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Function of revolute zygapophyses in the lumbar vertebrae of early placental mammals

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Abstract

The unique morphology of mammalian lumbar vertebrae allows the spine to flex and extend in the sagittal plane during locomotion. This movement increases stride length and allows mammals to efficiently breathe while running with an asymmetric gait. In extant mammals, the amount of flexion that occurs varies across different locomotor styles, with dorsostable runners relying more on movement of long limbs to run and dorsomobile runners incorporating more flexion of the back. Although long limbs and a stabilized lumbar region are commonly associated with each other in extant mammals, many "archaic" placental mammals with short limbs had lumbar vertebrae with revolute zygapophyses. These articulations with an interlocking S-shape are found only in artiodactyls among extant mammals and have been hypothesized to stabilize against flexion of the back. This would suggest that archaic placental mammals may not have incorporated dorsoventral flexion into locomotion to the same extent as extant mammals with similar proportions. We tested the relative mobility of fossil lumbar vertebrae from two early placental mammals, the creodonts *Patriofelis* and *Limnocyon*, to see how these vertebrae may have functioned. We compared range of motion (ROM) between the original vertebrae, with revolute morphology and digitally altered vertebrae with a flat morphology. We found that the revolute morphology had relatively little effect on dorsoventral flexion and instead that it likely prevented disarticulation due to shear forces on the spine. These results show that flexion of the spine has been an important part of mammalian locomotion for at least 50 million years.

Introduction

Extant mammals can dorsoventrally flex and extend the spine, which facilitates breathing and increases stride length during locomotion (Hildebrand, 1959; Gambaryan, 1974; Carrier, 1987; Schilling and Hackert, 2006). The extent to which the spine bends varies across extant mammals, corresponding to locomotor style and gait, but the evolution of this function has not been explored in the fossil record (Slijper, 1946; Gambaryan, 1974; Boszczyk et al., 2001). Placental mammals, the dominant extant group, first experienced a major radiation after the end Cretaceous mass extinction as they rapidly increased in size and diversified into new niches (Alroy, 1999; Grossnickle et al., 2019). This radiation included first appearances of many crown orders, but the first placental mammals to reach large size belonged to "archaic" groups, such as the carnivorous creodonts. Many archaic mammals had lumbar vertebrae with unusual, interlocking zygapophyses (Halpert et al., 1987; Zhou et al., 1992; Wood et al., 2010; Argot, 2013; Rose et al., 2019; Kort et al., 2022). This feature could indicate unique lumbar function in early Paleogene mammals and a shift in function later in the Cenozoic. To assess the function of lumbar vertebrae in archaic Paleogene mammals, we tested the relative mobility of fossil lumbar vertebral joints from two creodonts from this time.

Lumbar vertebrae sit between the thoracic vertebrae, which support the ribcage, and the sacrum, which supports the pelvis. Unlike the posterior dorsal vertebrae of non-mammalian synapsids, lumbar vertebrae in mammals lack ribs, and tend to have elongated centra and vertically oriented zygapophyses, making this region more mobile in the sagittal plane (Slijper, 1946; Jenkins, 1970). Lumbar joints have a higher range of motion in the sagittal plane than those of thoracic or sacral vertebrae, allowing the lumbar region to act as a hinge between the fore and hindlimbs during locomotion. Because lumbar vertebrae dually function in locomotion and body support, their relative mobility varies with body size and locomotor style across extant mammals (Slijper, 1946; Hildebrand, 1959; Boszczyk et al., 2001; Alvarez et al., 2013; Jones, 2015). Smaller animals and sprinters tend to have a more mobile lumbar

region, while larger animals and long-distance runners tend to have a more stabilized lumbar region (Hildebrand, 1959; Gambaryan, 1974). Many morphological changes can affect the relative mobility of lumbar vertebrae, like shortening of the centra or widening of the transverse processes (Boszczyk et al., 2001). Though many of these changes have evolved multiple times across Mammalia, revolute zygapophyses, articulations with an interlocking S-shape appear to have only evolved once (Kort, 2023).

In most mammals, lumbar zygapophyses are relatively planar, with a slight overall curve to the surface. In contrast, revolute zygapophyses are more strongly curved with a distinct dorsal ridge (Slijper, 1947; Halpert et al., 1987). The prezygapophyses of the more posterior vertebra wrap around peg-shaped postzygapophysis of the more anterior vertebra. Additionally, the postzygapophyses have a ridge along the dorsal edge that matches the shape of the prezygapophyses, creating a kind of lock and key (Figure 1). Historically, this shape has been referred to as revolute, referring to the leaf-like, S-shape of the postzygapophysis when viewed head-on (Wortman, 1894; Slijper, 1946). Among extant mammals, only artiodactyls, and possibly pangolins, have retained revolute zygapophyses (Slijper, 1946; Halpert et al., 1987; Kawashima et al., 2015). The interlocking configuration of revolute zygapophyses has been hypothesized to restrict movement of the lumbar vertebrae (Halpert et al., 1987). Large bovids have especially dorsostable running gaits, with most dorsoventral movement of the spine seemingly restricted to the lumbosacral joint by exceptionally tall postzygapophyseal ridges (Halpert et al., 1987; Belyaev et al., 2021; Belyaev et al., 2022).

Revolute zygapophyses have been documented in a variety of Paleogene taxa, but their development in creodonts is especially unexpected (Mellett, 1977; Zhou et al., 1992; Wood et al., 2010; Argot, 2013; Gould and Rose, 2014; Kort et al., 2022). Creodonts are a group of carnivorous placental mammals with uncertain phylogenetic affinity and questionable monophyly that appeared in the Paleocene and went extinct in the Miocene (Gunnell, 1998; Halliday et al., 2017; Polly, 1996). They are often placed within Ferae, the clade encompassing extant carnivorans and pangolins, as a basal polyphyletic or paraphyletic group, though the exact placement varies (Gunnell, 1998; Zack, 2019; Halliday, 2017; Beck and Baillie, 2018). The two major groups of creodonts commonly recognized are the dog-like hyaenodonts and the cat-like oxyaenodonts (Gunnell, 1998). Compared to extant carnivorans, creodonts tended to have shorter, more robust limbs and more plantigrade posture (Ahrens, 2017; Van Valkenburgh, 1985; Janis and Wilhelm, 1993).

The combination of short limbs and a seemingly stabilized spine presents an apparent contradiction in the interpretation of creodont locomotion. In artiodactyls, the only living analog for revolute zygapophyses, the combination of long legs and a stabilized spine has been interpreted as an adaptation for efficient long distance running across open environments (Halpert et al., 1987; Boszczyk et al., 2001; Galis et al., 2014). On the other hand, most creodonts are decidedly not cursorial (Gunnell, 1998; Morlo, 1999). Even *Hyaenodon*, a later creodont that was somewhat cursorial, did not reach the same limb proportions as living dogs (Mellett, 1977; Van Valkenburgh, 1985; Janis and Wilhelm, 1993). In extant carnivores with short legs, like mustelids, flexion and extension of the spine are a large component of the stride length (Gambaryan, 1974). The relative mobility of the lumbar region would therefore have a major impact on the locomotor capabilities of creodonts.

Three primary functions have been proposed for revolute zygapophyses based on the observations of spine movement in living mammals with this feature: 1.) stabilizing against dorsoventral flexion, 2.) resisting dorsoventral shear and disarticulation, and 3.) resisting torsion or axial rotation (Halpert et al.,

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1987; Boszczyk et al., 2001; Jones, 2015). We tested each of these functions in exceptionally preserved lumbar vertebrae from two creodont specimens by running digital range of motion (ROM) tests. ROM measures how much bones at a joint can rotate and move relative to each other. In lumbar vertebrae, the primary axes of rotation are defined as dorsoventral flexion (up and down bending), lateral flexion (side to side bending), and axial rotation (twisting). To isolate the effect of revolute zygapophyses, we created edited versions of the vertebrae with flattened zygapophyses and compared ROM between revolute and flattened zygapophyses.

