Fast running restricts evolutionary change of the vertebral column in mammals

Frietson Galis[a,1,2], David R. Carrier[b], Joris van Alphen[a], Steven D. van der Mije[a], Tom J. M. Van Dooren[a,c], Johan A. J. Metz[a,d], and Clara M. A. ten Broek[a,e,1]

[a]Naturalis Biodiversity Center, 2333 CR, Leiden, The Netherlands; [b]Department of Biology, University of Utah, Salt Lake City, UT 84112; [c]Centre National de la Recherche Scientifique/Université Pierre et Marie Curie/Université Paris-Est Créteil Val de Marne/Université Paris Diderot/Institut de Recherche pour le Développement/Institut National de la Recherche Agronomique–Unité Mixte de Recherche 7618, Institute of Ecology and Environmental Sciences Paris, Université Pierre et Marie Curie, 75005 Paris, France; [d]International Institute for Applied Systems Analysis, Laxenburg, Austria; and [e]Group of Evolutionary Ecology, University of Antwerp, 2020 Antwerp, Belgium

The mammalian vertebral column is highly variable, reflecting adaptations to a wide range of lifestyles, from burrowing in moles to flying in bats. However, in many taxa, the number of trunk vertebrae is surprisingly constant. We argue that this constancy results from strong selection against initial changes of these numbers in fast running and agile mammals, whereas 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 toward 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 count of trunk vertebrae stem from a combination of developmental and biomechanical constraints.

body plans | evolutionary conservation | stabilizing selection | locomotion | Eco-Evo-Devo | musculoskeletal system

Many mammalian taxa show a remarkable conservation of the presacral vertebral count (the sum of cervical, thoracic, and lumbar vertebrae; Fig. 1). For instance, carnivores almost invariably have 27 vertebrae, and artiodactyls have 26 presacral vertebrae. However, in some taxa, in particular afrotherians, there is considerable interspecific variation (1, 2). Narita and Kuratani (1) proposed that the presacral vertebral count is conserved because of developmental constraints, as is also the case for the cervical vertebral count (3, 4). We propose that developmental constraints are indeed involved and that they are interacting with biomechanical problems resulting from homeotic transformations. Homeotic transformations are necessary for changes of the presacral vertebral count (5), although it is often incorrectly thought that these changes can be solely the result of increases or decreases in the number of vertebrae of a certain region. This assumption is not true, except for vertebrae in the tail region, which is the part of the vertebral column formed last. Homeotic transformations are necessarily involved, because of the sequential head-to-tail generation of the embryonal segments from which the vertebrae develop (somites) and the patterning of these segments under the influence of head-to-tail signaling gradients (6–8). This process implies that if there is an increase in the presacral count, for instance from 26 to 27, this is caused by a homeotic transformation of the 27th vertebra from a sacral into a lumbar one, regardless of whether the total count of vertebrae changes or not (see ref. 5 for a more detailed

discussion). We expect biomechanical problems to be important because initial mutations for homeotic transformations of vertebrae usually lead to incomplete and often asymmetric transitional vertebrae (9). In the case of lumbosacral transitional vertebrae, this implies incomplete (Fig. 1 B and C) and often asymmetric fusions to the sacrum (Fig. 1B), which hamper the flexibility of the lumbosacral joint and may decrease strength of this portion of the vertebral column. Flexibility of this joint is crucial for speed and agility in running, swerving, and jumping (10–12). Hence, transitional lumbosacral vertebrae are expected to impair the performance and survival of fast and agile mammals (10–12). Conversely, we expect the abnormal fusions to affect performance in slow and sturdy species only marginally.

In this study, we test our hypothesis that biomechanical problems associated with initial homeotic transformations result in strong selection against changes of the presacral vertebral count in fast and agile species, but only weak selection in slow and sturdy ones. Hence, we predict that slower-running species will have more variable presacral counts than fast ones, both within and between taxa. Furthermore, on the assumption that there are no other causes for variation, we predict that afrotherians are not more variable than similarly slow species of other taxa. To this aim, we compare the frequencies of abnormal (i.e., nonmodal for the considered taxon) presacral vertebral counts in fast running artiodactyls and carnivores vs.

