Anatomical transformation in mammals: developmental origin of aberrant cervical anatomy in tree sloths

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SUMMARY Mammalian cervical count has been fixed at seven for more than 200 million years. The rare exceptions to this evolutionary constraint have intrigued anatomists since the time of Cuvier, but the developmental processes that generate them are unknown. Here we evaluate competing hypotheses for the evolutionary origin of cervical variants in *Bradypus* and *Choloepus*, tree sloths that have broken the seven cervical vertebrae barrier independently and in opposite directions. Transitional and mediolaterally disjunct anatomy characterizes the cervicothoracic vertebral boundary in each genus, although polarities are reversed. The thoracolumbar, lumbosacral, and sacrocaudal boundaries are also disrupted,

INTRODUCTION

Morphological stasis across geological time spans and diverse environments strongly suggests bias in the generation of variation (Maynard-Smith et al. 1985; Narita and Kuratani 2005; Hendrikse et al. 2007). Rare exceptions to stasis provide insights into possible underlying developmental constraints, as well as to the processes by which they can be circumvented. Among the most famous examples of morphological stasis is the vertebral count of the mammalian neck, which is fixed at seven despite variable counts in other regions of the vertebral column and in other vertebrate classes (Galis 1999; Narita and Kuratani 2005; Galis et al. 2006). The cervical constant has been in place for at least 200 million years (Jenkins 1971; Crompton and Jenkins 1973), and its origin and rare exceptions have intrigued anatomists since its first description by Buffon in 1769. Exceptions to the cervical constant include the genera Bradypus (three-toed sloths), Choloepus (two-toed sloths), and Trichechus (manatees).

Tree sloths are members of the Order Xenarthra, which recent analyses (e.g., Kriegs et al. 2006; Bininda-Emonds et al. 2007) assign a basal position within Eutheria. Xenarthrans are distinguished by the presence of accessory xenarthral vertebral articulations, reduced dentition, and the fusion of posterior sacral vertebrae to the ischium (Engelmann 1985; Gaudin and are more extreme in individuals with more extreme cervical counts. Hypotheses of homologous, homeotic, meristic, or associational transformations of traditional vertebral column anatomy are not supported by these data. We identify global homeotic repatterning of abaxial relative to primaxial mesodermal derivatives as the origin of the anomalous cervical counts of tree sloths. This interpretation emphasizes the strong resistance of the "rule of seven" to evolutionary change, as morphological stasis has been maintained primaxially coincident with the generation of a functionally longer (*Bradypus*) or shorter (*Choloepus*) neck.

2003). They are also unusual among mammals in their retention of ossified sternal ribs (Gaudin 2003). Phylogenetic analyses indicate that Bradypus and Choloepus are only distantly related, and are separately nested within clades with both terrestrial habits and traditional cervical counts (Höss et al. 1996; White and MacPhee 2001; Delsuc et al. 2002; Gaudin 2003). They thus evolved both their unusual suspensory lifestyles and their aberrant cervical anatomies independently, possibly in response to increased selection pressures from North American predators following the "Great Biotic Fauna Interchange" (Webb 1991). The two genera display extremes in a range of possible evolutionary "solutions" to mechanical support of the head while inverted. The elongate neck of Bradypus possesses 8-10 cervical vertebrae. It is weakly muscled and the clavicle is reduced. The neck's great mobility allows the head to rotate 270° and to rest on the chest (Miller 1935). In contrast, the short neck of Choloepus possesses 5-8 cervical vertebrae. It is thickly muscled, with a robust clavicle. It has limited flexibility, and is generally held in an extended position (Miller 1935).

The structure of the mammalian vertebral column is highly conserved across all mammalian orders. It is composed of serially homologous units (the vertebrae) that are subdivided into five distinctive series (cervical, thoracic, lumbar, sacral, caudal) by morphological discontinuities (Narita and Kuratani

70 EVOLUTION & DEVELOPMENT Vol. 11, No. 1, January-February 2009

2005). Series boundaries are coincident with the location of the appendages: the forelimb occurs at the cervicothoracic transition and the hind limb occurs at the lumbosacral transition (Winslow et al. 2007). Because the embryonic limb buds recruit the nerves that will supply them from the spinal cord during development (Detweiler 1919; Hamburger 1934), the brachial and lumbosacral plexuses also mark the cervicothoracic and lumbosacral transitions, respectively. Experimental relocation of the limb bud is accompanied by relocation of the plexus (Cohn et al. 1997). A characteristic brachial plexus is also found at the osteologically defined relocated cervicothoracic boundary of each genus with an anomalous cervical count, ruling out the possibility of independent osteological and neurological definitions of the term "cervical" (Giffin and Gillett 1996).

