



Differential occupation of axial morphospace[☆]

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ABSTRACT

The postcranial system is composed of the axial and appendicular skeletons. The axial skeleton, which consists of serially repeating segments commonly known as vertebrae, protects and provides leverage for movement of the body. Across the vertebral column, much numerical and morphological diversity can be observed, which is associated with axial regionalization. The present article discusses this basic diversity and the early developmental mechanisms that guide vertebral formation and regionalization. An examination of vertebral numbers across the major vertebrate clades finds that actinopterygian and chondrichthyan fishes tend to increase vertebral number in the caudal region whereas Sarcopterygii increase the number of vertebrae in the precaudal region, although exceptions to each trend exist. Given the different regions of axial morphospace that are occupied by these groups, differential developmental processes control the axial patterning of actinopterygian and sarcopterygian species. It is possible that, among a variety of factors, the differential selective regimes for aquatic versus terrestrial locomotion have led to the differential use of axial morphospace in vertebrates.

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1. Introduction

One of the major defining characteristics of Vertebrata is having a vertebral column that is composed of serially repeating ossified, cartilaginous, and ligamentous elements that surround the spinal cord and notochord (Schultze and Arratia, 1988; Janvier, 1997). These repeating structures are known as vertebrae. Over evolutionary history, vertebrae have taken on many functional roles including protection of the spinal cord and dorsal aorta as well as providing attachment points for the axial musculature. These functional differences correspond to the structural and numerical diversity that we observe across the vertebrate axial skeleton.

For centuries, vertebrae were and continue to be a potent morphological character for taxonomic and comparative studies. Since the 1800s, studies have pointed out the effects of environmental variation on vertebral characteristics (Jordan, 1891; Lindsey, 1975; McDowall, 2008 and references therein). However, only during the last fifty years have vertebrae become the focus of functional biology research. For example, vertebral number has provided insight into maximum body length and shape in fishes (Lindsey, 1975), which, in turn, has been shown to have functional consequences on important survival traits such as swimming

performance (Tytell et al., 2010). Both vertebral number and shape of the individual vertebrae have been shown to be useful in understanding the evolution of body shape variation within (Ward and Brainerd, 2007; Bergmann and Irschick, 2012) and across vertebrate groups (Collar et al., 2013). Furthermore, movement of individual vertebrae within the vertebral column has provided insight into diverse behavior patterns of vertebrates (Moon, 1999). Despite these recent studies, the functional implications of vertebral variation still need to be explored.

In the present article, our aim is to provide a brief overview of the morphological diversity of vertebrae and the early developmental mechanisms that guide vertebral formation. While doing so, we discuss differentiation of the axial skeleton and the mechanisms underlying regionalization. We also search for broad-scale patterns in the axial skeleton by assembling a large data set consisting of vertebral numbers in the precaudal and caudal regions from diverse members of the major vertebrate clades. Lastly, we highlight exciting avenues for future research.

2. Postcranial axial skeleton and variation in vertebral articulation

Although often overshadowed by attention given to the skull, the postcranial system, composed of the axial and appendicular skeletons, provides the skull with movement, suspends the body, and propels the body through different media. In Chordata, the notochord is the primary axial structure. In Agnatha and some early Actinopterygii, the notochord continues to be the primary axial structure in embryos and adults. It is not until the arrival of

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the Gnathostomata that the notochord is replaced by a vertebral column with centra in adults.

On examining the axial skeleton, the morphological diversity of individual vertebrae is apparent. Vertebrae are composed of multiple components: centra, arches, and intervertebral discs (Schultze and Arratia, 1988). Any of these components can contribute to the morphological and functional diversity of the axial skeleton. Vertebrae have also undergone enlargements, reductions, and fusions, which have added to the challenge of tracking development and evolution.

The centrum is the main body of the vertebra; it surrounds and replaces or incorporates the notochord. Vertebrae vary in type based on the relationship of the arches to the centrum, the number of elements forming each centrum, and the embryonic origin of the centrum. Based on embryonic origin, centra may be categorized into four different types: chordacentra (when present as mineralized or calcified rings within the notochordal sheath in actinopterygians), arco centra (when formed by ossification of cartilage extending from the arches around the notochord), auto-centra (when formed independent of the dorsal and ventral arches), or holocentra (when formed by proliferation of cartilage cells around the notochord that then ossify to form amphicoelous centra) (Gadow, 1933; Arratia et al., 2001).