Significance

Our study explains one of the riddles of mammal evolution: the strong conservation of the number of trunk vertebrae. The vertebral column and its high evolvability are considered to be of central importance for the evolution of vertebrates, which is why the constancy is both puzzling and important. We hypothesize, on biomechanical and developmental grounds, that evolutionary change is virtually impossible in fast running and agile mammals. The rationale is that several mutations are necessary to change trunk vertebral counts, with single mutations usually leading to irregular lumbosacral joints that severely hamper running and jumping capability. Our observations indeed show that selection against these initial changes is strong in fast and agile mammals and weak in slower and sturdier ones.
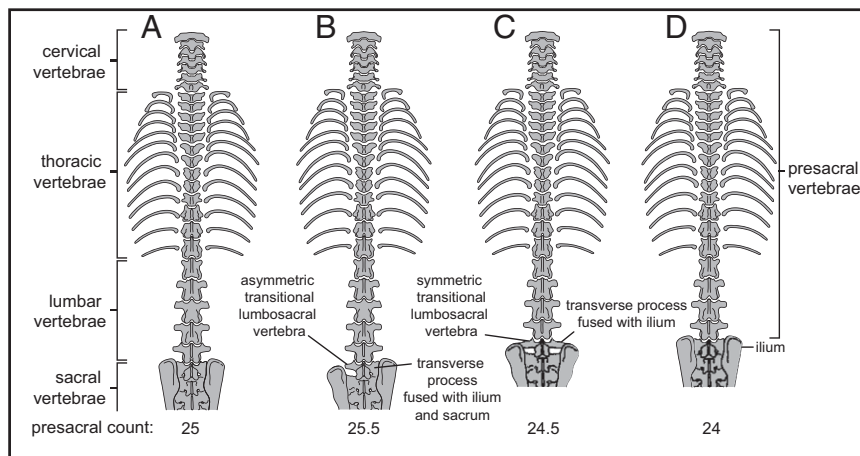
Fig. 1. Schematic view of vertebral columns illustrating different presacral counts and transitional vertebrae. (*A*) Individual with a modal number of 25 presacral vertebrae (7 cervical, 13 thoracic, and 5 lumbar vertebrae). (*B*) Individual with an asymmetric transitional lumbosacral vertebra (counted as 0.5 lumbar and 0.5 sacral; *Materials and Methods*) and an abnormal (i.e., nonmodal) presacral count of 25.5. Note the asymmetry in the position of the ilia (anterior parts of the pelvic girdle). (*C*) Individual with a symmetric transitional lumbosacral vertebra and an abnormal presacral count of 24.5. (*D*) Individual with one lumbar vertebra missing, due to a sacralization of the fifth lumbar vertebra into a sacral one (i.e., a complete homeotic transformation), resulting in an abnormal presacral count of 24.

slower-running species in the same taxa and slower-running afrotherians and monotremes.

Results and Discussion

Fast vs. Slow. Variation in the presacral vertebral count in fast running artiodactyls and carnivores was almost absent in our dataset (Table 1; <2%), both in sprinters (felids) and endurance runners (canids and artiodactyls). We found only three abnormal counts (≠26) in 161 artiodactyl specimens (*Saiga tartarica*, *Eudorcas rufifrons*, and *Kobus vardoni*; Dataset S1) and one (≠27) in 271 carnivore specimens (*Leptailurus serval*; Dataset S2). In contrast, variation was common in slower running artiodactyls and carnivores, ranging from ±25% abnormal counts in badgers, muskoxen, and bay duikers to >50% in water chevrotains and *Hippopotamus*. Most abnormal presacral counts were due to transitional lumbosacral vertebrae, i.e., incomplete homeotic transformations (80 of 117; Table 1). However, complete homeotic transformations also occurred and were responsible for almost a third of the abnormal presacral counts (37 of 117; Table 1). Within the Artiodactyla, the differences between fast and slower runners were significant for the frequency of abnormal presacral counts and for the presence of transitional vertebrae separately (Dataset S3). This outcome held at the family level for the Bovidae and for all nonbovid taxa together. Similarly, in the Carnivora, fast and slower runners differed significantly as, at the family level, did the short-limbed mustelids (fast half-bound vs. slow).

