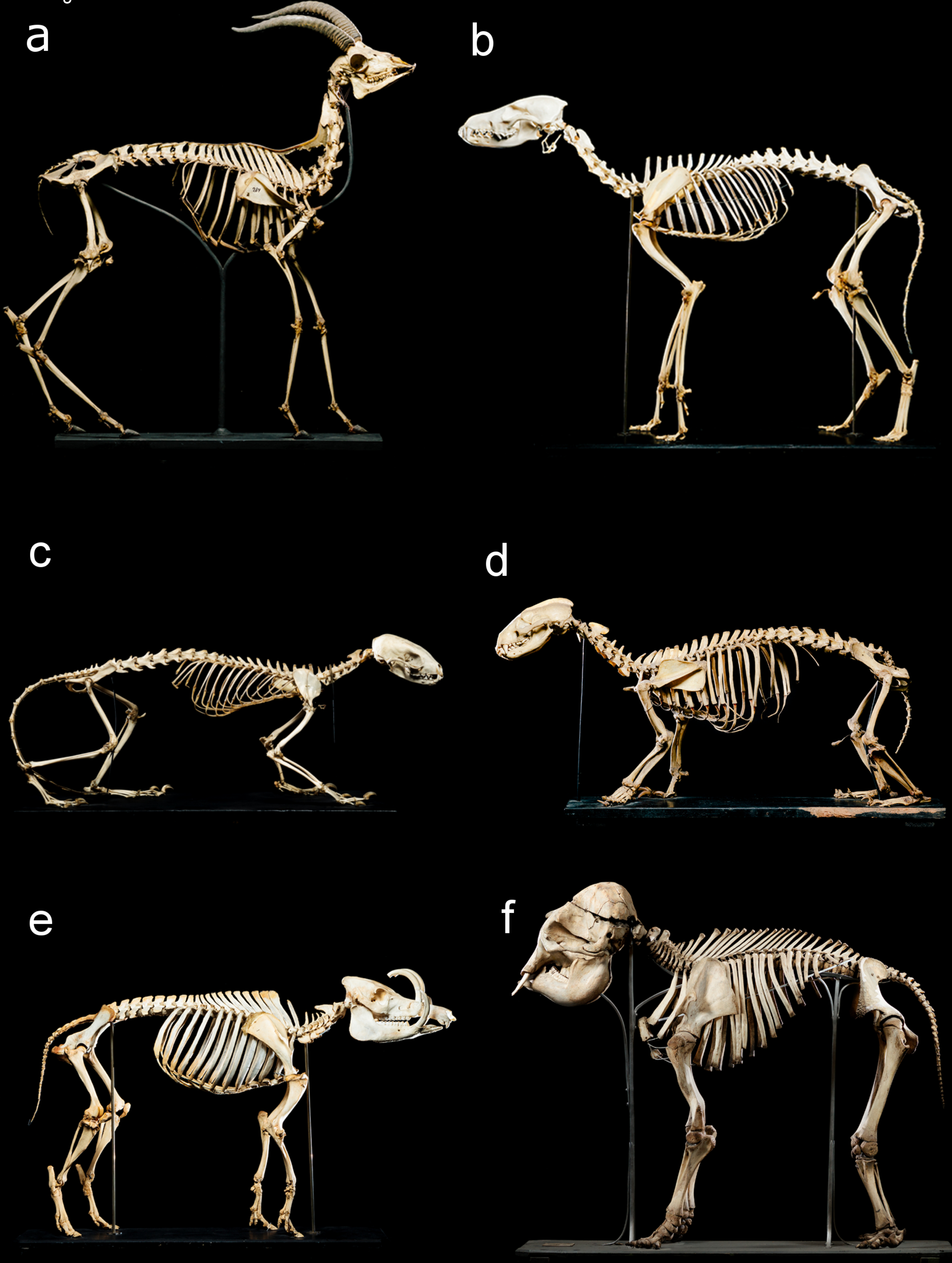

Fig.2

Table 1.

	Normal number presacral vertebrae	Abnormal number non-transitional vertebrae	Abnormal number transitional vertebrae
Carnivora			
fast	260 (99.6%)	1 (0.4%)	0 (0.0%)
fast half-bound	95 (95.0%)	2 (2.0%)	3 (3.0%)
slow	38 (74.5%)	4 (7.8%)	9 (17.6%)
Artiodactyla			
fast	158 (98.1%)	2 (1.2%)	1 (0.6%)
slow	62 (59.0%)	12 (11.4%)	31 (29.5%)
Bovidae			
fast	134 (97.8%)	2 (1.5%)	1 (0.7%)
slow	21 (75.0%)	2 (7.1%)	5 (17.9%)
Afrotheria (slow)	15 (31.3%)	11 (22.9%)	22 (45.8%)

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Table 2.

