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# Crossing the Frontier: a Hypothesis for the Origins of Meristic Constraint in Mammalian Axial Patterning

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Title: Crossing the frontier: a hypothesis for the origins of meristic constraint in mammalian axial patterning

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**Abstract**

Serially homologous systems with high internal differentiation frequently exhibit meristic constraints, although the developmental basis for constraint is unknown. Constraints in the counts of the cervical and lumbosacral vertebral series are unique to mammals, and appeared in the Triassic, early in their history. Concurrent adaptive modifications of the mammalian respiratory and locomotor systems involved a novel source of cells for muscularization of the diaphragm from cervical somites, and the loss of ribs from lumbar vertebrae. Each of these innovations increased the modularity of the somitic mesoderm, and altered somitic and lateral plate mesodermal interactions across the lateral somitic frontier. These developmental innovations are hypothesized here to constrain the anteroposterior transposition of the limbs along the column, and thus also cervical and thoracolumbar count. Meristic constraints are therefore regarded here as the nonadaptive, secondary consequences of adaptive respiratory and locomotor traits.

**Introduction**

The evolution of adaptive morphology by natural selection is the major theme and guiding principle of comparative morphology. But a strict correlation of structure and function is complicated by structures that appear to be resistant to natural selection, even over long evolutionary time periods and under extremely different selection regimes. The existence of static or poorly adaptive structures is commonly attributed to the origin of evolutionary novelties by the modification of already existing structures, a process that François Jacob (1977) memorably compared to “tinkering.” In this tinkering, ancestral developmental programs and morphology are incompletely and imperfectly re-engineered for new functions. They can therefore exert a bias in the production of the phenotypes on which natural selection acts (Brakefield 2006). Here, a rich fossil record documenting the synapsid / mammal transition, adaptively diverse

living mammalian taxa, and a large body of recent developmental work on axial patterning are integrated to propose a synthetic hypothesis for the origin of a well-known example of evolutionary stasis, vertebral count in mammals.

Structures composed of multiple serially homologous subunits can provide accessible and discrete systems for addressing the imposition of evolutionary stasis. In many but not all cases, the elements of ancestral series are “primordially similar,” while the elements of descendant series have been adapted “to the most diverse purposes” (Darwin 1859). In *Materials for the Study of Variation*, William Bateson (1894) noted that, “on the whole, series containing large numbers of undifferentiated parts more often show Meristic Variation than series made up of a few parts much differentiated.” An example of the evolutionary transition from undifferentiated segments with high and variable counts to differentiated segments with lower and more constrained counts has been elegantly traced in arthropods using the 250 million year fossil record of trilobites (Hughes 2007).

The developmental link between morphological differentiation and count constraint is only poorly understood. A general hypothesis suggests that the same developmental program generates each serially homologous subunit ancestrally, identifying the entire composite structure as a single morphological and developmental module. As evolution proceeds, downstream developmental steps are added in isolated areas of the series, producing regionally unique morphology that is selectively adaptive. After multiple generations of differentiation, each developmentally and morphologically differentiated region of the series becomes an internally integrated submodule that is resistant to incorporation or expansion into adjacent, differently specialized submodules without significant adaptive cost. This developmental entrenchment leads to stasis of count.



Vertebrates vary in the extent of their column regionalization. Minimal morphological regionalization and high count variability are found in the columns of ancestral vertebrates and many living teleosts (e.g. Ward and Brainerd 2007). The columns of tetrapods are regionalized by their integration with limb girdles into cervical, dorsal, sacral, and caudal series, but series counts in many taxa are variable (Müller et al. 2010). In contrast, the axial skeleton of mammals exhibits both high morphological differentiation and minimal meristic variation. Five specialized series (cervical, thoracic, lumbar, sacral, caudal), several with intra-regional differentiation, are usually recognized. Cervical count is effectively fixed at seven, total thoracolumbar count is more loosely constrained at nineteen or twenty, and sacral count is typically three or four. Only the caudal series varies widely in count. The adaptation of mammalian axial anatomy to a wide diversity of lifestyles over a long geologic history (Buchholtz 2012) has thus been limited primarily to changes in vertebral shape and caudal count.