Serially homologous structures display characteristic patterns of variation, first studied systematically by Bateson (1894). The repeated occurrence of distinctive variants is now recognized as the morphological consequence of the modular organization of development (Lovejoy et al. 1999; Bolker 2002; Carroll et al. 2005). Particular alterations in developmental processes can often be linked tentatively to these homologous, homeotic, meristic, and associational categories (Raff 1996; Polly et al. 2001; Carroll et al. 2005; Buchholtz 2007).

Homologous or *diversifying* variations alter the size and/or shape of particular column subunits that retain traditional count and identity. A variety of *Hox* genes and/or growth factors may contribute to these changes (Johnson and O'Higgins 1996; McPherron et al. 1999; Oostra et al. 2005), probably acting after vertebral count and series boundaries are set during development.

Axial *homeotic* variations alter count in one column series at the expense of an adjacent series, but total column count remains fixed. Homeotic changes are the result of changes in the expression domains of *Hox* and other patterning genes that specify the identity of different axial regions (Burke et al. 1995). They can be induced experimentally by application of retinoic acid (Kessel 1992), by changes in the temperature of development (Li et al. 1997; Li and Shiota 1999), and by the overexpression, dosage alteration, or knockout of patterning genes (e.g., Horan et al. 1994; Krumlauf 1994).

Meristic variations alter total count. They reflect the subdivision of the presomitic mesoderm into a different number of units during somitogenesis, probably caused by changing the rate of the molecular oscillators that control somite (and vertebral) number (Hirsinger et al. 2000; Pourquié 2003; Sanger and Gibson-Brown 2004).

Associational variations alter the pattern of the developmental hierarchy at any level, constraining or freeing the development of one column series with respect to another. Examples include the addition, loss, or novel association of series. Their cause is unknown (Raff 1996; Lovejoy et al. 1999; Polly et al. 2001; Buchholtz 2007).

Enhanced knowledge of the developmental processes that underlie axial patterning invites the reexamination of the enigma of cervical stasis. In a series of innovative papers, Galis and her coworkers (e.g., Galis 1999; Galis et al. 2006) suggest that cervical count is held at seven by the pleiotropic linkage of Hox (and possibly other) axial patterning genes with genes that cause major developmental abnormalities and/or susceptibility to juvenile cancers. They support their hypothesis with the demonstration (Galis et al. 2006) that human individuals with incomplete proximal ribs on the seventh cervical vertebra are represented in unusually high numbers among stillborns in a large data set. Their hypothesis of axial mispatterning is supported by recent documentation that proximal ribs are patterned with the axial skeleton (Huang et al. 2000; Aoyama et al. 2005; Durland et al. 2008). Galis (1999) further hypothesizes that the low metabolic rates characteristic of all three aberrant genera limit the occurrence of the linked developmental abnormalities (Galis 1999), permitting escape from the cervical constant. In stark contrast to the cited human examples, however, the aberrant genera display the addition or deletion of one or even multiple complete ribs with sternal connections. It seems unlikely that they are the products of the same disruptive process as that described by Galis.

Here we examine vertebral morphology in *Bradypus* and *Choloepus* with the goal of identifying the developmental processes responsible for their unusual cervical traits. Extensive anatomical variation in the axial skeleton of sloths (de Comte Buffon 1769; Bell 1835; Bateson 1894) makes them ideal case studies for an analysis of developmental constraints and anatomical transformation.

MATERIALS AND METHODS

Postcranial skeletons of two of the four extant species (Anderson and Handley 2001) of the genus Bradypus (B. variegatus and B. tridactylus) and both extant species of the genus Choloepus (C. didactvlus, C. hoffmanni) were available in sufficient numbers to allow analysis. Species level identifications of institutional specimens were corroborated with cranial characters (Wetzel 1985) except in the rare cases where skulls were not available. Individual vertebrae were assigned to series based on classic criteria: The cervical (C) series consists of the anterior atlas and axis and the (traditionally five) successive vertebrae bearing reduced, synostosed cervical ribs. Transverse foramina, ventrally oriented transverse processes, and vertical neural spines are also typical of cervical vertebrae. The presence of at least one full, moveable rib that articulates directly with the sternum was used as the indicator of the first thoracic (T) vertebra following the traditional definition of Turner (1847) and Flower (1884). The ribs of posterior thoracic vertebrae either articulate indirectly with the sternum via more anterior ribs or lack sternal connections entirely. Thoracics also bear posteriorly oriented neural spines, horizontal transverse

processes, and intervertebral articular surfaces (zygopophyses) with predominantly horizontal orientations. Lumbar (L) vertebrae lack moveable ribs, but classically exhibit robust fixed transverse processes, zygopophyses with sagittal orientations, and anteriorly oriented neural spines. The presence of multiple anatomical transitions makes the thoracolumbar transition "complex" instead of "discrete" (Filler 1986, 2007) and therefore ambiguous when markers change at different axial locations. In many mammalian orders, lumbar transverse processes and ribs are homologous, but in sloths they have separate origins (Filler 1986, 2007). As a result, both ribs and lumbar transverse processes may be present on the same vertebra. Thoracolumbar vertebrae with at least one (often very small) fused rib in addition to lumbar transverse process(es) were coded as transitional (TL). The first vertebra with transverse processes fused to the ilium is the first sacral (S) vertebra. Sacral centra and transverse processes are typically synostosed; the intervertebral zygopophyseal articulations found in the rest of the column are absent. The first postsacral vertebra lacking ischial and syncentral fusion is the first caudal (Cd) vertebra. Vertebrae with only ischial or only central fusion were coded as transitional sacrocaudals (SCd). Vertebral numbers (V1, V2, etc.) were used to identify axial positions.