Materials and Methods

Specimens

We acquired pairs of fossil lumbar vertebrae in articulation from two creodonts: *Patriofelis ulta* (UMNH VP 550) and *Limnocyon potens* (AMNH 13138) from the Natural History Museum of Utah (UMNH) and the American Museum of Natural History (AMNH), respectively (Figure 1). These specimens were selected because both had intact, in articulation vertebrae that were identifiable as lumbar vertebrae based on morphology and comparison to other vertebrae within the specimens. Additionally, unlike many of the partial skeletons with articulated vertebrae from this time period, neither specimen was in a slab, which presents substantial challenges for CT scanning. Both creodonts are thought to have six lumbar vertebrae, but this is difficult to confirm from the specimens preserved (Matthew, 1909; Kort et al., 2022). The pairs selected are the anterior-most vertebrae pairs that are intact, estimated to be L2 and L3 for both specimens.

Both specimens come from the Bridger Formation of Wyoming, a series of limestones and siltstones deposited around 50-45 million years ago in a wet, forested environment (Buchheim et al., 2000). *Patriofelis* is a large oxyaenodont, approximately the size of a cougar, with a cat-like skull and short, robust limbs. It was likely one of the largest predators in the ecosystem (Gazin, 1957; Kort et al., 2022). *Limnocyon* is a smaller, basal hyaenodont, around the size of a raccoon, with relatively short limbs. It had a longer snout than *Patriofelis* and was likely more omnivorous and insectivorous (Borths et al., 2016; Matthew, 1909).

Scanning and Model Preparation

To digitize the vertebrae, we CT scanned both specimens. *Patriofelis* was CT scanned with a slice thickness of 0.6 mm on the Siemens Dual Source medical CT scanner at the University of Utah Hospital in Salt Lake City, Utah. *Limnocyon* was CT scanned at a resolution of 38 microns on the X5000 high resolution micro-CT system at the University of Minnesota in Minneapolis, Minnesota. We used 3D Slicer to segment the two most intact adjacent vertebrae from each specimen (Fedorov et al., 2012). For both these were a more anterior pair, likely L2 and L3. We exported the models as OBJ files for further editing (Table S1).

We used Geomagic Design to clean and smooth the models (Figure 2). We decimated each model to around 25k faces. Because of minor deformation in the *Patriofelis* vertebrae, we used the shear tool to manually make the right and left halves of the vertebrae symmetrical. For *Limnocyon*, the zygapophyses were only fully intact on one side of the vertebrae. We created a fully intact set of vertebrae by deleting the broken half and mirroring the intact half. Finally, we used the smooth tool to reduce any rough geometry that came from the segmentation process.

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To test the effect of revolute zygapophyses, we created an edited set of vertebrae for both specimens by flattening the zygapophyses. The morphology of these edited vertebrae is entirely hypothetical for the purposes of experimentation (Jurestovsky et al., 2020). Although measurements of ROM in the edited vertebrae do not reflect the function of any real animal, experimenting with the morphology in this way allowed us to isolate the effects of the zygapophysis shape. This experimental approach is particularly useful for fossils, given the possible effects of deformation on any absolute measurements.

We primarily focused on flattening the prezygapophyses. In *Patriofelis*, we also flattened the postzygapophyses to look more like those of a carnivoran, though we did not adjust the angle of projection. For *Limnocyon*, we did not flatten the postzygapophyses extensively because the articulating surface was already relatively flat. In total, this resulted in four sets of two vertebrae each: an unedited set with revolute zygapophyses for both *Patriofelis* and *Limnocyon* and an edited set with flattened zygapophyses for both species (Figure 2).

Range of Motion Test

We used the 3D animation software Maya and Python package AutoBend to measure the range of motion (ROM) for each set of vertebrae (Jones et al., 2021). AutoBend systematically rotates vertebrae around 3 axes of the joint: anteroposterior (axial rotation), dorsoventral (lateral bending), and mediolateral (sagittal bending). Although ROM traditionally uses only bony intersection to determine the limit of rotation, in the spine, ligaments play an especially important role in preventing disarticulation of the joint (Slijper, 1946; Evans, 1993). To estimate the role of ligaments in ROM, AutoBend measures distances between landmarks on the centra and zygapophyses of the vertebrae to estimate strain on the intervertebral joint. Excessive strain, which would stop rotation of the joint, was validated against data from a tegu and cat (Jones et al., 2021). When either joint strain is exceeded or bony intersection occurs, rotation stops.

We set up each pair of vertebrae using the recommended protocol, placing landmarks on the centra and zygapophyses and setting the center of rotation in between the centrum faces of the articulating vertebrae (Figure S1). We set the neutral starting position by starting with the articulated position in which each pair of vertebrae was preserved. We then adjusted the alignment of each pair so centra faces were parallel and the zygapophyses were maximumly overlapped (Jones et al., 2021). We then set the center of rotation (COR) using a sphere mesh centered between the faces of the centra. Because the zygapophyses of *Patriofelis* had a slight curve, we adjusted the position of the COR using a sphere fit to this curve.

AutoBend rotates the vertebra around one axis at a time, but any joint in a living animal will often experience rotation and potentially translation in more than one direction at a time. To test how different combinations of rotation and translation affected ROM, we created six alternate starting positions that deviated from the neutral position (Figure 3). Three positions varied the starting rotation of the vertebrae and three varied the relative position of the vertebrae. Seven starting positions, including the neutral position, for each of the four sets of vertebrae resulted in 28 models total (Table S2). For each of these models, we ran 23 trials, varying parameters for tolerance of bony intersection, the exact position of the center of rotation, and the amount of joint strain allowed for the zygapophyses and the centra (Table S3).

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ANOVA

We performed a set of two way analysis of variance (ANOVA) tests on the resulting range of motion data to compare edited and unedited zygapophyses for each species. We used starting position and shape of zygapophysis as the two factors affecting range of motion, treating each trial as a data point. This resulted in 280 trials for each species, after removing the three trials that failed to intersect. We then performed eight total ANOVAs, one for each direction of rotation (dorsal, ventral, lateral, and axial) for each species (See Supplementary Data Code).

Dorsal and ventral directions of rotation were treated separately because vertebrae are dorsoventrally asymmetric. For lateral and axial rotation, left and right rotation were averaged for each trial, and the ANOVAs were performed on these average rotations from the neutral position. Because the data generated by AutoBend is non-parametric, we used the RRPP package for R to perform the ANOVAs (Collyer and Adams, 2018). We used the *lm.rrpp* function to fit a linear model to the data and calculate effect sizes using randomized residuals of the data. We then used the *pairwise* function in the same package to compare effects between zygapophysis shape within each starting position.

Results

Dorsoventral Flexion

In *Patriofelis*, revolute zygapophyses showed significant differences in both dorsal and ventral flexion ($p<0.001$) (Table S4). However, pairwise comparison of zygapophysis shape within each starting position showed significant difference only for the trials where the posterior vertebra was translated either ventrally or posteriorly (Table S5, S6). When the posterior vertebra started ventrally translated from the neutral position, dorsal flexion was substantially reduced in the unedited, revolute zygapophyses but not in the flattened zygapophyses. Similarly, when the posterior vertebra started posteriorly translated, both dorsal and ventral flexion were reduced in the revolute zygapophyses but not in the flattened.

In *Limnocyon*, revolute zygapophyses had little effect on dorsoventral flexion (Figure 4). The ANOVA showed significant difference between zygapophysis shape in the level of dorsal flexion ($p<0.001$), and no significant difference in level of ventral flexion ($p=0.605$) (Table S4). Pairwise comparison of zygapophysis shape for each starting position showed significant differences in the neutral starting position for each of these directions of rotation (Table S7, S8; Figure 4). Unexpectedly, the revolute zygapophsyes showed higher ROM than the flattened zygapophyses for these trials.