The historically remote and subcellular origins of the developmental innovations responsible for vertebral constraint complicate its analysis. The reappearance of ancestral structures following experimental expansion of *Hox* gene expression domains led Pollock et al. (1995) to hypothesize that morphological differentiation of the vertebral column reflects the progressive restriction of expression domains that were originally expressed over much, or all, of the column. Although this pattern is not universally observed (e.g. Mallo et al. 2010), an evolutionary increase in developmental modularity is clear. On a morphological level, the adaptive clues typically provided by the structure / function paradigm are absent, as count is fixed in mammals with widely different adaptations. Additionally, variable counts in non-mammalian taxa argue against the adaptive superiority of any particular count. However, if regionalization was the product

of a developmental program that generated a trait with high adaptive value, and secondarily also limited changes in vertebral count, stasis could be imposed without any inherent adaptive advantage. This scenario demands a developmental tie between the adaptive trait and meristic constraint, as well as their synchronous origin.

Below I present a hypothesis for the origin of cervical and thoracolumbar constraints in mammalian axial patterning as secondary consequences of developmental innovations tied to the highly adaptive mammalian respiratory / locomotor complex. Each innovation is associated with increased modularity of the column, has a developmental link to limb placement, and first appeared roughly synchronously with constraint imposition.

### **Mammalian respiration and locomotion**

Living mammals possess a unique suite of integrated characters that enhance respiratory and locomotor performance. This character complex includes elevated body temperature, a high resting metabolic rate, the capacity for sustained activity, parasagittal excursion of the limbs, and dorso-ventral movement of the vertebral column (Carrier 1987, Ruben et al. 1987). A muscularized diaphragm located posterior to the lungs is a key soft-tissue character that integrates components of the complex. On contraction of the diaphragm, thoracic cavity volume increases, reducing intrathoracic pressure and enhancing inspiration and gas exchange. The diaphragm also limits anterior movement of the viscera into the thorax (Klein and Owerkowicz 2006). Instead, abdominal viscera are displaced outwardly in the rib-free abdominal / lumbar region during inspiration (Perry et al. 2010). In contrast to non-mammalian amniotes, whose lateral column flexion during locomotion limits costal-based aspiration, the sagittal locomotor movements of the mammalian column allow continuous ventilation, supporting

the elevated body temperature and aerobic demands of the sustained activity so characteristic of mammals (Carrier 1987, Ruben et al. 1987, Perry et al. 2010).

The sequence and antiquity of mammalian respiratory / locomotor traits can be estimated by mapping those with osteological correlates onto a consensus phylogeny. Respiratory turbinates, heat and moisture conserving structures present only in living endotherms, were present in the Middle Triassic cynodont *Massetognathus* (Hellenius and Ruben 2004). The Middle Triassic dicynodont *Wadiazuarus* (Bandyopadhyay 1988) possessed femoral and acetabular morphology interpreted as indicating upright hind limb posture; the co-occurrence of elongate vertebral transverse processes that allow the separation of respiratory and locomotor muscle groups (Carrier 1987) supports this interpretation. The differentiation of an anticlinal vertebral region typical of dorsoventral column movement was present in the Late Triassic morganucodonts *Eozostrodon* and *Megazostrodon* (Sues and Jenkins 2006). The Early Triassic cynodont *Thrinaxodon* exhibited incipient differentiation of a lumbar vertebral series with shortened, moveable ribs (Jenkins 1970). The loss of lumbar ribs and the stabilization of lumbar count at 19-20 are somewhat later and more variable in occurrence, and likely homoplastic in origin (Luo et al. 2007). Multiple authors (e.g. Brink 1956, Perry et al. 2010) have argued that shortened or absent lumbar ribs are indicators of mammalian style locomotion and/or the presence of diaphragm-assisted inspiration, and the transition from thoracic to lumbar morphology is associated with the location of the diaphragm in almost all living mammals (Buchholtz et al. 2012). What evidence is available therefore indicates that the mammalian respiratory / locomotor complex, including a muscularized diaphragm, was assembled very early in mammalian history, during the Triassic Period. This transition is roughly coincident with the origin of fixed cervical count, seen in *Thrinaxodon* and all more derived synapsids and mammaliamorphs. In contrast, the reduction and loss of

free ribs on cervical vertebrae are restricted to monotremes and more crownward mammalian taxa, and significantly postdate the onset of cervical count stasis (Fig. 1).