We documented vertebral anatomy in a total of 113 specimens, of which 101 were complete enough for statistical analysis (Table 1 and Supporting Table S1). Traits observed included series counts, centrum lengths, rib count and sternal articulation pattern, neural spine shape and orientation, transverse foramen presence, thoracolumbar changes in zygopophysis orientation, lumbar transverse process differentiation, and the pattern of sacral central and transverse process synostosis. Cervical counts were tested for correlation with thoracic, lumbar, sacral, and presacral counts using leastsquares linear regressions and an α level of 0.05 (Table 1). The diminutive size and frequent absence of terminal caudal vertebrae in museum specimens prohibited analysis of caudal and total counts.

RESULTS

Anomalous vertebral traits common to both genera

Vertebral anatomy in tree sloths varies between genera and from individual to individual; virtually no two specimens show identical patterns. Nevertheless, multiple generalizations can be made that apply to all four species of the two genera studied: Cervical counts are highly variable in each species with the exception of *C. didactylus*, of which a single individual with a nontraditional count was observed (Table 1 and Supporting Table S1). Vertebrae with unconventional anatomy (cervical vertebrae at axial positions 8–10 in *Bradypus*, thoracic vertebrae at axial positions 6–7 in *C. hoffmanni*) have transitional instead of discrete identities, displaying components of both cervical and thoracic series. Rib homologs on transitional vertebrae vary widely with respect to length, shape, articulation with the vertebral column, and sternal contact. Left/right asymmetry is common. Most unexpectedly

Table 1. Cervical counts in Bradypus and Choloepus

Species	п	Count mean, SD	Significance of cervical count regression			
			C:T	C:L	C:S	C:preS
B. tridactylus	15	9.2, 0.4	NS	NS	NS	NS
B. variegatus	25	8.8, 0.6	NS	NS	NS	P<0.0001
C. didactylus	14	7.0, 0.4	NS	NS	NS	NS
C. hoffmanni	49	5.9, 0.4	NS	NS	NS	P < 0.005

Least-squares linear regressions show no relationship between cervical (C) count and thoracic (T), lumbar (L) or sacral (S) counts (α level of 0.5) in any species, but a positive relationship between cervical count and presacral (PreS) count in *B. variegatus* and *C. hoffmanni*. Equations for the significant C: PreS regressions were y = 1.10x+17.61, $R^2 = 0.58$ in *B. variegatus* and y = 0.88x+26.45, $R^2 = 0.21$ in *C. hoffmanni*.

and revealingly, cervical disruptions occur coincident with abnormalities at the thoracolumbar, lumbosacral, and sacrocaudal boundaries. Disruptions are most extreme at the cervicothoracic and lumbosacral boundaries and in individuals with greater deviations from traditional cervical count. Midseries (nonborder) vertebrae display normal anatomy. Series counts (Supporting Table S1) are based on formal series definitions (above), so they do not reflect the full range of observed variation, described and illustrated below. Cervical counts are not correlated statistically with thoracic, lumbar, or sacral count in any of the four species (Table 1), but are positively correlated with presacral count in *C. hoffmanni* (P<0.005) and *B. variegatus* (P<0.001), and nearly so in *B. tridactylus* (P = 0.062).

Bradypus vertebral anatomy

Cervical count in Bradypus varies from eight to 10 (Fig. 1, A-C). The most frequent cervical count in both *B. tridactylus* and B. variegatus in this database is nine. More than 90% of all individuals display mediolaterally discordant anatomy at one or more vertebral series transitions. Most characteristic of these are miniature riblets (Fig. 1, A-E), often truncated at or near the rib neck, on the terminal cervical vertebrae of three quarters of the specimens. These riblets articulate with (Fig. 1, B-D left, E left) or occasionally fuse to (Fig. 1, A and D right, E right) the vertebra's transverse processes, rarely enclosing transverse foramina (Fig. 1E right). Both dorsal vertebral and ventral sternal ossified ribs are clearly present on all of the vertebrae in the short Bradypus thorax (Fig. 1, F and G), although the sternal component is exceptionally short on T1 (Fig. 1D, vertebra 10). Transitional thoracolumbar vertebrae (Fig. 1, H and I) exhibit small lumbar transverse processes in addition to very short fused or articulating ribs, often with left/right asymmetry. The vertebral versus sternal identity of these thoracolumbar ribs is uncertain. The centrum of the first