Lateral Flexion and Axial Rotation

Patriofelis shows consistently higher ROM in the flattened zygapophyses than the unedited zygapophyses for these axes of rotation. Like with dorsoventral flexion, the only pairwise comparisons of zygapophysis shape that show significant difference are the ventrally and posteriorly translated starting positions (Table S9, S10; Figure 4). *Limnocyon*, on the other hand, showed significant difference ($p<0.05$) between edited and unedited zygapophyses for range of axial rotation (torsion) and lateral flexion only when starting from the neutral position (Table S11, S12; Figure 4). Again the unedited zygapophyses show slightly higher values for ROM than the flattened zygapophyses for both axial rotation and lateral flexion.

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Discussion

Function of Revolute Zygapophyses

In both creodonts, the revolute zygapophyses appear to prevent disarticulation rather than heavily stabilize against dorsoventral flexion. In *Patriofelis*, the S-shape of the articulation acts as a track, restricting axial rotation and lateral flexion, and only allowing rotation in the sagittal plane. The large ridge of the postzygapophysis can move only along the anteroposterior axis, sliding by the overhang of the prezygapophysis. Because the postzygapophysis has a slight curve in the sagittal plane, anteroposterior movement along this "track" becomes rotation in the sagittal plane (Figure 5). The limit of sagittal rotation is set by the length of the zygapophyses, as a longer "track" allows for more movement before the zygapophyses separate.

Limnocyon shows a higher ROM in all directions compared to *Patriofelis*, and the tracked effect is not present, likely due to the reduced dorsal ridge along the postzygapophysis. Instead, lateral flexion and axial rotation are slightly higher in the original zygapophyses compared to the flattened zygapophyses. This may be due to the curved, bulging shape of the original zygapophyses having a ball and socket like effect (Figure 6). Like in *Patriofelis*, the lower part of the postzygapophysis is more curved than in the zygapophyses of most mammals, and the prezygapophysis matches this curve with a more cupped, indented shape (Figure 2). By flattening the prezygapophysis, the surface of the prezygapophysis was raised slightly relative to original curved morphology. This appears to have caused the flattened zygapophyses to intersect after less rotation than the revolute zygapophyses. Additionally, the smaller ridge along the dorsal edge of the postzygapophysis in *Limnocyon* does not track movement into one direction as in *Patriofelis*. However, the overhang on the prezygapophysis would still act as a bony stop that would prevent dorsoventral disarticulation, unlike with flattened zygapophyses.

Although revolute zygapophyses have been long hypothesized to restrict dorsoventral flexion, these results suggest that other features are more important in the mobility of the lumbar region (Slijper, 1946; Halpert et al., 1987). For instance, taller centra in *Patriofelis* compared with *Limnocyon* may be responsible for the decreased ROM, as the edges of the centra farther out from the center of rotation would come into contact more quickly than in dorsoventrally shallow centra (Gambaryan, 1974). Revolute zygapophyses may have instead functioned to decrease the chances of disarticulation, by increasing surface area and spreading out forces acting on the joint (Jones, 2016). Notably, *Patriofelis* has taller dorsal ridges along the postzygapophysis compared with the reduced ridges of *Limnocyon*. This matches the pattern found in extant artiodactyls, where larger animals, like *Bison*, have prominent ridges that create the distinctive S-shape, and smaller animals, like the dik-dik (*Madoqua kirkii*) have reduced ridges (Halpert et al., 1987). This suggests that the ridge may play a role in resisting breakage, though this cannot be confirmed with a simple range of motion test.

Locomotor Function

When comparing the ROM from the creodont vertebrae to measurements from extant mammals, *Patriofelis* is comparable to a sheep, while *Limnocyon* is comparable to a cat (Gál, 1993; Jones et al., 2020). The dorsoventral ROM for *Patriofelis* falls in line with *ex vivo* measurements from a dissected sheep (*Ovis aries*) spine, where the more anterior lumbar vertebrae show around 5 degrees of dorsal and ventral flexion from the neutral position (Gál, 1993). Sheep, like other artiodactyls tend to be more dorsostable when running, though not to the extremes of larger bovids (Belyaev et al., 2021). On the other hand, the higher ROM, particularly in the dorsoventral direction, of *Limnocyon*, is closer to the 10-

15 degrees measured from the dissected spine of a cat (*Felis catus*) (Jones et al., 2020). Cats are generally considered dorsoflexible, incorporating more dorsoventral flexion into their running gaits (Gambaryan, 1974).

Previous locomotor interpretations of *Patriofelis* and *Limnocyon* limb morphology suggest that both animals were likely ambulatory terrestrial (Matthew, 1909; Morlo, 1999; Kort et al., 2022). Given the mobility of its vertebrae, *Limnocyon* may have been able to run with a bounding gait, similar to living mustelids (Gambaryan, 1974; Schutz and Guralnick, 2007). Mobility in lateral and axial rotation may have also allowed for a high amount of agility in its movements. *Patriofelis*, on the other hand, shows more restriction in dorsoventral flexion, and little to no lateral or axial rotation. Though it would have likely had some capacity to run with a bounding gait or gallop, it would have not had the same level of bending as *Limnocyon*.

The increased stabilization of the lumbar region in *Patriofelis* may be related to its large size compared with *Limnocyon*. *Patriofelis* is estimated to have weighed between 30-100 kg, around the size of a cougar, while *Limnocyon* is estimated to have weighed 3-10kg, around the size of a raccoon (Morlo, 1999). Lumbar vertebrae typically experience a functional trade-off between mobility and stability as mammals increase in size and the relative force of body weight on the spine increases (Halpert et al., 1987; Jones, 2015). Creodonts were some of the first large placental mammals and, *Patriofelis* especially, would have experienced increased forces on the spine compared to earlier placentals (Bi et al., 2018; Lyson et al., 2019). By preventing disarticulation while allowing dorsoventral flexion, revolute zygapophyses seem to, at least partially, bypass the trade-off between mobility and body support. In domestic cats and dogs, disarticulation of the posterior thoracic and anterior lumbar is the most common spinal injury (Grasmueck and Steffen, 2004; Bali et al., 2009). This region is where the most flexion occurs, and in cats especially, muscle and ligaments are primarily responsible for preventing vertebrae from disarticulating in response to shear stresses (Slijper, 1946; Evans, 1993; Boszczyk et al., 2001). Because spinal injuries are so often fatal, it is plausible that the revolute zygapophyses evolved in some early placental groups that increased in size and experienced increased forces on the spine. This is further supported by the elaboration of the revolute zygapophyses in *Patriofelis*, the larger of the two creodonts, which results in a more stabilized joint.

Evolution of Lumbar Vertebrae Function

These results demonstrate that dorsoventral flexion of the spine has been a component of mammalian locomotion for at least the past 50 million years. Both creodont vertebrae have morphological features that enhance dorsoventral flexion: elongated centra, vertically oriented zygapophyses, and anteriorly pointed neural spines (Slijper, 1946; Gambaryan, 1974).

At the beginning of the Cenozoic, mammals rapidly increased in body size (Lyson et al., 2019; Bertrand et al., 2022). This increased forces on the spine and may have led to the evolution of stabilizing features that overwrote earlier, mobile features, like shortening of the elongated centra. Revolute zygapophyses appear to a unique, derived feature, that stabilized the spine against disarticulation while still allowing flexion. Among Paleogene mammals, revolute zygapophyses appear widespread in taxa thought to fall under Ferungulata, the superorder that includes living ungulates and carnivorans, while many other groups, including those more basal to Placentalia, have relatively flat zygapophyses (Zhou et al., 1992; Wood et al., 2010; Argot, 2013; Rose et al., 2019; Kort et al., 2022; Kort, 2023). Paleogene mammals that have revolute zygapophyses include some of the largest mammals for the time period, like mesonychids,

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but many large mammals, like uintatheriids, lacked this feature. Thus, as mammals diversified in size and locomotor style, lumbar function also diversified.