### **Patterning of somitic and lateral plate mesodermal structures.**

The vertebrate postcranial musculoskeletal system is composed of mesodermal tissues that have two different developmental origins, the somites and the lateral plate mesoderm. The somitic mesoderm forms as paraxial bands lateral to the neural tube. These bands are later subdivided into segments in the process of somitogenesis. Segment number is controlled by the speed of a molecular “segmentation clock” (e.g. Dequeant and Pourquie 2008), while somite differentiation is controlled by *Hox* and other transcription factors (e.g. Wellik 2009). Expression domains of the *Hox* genes overlap anteroposteriorly along the body axis, and are colinear with respect to their locations on the chromosome. In contrast, the laterally located lateral plate mesoderm (LPM) is not segmented. Like the somites, it is patterned by *Hox* genes during development, but LPM patterning is independent of that of the somites and is not colinear (McIntyre et al. 2007).

The tissues that comprise the postcranial musculoskeletal system can be placed into three categories based on their developmental histories. The axial musculature, vertebrae, proximal ribs, and intermediate ribs are somitic in origin, and are patterned in a somitic or primaxial environment (Burke and Nowicki 2003). The limb bones, sternum and the pelvis have developmental origin in the lateral plate mesoderm, and are patterned in a lateral plate or abaxial environment. A third group of tissues are of somitic origin, but enter the abaxial environment during early development and are patterned there with tissues of lateral plate origin (Burke and Nowicki 2003). These tissues include the distal portions of ribs (origin in the sclerotome of the somites) and the migrating muscle precursor cells (MMPs, origin in the dermomyotome of the somites) that enter

and differentiate within their destinations as the musculature of the tongue, limbs, and diaphragm (Birchmeier and Brohmann 2000). A reporter expressed in the lateral plate mesoderm in transgenic Prx1/Cre/Z/AP mice has been used to identify abaxially patterned tissues, including those of somitic origin (Durland et al. 2008).

The integration of mesodermal tissues with different developmental histories is critical to the construction of the musculoskeletal system, and is accomplished by a complex signaling process across their interface, the lateral somitic frontier (Burke and Nowicki 2003). Components of the signaling system between somitic MMPs and their forelimb and diaphragm targets have been studied intensively, and include the ligand SF/HGF (scatter factor/hepatic growth factor) and the c-Met tyrosine kinase receptor (Birchmeier and Brohmann 2000, Vasyutina and Birchmeier 2006). The morphological interactions between the developing distal ribs and the sternal plate (Chen 1952), and between the sacrum and the pelvic element (Pomikal and Streicher 2010) have been described, but the signaling cascades that direct them have not yet been identified. The known examples of integration across the lateral somitic frontier occur between two abaxial tissues: a somitic tissue that has been patterned abaxially and a LPM tissue (Chen 1952, Birchmeier and Brohmann 2000, Burke and Nowicki 2003, Pomikal and Streicher 2010).

### **Hypothesis of constraint origins**

The diaphragm and the lumbar vertebral series are key components of the mammalian ventilation / respiratory pattern. Examination of the developmental bases of their origins suggests that each was also associated with an innovation in somite / LPM patterning and with constraint of a vertebral series count.

**Origin of the cervical constraint.** Like many tetrapods, mammals possess a post-pulmonary septum, the septum transversum. This structure is of lateral plate origin, and acts as a visceral stabilizer. Uniquely in mammals, however, the septum transversum is muscularized, allowing it to enhance changes in thoracic cavity size and thus inspiration. The cells that muscularize the diaphragm are somitic MMPs that originate in the dermomyotome of cervical somites 3, 4 and 5 and migrate in response to a SF/HGF-*cmet* signaling system (Dietrich et al. 1999, Birchmeier and Brohmann 2000). Their migration pathway can be visualized by tracking the expression of *Lbx1*, a transcription factor restricted to MMPs (Dietrich et al. 1999). MMPs destined for the diaphragm travel originally with MMPs from immediately adjacent posterior somites that are destined for the forelimb. As the two cell populations migrate they respond to different downstream guidance cues, and only MMPs from somites 3, 4 and 5 enter the septum transversum to muscularize the diaphragm (Vasyutina et al. 2005). The unique migration and fate of the C3-C5 MMPs indicates that they are uniquely patterned, forming a discrete developmental module within the cervical series. This interpretation is supported by the presence of a unique C3-C5 suite of *Hox* axial patterning genes, distinct from those of the more anterior atlas and axis (C1-C2) and of the immediately posterior cervical somites, in *Mus* (Kessel 1992).