Fast carnivores and fast artiodactyls did not differ significantly, nor did slow carnivores and slow artiodactyls (Dataset S3). The slow carnivores, artiodactyls, monotremes, and afrotherians, differed significantly; however, post hoc pairwise comparisons show that only the afrotherians differed from slow carnivores and slow artiodactyls. The other differences were not significant (Dataset S3). The afrotherians did not differ significantly from the slowest artiodactyls, *Hyemoschus* and *Hippopotamus*. *Hippopotamus* had the highest frequency of abnormal presacral counts, a striking 82% (Dataset S1). However, the range of counts (25.5–26) was smaller than in other species, such as *Hyemoschus* (24.5–26; Dataset S1) and *Elephas* (28.5–31; Dataset S4).

Flexible vs. Stiff Trunk. The fast running taxa with the lowest frequency of transitional vertebrae gallop at relatively high speeds and are generally long-limbed (Fig. 2 *A* and *B* and Datasets S1 and S2). The spine of these species is flexible dorsoventrally and laterally, the rigid ribcage is rather short and narrow, and the lumbar spine is relatively long and slender (11–13). The mobility of the trunk is largest at the lumbosacral transition (10–12, 14). The laterally projecting transverse processes are slender and point forward, clearly separated from the sacrum and ilium (Fig. 3 *H–J*). The dorsal spinous processes of the thorax point backward up to the anticlinal vertebra, which usually has a straight spinous process (Fig. 2 *A–C*). Posterior to the anticlinal vertebra,

Table 1. Frequency of abnormal presacral vertebral count among individuals of different species

Locomotory classification per taxon	No. of individuals with normal presacral vertebral count	No. of individuals with abnormal presacral count and no transitional L/S vertebra	No. of individuals with abnormal presacral count and transitional L/S vertebra	Total no. of individuals (*N*)
Carnivora				
Fast	270 (99.6%)	1 (0.4%)	0 (0.0%)	271
Fast half-bound	96 (95.0%)	2 (2.0%)	3 (3.0%)	101
Slow	38 (74.5%)	4 (7.8%)	9 (17.6%)	51
Mustelidae				
Fast half-bound	96 (95.0%)	2 (2.0%)	3 (3.0%)	101
Slow	25 (75.8%)	2 (6.1%)	6 (18.2%)	33
Artiodactyla				
Fast	158 (98.1%)	2 (1.2%)	1 (0.6%)	161
Slow	63 (58.3%)	12 (11.1%)	33 (30.6%)	108
Bovidae				
Fast	134 (97.8%)	2 (1.5%)	1 (0.7%)	137
Slow	21 (75.0%)	2 (7.1%)	5 (17.9%)	28
Afrotheria (slow)	15 (30.6%)	12 (24.5%)	22 (44.9%)	49
Monotremata (slow)	17 (51.5%)	4 (12.1%)	12 (36.4%)	33