Fig. 1. Key features of vertebral anatomy in Bradypus. (A-C) Cervical series (dorsal view) of individuals with eight (A, B. variegatus AMNH 209940), nine (B, B. variegatus FMNH 69587) and 10 (C, B. tridactylus AMNH 74137) cervical vertebrae. Terminal cervicals bear horizontal transverse processes that articulate with or fuse to miniature riblets (white arrows). (D) Anterior views of vertebrae 7, 8, 9, and 10 in a C9 individual (B. variegatus FMNH 69588). V7 is a typical cervical with synostosed cervical ribs and transverse foramina. V8 and V9 have horizontal transverse processes, and V9 bears a fused right riblet and an articulating left riblet. V10 bears full ribs that articulate with the sternum; its sternal rib component (red arrow) is extremely short. (E) Anterior view of the cervicothoracic transition in a C9 individual (B. variegatus FMNH 60164). Ribs truncated at the rib neck are present on V9. The right rib is fused enclosing a small transverse foramen, but the left rib is moveable. The ribs of V10 are missing in this individual. Articular surfaces for sternal ribs are visible on the terminal ends of vertebral ribs V11 and V12. (F) Mounted skeleton of a C8 individual (B. variegatus YPM 11647) showing the long neck and short thorax of Bradypus. (G) Posterior view of the thorax of a C9 individual (B. variegatus USNM 281352) showing the clear distinction between dorsal vertebral and ventral sternal ribs (red dots). (H) Dorsal view of the terminal thoracic (T14-15 = V22-23) and lumbar (L1-3 = V24-26) vertebrae of a C8 individual (B. variegatus USNM 13054). Vertebra 23 has short articulating ribs and small

lumbar transverse processes. (I) Dorsal view of the two terminal thoracic (T14–15 = V24–25), a thoracolumbar (V26), and two lumbar (L1–2 = V27-28) vertebrae of a C10 individual (*B. variegatus* USNM 241363). Vertebra 26 has short fused ribs and incipient lumbar transverse processes. (J–L) Dorsal views of the lumbosacral and sacrocaudal transitions in *B. variegatus* individuals with eight (J, USNM 13054), nine (K, USNM 281352), and 10 (L, USNM 241363) cervical vertebrae. In the C8 individual, S1 extends anterior to the ilia. In the C9 and C10 individuals, terminal lumbar vertebrae fuse to the sacrals via their posteriorly oriented transverse processes dorsally and via their centra ventrally. In the C10 individual, small ossifications isolate these vertebrae from direct contact with the ilium (blue arrows). Terminal sacrocaudal transitional vertebrae (purple arrows) are marginally and variably incorporated into the sacrum. They possess zygopophyseal articulations, which are typically suppressed in the sacrum. Numbers indicate the axial position of each vertebra. Institutional abbreviations: AMNH, American Museum of Natural History, New York, NY; FMNH, Field Museum of Natural History, Chicago, IL; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, MA; USNM, National Museum of Natural History, Washington, DC; YPM, Peabody Museum of Natural History, Yale University, New Haven, CT.

sacral vertebra (S1) typically extends anterior to the ilial wings in individuals with 8 cervical vertebrae (Fig. 1J). The fusion of the transverse processes of this vertebra to the ilium is often irregular and separated dorsally from S2 by gaps. The last sacral vertebra articulates with the ischium; it typically lacks synostosis with either the transverse processes or the centrum of the penultimate sacral, articulating with it instead via zygopophyses, anomalous in the sacrum. This unusual anatomical suite is exaggerated in specimens with nine (Fig. 1K) or 10 (Fig. 1L) cervical vertebrae, where one or more lumbosacral transitional vertebrae lying anterior to the ilium are incorporated into the anterior sacrum via transverse process fusion. Small ossifications may isolate these vertebrae from direct contact with the ilium. Posteriorly, at least one and