A secondary consequence of the novel patterning of C3-5 is that all mammalian cervical segments are committed to specialized fates: skull articulation (C1, C2), diaphragm (C3-C5), or forelimb (C6, C7). The presence of a common somitic origin and early migration of diaphragm and forelimb MMPs suggests the possibility of developmental interference. Such interference has been documented in chicks, where ectopic limb placement diverts somitic cells from their default abaxial destinations to the limbs (Liem and Aoyama 2009). Any anterior movement of the forelimb from its axial location in mammals may

therefore limit myoblast precursors available for the muscularization of the diaphragm, the forelimb, or both. Additionally, posterior movement of the limb would displace the lateral plate but not the somitic components of the diaphragm's unique SF/HGF - *cmet* signaling system across the lateral somitic frontier, disrupting myoblast migration and therefore diaphragm muscularization (Buchholtz et al. 2012, Fig. 2 A-D). To the extent that the muscularized diaphragm is selectively advantageous for ventilation and locomotion, both anterior and posterior transposition of the limb field would be nonadaptive.

Three mammalian species do have atypical cervical counts, apparently tolerating the postulated adaptive cost of forelimb transposition. Detailed study of the tree sloths *Choloepus* and *Bradypus* suggests that all of their abaxial skeletal tissues (sternum, distal ribs, pelvis) are mispatterned either anteriorly or posteriorly in concert, generating mismatched structures across the lateral somitic frontier at all segmental levels (Buchholtz and Stepien 2009). This interpretation is challenged by the difficulty of identifying the primaxial cervicothoracic boundary without reference to associated abaxial structures in non-model taxa. However, vertebral ossification during early ontogeny appears to be modular, and preliminary analysis of ossification patterns supports the presence of primaxial/abaxial offsets in *Bradypus* (Hautier et al. 2010). An alternative analysis (Varela-Lasheras et al. 2012) identifies homeotic mispatterning as the source of sloth cervicothoracic anatomy, and also suggests that cervical ribs are associated pleiotropic developmental defects and cancer. The conclusion that cervical count is constrained by these pleiotropic effects is supported by the frequent occurrence of cervical ribs in perinatal deaths in humans, but challenged by the presence of cervical ribs and cervical count stasis over more than 150 million years of early mammalian history. Both analyses posit a connection between the low metabolic rates of exceptional

taxa and their apparent tolerance for unusual cervical anatomy, and both may be consistent with a hypothesis of a diaphragm-linked origin of the cervical constraint. Cervical anatomy of the third aberrant genus, the manatee *Trichechus*, is less well-studied, but the genus exhibits both a low metabolic rate and a uniquely structured diaphragm (Rommel and Reynolds 2000).

**Origin of the lumbosacral constraint.** In non-mammalian amniotes, each interlimb (or dorsal) vertebra articulates with a pair of moveable ribs. In mammals, moveable ribs are present only anteriorly, in the thorax. Posterior to the thorax, lumbar vertebrae bear fixed transverse process but lack moveable ribs. In *Mus*, thoracic ribs are derived from the sclerotome of somites, but only their proximal and intermediate units are patterned primaxially; the distal rib unit, which interacts with the abaxial sternum (Chen 1952) is patterned abaxially (Aoyama et al. 2005, Durland et al. 2008). Lumbar transverse processes have been variably interpreted as modified ribs or as vertebral outgrowths (Filler 2007). Detailed morphological analysis now indicates that, with a few exceptions, lumbar transverse processes arose independently from a variety of vertebral processes in different mammalian orders (Filler, 2007). Like vertebrae they are therefore somitic and developmentally primaxial. Primaxial lumbar transverse process patterning is also indicated by the absence of reporter expression in the *Prx1/Cre/Z/AP* mouse (Durland et al. 2008). A secondary consequence of the origin of the lumbar series is thus that axial segments lying between the thorax and the sacrum lack not only ribs but any abaxially patterned tissues. Lumbar vertebrae are also characterized by a unique (though somewhat internally variable) suite of *Hox* genes (Kessel 1992, Wellik and Capecchi 2003), and therefore constitute a new modular unit within the mammalian column.