Multiple centra articulate to form the axial column. The articulating surfaces of the centra vary and this variation can affect the movement and the roles of the axial skeleton. Centra with flat ends are termed acoelous whereas centra with concave ends are amphicoelous. Acoelous centra are found in precaudal vertebrae, i.e., mainly the trunk, of early reptiles, birds, and mammals. Amphicoelous centra are found in Chondrichthyes, Actinopterygii, and some amphibians, such as the salamander *Necturus*. Centra that are concave anteriorly and convex posteriorly are called procoelous while the reverse of this design is known as opisthocoelous. In the procoelous and opisthocoelous conditions, the centra articulate like a ball-and-socket joint and rotation is at the center of the joint, reducing the possibility of vertebral dislocation. Examples of vertebrae with procoelus and opisthocoelous centra can be found in Lissamphibia and early reptiles. Lastly, heterocoelous centra, which are found in the cervical region of birds and turtles, have saddle-shaped articular surfaces at both ends (Liem et al., 2001).

Projections known as apophyses may also be found extending from the centra and vertebral arches. Depending on their size and location, apophyses can articulate with other bones, such as ribs, or form interlocking processes, known as zygapophyses, between vertebrae. In some elongate terrestrial amniotes where torsion of the body may be extreme, such as snakes, additional zygapophyses, called zygosphenes and zygantra, may be present on the anterior and posterior margins, respectively, of the neural spine. It was proposed that torsion at the vertebral joints was not possible due to the zygosphenes–zygantrum articulations (Mosauer, 1932). However, in snakes, flexion of the vertebral column is extremely important for many survival behaviors such as locomotion, defensive behaviors, and feeding (see Moon, 1999 for a discussion of axial torsion in different snake taxa during diverse behaviors). Despite the additional morphological restrictions imposed by zygosphenes–zygantrum articulations, considerable torsion is possible in the snake axial skeleton. In fact, the trunk vertebrae of gopher snakes, *Pituophis melanoleucus*, were found to twist by up to 2.19 degrees and it was noted that torsion up to 2.89° was possible per vertebral joint (Moon, 1999).

3. Regionality in the axial skeleton

In addition to variation in the morphology of individual vertebrae, there is tremendous diversity in the degree of vertebral