Fig. 2. Key features of vertebral anatomy in Choloepus hoffmanni. (A-C) Individuals with six cervical vertebrae (A, FMNH 165374, lateral view; B, FMNH 60108, dorsal view; C, FMNH 137420, anterior view) usually bear irregular fused riblets (white arrows) on V6 and a moveable rib that articulates with typical rib facets on V7. (D-F) Individuals with five cervical vertebrae (D, FMNH 127420, lateral view; E, FMNH 121521, dorsal view; and F, FMNH 121521, anterior view) bear a complete, articulating rib on both V6 and V7. These additional ribs are typically flat and irregular in shape (A, D, G, I). (G) Ventral view of the rib cage in a C6 individual (FMNH 15621). With the exception of V7 and V8, anterior ribs have separate vertebral and sternal components (small red arrows indicate suture lines). (H) The mounted skeleton of a C6 individual (FMNH 15621) showing the short neck and long thorax typical of Choloepus. (I) Ventral view of the anterior rib cage of a C5 individual (FMNH 156655). The C6 rib articulates with the sternum on the left (large red arrow) but not on the right. V8 bears a sternal rib (small red arrow), but the presence of a V7 sternal component is ambiguous. (J) Dorsal view of posterior thoracic to anterior sacral vertebra in a C5 individual (FMNH 186917). The green arrow marks the asymmetrical, short, fused ribs of the transitional thoracolumbar vertebra. (K-M) Lumbosacral anatomy in individuals with different cervical counts. The anterior sacral profile is concave in the only C7 individual (K, YPM 6781), and more deeply inset in most C6 individuals (L, FMNH 137420). Multiple anterior sacral

vertebrae are disrupted in individuals with five cervical vertebrae (M, FMNH 60455). Blue arrows mark the slip of bone that isolates vertebra 32 from the ilium. A single terminal sacral vertebra fuses to the ischium in the only C7 individual (K, purple arrow); two terminal sacrals fuse in most C6 and C5 individuals (L, M). Numbers indicate the axial position of each vertebra.

sometimes two terminal sacral vertebrae bear zygopophyses and articulate with the ischium.

Choloepus vertebral anatomy

In this data set, almost all *C. didactylus* specimens, but only a single *C. hoffinanni* specimen, possess seven cervical vertebrae. In contrast, the great majority of the *C. hoffinanni* individuals (42, 86%) have six cervical vertebrae, and a few (six, 12%) have only five. In C6 individuals (Fig. 2, A–C), incomplete, fused riblets are typical on V6 in front of the anomalous full rib on V7. In C5 individuals (Fig. 2, D–F), C5 displays typical cervical anatomy, but the V6 rib is moveable and reaches the

sternum on at least one side, although fusion to the sternum is often asymmetrical (Fig. 1I). Complete ribs on V6 or V7 are typically flattened, short, and irregularly shaped (Fig. 2, A, D, G, and I). The anterior 10–11 ribs of the elongate *C. hoffimanni* thorax (Fig. 2, G and H) articulate directly with the sternum, and another four or five articulate indirectly via contacts with anterior ribs. Almost all of these have distinct vertebral and sternal components; V6 and V7 lack sternal ribs, while a very small V8 sternal rib is visible on some specimens (Fig. 2I) but not others (Fig. 2G). Costal cartilages connect the posterior ribs (Fig. 2H). At the thoracolumbar boundary, the terminal thoracic vertebra often shows enlarged lumbar transverse processes as well as ribs. In C6 individuals, these ribs are usually of normal length and articulate freely. Short and/or asymmetrical ribs are typical of C5 individuals (Fig. 2J), and these often fuse instead of freely articulate. The anterior margin of the sacrum is modestly inset in the individual with seven cervical vertebrae (Fig. 2K), but is incised around terminal lumbars or incompletely fused to lumbosacral transitional vertebrae when only six cervical vertebrae are present (Fig. 2, I and L). Sacral disruption extends still further into the sacrum in the rare C. hoffmanni individuals with only five cervicals (Fig. 2M), where up to three lumbosacral transitional vertebrae are incompletely fused to the ilium. The transverse processes of the first lumbosacral may be isolated from the ilium by a slip of the transverse processes of the second (Fig. 2M). Lumbosacrals typically articulate with axially adjacent vertebrae via zygopophyses, traditionally absent from the sacrum. A single sacral vertebra contacts the ischium of the pelvis in the single C7 example. A transitional sacrocaudal fuses to it asymmetrically by its transverse processes (Fig. 2K). C6 and C5 individuals typically have two sacrals with ischial contacts as well as one or more sacrocaudal transitional vertebrae.

DISCUSSION

The highly variable vertebral anatomy of tree sloths can be summarized by three critical traits: (1) deviation from traditional cervical count that is independent of thoracic, lumbar, and sacral, but not presacral count, (2) vertebrae with disrupted anatomy at all series borders but not within series, and (3) the transitional, mediolateral pattern of vertebral disruption. The anatomy of each genus was compared with the four patterns of variation typical of serially homologous structures, bearing in mind the possibility that different developmental mechanisms could have been active in the two genera. Extensive intraspecific variation and the large size of the database greatly facilitated comparisons.

Common mechanisms of vertebral column transformation must be rejected

The vertebral anatomy of *Bradypus* and *Choloepus* is clearly inconsistent with homologous and associational categories of variation. Homologous variants are ubiquitous across Mammalia, but are not associated with changes in series counts, the primary character trait observed in sloths. Associational variations result in the addition, loss, or novel association of entire vertebral series. Like almost all mammals, tree sloths retain the ancestral five series of the vertebral column.