Conclusion

Revolute, interlocking zygapophyses have previously been interpreted as an adaptation for efficient long-distance running, restricting dorsoventral flexion (Halpert et al., 1987; Zhou et al., 1992). However, among extant mammals, these zygapophyses are only found in artiodactyls, limiting functional comparisons to extinct mammals with dissimilar ecology (Slijper, 1946). These range of motion tests show that the revolute zygapophsyes found in the lumbar vertebrae of early placental mammals did not restrict dorsoventral flexion and extension of the spine. Instead, this feature stabilizes lumbar vertebrae against shear forces and disarticulation. The capacity for dorsoventral flexion in these creodonts demonstrates that flexion of the spine was a component of mammalian locomotion, even as placentals diversified and increased in size. Stabilizing features likely evolved secondarily in response to increased forces on the spine during locomotion as mammals rapidly increased in body size at the beginning of the Cenozoic (Lyson et al., 2019).

Though the fossil record contains valuable information about the complex evolution of mammalian locomotion, interpreting the function of fossil organisms by comparison with the anatomy of extant organisms is limited. Fossil organisms may have unique ecology and functional adaptations unlike those of any living animal. Functional experiments, like ROM and modifying morphology, can therefore provide deeper insight to how ancient animals moved.

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Bibliography

Ahrens, H. E. 2017. Phylogeny and locomotor ecomorphology of Oxyaenidae and macroevolutionary patterns in North American "Creodonta" (Mammalia, Placentalia). Ph.D. dissertation, John Hopkins University, Baltimore, MD.

Alroy, J. 1999. The fossil record of North American mammals: Evidence for a Paleocene evolutionary radiation. Systematic Biology 48:107–118.

Alvarez, A., M. D. Ercoli, and F. J. Prevosti. 2013. Locomotion in some small to medium-sized mammals: a geometric morphometric analysis of the penultimate lumbar vertebra, pelvis and hindlimbs. Zoology (Jena) 116:356–371.

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Argot, C. 2013. Postcranial analysis of a carnivoran-like archaic ungulate: The case of Arctocyon primaevus (Arctocyonidae, Mammalia) from the late Paleocene of France. Journal of Mammalian Evolution 20:83–114.

Bali, M. S., J. Lang, A. Jaggy, D. Spreng, M. G. Doherr, and F. Forterre. 2009. Comparative study of vertebral fractures and luxations in dogs and cats. Veterinary and Comparative Orthopaedics and Traumatology 22:47–53.

Beck, R. M. D., and C. Baillie. 2018. Improvements in the fossil record may largely resolve current conflicts between morphological and molecular estimates of mammal phylogeny. Proceedings of the Royal Society B: Biological Sciences 285.

Belyaev, R. I., A. N. Kuznetsov, and N. E. Prilepskaya. 2021. How the even-toed ungulate vertebral column works: Comparison of intervertebral mobility in 33 genera. Journal of Anatomy 239:1370–1399.

Belyaev, R. I., A. N. Kuznetsov, and N. E. Prilepskaya. 2022. From dorsomobility to dorsostability: A study of lumbosacral joint range of motion in artiodactyls. Journal of Anatomy joa.13688.

Bertrand, O. C., S. L. Shelley, T. E. Williamson, J. R. Wible, S. G. B. Chester, J. J. Flynn, L. T. Holbrook, T. R. Lyson, J. Meng, I. M. Miller, H. P. Püschel, T. Smith, M. Spaulding, Z. J. Tseng, and S. L. Brusatte. 2022. Brawn before brains in placental mammals after the end-Cretaceous extinction. Science 376:80–85.

Bi, S., X. Zheng, X. Wang, N. E. Cignetti, S. Yang, and J. R. Wible. 2018. An Early Cretaceous eutherian and the placental-marsupial dichotomy. Nature 558:390–395.

Borths, M. R., P. A. Holroyd, and E. R. Seiffert. 2016. Hyainailourine and teratodontine cranial material from the late Eocene of Egypt and the application of parsimony and Bayesian methods to the phylogeny and biogeography of Hyaenodonta (Placentalia, Mammalia). PeerJ 4:e2639–e2639.

Boszczyk, B. M., A. A. Boszczyk, and R. Putz. 2001. Comparative and functional anatomy of the mammalian lumbar spine. The Anatomical Record 264:157–168.

Buchheim, H. P., L. R. Brand, and H. T. Goodwin. 2000. Lacustrine to fluvial floodplain deposition in the Eocene Bridger Formation. Palaeogeography, Palaeoclimatology, Palaeoecology 162:191–209.

Carrier, D. R. 1987. The evolution of locomotor stamina in tetrapods: Circumventing a mechanical constraint. Paleobiology 13:326–341.

Collyer, M. L., and D. C. Adams. 2018. RRPP: An r package for fitting linear models to high-dimensional data using residual randomization. Methods in Ecology and Evolution 9:1772–1779.

Evans, H. E. 1993. Miller's Anatomy of the Dog, 3rd ed. /. (M. E. Miller (ed.)). W.B. Saunders, Philadelphia, PA.

Fedorov, A., R. Beichel, J. Kalpathy-Cramer, J. Finet, J.-C. Fillion-Robin, S. Pujol, C. Bauer, D. Jennings, F. Fennessy, and M. Sonka. 2012. 3D Slicer as an image computing platform for the Quantitative Imaging Network. Magnetic Resonance Imaging 30:1323–1341.

Gál, J. M. 1993. Mammalian spinal biomechanics. I. Static and dynamic mechanical properties of intact intervertebral joints. Journal of Experimental Biology 174:247–280.

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Galis, F., D. R. Carrier, J. van Alphen, S. D. van der Mije, T. J. Van Dooren, J. A. Metz, and C. M. ten Broek. 2014. Fast running restricts evolutionary change of the vertebral column in mammals. Proceedings of the National Academy of Sciences 111:11401–11406.

Gambaryan, P. P. 1974. How Mammals Run: Anatomical Adaptations. Halsted Press, New

York, NY. Translated from Russian by Hilary Hardin.

Gazin, C. L. 1957. A skull of the Bridger middle Eocene creodont, *Patriofelis ulta* Leidy. Smithsonian Miscellaneous Collections 134:1–20.

Gould, F. D. H., and K. D. Rose. 2014. Gnathic and postcranial skeleton of the largest known arctocyonid 'condylarth' Arctocyon mumak (Mammalia, Procreodi) and ecomorphological diversity in Procreodi. Journal of Vertebrate Paleontology 34:1180–1202.

Grasmueck, S., and F. Steffen. 2004. Survival rates and outcomes in cats with thoracic and lumbar spinal cord injuries due to external trauma. Journal of Small Animal Practice 45:284–288.

Grossnickle, D. M., S. M. Smith, and G. P. Wilson. 2019. Untangling the multiple ecological radiations of early mammals. Trends in Ecology & Evolution 34:936–949.

Gunnell, G. F. 1998. Creodonta; pp. 91–109 in C. M. Janis, K. M. Scott, and L. L. Jacobs (eds.), Evolution of Tertiary Mammals of North America. vol. 1. Cambridge University Press, Cambridge, United Kingdom.

Halliday, T. J., P. Upchurch, and A. Goswami. 2017. Resolving the relationships of Paleocene placental mammals. Biological Reviews of the Cambridge Philosophical Society 92:521–550.

Halpert, A. P., F. A. Jenkins, and H. Franks. 1987. Structure and scaling of the lumbar vertebrae in African bovids (Mammalia: Artiodactyla). Journal of Zoology 211:239–258.