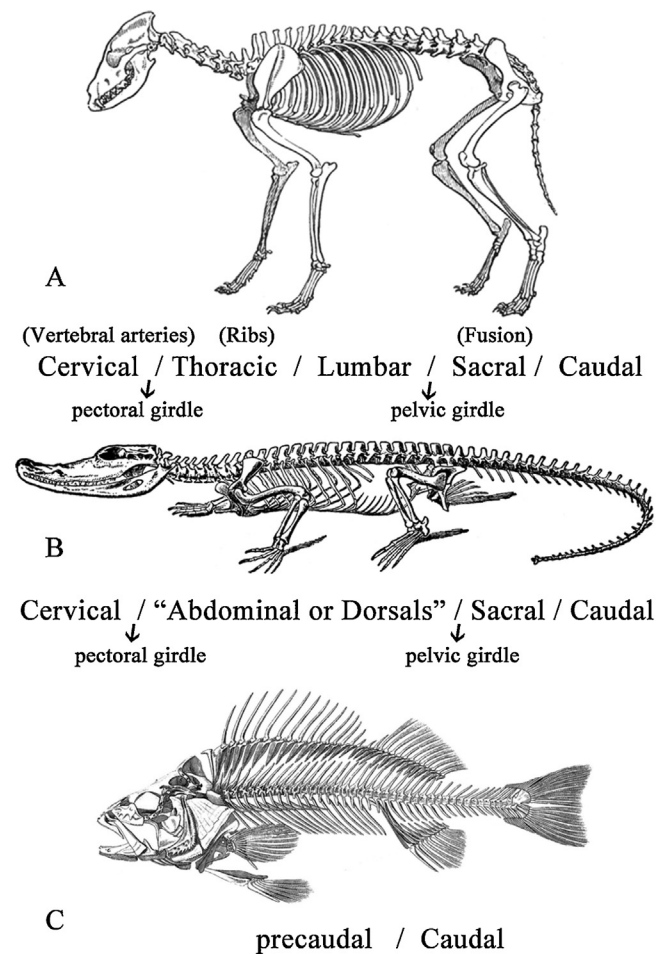


Fig. 1. Regionalization of the vertebral column in Vertebrata. While the vertebral columns of Chondrichthyes and Actinopterygii are divided into a precaudal and caudal region, all other groups have more than two regions. In amniotes, regionalization seems to be based upon the presence or absence of ribs or the variation in ribs along the vertebral column. (A) Mammals have the greatest diversity across the axial skeleton with five distinct regions. Also, despite elongation in specific regions, mammals still maintain 7 cervical vertebrae and 19–20 thoracolumbar vertebrae. Mammalian precaudal elongation is mostly due to elongation of the individual centra. (B) Sauropsids have four distinct regions with the thoracolumbar region (also known as abdominal or dorsal) having the greatest number of vertebrae. (C) A bony fish skeleton illustrating the simple divisions of the axial skeleton with the precaudal and caudal region. Vertebrae in the caudal region are distinguished from those in the precaudal region by the presence of hemal arches.

Line drawings are modified from Parker (1900), Ellenberger et al. (1956), and Romer (1966).

regionalization across vertebrates (Fig. 1). The vertebral column may be divided into 2–5 regions depending on the taxon; these regional designations are based on anatomical features of the vertebrae as well as their relative placement in the body (Goodrich, 1958). In Chondrichthyes and Actinopterygii, axial regions are frequently divided in two: precaudal and caudal (exceptions will be discussed below). In Tetrapoda, the axial skeleton may contain up to five distinct regions: cervical, thoracic, lumbar, sacral, and caudal (Fig. 1).

3.1. Cervical region

The cervical region is the most anterior region of the vertebral column in tetrapods (Goodrich, 1958). The limit of the posterior extension of the cervical region has been described as the anterior limit of *hoxc6* expression (Burke et al., 1995). Anatomically, cervical vertebrae can be distinguished as having a foramen for the vertebral

blood vessels (Liem et al., 2001). In amniotes, the first two cervical vertebrae are referred to as the atlas and axis, which are modified to support and move the skull. Mammals have the most well-defined cervical region, with a vast majority of mammals having seven cervical vertebrae. The most well known exceptions to this rule are sloths and manatees (Galis, 1999; see also Buchholtz, this issue). Having seven cervical vertebrae does not constrain diversity in the cervical region as vertebral shape can vary greatly in mammals. For example, giraffes have extremely long cervical vertebrae that grow disproportionately long throughout ontogeny (van Sittert et al., 2010) and many cetaceans have compact and fused cervical vertebrae (Thewissen, 1988). Variation in cervical vertebral number is more varied in other tetrapod groups, with Aves having the greatest range in vertebral number for extant groups (Galis, 1999). While a large part of the avian vertebral column is fused, the cervical region is not, permitting a high degree of flexibility.

Although not often described, actinopterygian and chondrichthyan fishes show modifications of the anterior vertebrae. Nowroozi et al. (2012) described the morphological differences between the four anterior-most vertebrae and those in the remaining precaudal region in striped bass (*Morone saxatilis*); the first four vertebrae have a stouter shape than the rest of the precaudal vertebrae. In trumpet fishes (*Aulostomus* sp.), the first four vertebrae are elongate and fused with the transverse processes, forming a continuous shelf (Wheeler, 1955). Sygnathiformes also have modifications of the anterior-most vertebrae. The first 3–5 are highly elongated and fused (Pietsch, 1978). The Weberian apparatus in otophysan fishes, a large group including approximately 8000 species, is a modification of the first five anterior vertebrae that has often been suggested to be a major reason for the increased speciation of that group (Bird and Mabee, 2003; Nelson, 2006; Bird and Hernandez, 2009). Recently, Sallan (2012) described a tetrapodal-like cervical region for a fossil actinopterygian fish, *Tarasius*. Claeson and Hilger (2011) have described modifications of the anterior vertebral column in Squamitiformes, a lineage of chondrichthyan fishes. Given the recent evidence of modification of anterior vertebrae, fishes should be considered to have a cervical region just like the other groups of vertebrates.

Overall, it is likely that the functional biology of the cervical region will be increasingly important to consider when attempting to understand regionality across Vertebrata.