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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. 2*A*), especially in larger species, with dorsoventral flexibility at the lumbosacral joint considerably more important than around the anticlinal vertebra (11, 15). Dorsoventral flexibility significantly contributes to speed as it increases stride length (10, 11). 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 transitional lumbosacral vertebrae to the sacrum necessarily reduce flexibility of the lumbosacral joint (Fig. 3 *K–N*). Furthermore, in wolves, dogs, and humans, transitional lumbosacral vertebrae are associated with additional biomechanical problems in adjacent tissues, including increased pressure on blood vessels and nerves, intervertebral disk degeneration, iliolumbar ligament degeneration, scoliosis, and hip dysplasia (16–18). Hence, such transitional vertebrae are likely to 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 counts (>48%, echidnas, afrotherians, and slow artiodactyls; Datasets S1, S4, and S5) do not gallop, and their locomotion is cautious, with usually three or four and minimally two feet on the ground, thus avoiding great transitory stresses on the joints (14, 19–23). The trunk of these species 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 (Figs. 2*F* and 3 *B–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. 2*F*). Additionally, the lumbar region is short and wedged between the rigid ribcage and sacrum (Figs. 2*F* and 3*A*). 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. 3 *B–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 (10, 24). The restricted mobility of the lumbosacral transition and the usually slow movements of these species mean
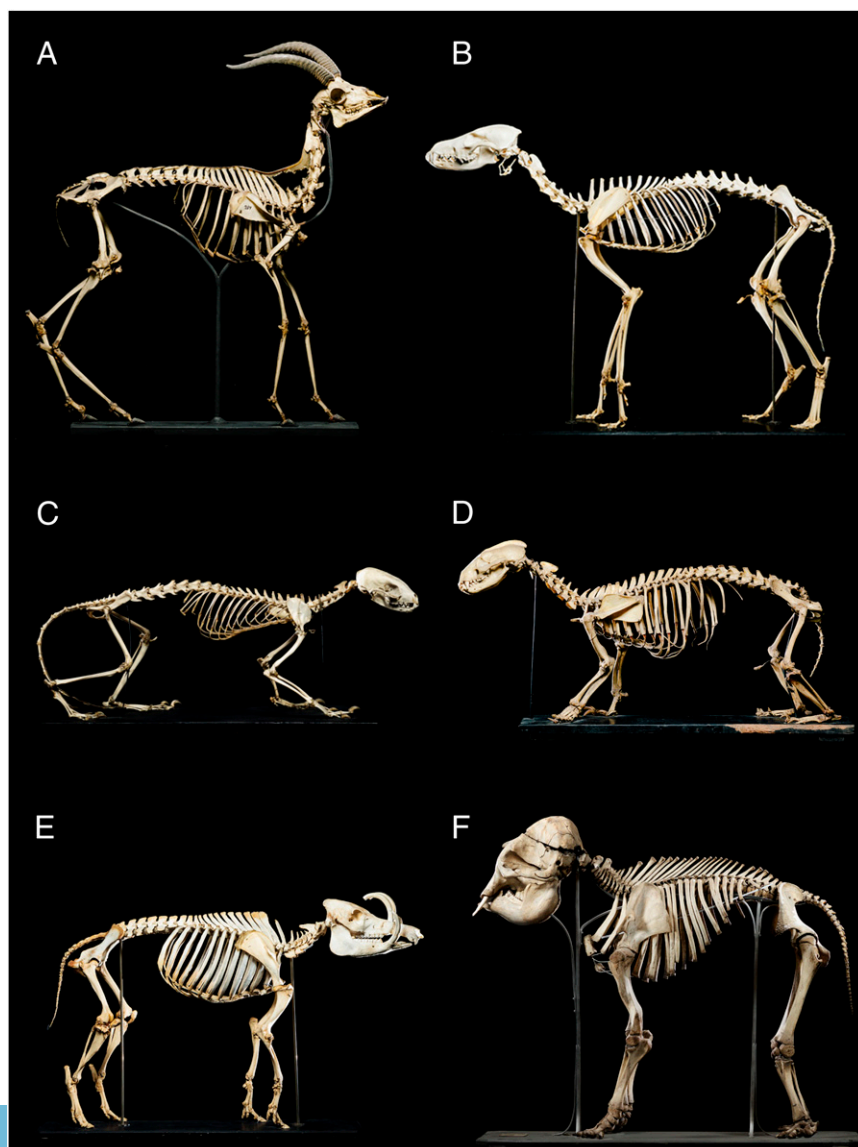
Fig. 2. Skeletons of fast and slower running mammals, lateral views. (*A–C*) Fast running and agile species with slender vertebral columns with a relatively short thoracic region (ribs provide rigidity), a relatively long lumbar region, and a highly flexible lumbosacral transition: long-limbed gallopers, (*A*) *Gazelle dorcas* and (*B*) *Canis latrans* (coyote) and (*C*) short-limbed and half-bounding marten (*Martes martes*). (*D–F*) Slower running species with more sturdy skeletons, longer thoracic and shorter lumbar regions, and stiffer lumbosacral transitions: (*D*) badger (*Meles meles*) and (*E*) Babyrusa swine (*Babyrousa babyrussa*), species that occasionally run fast (galloping and half-bounding, respectively), and (*F*) Asian elephant, *Elephas maximus* (juvenile specimen). Elephants never use the gallop and have a particularly stiff lumbosacral transition. The stiffness of their spine comes from the dorsal spinous processes that are all backward pointing (no anticlinality; *F*) and a particularly short lumbar region that is wedged between the long and sturdy ribcage and rigid sacrum. (*C* and *D*) For a comparison of fast running and slower running species within one family, compare 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. 3 *F* and *G* for dorsal views of the lumbosacral spines). Anticlinality is particularly pronounced in fast carnivores (*B* and *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. Photographs by J.v.A.