Sacral vertebrae, which lie posterior to the lumbar region, have a lateral ala that articulates with the abaxial pelvis. Although sometimes identified as a transverse process, both paleontological and molecular evidence identifies the ala as a rib homolog. Fossils of Devonian stem tetrapods (e.g. *Acanthostega*) possess a single sacral rib that articulates with the pelvis. This rib mimics other ribs morphologically and lies in series with ribs found both anteriorly and posteriorly (Coates 1996), strongly suggesting homology. Further, distal portions of the ala express the reporter that tracks abaxially patterned tissues in the transgenic Prx1/Cre/Z/AP mouse (Durland et al. 2008). As vertebrae and their transverse processes are exclusively somitic, this marker also identifies at least the distal portion of the ala as a distal rib homolog.

Assembly of the mammalian sacrum is the product of the lateral formation and medial migration of the LPM pelvic element (Pomikal and Streicher 2010). The signaling cascade that results in the “docking” of the abaxial pelvis with the abaxial distal components of ribs is unknown. However, the concurrent posterior transposition of both sacral vertebrae and the pelvis in *Hox9* quadrupal mutants (McIntyre et al. 2007) argues for the importance of distal rib / pelvis interaction across the lateral somitic frontier to pelvic assembly. To the extent that a ribless lumbar region is selectively advantageous for abdominal expansion during inspiration and parasagittal locomotion, anterior displacement of the pelvis would be nonadaptive, and would limit reduction of presacral vertebral count in mammals (Fig. 2 E-H). The morphological effects of aberrant anterior displacement of the abaxial ilium relative to the primaxial lumbosacral transition are demonstrated by the very unusual pelvic morphology of *Choloepus hoffmanni*. In this species, both sternum and ilium lie anterior to their outgroup axial locations, suggesting primaxial / abaxial displacement (Buchholtz and Stepien 2009). The apparently lumbar vertebrae lying between the ilia either “refuse” incorporation into the sacrum, are poorly

incorporated into the sacrum, or in extreme cases “escape” sacral integration anteriorly (Fig. 3).

Posterior translocation of the pelvis (thoracolumbar count > 20) presents no obvious selective disadvantage and does occur, most frequently in afrotherians (Sanchez-Villagra et al. 2007). At least one afrotherian, the manatee *Trichechus*, is known to possess rib-derived transverse processes (Filler 2007), raising the possibility that some or all of the taxa with exceptional thoracolumbar counts may possess abaxially patterned components compatible with pelvic element articulation in their lumbar or anterior caudal segments.

### **Overview**

The evolution of the vertebral column is seen here as a case study in the evolution of structures with serially homologous elements. These structures typically show an increase in differentiation or modularity over evolutionary time, concurrent with a reduction in the variability of element count. Despite the commonality of the pattern, the specific developmental steps involved in the imposition of count constraint are likely to be unique in each case. The hypothesis presented above suggests that meristic constraints in the mammalian cervical and thoracolumbar column were not in themselves adaptively selected, but were the secondary consequences of respiratory and locomotor adaptations. In this hypothesis, the ancestral synapsid column was a permissive, low modularity system, with anteroposterior movement of the limbs unrestricted by specializations of adjacent segments. Developmental specializations of mid-cervical segments associated with the muscularization of the diaphragm by MMPs and of the lumbar segments associated with the loss of abaxially patterned rib components generated non-permissive or high modularity systems in mammals. Novel

developmental relationships of somitic and LPM tissues across the lateral somitic frontier characterize these new modular units, and tie them to limits on the anteroposterior translocation of the limbs.

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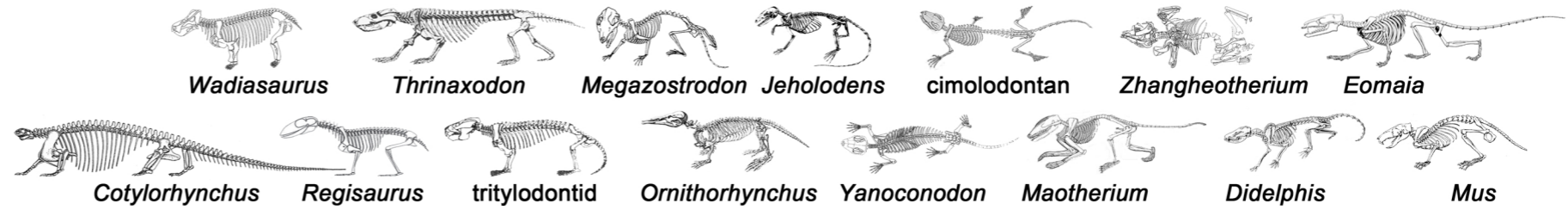
## Figure Captions

Fig. 1. The distribution of cervical and thoracic vertebral innovations across the synapsid / mammal transition as described in the literature (see text). The phylogeny is based primarily on Luo et al. (2007), and characters are polarized by reference to the Permian synapsid *Cotylorhynchus*. Colored boxes indicate the presence of derived traits. Question marks indicate that trait status is unknown.