Axial homeotic repatterning is the default hypothesis for changes in vertebral series boundaries, and displacement of the cervicothoracic border is the hypothesis suggested by Galis (1999) and Galis et al. (2006). It must be rejected here for sloths because the predicted negative correlation between cervical and thoracic counts was not observed in either genus (Table 1). Additionally, axial homeotic repatterning is typically local, and does not explain either the occurrence of transitional vertebrae at multiple series boundaries or its mediolateral character.

Developmental changes in somitogenesis with resulting meristic variation are consistent with the observed correlation of cervical and presacral vertebral counts in *B. variegatus* and *C. hoffmanni*. In both species, however, changes in the number of cervical vertebrae account for almost all change in presacral counts (slopes of regressions are close to 1.0). This suggests that any meristic addition has been limited to the cervical series, and therefore cannot explain the occurrence of transitional vertebrae at other series boundaries. Additionally, meristic variations predict anteroposterior instead of the observed mediolateral discordances (Oostra et al. 2005). Cervical poly- or oligosegmentation might further be expected to generate defects in the atlas or serially homologous occiput as it does in human meristic variants (Oostra et al. 2005). These defects were not observed.

Tree sloth anatomy supports a hypothesis of a frame shift in primaxial/abaxial patterning

Rejection of the commonly recognized patterns of serial structure transformation requires the proposal of an alternate hypothesis as the source of the anatomical suites observed in *Bradypus* and *Choloepus*. We propose that an additional category of variation may occur in serially homologous structures constructed from subunits with different mesodermal patterning histories: a frame shift in the patterning of primaxial relative to abaxial structures. While novel, this hypothesis is consistent with the observed anatomy in each genus and draws additional support from recent experimental work that addresses mesodermal patterning.

The musculoskeletal system of vertebrates develops from both somitic and lateral plate mesoderm precursors. The vertebrae and ribs are of somitic origin, while the sternum, limbs, and limb girdles are of lateral plate origin (Kato and Aoyama 1998; Huang et al. 2000). Sternal ribs, appendicular muscles and ventrolateral body wall muscles have a somitic origin, but their precursor cells subsequently migrate into lateral plate tissue, differentiating there within a lateral plate connective tissue environment (Burke and Nowicki 2003; Wellik 2007; Durland et al. 2008). The somitic and lateral plate connective tissue environments, now identified as primaxial and abaxial domains, are separated by a "lateral somitic frontier" (Burke and Nowicki 2003). Although many of the same Hox gene paralog groups are involved in patterning elements in the two domains (McIntyre et al. 2007), recent experimental data (McIntyre et al. 2007; Wellik 2007; Durland et al. 2008) indicate that their expression patterns and functions are

independent across the frontier. Further, patterning in the primaxial domain is colinear, while patterning in the abaxial domain is not (McIntyre et al. 2007). Primaxial/abaxial developmental independence is also supported by the results of limb induction experiments. Ectopic induction of limbs in chicks is followed by reprogrammed *Hox* gene expression domains in the lateral plate but not in the somitic mesoderm (Cohn et al. 1997).

The boundary separating the primaxial and abaxial domains differs in the two experimental animals (chick, mouse) in which it has been mapped (Nowicki et al. 2003; Durland et al. 2008), possibly reflecting anatomical differences in the taxa. Chicks have ossified sternal, as well as vertebral, ribs. The dorsally lying vertebral ribs are patterned primaxially, while the ventrally lying sternal ribs are patterned abaxially. The dual patterning of chick ribs is supported by the results of somite transplant experiments: somites transplanted from thoracic to cervical levels form primaxial vertebral ribs but not abaxial sternal ribs (Kieny et al. 1972; Nowicki et al. 2003). Mice, as most mammals, lack ossified sternal ribs. With the exception of the first rib, mouse vertebral ribs as well as their cartilaginous ventral extensions exhibit primaxial patterning (Wellik 2007; Durland et al. 2008). Sloths are rare among mammals in the presence of ossified sternal rib components (Gaudin 2003), but the position of the lateral somitic frontier in sloths is unknown.

Experimental evidence for the existence of two independently patterned mesodermal fields is critical to the analysis of the cervical constant, as the component parts of the vertebral body/vertebral rib/sternal rib/sternum unit used to define the cervicothoracic boundary have different patterning histories. Similarly, the lumbosacral and sacrocaudal boundaries both involve the primaxial vertebral column and the abaxial pelvis. The thoracolumbar boundary is more ambiguous, as it may include only primaxial (vertebrae, vertebral ribs) or both primaxial and abaxial (vertebrae, vertebral ribs, sternal ribs) components depending on the taxon.

The hypothesis of a frame shift in the patterning of primaxial versus abaxial structures as the developmental origin of aberrant tree sloth anatomy is consistent with each of the three categories of anatomical disruption observed if it is assumed, as we do below, that xenarthran ossified sternal ribs are abaxially patterned.