3.2. Thoracolumbar region

Technically, the thoracolumbar region is only present in mammals, with the thoracic region separated from the lumbar region by the presence of ribs (Romer, 1970). The vertebrae in this region have also been referred to as abdominal or dorsal. However, we consider all non-cervical precaudal and presacral/precaudal vertebrae to be members of this region. This region has also been referred to as the dorsal or the abdominal region (Romer, 1970). In tetrapods, the vertebrae in this region lie between the pectoral and pelvic girdles, when present (Goodrich, 1958). In most fishes, amphibians, and lepidosaurs there is little distinction between the various postcervical and precaudal vertebrae (Romer, 1970). Within these groups, the number of precaudal vertebrae can vary dramatically (Fig. 2). In birds, the lumbar vertebrae fuse with the sacral vertebrae to form the synsacrum. Turtles fuse the thoracolumbar vertebrae into the shell (Romer, 1970). Although lacking a complete carapace, the extinct parareptile *Eunotosaurus* demonstrates the transitions between typical slender ribs and the shell morphology of modern turtles (Lyson et al., 2013). Most mammals have 19 thoracolumbar vertebrae (Narita and Kuratani, 2005), although their number can range as high as 30.

3.3. Sacral region

True sacral vertebrae are only found in tetrapods. This region provides an important anchor for the pelvic girdle to transfer energy from the hindlimbs contacting the ground to the body axis. Frogs have a single sacral vertebra connected to a long urostyle, which is derived from several fused postsacral vertebrae. Jorgensen and Reilly (2013) demonstrated that size of the sacral diapophysis, which connects the sacrum to the elongated ilia, is a major predictor of locomotory mode, even more so than relative length of the hindlimbs. Birds have the greatest number of sacral vertebrae; here, the lumbar and sacral vertebrae are fused into the synsacrum (Gadow, 1933). The number of sacral vertebrae in mammals ranges from 2 to 9 (Narita and Kuratani, 2005).

3.4. Caudal region

The caudal region is the most posterior region of the vertebral column. In fishes, caudal vertebrae have fused hemal spines with the exception of the ural vertebra. In sarcopterygians, the caudal region is posterior to the sacral vertebrae (Gadow, 1933). The caudal region varies dramatically in vertebral number, especially in actinopterygian and chondrichthyan fishes (Fig. 2). There is much variation in caudal vertebral number in Actinopterygii, ranging from 9 in several members of the Tetraodontiformes (pufferfish and their allies) to over 200 in electric eels (*Electrophorus*). While the low end of the range is not present in Chondrichthyes, *Alopias* (thresher sharks) can have well over 200 caudal vertebrae (Ward and Brainerd, 2007). Sarcopterygii also have a wide range in caudal vertebral number although the maximum number of vertebrae does not come close to that of the former two lineages (Fig. 2).

Of all of the axial regions, the caudal region has likely experienced the greatest range of functional specializations; for example, the tail can be used for aquatic propulsion (both lateral and dorsoventral), for prehensibility, for support as an additional limb or for balance in cursoriality, and for defense mechanisms (caudal autotomy). Prehensibility is especially interesting as it is a behavior that has evolved in almost every vertebrate class (Liem et al., 2001). Investigating the caudal region in prehensile taxa would be particularly informative for understanding functional convergence as well as phylogenetic constraints on vertebral morphology.

4. Vertebral development

The adult vertebra develops from the paraxial mesoderm. Early in development, the paraxial mesoderm segments through a process called somitogenesis. Somites separate from the unsegmented paraxial mesoderm when genes from the Notch signaling pathway are activated in a group of cells, resulting in epithelialization of cells at the boundary of the segment. The periodicity of *notch* is partially set by its ability to activate an inhibitory protein (such as Mesp), which causes *notch* activity to cease. The inhibitory proteins are unstable and, once degraded, *notch* activity will continue (Gilbert, 2014). The period of this activity and of inhibition appears to be set within a species although there is evidence that this period is not constant throughout somitogenesis (e.g., Woltering et al., 2009; Gomez et al., 2008). For example, the first 5–6 somites in zebrafish form every 20 min. The remaining somites form every 30 min (Kimmel et al., 1995).