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Fig. 3. Lumbosacral spines of fast and slower running mammals, dorsal views. (*A–D*) Relatively slow and cautiously moving species with a stiff lumbosacral transition. (*A*) *Elephas maximus* (Asian elephant) 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 (absence of anticlinality; Fig. 2*F*). (*B–D*) Stiff lumbosacral transitions 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: (*B*) *Orycteropus afer* (aardvark), (*C*) *Hippopotomus amphibius*, and (*D*) *Hyemoschus aquaticus* (water chevrotain). (*E* and *F*) Intermediately stiff lumbosacral transitions in species that occasionally run: (*E*) *Babyrousa babyrussa* (Babyrusa swine) and (*F*) *Meles meles* (badger). The transverse processes of the lumbar vertebrae are clearly separated from each other and less robust compared with *A–D* but more robust than 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: (*G*) the short-limbed half-bounding *Martes martes* (pine marten) and (*H–J*) long limbed gallopers: (*H*) *Acinonyx jubatus* (cheetah), (*I*) *Canis latrans* (coyote), and (*J*) *Gazella dorcas*. 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 (*K* and *L*) and symmetrical (*M* and *N*) transitional lumbosacral vertebrae in (*K*) *Meles meles* (badger), (*L*) *Saiga tatarica*, (*M*) *Orycteropus afer* (aardvark), and (*N*) *Hyemoschus aquaticus* (water chevrotain). The partial fusions with the sacrum drastically limit the flexibility of the lumbosacral joint, which is especially problematic in fast and agile mammals.

that structural abnormalities will only minimally affect performance, and therefore, indirect selection against change in vertebral numbers should be weak.

Species with an intermediate number of abnormal presacral counts (24–33% in swine, badgers, musk oxen, and bay divers; Datasets S1 and S2) are also intermediate in running speed, agility, and trunk stiffness (as shown by shape, size, and position of transverse and spinous processes, relative lengths of thoracic and lumbar regions; Figs. 2 *D* and *E* and 3 *E* and *F*). These species gallop, but only infrequently. The variability in presacral numbers that we found in different taxa thus agrees well with the hypothesized strength of selection against homeotic transformations.

Gallop vs. Half-Bound. The fast, short-limbed mustelids have a somewhat higher incidence of abnormal presacral counts than fast long-limbed carnivores and artiodactyls (∼5% vs. ∼1%), notwithstanding the flexibility of their lumbosacral spine (Figs. 2*C* and 3*G*). These mustelids do not gallop, but use a half-bounding gait with the left and right hind limb striking the ground simultaneously. 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 parasagittally oriented stresses, further increasing the biomechanical adversity of abnormal lumbosacral joints.

Within-Species Variation. In most species in the fast galloping group, we found no within-species variation (42 of 46). For all taxonomic groups combined, we found no variation for most species in the fast galloping group (42 of 46), even in species with *n* > 20 (Fig. 4). In contrast, all species of the fast half-bounding group and almost all of the slow group showed intraspecific variation (3 of 3 and 18 of 19, respectively). When we estimated relative magnitudes of within-species variances among species

with nonzero variances only, the CI for the ratio of the variances of the fast galloping group and that of the slow group is 0.402–0.633 and 0.298–0.419 for the fast half-bounding group relative to the slow group. Hence, even for the variable species, the within-species variances of the fast galloping and fast half-bounding groups are significantly lower than that of the slow group. If we include the nonvariable species in this analysis as well, the fast galloping group also has significantly lower within-species variance than the fast half-bounding group.

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; Datasets S1, S4, and S5). Naturally, weight plays a role in that extremely heavy mammals always have stiff lumbar spines, to prevent structural damage and minimize muscular stabilization costs (10, 11, 14).