Hildebrand, M. 1959. Motions of the running cheetah and horse. Journal of Mammalogy 40:481–495.

Janis, C. M., and P. B. Wilhelm. 1993. Were there mammalian pursuit predators in the tertiary? Dances with wolf avatars. Journal of Mammalian Evolution 1:103–125.

Jenkins, F. A. 1970. Cynodont Postcranial Anatomy and the "Prototherian" Level of Mammalian Organization. Evolution 24:230–252.

Jones, K. E. 2015. Evolutionary allometry of lumbar shape in Felidae and Bovidae. Biological Journal of the Linnean Society 116:721–740.

Jones, K. E. 2016. New insights on equid locomotor evolution from the lumbar region of fossil horses. Proc Biol Sci 283.

Jones, K. E., R. J. Brocklehurst, and S. E. Pierce. 2021. AutoBend: An automated approach for estimating intervertebral joint function from bone-only digital models. Integrative Organismal Biology obab026.

Jones, K. E., S. Gonzalez, K. D. Angielczyk, and S. E. Pierce. 2020. Regionalization of the axial skeleton predates functional adaptation in the forerunners of mammals. Nature Ecology & Evolution 4:470–478.

Author Manuscript

Jurestovsky, D. J., B. C. Jayne, and H. C. Astley. 2020. Experimental modification of morphology reveals the effects of the zygosphene-zygantrum joint on the range of motion of snake vertebrae. Journal of Experimental Biology jeb.216531.

Kawashima, T., R. W. Thorington, P. W. Bohaska, Y.-J. Chen, and F. Sato. 2015. Anatomy of Shoulder Girdle Muscle Modifications and Walking Adaptation in the Scaly Chinese Pangolin (Manis pentadactyla, Pholidota) Compared with the Partially Osteoderm-Clad Armadillos (Dasypodidae). The Anatomical Record 298:1217–1236.

Kort, A. E., H. Ahrens, P. David Polly, and M. Morlo. 2022. Postcrania and paleobiology of *Patriofelis ulta* (Mammalia, Oxyaenodonta) of the Bridgerian (lower-middle Eocene) of North America. Journal of Vertebrate Paleontology e2045491.

Kort, A. E. 2023. The ancestral morphology of lumbar vertebrae and its diversification in early Cenozoic mammals. Journal of Mammalian Evolution. https://doi.org/10.1007/s10914-023-09676-2

Lyson, T., L. M. Miller, A. Bercovici, K. Weissenburger, A. J. Fuentes, W. C. Clyde, H. Hagdorn, M. J. Butrim, K. R. Johnson, R. F. Fleming, R. S. Barclay, S. A. Maccracken, B. Lloyd, G. P. Wilson, D. W. Krause, and S. G. Chester. 2019. Exceptional continental record of biotic recovery after the Cretaceous–Paleogene mass extinction. Science 366:977–983.

Matthew, W. D. 1909. The Carnivora and Insectivora of the Bridger Basin, middle Eocene. Memoirs of the American Museum of National History 9:291–576.

Mellett, J. S. 1977. Paleobiology of North American Hyaenodon (Mammalia, Creodonta). Contributions to Vertebrate Evolution 1:1–134.

Morlo, M. 1999. Niche structure and evolution in creodont (Mammalia) faunas of the European and North American Eocene. Geobios 32:297–305.

Polly, P. D. 1996. The skeleton of Gazinocyon vulpeculus gen. et comb. nov. and the cladistic relationships of Hyaenodontidae (Eutheria, Mammalia). Journal of Vertebrate Paleontology 16:303–319.

Rose, K. D., L. T. Holbrook, K. Kumar, R. S. Rana, H. E. Ahrens, R. H. Dunn, A. Folie, K. E. Jones, and T. Smith. 2019. Anatomy, Relationships, and Paleobiology of Cambaytherium (Mammalia, Perissodactylamorpha, Anthracobunia) from the lower Eocene of western India. Journal of Vertebrate Paleontology 39:1–147.

Schilling, N., and R. Hackert. 2006. Sagittal spine movements of small therian mammals during asymmetrical gaits. J Exp Biol 209:3925–3939.

Schutz, H., and R. P. Guralnick. 2007. Postcranial element shape and function: assessing locomotor mode in extant and extinct mustelid carnivorans. Zoological Journal of the Linnean Society 150:895–914.

Slijper, E. J. 1946. Comparative Biologic-Anatomical Investigations on the Vertebral Column and Spinal Musculature of Mammals. North-Holland Publishing Company, Amsterdam, Netherlands.

Slijper, E. J. 1947. Observations on the Vertebral Column of the Domestic Animals. The Veterinary Journal (1900) 103:376–387.

Author Manuscript

Van Valkenburgh, B. 1985. Locomotor diversity within past and present guilds of large predatory mammals. Paleobiology 11:406–428.

Wood, A. R., R. M. Bebej, C. L. Manz, D. L. Begun, and P. D. Gingerich. 2010. Postcranial functional morphology of Hyracotherium (Equidae, Perissodactyla) and locomotion in the earliest horses. Journal of Mammalian Evolution 18:1–32.

Wortman, J. L. 1894. Osteology of *Patriofelis*, a middle Eocene creodont. Bulletin of the American Museum of Natural History 6:129–164.

Zack, S. P. 2019. A skeleton of a Uintan machaeroidine 'creodont' and the phylogeny of carnivorous eutherian mammals. Journal of Systematic Palaeontology 17:653–689.

Zhou, X., W. J. Sanders, and P. D. Gingerich. 1992. Functional and behavioral implications of vertebral structure in Pachyaena ossifraga (Mammalia, Mesonychia). Contributions from the Museum of Paleontology the University of Michigan 28:289–319.

Figure Captions

Figure 1.) Fossil vertebrae scanned for bending experiments. All scale bars are 1 cm. **A.)** Lateral view of lumbar vertebrae from *Patriofelis ulta* (UMNH-VP 550) with anterior to the right. Likely L2 and L3, though, this was not possible to confirm. The black line shows the approximate location of **B.)** transverse CT slice through the zygapophyses in articulation. Note the revolute, S-shape of the zygapophyses. **C.)** Lateral view of lumbar vertebrae L1 through L3 from *Limnocyon potens* (AMNH FM 13138), with anterior to the right. The black line shows the approximate location of **D.)** transverse CT slice through the zygapophyses in articulation. Note the less pronounced ridge compared to *Patriofelis*. **Abbreviations: pre**, prezygapophysis; **post**, postzygapophysis.

Figure 2.) Final models of posterior vertebrae used for ROM tests show from anterolateral view Red axis is anteroposterior, with light red pointing towards anterior. Prezygapophyses are indicated with black arrows. All scale bars 1 cm. **A.)** L3 from Patriofelis cleaned and retrodeformed to be as symmetrical in articulating elements. **B.)** Edited L3 from *Patriofelis* with prezygapophyses flattened. **C.)** L3 from *Limnocyon* cleaned and retrodeformed to be symmetrical in articulating elements. **D.)** Edited L3 from *Limnocyon* with flattened prezygapophyses.

Figure 3.) Variations on starting pose for ROM trials illustrated with *Limnocyon* vertebrae pair. The neural pose is shown in gray, from a posterolateral angle (posterior is to the left). The deviation from the neutral pose is shown by the blue vertebra. Axes of rotation are dorsoventral (blue), axial (red), and lateral (green). Variations are **A.)** posterior vertebra flexed dorsally, **B.)** posterior vertebra flexed ventrally, **C.)** posterior vertebra flexed laterally, **D.)** posterior vertebrae translated dorsally, **E.)** posterior vertebra translated ventrally, and **F.)** posterior vertebra translated posteriorly.