Ultimately somite formation occurs due to an interaction between “clock” (*notch*) and “wavefront” (*fgf*) genes. The wavefront genes are expressed in a gradient from posterior to anterior. The

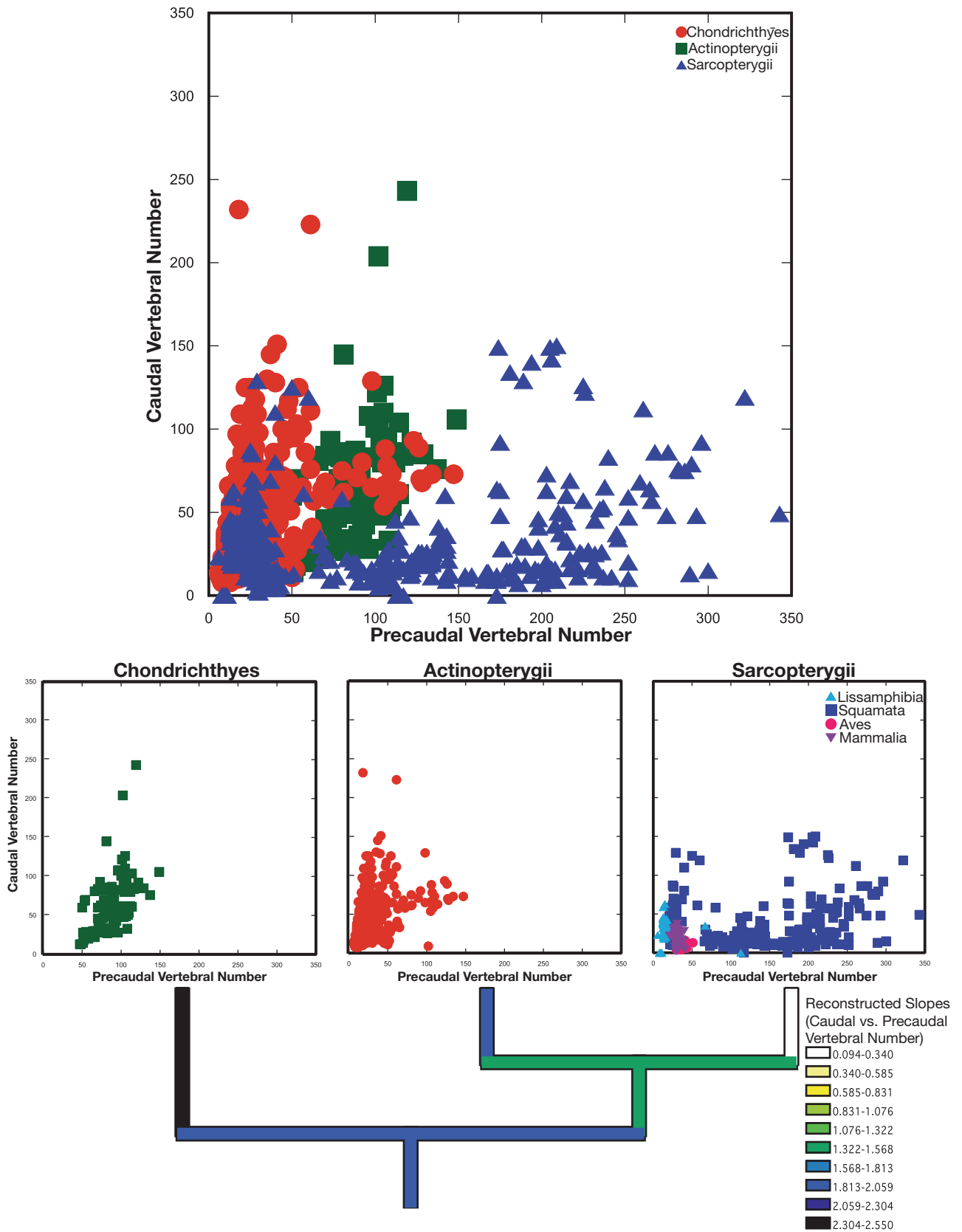


Fig. 2. Vertebral numbers were collected from the literature as well as from museum and personal collections. When a range was given, the maximal value was used. Numbers were only included if the number of vertebrae in each region was given. The precaudal region was defined as containing all vertebrae anterior to the caudal vertebrae including the cervical, thoracic, lumbar, and sacral regions (Collar et al., 2013). The anterior border of the caudal region was defined as the first vertebra with fused hemal spines (Actinopterygii, Chondrichthyes) or as the first post-sacral vertebra (Sarcopterygii). References that were used for the vertebral numbers as well as the species and vertebral numbers included can be found in Appendix A (supplementary online documents S1 and S2). The slopes from the regression analysis (caudal versus precaudal vertebral numbers) were traced onto the phylogeny by minimizing the sum of squares using MacClade 4.06. Darker bars (black and blue) indicate a relatively higher number of caudal vertebrae and lighter bars (white and yellow) indicate fewer caudal vertebrae in relation to precaudal vertebrae.

clock is not functional in areas of high *fgf* concentration. As the tail bud extends posteriorly, cells in the anterior presomitic mesoderm have a decreased *fgf* concentration and thus *notch* cycling can be activated (reviewed by Oates et al., 2012).