Domestication and Inbreeding. Domesticated species usually harbor high numbers of transitional lumbosacral vertebrae, including those that originate from fast and agile wild counterparts (e.g., cats, dogs, and horses) (17, 25, 26) 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 (18, 27). The *Saiga tatarica* with a transitional vertebra may well be the product of the strong inbreeding in this endangered species (28, 29).

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 head-to-tail patterning of the embryonic axis, buffering mechanisms
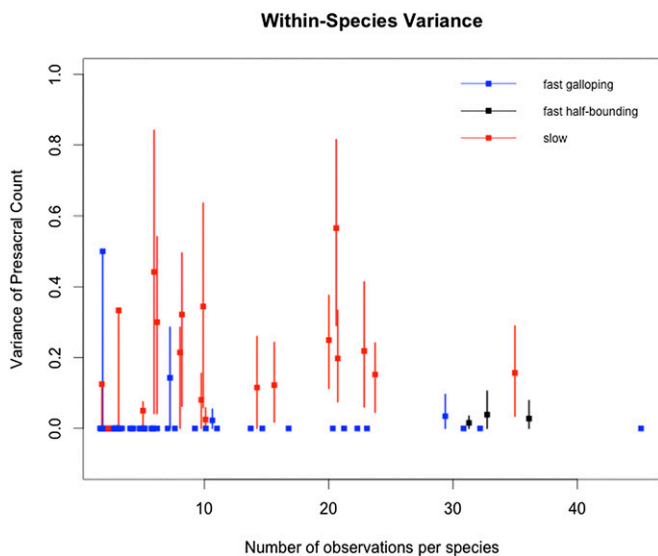
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Within-Species Variance

Fig. 4. Plot of the within-species variances. Blue, fast galloping species; black, fast half-bounding species; red, slow species. Estimates per species are indicated by points at their respective sample sizes. Line segments indicate bootstrap percentile CIs per species. A small random number is added to each number of observations to prevent overlap in the figure between CIs. We found no variation for most of the fast galloping species (42 of 46). In contrast, all of the fast half-bounding (3 of 3) and almost all of the slow species (18 of 19) show intraspecific variation.

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 (30–32). In contrast, in fast running species, the transition at the lumbosacral boundary is sharp and vertebral shape is regular (Fig. 3 *G–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 sharp decrease can in part be explained by the high interactivity and low modularity of the vulnerable early organogenesis stage, when lumbosacral vertebral identities are determined (33, 34). Moreover, the early irreversibility of the determination of vertebral identity further limits the buffering potential (5).

Fast and Inbred Cheetahs. Unexpectedly, we did not find any abnormal lumbosacral transitions in cheetahs (Dataset S2), despite their dramatically low genetic diversity (35) and our (exceptional) inclusion of captive-born specimens (9 of 42 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 strong canalization of 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 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, muscles, vasculature, and bones (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.

Materials and Methods

For information on the skeletons, see *SI Materials and Methods*.

Vertebral Formula and Presacral Count. We determined the vertebral formula of the skeletons by determining the number of cervical, thoracic, lumbar, sacral, and coccygeal vertebrae (Fig. 1). Transitional vertebrae at boundaries were counted as half for each of the neighboring regions, e.g., half thoracic and half lumbar (e.g., 5.5 lumbar and 3.5 sacral vertebrae as a change from 5 lumbar and 4 sacral vertebrae). The thoracolumbar boundary was sometimes difficult to establish with precision, because transitional thoracolumbar vertebrae have one or two rudimentary ribs, and these are often lost, making the detection of their small articulations on the vertebra difficult, especially when the vertebrae were worn or damaged by strong maceration during preparation. Therefore, the sum of the thoracic and lumbar vertebrae was more precise than the separate numbers, but this did not affect the precision of the presacral count (sum of cervical, thoracic, and lumbar vertebrae). We considered the most frequent whole presacral number (mode) as the normal presacral count. For carnivore species, the normal presacral count is 27 and for artiodactyl species it is 26 (Datasets S1 and S2). For Afrotherians, the normal presacral count is 28 for *Orycteropus*, 30 for *Elephas*, and 31 for *Loxodonta* (Dataset S4). For the monotremes, it is 26 for *Tachyglossus* and 27 for *Zaglossus* (Dataset S5). Abnormal presacral vertebral counts were divided into two groups: (*i*) with a transitional lumbosacral vertebra, e.g., 26.5 or 25.5 instead of 25 and (*ii*) without a transitional lumbosacral vertebra, e.g., 27 or 25 instead of 26 for artiodactyl species.