Figure 4.) Comparison of maximum degrees of rotation for each axis in ROM trials between revolute and digitally flattened zygapophyses. Pairwise comparisons between zygapophysis shape with the same starting position that show significant difference in rotation ($p<0.05$) are highlighted in red. Starting positions for each trial are labelled at the bottom of the chart from left to right: neutral, dorsal flexion (**DF**), ventral flexion (**VF**), lateral flexion (**LF**), dorsal translation (**DT**), ventral translation (**VT**), and

posterior translation (**PT**). Each bar represents the mean value for trials run for that model and starting conditions with error bars showing one standard deviation. *Limnocyon* shows little difference between the two types of zygapophyses. *Patriofelis* shows similar values across most trials, but reduced flexion in the revolute zygapophyses when the posterior vertebra starts with some ventral translation or some posterior translation. Note that in *Patriofelis*, values for both lateral flexion and axial rotation are consistently higher in the flattened zygapophyses compared with the revolute zygapophyses. However, in *Limnocyon*, the unedited zygapophyses show slightly higher degrees of rotation.

Figure 5.) Illustration showing how the interlocking morphology of *Patriofelis* zygapophyses acts to allow dorsoventral flexion. The prezyapophysis of the posterior (**gray**) vertebra is drawn with the middle section cut away, so that the postzygapophysis of the anterior (**red**) vertebra is visible. The red and black dotted line outlines the surface where the prezygapophysis and postzygapophysis slide past each other The posterior (left) vertebra isshown in maximum dorsal flexion (**top**) and maximum ventral flexion (**bottom**) relative to the anterior (right) vertebra, which is stationary. Note how the rotation of the posterior vertebra follows the curve of the postzygapophysis of the anterior vertebra. This "track" allows dorsoventral flexion while restricting movement in other directions.

Figure 6.) Illustration of *Limnocyon* in lateral (A-C) and ventral flexion (D-F). Anterior vertebrae is shown in grey and posterior vertebrae is shown in blue. A.) *Limnocyon* vertebrae shown from a dorsoposterior angle in the neutral position. B.) *Limnocyon* vertebrae with revolute zygapophyses shown in maximum lateral flexion. C.) *Limnocyon* vertebrae with flattened zygapophyses shown in maximum lateral flexion. Note how the flattened face of the prezygapophysis touches the lateral most point of the postzygapophysis. D.) *Limnocyon* vertebrae shown from a dorsal angle in the neutral position. E.) *Limnocyon* vertebrae with revolute zygapophyses shown in ventral flexion. F.) *Limnocyon* vertebrae with flattened zygapophyses shown in ventral flexion.

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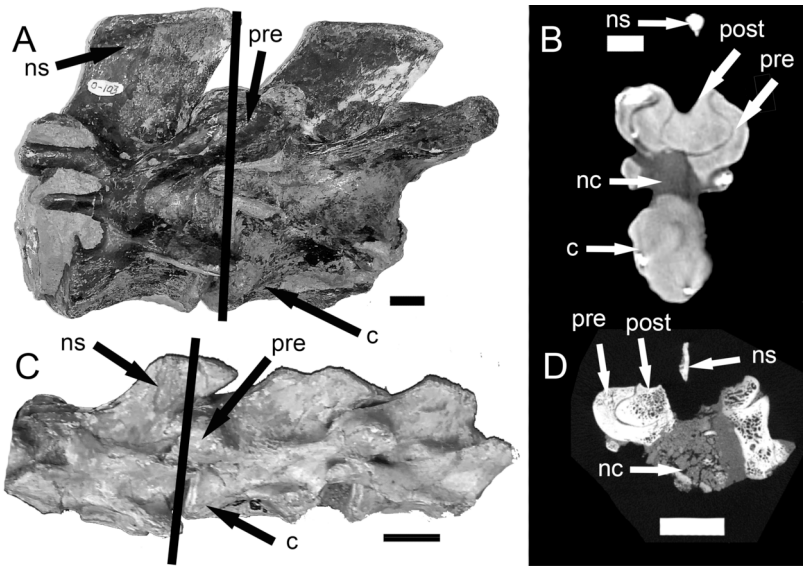


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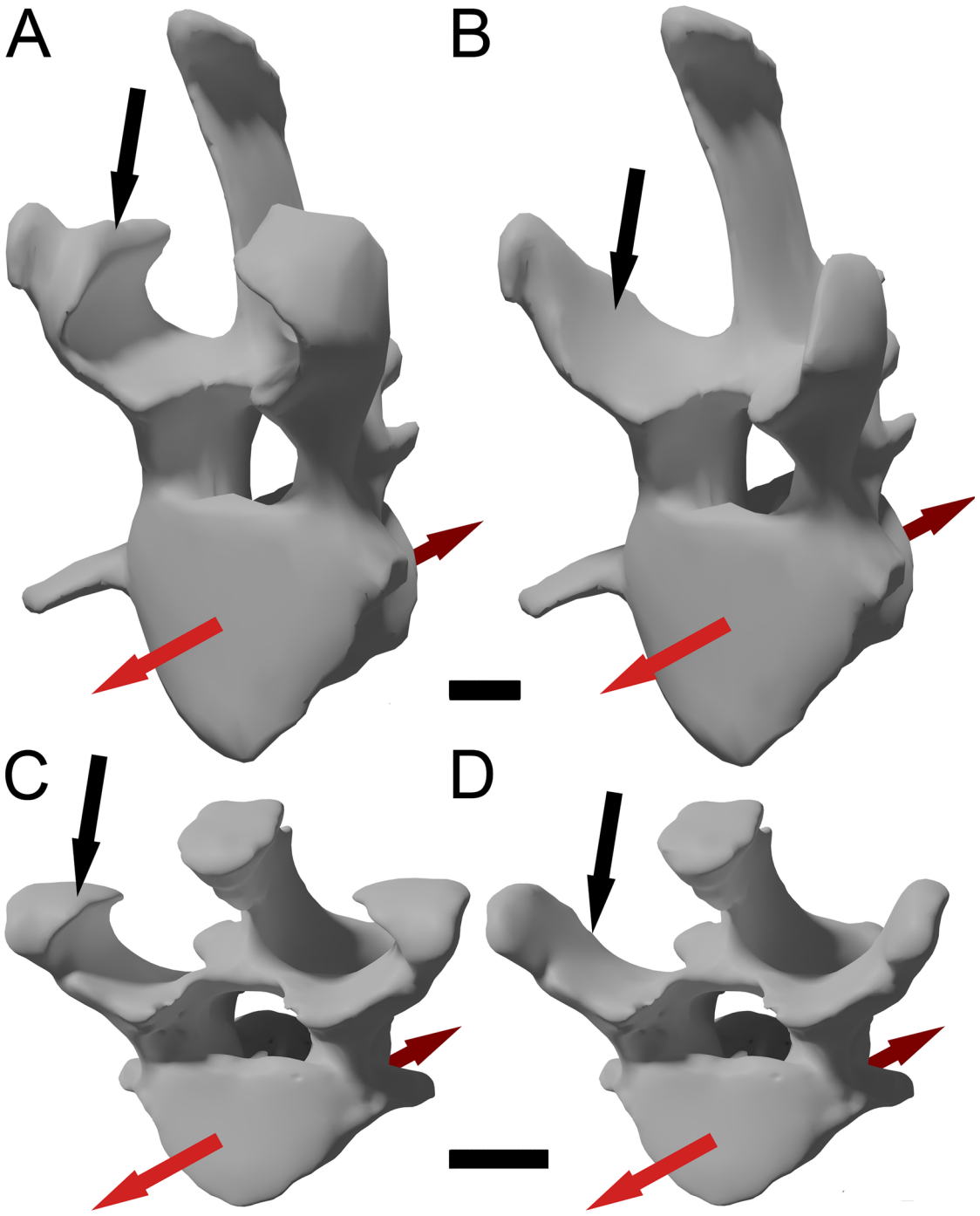