The number of somites could be altered by changing either the timing of the clock or the retreat speed of the wavefront. If the clock period increases, fewer somites will form, while each somite will be longer. The same effect would be caused by increasing the retreat speed of the wavefront (reviewed by Oates et al., 2012). However, in both of these cases the overall length of the axial skeleton will not differ. Gomez et al. (2008) demonstrated that axial elongation may occur through a change in the rate of somitogenesis relative to the rate of overall development. Corn snakes, for example, increase the number of vertebrae by increasing the rate of segmentation relative to the overall developmental rate (Gomez et al., 2008; Gomez and Pourquié, 2009).

Vertebrae develop from the sclerotomal portion of the somite. Zebrafish and amniotes go through a process of resegmentation by which the anterior part of the sclerotomal portion of a somite will join with the posterior portion of the next anterior somite, although zebrafish are considered to have “leaky” resegmentation (Morin-Kensicki et al., 2002; Dequéant and Pourquié, 2008). In anamniotes, the formation of vertebrae through resegmentation is more controversial (Buckley et al., 2013). For example, Wake and Lawson (1973) suggested that since salamanders and frogs have only a minimal sclerotome, resegmentation does not occur.

5. Genetic basis for establishing regionality

As discussed, the axial skeleton has varying levels of regionality, with each region delineated by a change in morphology (Goodrich, 1958; Fig. 1). The morphological differences that are associated with the different regions are thought to be due to *hox* expression domains that are set up during early development (Burke et al., 1995; Buchholtz, this issue). Despite the difference in the number of cervical vertebrae between chicks and mice, Burke et al. (1995) showed that the anterior expression domain of *hoxc6* always occurs at the transition between cervical and thoracic vertebrae.

The gene *hox10* is responsible for the transition between the rib-bearing thoracic region and the lumbar region, which contains vertebrae that do not bear ribs (Romer, 1970; Wellik and Capecchi, 2003). Despite this, in snakes *hox10* is expressed in rib-bearing somites which are the result of a single base pair change in the Hox binding site upstream of *myf5*, a gene required for rib formation (Guerreiro et al., 2013). Interestingly, this same polymorphism is seen in mammalian species that are characterized by longer ribcages due to additional thoracic segments (Guerreiro et al., 2013).

The transition between precaudal and caudal vertebrae also has a conserved expression pattern; this transition occurs at the anterior expression domain of *hoxd12* in chicks and mice as well as in zebrafish (Burke et al., 1995; van der Hoeven et al., 1996). In zebrafish, there is strong expression of *hoxd13* in the posterior hindgut. This might be important for setting up the transition between the precaudal and caudal regions by defining the end of the gut tube (van der Hoeven et al., 1996). In the caecilian *Ichthyophis kohtaoensis*, *hoxc13* is expressed at the boundary between the precaudal and caudal vertebrae (Woltering et al., 2009). It is currently unknown whether any *hox12* or *hox13* genes are expressed at the boundary between precaudal and caudal regions in chondrichthyans and lepidosaurs. Given the major distinction that occurs at this boundary, a better understanding of what controls this regional distinction will be critical for understanding axial patterning.

6. Evolution of vertebral number and axial patterning

Variation in vertebral number is one of the more striking differences in the axial skeleton of vertebrates. While a handful of studies have focused on the variation in vertebral numbers within specific vertebrate clades (Ward and Brainerd, 2007; McDowall, 2008; Mehta et al., 2010; Bergmann and Irschick, 2012), our interest here was to examine the diversity of vertebral numbers, specifically the relationship between precaudal and caudal vertebral numbers across Vertebrata. To do this, we collected regional vertebral numbers for over 1400 species of vertebrates. Vertebral numbers were either mined from the literature or collected from specimens borrowed from museums or private collections. Vertebral data were analyzed using reduced major axis (RMA) regression with variances set as equal (JMP 8; SAS Institute, Cary, NC, USA). What we found is that the relationship between precaudal and caudal vertebral numbers varies across the major clades. In all three groups (Chondrichthyes, Actinopterygii, and Sarcopterygii), there is a strong relationship between the numbers of precaudal and caudal vertebrae, although the slopes differ (Fig. 2). In actinopterygians and chondrichthyans, the slope of the relationship between precaudal and caudal vertebrae is >1 , indicating that increasing overall vertebral number occurs primarily through the addition of caudal vertebrae (95% confidence interval of slope; Chondrichthyes: 1.95–3.58, $R=0.54$; Actinopterygii: 1.75–2.22, $R=0.50$). In other words, when vertebral number increases, it tends to increase in the caudal region. In Sarcopterygii, the slope was significantly lower than in the other two groups (95% confidence interval: 0.07–0.12; $R=0.27$) indicating that increases in vertebral number tend to occur in the precaudal region (Fig. 2).