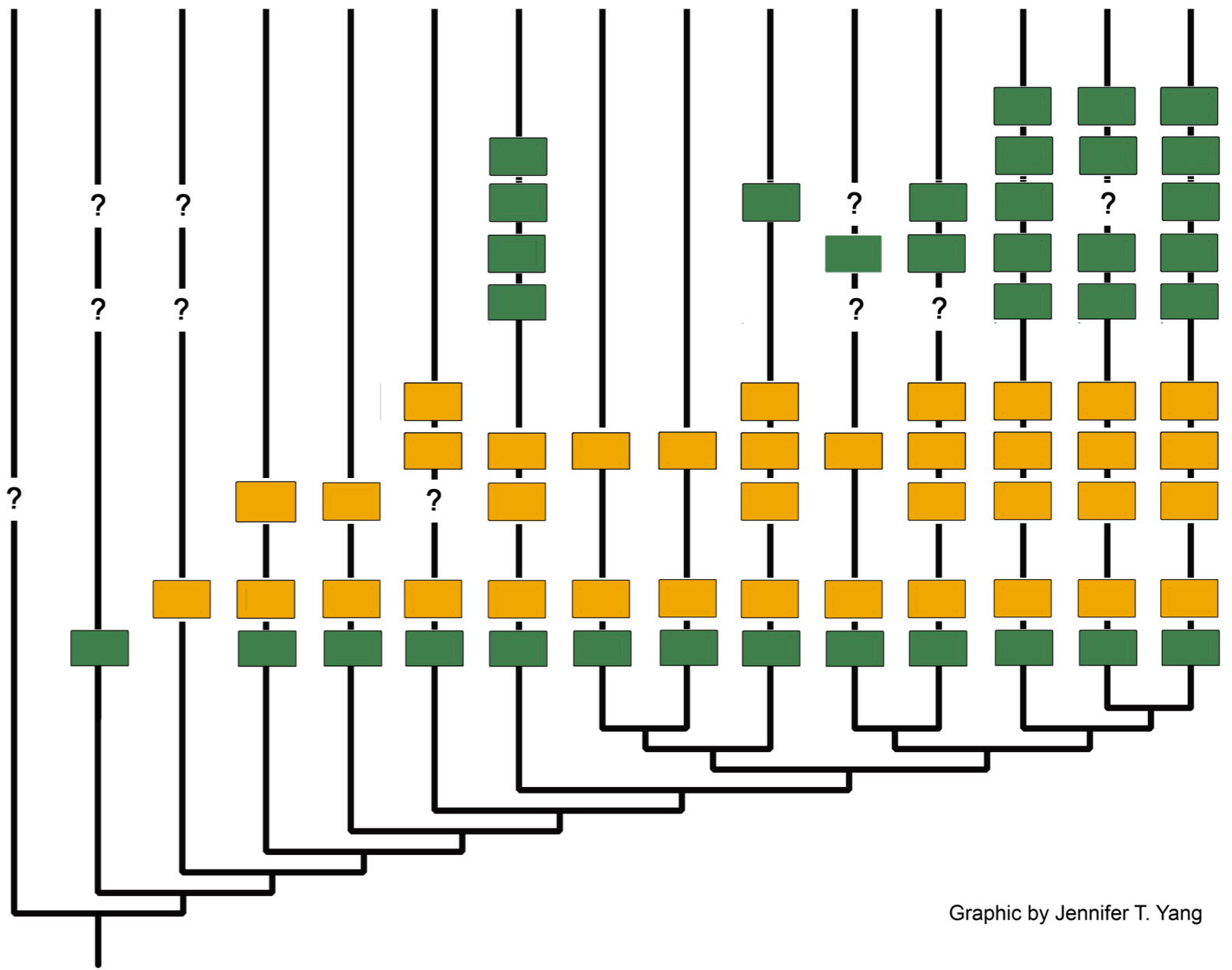
Fig. 2. Hypothesis for the role of developmental interactions across the lateral somitic frontier in the origin of meristic constraints in mammals. (A-D), cervical constraint; (E-H), thoracolumbar constraint. Vertebral patterning inferred for ancestral taxa is represented in the far left column (A, E) in light gray. Novel mammalian morphological modules (mid-cervical, lumbar) are represented in white in the remaining columns (B-D and F-H). Abaxial tissues (MMPs migrating from the limb-level and mid-cervical somites, distal rib components, sternum, pelvic element) are in dark gray. Both anterior (C) and posterior (D) transposition of the fore limb are predicted to generate a poorly muscularized diaphragm, forelimb, or both. Anterior transposition (G) of the pelvis is predicted to be limited by the importance of a ribless lumbar region to abdominal expansion during inspiration and to parasagittal locomotion. No non-adaptive consequence of posterior transposition (H) of the pelvis is known. C = cervical, Cd = caudal, D = dorsal, Di = diaphragm, P = pelvic element, PPS = post-pulmonary septum, S = sacral, St = sternum, T = thoracic, ? = patterning uncertain. A-D modified from Fig. 5 of Buchholtz et al. 2012.