(1) Deviation from traditional cervical count is independent of thoracic, but not presacral count. Displacement of the abaxial sternal ribs and sternum relative to the primaxial vertebral ribs predicts a change in cervical count, because anterior-most sternal fusion is used as the criterion for the identification of T1. Coordinated global displacement of abaxial structures should offset the boundaries of more posterior series without a change in count, as reciprocal changes occur at the anterior and posterior ends of each of these series. The resulting presacral count, however, will vary by the number that the cervical series varies, as there is no reciprocal change at the column's anterior border.

- (2) Vertebrae with disrupted anatomy occur at all series borders but not within series. Global displacement of abaxial relative to primaxial structures will result in the overlap of elements of two vertebral series at both the anterior and posterior border of each series. The segmental extent of border disruption throughout the column should reflect the size of the original (cervical) offset. Nonboundary vertebrae do not display disrupted anatomy because they retain primaxial and abaxial patterning of a single series. The observed lengthening of the zone of sacral disruption with greater deviance from cervical counts of seven supports this prediction in both genera.
- (3) The pattern of vertebral disruption is transitional and mediolateral. In contrast to the anteroposterior disruption of series identities that can occur as a result of axial repatterning (Oostra et al. 2005), a shift in primaxial relative to abaxial patterning should produce mediolateral juxtaposition of structures of disparate series identity. Clear mediolateral disjunctions occur at cervicothoracic, lumbosacral, and sacrocaudal boundaries. The frequent occurrence of partial cervicothoracic ribs suggests the existence of more than one rib-patterning domain, as in chicks. Further, the truncation of partial ribs at or near the rib neck in both genera suggests the possible location of the sloth patterning frontier. This location is further dorsal than the boundary mapped experimentally in chicks, but coincides approximately with a boundary between two differently patterned primaxial vertebral rib regions (Sudo et al. 2001; Aoyama et al. 2005). The existence of mediolateral thoracolumbar disjunctions is more ambiguous, and is complicated by the difficulty of identifying the part of the rib represented by small thoracolumbar riblets. The lack of a consistent pattern of thoracolumbar disruption in Choloepus may reflect the absence of identifiable sternal rib components on posterior thoracics.

Three additional lines of evidence suggest that the repatterning in each sloth genus has been of abaxial, and not primaxial, structures (Fig. 3). Firstly, primaxial repatterning predicts no change in traditional sacrum location and thus should not be associated a change in presacral count. The observed positive correlation between cervical and presacral counts implies coordinated movement of the defining abaxial sternal and pelvic structures. Secondly, primaxial repatterning predicts no change in the location of the brachial plexus relative to the vertebral column. The brachial plexus is recruited from spinal cord neurons by the abaxial limb bud, and so reflects the segmental location of abaxial instead of primaxial structures. Primaxial repatterning and abaxial stasis should therefore be



normal patterning primaxial repatterning abaxial repatterning

Fig. 3. Primaxial repatterning and abaxial repatterning generate different phenotypes if the axial positions of transitional vertebrae are fixed (at V6 and V7 in this example). (A) Normal patterning, with the primaxial cervicothoracic transition (hatched line) between vertebrae 7 and 8. (B) Primaxial repatterning of V6-7 generates composite vertebrae with thoracic primaxial components (vertebrae with posteriorly inclined neural spines and articulating proximal ribs) and cervical abaxial traits (lack of sternal ribs and sternum). Primaxial repatterning moves the primaxial cervicothoracic transition, but the anatomical cervicothoracic boundary, which is defined by the first rib with sternal articulation, is unchanged. (C) Abaxial repatterning of V6-7 generates composite vertebrae with cervical primaxial components (vertebrae with vertical neural spines and synostosed proximal ribs) and thoracic abaxial components (sternal ribs and sternum). Abaxial repatterning does not alter the primaxial cervicothoracic transition between vertebrae 7 and 8, but the first full rib and the anatomical cervicothoracic boundary are shifted anteriorly, consistent with observations of this study. Primaxial structures are represented in white and abaxial structures are represented in black. Shaded structures are of uncertain patterning regime. They correspond to the distal vertebral ribs (with primaxial patterning) in mice, but their patterning appears to be coincident with that of the sternal ribs (abaxial patterning) in sloths. Numbers (V1, V2, etc.) indicate the axial position of each vertebra.

associated with a brachial plexus at the unaltered (traditional) segmental location. However, the brachial plexus of both *Bradypus* and *Choloepus* is known to be displaced along with the abaxially patterned limbs (Giffin and Gillett 1996). Finally, the polarities of the phenotypic disruptions observed are consistent with only abaxial repatterning. The generation of an animal with five cervical vertebrae (as in Choloepus) via primaxial repatterning predicts V6-V7 composite vertebrae that juxtapose thoracic primaxial anatomy (posteriorly oriented neural spines, proximal vertebral ribs) and unaltered cervical abaxial anatomy (no sternal ribs or sternum) (Fig. 3B). This was not observed. In contrast, abaxial repatterning predicts V6–V7 composite vertebrae with primaxial cervical patterning (upright neural spines) and abaxial thoracic patterning (sternal ribs and sternum), which was observed. A similar polarity mismatch occurs if primaxial repatterning is proposed for V8-V9 in Bradypus.