Lumbosacral Transitional Vertebrae. Lumbosacral transitional vertebrae were considered to be vertebrae that had both lumbar and sacral characteristics (Figs. 1 *B* and *C* and 2 *K–N*) and resulted from an incomplete homeotic transformation of a lumbar vertebra into a sacral vertebra (sacralization) or a sacral vertebra into a lumbar vertebra (lumbarization) (36). The left and right halves of transitional vertebrae were often transformed to a different extent, leading to strong left-right asymmetry (Fig. 2 *K* and *L*) (5, 9). Partially sacralized lumbar vertebrae were considered to be transitional lumbosacral vertebrae when at least on one side the transverse process was enlarged to such an extent that it was fused with, or touching, the adjacent sacral vertebra or the ilium (Fig. 1*C*). Partially lumbarized sacral vertebrae were considered to be transitional lumbosacral when at least on one side the transverse process was not osseously connected with the adjacent sacral vertebra or ilium (Fig. 1*B*).

Classification of Fast Running vs. Slower Running. Predictions regarding running speed and gait were based on references found in the literature and surmised from the anatomy and observations before the analysis of the vertebral columns. To avoid classification mistakes, we selected as much as possible the fastest and most agile of galloping, long-limbed species vs. the slowest and sturdiest species within the taxon. For the fast, long-limbed gallopers, we included both sprinters (felids; refs. 23, 37–43) and persistence runners (canids and artiodactyls; refs. 23, 37–43). The slower-running species consist of (*i*) those that never gallop on land: the afrotherian *Loxodonta* and *Elephas* (19, 44, 45), *Orycteropus* (21, 46), the monotreme echidnas (41), and the artiodactyl *Hyemoschus* (20, 47) and *Hippopotamus* (which only gallops in the water, ref. 22); and (*ii*) those that rarely, or infrequently, gallop: the artiodactyl *Ovibos* (48, 49), *Cephalophus dorsalis* (20, 47, 50), and *Tragulus* (little known, but supposed to be slow, refs. 20, 51, 52), the suid species (able to gallop, but rarely do so, refs. 53–55), and within the carnivores, *Meles* (38, 56, 57) and *Procyon* (able to gallop, but rarely do so, refs. 58–61). Classifications can be found in Datasets S1–S4. In total, we classified 252 specimens of carnivores as fast running and 51 as slower running. An exception was made for the family Mustelidae (Carnivora). Exclusively for the analysis at the family level, we included as fast species the short-limbed *Martes foina*, *Mustela erminea*, and *Mustela nivalis* (61). The rationale was that, in this set of mustelid species, there are no long-limbed and fast galloping species, but only fast and agile species that use a half-bound gait and have an elongate trunk and short limbs.

EVOLUTION

Statistical Tests. We analyzed overall contingency tables of the different slow and fast running taxa and their presacral counts using Fisher's exact tests (Dataset S3). Post hoc comparisons were performed by Fisher's exact tests and *P* values were Holm–Bonferroni adjusted. We compared the within-species variation in number of presacral vertebrae between fast galloping, fast half-bounding, and slow groups in the following manner. Per species, 95% bootstrap percentile CIs of the variance were made by resampling the data 1,000 times. Groups were compared in a graphical way, by plotting these CIs in dependence on sample size per species. For small sample sizes, it is more likely that we will underestimate within-species variation. Within-species variances were compared between these three groups in a second manner, by fitting general linear models with a separate estimate of the mean number of presacral vertebrae per species and different within-species variances for the three groups [function gls from the R nlme library (62)]. Count data have typical distributions such as the Poisson distribution. For Poisson distributions with a parameter greater than 10, the distributions tend to be well approximated by the normal distributions and we therefore based our calculations on this assumption.

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