Fig. 3. Pelvic morphology in *Choloepus hoffmanni* suggests disturbances across the lateral somitic frontier. Each individual has six cervical vertebrae and possesses ribs articulating with the sternum on vertebra 7. Vertebrae in axial position 32 are highlighted in purple; those in axial position 33 are highlighted in green. A, USNM (National Museum of Natural History, Washington, D.C) 137420; B, FMNH (Field Museum of Natural History, Chicago) 60544; C,

USNM 256181.



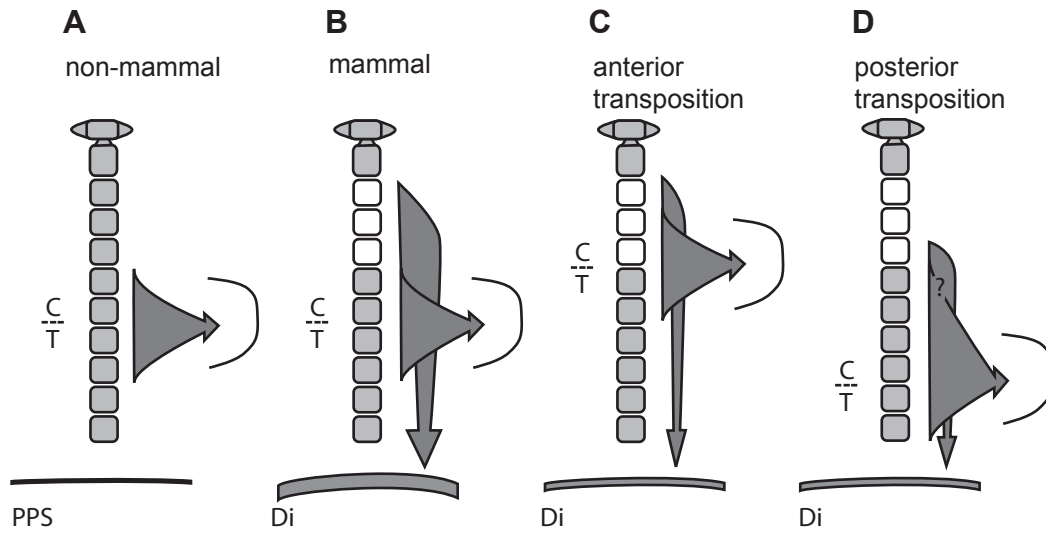
Character	primitive	derived
axis ribs	present	absent
postaxial cervical ribs	present	absent
dens and axis	separate	fused
atlas rib	present	absent
atlas neural arch and intercentrum	separate	fused
lumbar ribs	present	absent
anticlinal vertebra	absent	present
thoracolumbar count	≠ 19 or 20	= 19 or 20
thoracolumbar differentiation	absent	present
cervical count	≠ 7	=7



cervical trait
  thoracic trait

Graphic by Jennifer T. Yang

## Hypothesis of cervical constraint



## Hypothesis of thoracolumbar constraint