We interpret *Bradypus* cervical anatomy (Fig. 4B) as the result of the global posterior repatterning of abaxial structures. The observed extra "cervicals" (V8–V10) retain typical primaxial thoracic transverse process orientation and truncated proximal thoracic ribs, but lie adjacent to an abaxial

cervical domain that lacks the sternum and sternal ribs. The absence of more distal portions of the vertebral ribs suggests that they, too, may be patterned with abaxial structures. Analysis of the transitional thoracolumbar vertebrae is complicated because the portion of the rib represented by their small riblets is unclear. One possible interpretation is that these vertebrae are composites of primaxial lumbar anatomy with partially differentiated lumbar transverse processes and abaxial thoracic anatomy with short sternal ribs. We interpret lumbosacral vertebrae immediately anterior to and variably fused to the sacrum as primaxial sacrals. Their transverse processes appear to retain developmental signals promoting fusion to the more posteriorly lying sacral transverse processes but lie adjacent to a lumbar abaxial domain lacking girdle elements. The poorly integrated sacrocaudal vertebrae are interpreted as primaxial caudals that lie adjacent to the abaxial ischium.

Conversely, we interpret Choloepus anatomy (Fig. 4C) as the result of the anterior repatterning of abaxial elements. The first "thoracic" vertebrae (V6-V7) are the result of mediolateral juxtapositions of primaxial cervical vertebral bodies with reduced cervical ribs and abaxial thoracic sternal and/or distal vertebral ribs. The irregular shapes of these ribs may reflect this anomalous fusion of cervical and thoracic rib elements. Terminal thoracic vertebrae show incipient development of the transverse processes typical of lumbar vertebrae. Those seen in most C5 individuals may represent primaxial vertebral ribs of thoracic vertebrae lying adjacent to an abaxial lumbar field that lacks sternal ribs. Lumbosacral vertebrae lying within the anterior indentation of the sacrum are interpreted as primaxial lumbar vertebrae that appear to lack the developmental signals promoting fusion to the ilia of the adjacent sacral domain. When fusion does occur, it is disrupted and incomplete. The variably fused sacrocaudal vertebrae are interpreted as primaxial sacrals that lie posterior to their usual abaxial ischial fusion targets.

Anatomical transformation in mammals

The common axial identity of each vertebra and its articulating lateral structures has been an unspoken assumption in previous attempts to understand the developmental origin of the anomalous anatomy of tree sloths. However, the disjunct mediolateral anatomy observed at all series boundaries is not consistent with commonly recognized patterns of variation in serially homologous structures. It is, however, consistent with a frame shift between primaxial and abaxial structures and with the recent documentation of independent patterning of these two mesodermal domains. Each genus varies from the mammalian standard with respect to abaxial, but not primaxial, patterning. These posterior (*Bradypus*) and anterior (*Choloepus*) abaxial frame shifts have generated changes in neck architecture that appear to be selectively advantageous



Fig. 4. The hypothesis of abaxial repatterning as the source of aberrant vertebral anatomy in Bradypus and Choloepus. (A) Normal (ancestral) mammalian patterning. (B) Miniature V8 and V9 primaxial riblets and presacral vertebrae with transverse processes that turn posteriorly to fuse with the sacrum indicate a posterior abaxial shift in Bradypus. (C) Full C5 and C6 ribs and lumbar vertebrae deeply inset between the ilia without fusion indicate an anterior abaxial shift in Choloepus. Primaxial series transitions (hatched lines) are identical with those of traditional mammals. Primaxial structures are represented in white and abaxial structures are represented in black. Shaded structures are of uncertain patterning regime. They correspond to distal vertebral ribs (with primaxial patterning) in mice, but their patterning appears to be coincident with that of the sternal ribs (abaxial patterning) in sloths. Generalized column counts and a uniform abaxial frame shift of two segments are diagrammed for the sake of clarity. Numbers (V1, V2, etc.) indicate the axial position of each vertebra.

to animals with inverted posture, but coincidently produce multiple disruptions in the posterior vertebral column. This extremely unusual morphology highlights the strong developmental resistance to homeotic and meristic alterations of cervical seriation that must be in place in mammals, despite their frequent occurrence in other mammalian vertebral series and in other vertebrate classes. The mammalian primaxial "rule of seven" is more developmentally inflexible, and more in need of explanation, than ever.

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SUPPORTING INFORMATION

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

Table S1. Database of specimens with vertebral identifications at each axial position.

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