Fisher's exact tests
fast galloping vs fast half-bound Carnivora
fast half-bound vs slow Carnivora
fast galloping vs slow Carnivora
fast vs slow Artiodactyla
fast vs slow Bovidae
fast vs slow non-Bovidae
fast Carnivora vs fast Artiodactyla
slow carnivora, artiodactyla, afrotheria, monotremata

Posthoc pairwise comparisons
slow Carnivora vs slow Artiodactyla
slow Carnivora vs Monotremata
slow Carnivora vs Afrotheria
slow Artiodactyla vs Afrotheria
slow Artiodactyla vs Monotremata
Monotremata vs Afrotheria

P-value
< 0.01
< 0.001
< 0.001
< 0.001
< 0.001
< 0.001
0.24
0.01

P-value (Holm-Bonferroni adjusted)
0.44
0.40
< 0.001
< 0.01
0.69
0.40

Supplementary table 1.

Fast galloping Carnivora (N=267)			**Slow Carnivora (N=51)**			**Fast half-bound Carnivora (N=101)**		
Family			**Family**			**Family**		
Genus	Presacral No.	N	*Genus*	Presacral No.	N	*Genus*	Presacral No.	N
Canidae	0.0% abnormal	84	**Mustelidae**	24.2% abnormal	33	**Mustelidae**	5.0% abnormal	101
Canis	27	63	*Meles*	***25.5***	***1***	*Martes*	27	36
Chrysocyon	27	6		**26**	**2**		**28**	**1**
Lycaon	27	15		***26.5***	***3***	*Mustela*	***26.5***	***1***
				27	25		27	60
Felidae	0.5% abnormal	183		***27.5***	***2***		***27.5***	***2***
Acinonyx	27	38					**28**	**1**
Caracal	27	17	**Procyonidae**	27.8% abnormal	18			
Felis	27	20	*Procyon*	**26**	**2**			
Leopardus	27	8		***26.5***	***2***			
Leptailurus	**26**	**1**		27	13			
	27	28		***27.5***	***1***			
Lynx	27	22						
Panthera	27	30						
Prionailurus	27	11						
Profelis	27	8						

Supplementary table 2.

Fast Artiodactyla (N=161)		
Family		
Genus	Presacral No.	N
Antilocapridae	0.0% abnormal	3
Antilocapra	26	3
Bovidae	2,2% abnormal	137
Aepyceros	26	3
Alcelaphus	26	4
Antidorcas	26	10
Beatragus	26	1
Boselaphus	26	3
Capra	26	1
Connochetes	26	4
Damaliscus	26	4
Eudorcas	26	5
	25	**1**
Gazella	26	19
Kobus	26	27
	27	**1**
Litocranius	26	2
Nanger	26	2
Oryx	26	9
Pelea	26	1
Redunca	26	14
Rupicapra	26	1
Saiga	26	10
	26.5	**1**
Taurotragus	26	2
Tragelaphus	26	12
Cervidae	0.0% abnormal	21
Rangifer	26	21

Slow Artiodactyla (N=105)		
Family		
Genus	Presacral No.	N
Bovidae	25.0% abnormal	28
Cephalophus	**25.5**	**2**
	26	11
	27	**1**
Ovibos	**25**	**1**
	25.5	**2**
	26	10
	26.5	**1**
Hippopotamidae	70.0% abnormal	10
Hippopotamus	**25.5**	**7**
	26	3
Suidae	33.3% abnormal	33
Babyrousa	26	1
Phacochoerus	**25**	**1**
	26	2
Potamochoerus	**25**	**3**
	25.5	**3**
	26	16
	26.5	**1**
Sus	**25.5**	**2**
	26	3
	27	**1**
Tragulidae	50.0% abnormal	34
Hyemoschus	**24.5**	**2**
	25	**3**
	25.5	**7**
	26	8
Moschiola	**26.5**	**1**
Tragulus	**25**	**1**
	25.5	**1**
	26	9
	26.5	**1**
	27	**1**

Supplementary table 3.

Afrotheria (N=47)		
Family		
Genus	Presacral No.	N
Elephantidae	63.0% abnormal	27
Elephas	**28.5**	**3**
	29	**4**
	29.5	**3**
	30	7
	30.5	**1**
	31	**2**
Loxodonthas	**29.5**	**1**
	30	2
	30.5	**1**
	31	**2**
Orycteropodidae	76.2% abnormal	21
Orycteropus	**26.5**	**1**
	27	**3**
	27.5	**11**
	28	5
	28.5	**1**

Supplementary table 4.

Monotremata (N=30)		
Family		
Genus	Presacral No.	N
Tachyglossidae	46.7% abnormal	30
Tachyglossus	**25.5**	**3**
	26	13
	26.5	**4**
	27	**1**
	27.5	**1**
Zaglossus	27	3
	27.5	**4**
	28	**1**