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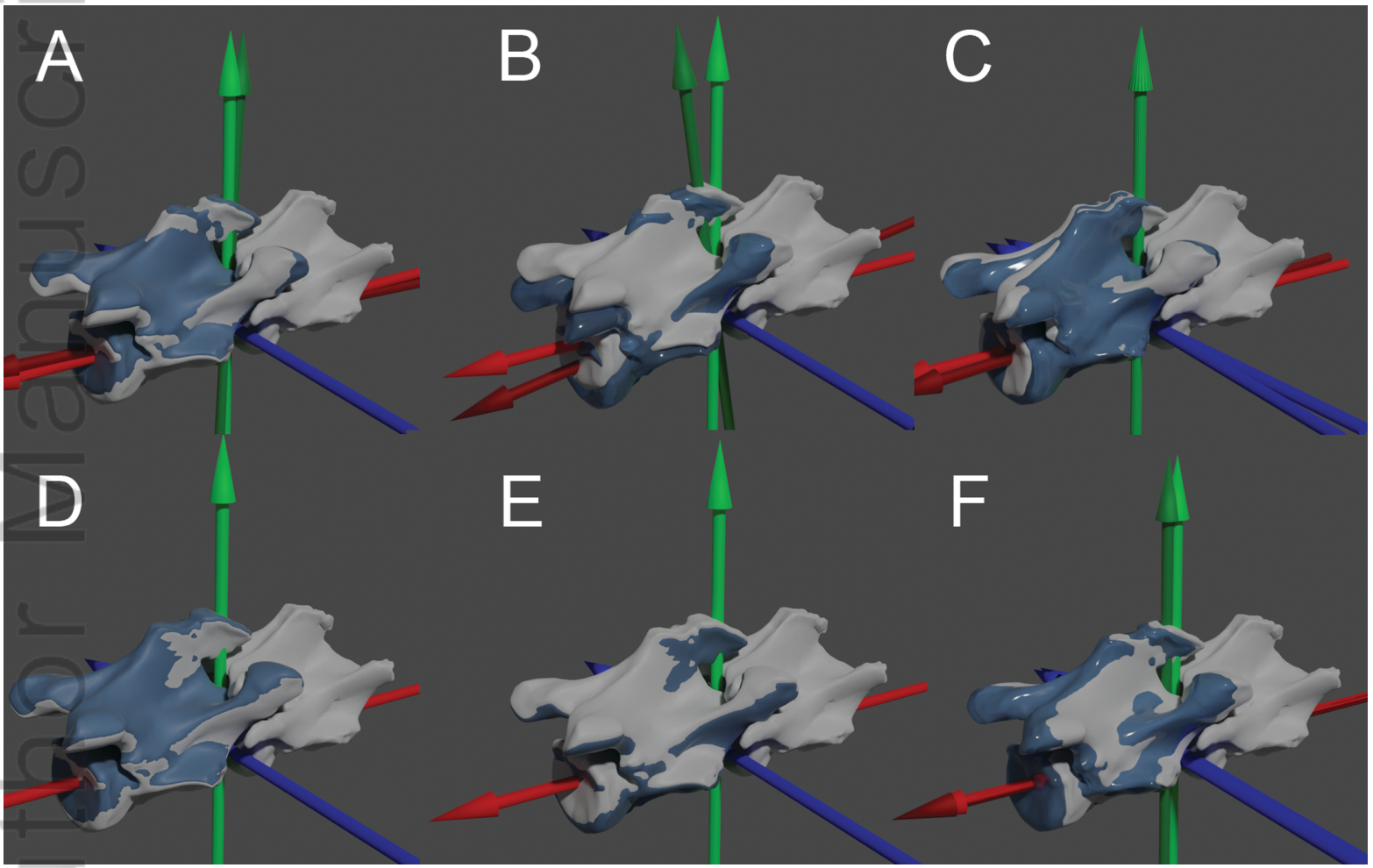


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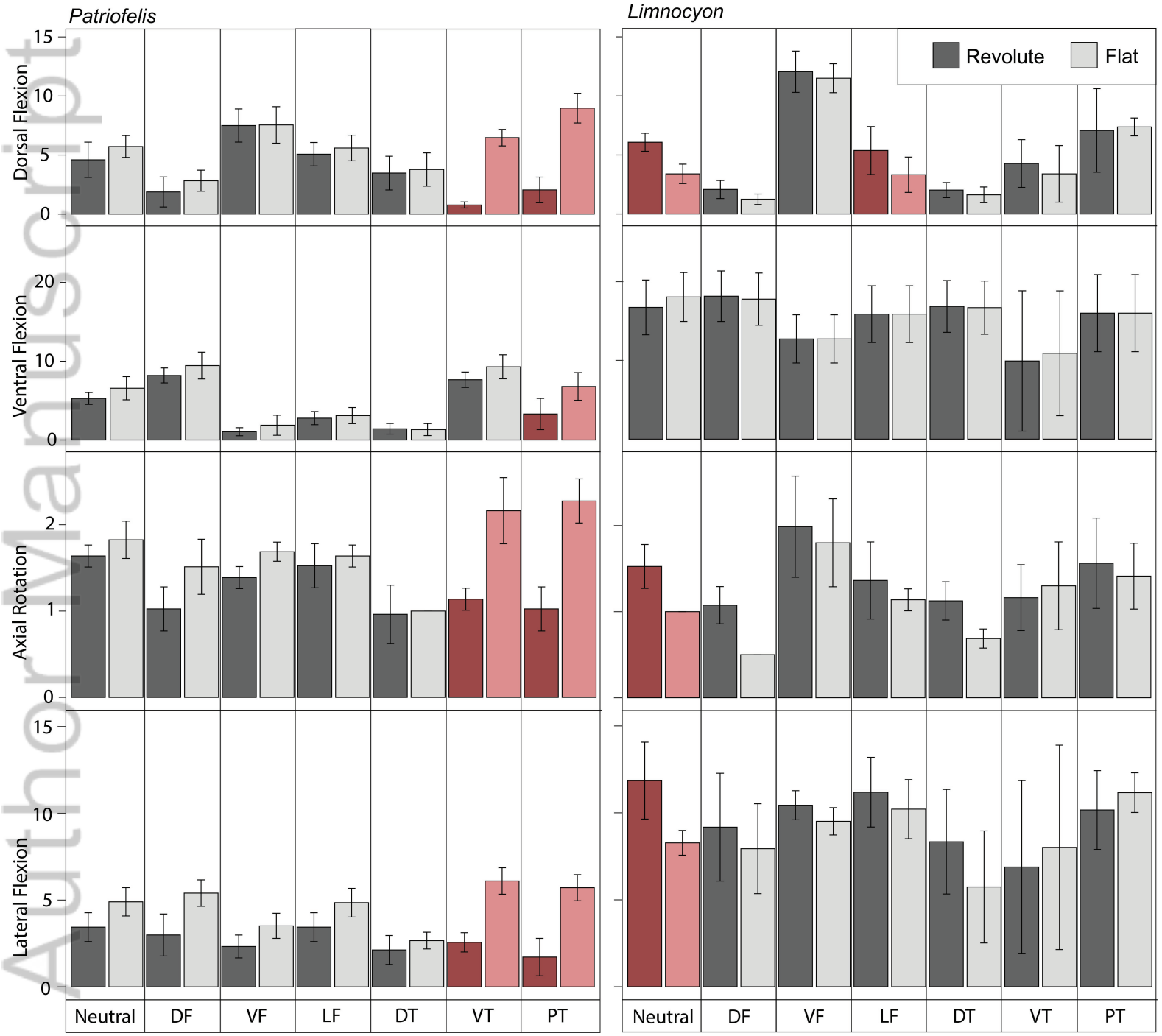