Thus, actinopterygian and chondrichthyan fishes tend to increase vertebral number in the caudal region whereas Sarcopterygii increase the number of precaudal vertebrae (Fig. 2). Based on our reconstruction, we hypothesize that the ancestral condition for vertebrates is to add caudal vertebrae, the character state of the common ancestor of gnathostomes (Fig. 2). The pattern of character state evolution is likely more complicated than what is shown in Fig. 2, however, because the most basal group of living actinopterygian fishes increases the number of precaudal vertebrae (Polypteriformes; Ward and Brainerd, 2007). There are also exceptions to this general trend within the sarcopterygian lineage; e.g., ichthyosaurs tend to have more caudal vertebrae (Buchholtz, 2001). Despite these exceptions, it is likely that different developmental processes control the axial patterning of actinopterygian and sarcopterygian species. Within the actinopterygian lineage, highly elongate body forms are probably due to changes in the regulation of the total number of vertebrae, in particular, control of axial or tail bud extension similar to the mechanism described for snakes (Gomez et al., 2008). In Sarcopterygii, there are likely two different mechanisms, (i) a *hox*-derived mechanism that affects placement of the boundary that defines the precaudal region and (ii) an axial elongation mechanism, which would affect the total number of vertebrae (Gomez et al., 2008; Ward and Mehta, 2010). What is unknown is whether fishes also possess a mechanism to grow the relatively large number of precaudal vertebrae seen in snakes. The greatest number of actinopterygian precaudal vertebrae in this study was 134 in *Callechelys melanotaenia*, a member of the Anguilliformes or true eels. This is significantly less than the typical 200+ precaudal vertebrae seen in snakes. It is possible that fishes have lost the ability to grow the long precaudal regions that are typical of snakes. Snipe eels (Anguilliformes) are the only clade reported to have over 600 vertebrae (Beebe and Crane, 1937). Snipe eels are extremely unusual as they are thought to add vertebrae throughout postnatal ontogeny. Whether these vertebrae are added to the precaudal, caudal, or both regions, is unknown.

7. Future research directions and questions

As with many data sets, this one also brings up several possible questions that can lead to exciting new avenues of research. Here are a few that we think should be considered:

- (i) While it is obvious that differential patterning occurs in actinopterygian and sarcopterygian lineages, we have yet to understand the developmental control required for building a longer precaudal region. Do actinopterygian fishes still maintain the ability to increase the precaudal region or has it been lost?
- (ii) The caudal region is often ignored in studies of tetrapod groups, in part due to the likelihood of damage to the posterior end of specimens. It is possible that there is greater range in the number of caudal vertebrae in sarcopterygians than has been reported here. What is the relationship between caudal vertebral number and functional specializations? Is there morphological convergence in the caudal region during repeated functional specializations across vertebrates?
- (iii) While we primarily discussed the broad patterns of axial regionalization in the different groups of vertebrates, there are exceptions such as in the squamate *Delma fraseri* (Pygopodidae) that has a relatively large number of caudal vertebrae relative to the precaudal vertebrae. Additionally, ichthyosaurs also have relatively large numbers of caudal vertebrae (Buchholtz, 2001). Which selective pressures have led to these exceptions?
- (iv) When an earlier version of Fig. 2 was initially published by Ward and Brainerd (2007), there was unfilled morphospace in the lower right quadrant, which is now filled by Sarcopterygii. The only remaining empty morphospace is in the upper right quadrant. Are there species that can be found in this area? If not, why are there no species in this region of morphospace; is this due to developmental constraints or to a lack of selection for equally large numbers of precaudal and caudal vertebrae?

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.zool.2013.10.006>.

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