figure4_rangeofmotion.eps

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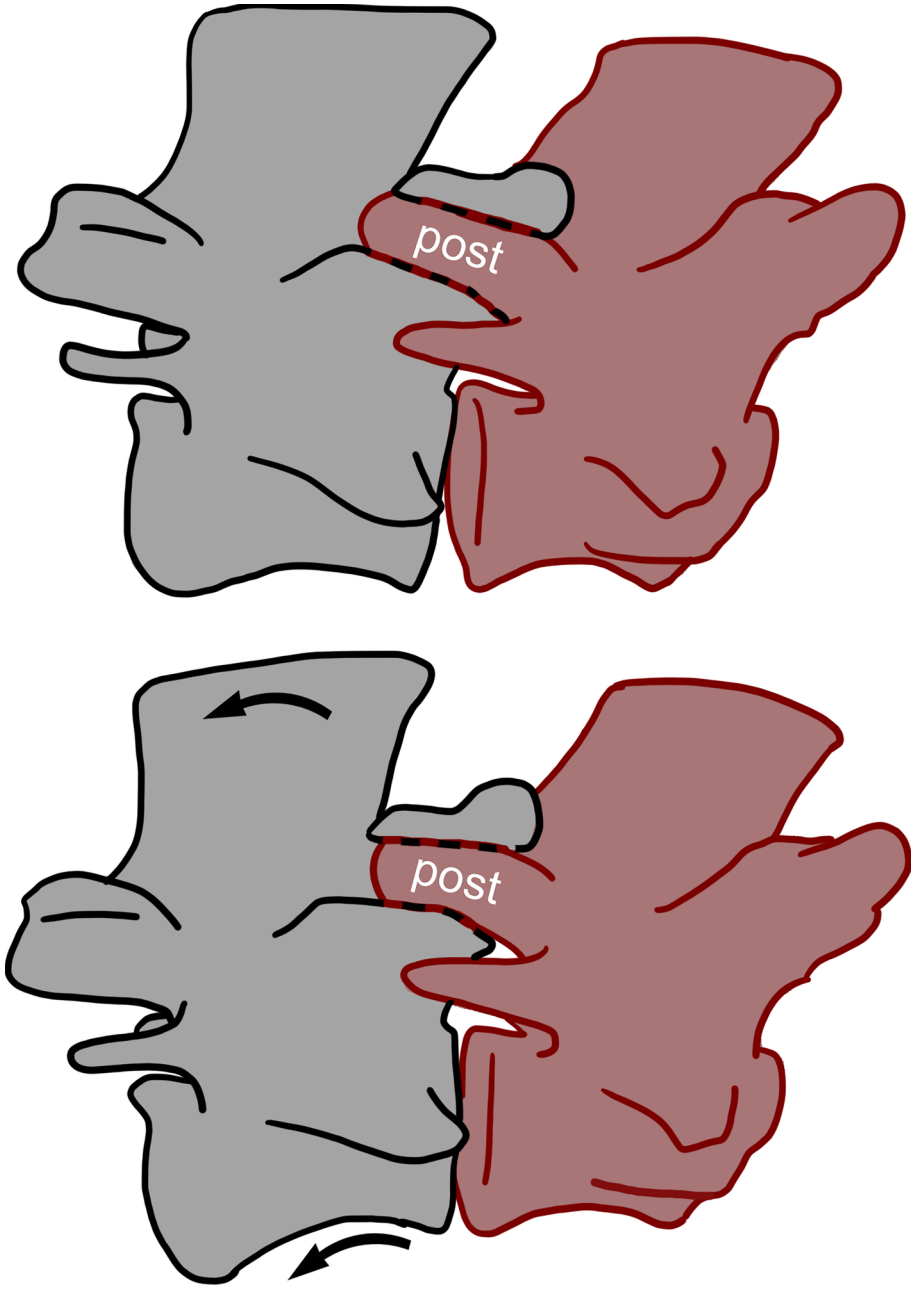


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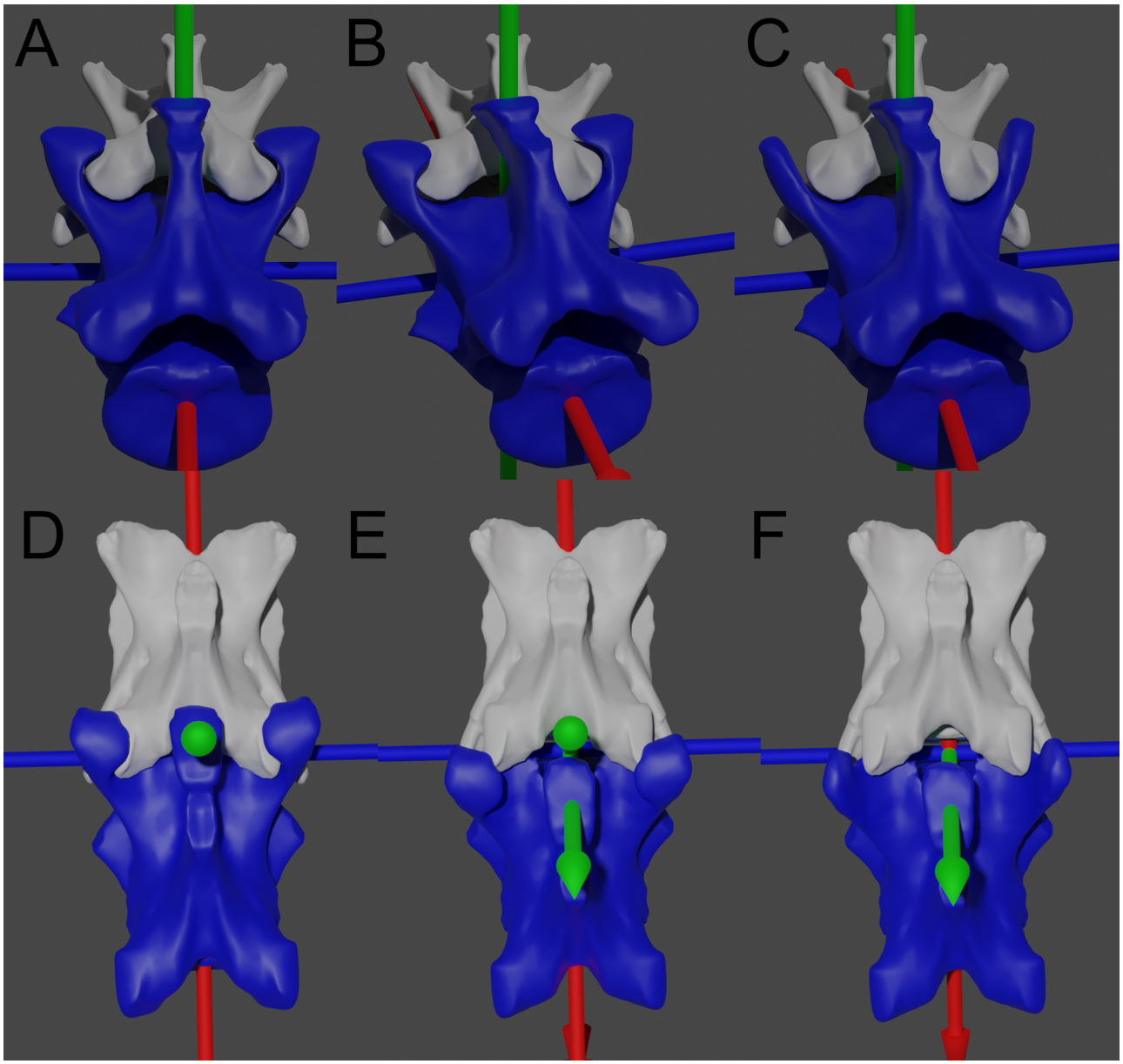


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