

Evolution of the Vertebral Formulae in Mammals: A Perspective on Developmental Constraints

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ABSTRACT Developmental constraints refer to biases that limit phenotypic changes during evolution. To examine the contribution of developmental constraints in the evolution of vertebrate morphology, we analyzed the distribution pattern of mammalian vertebral formulae. Data on mammalian vertebral formulae were collected from the *Descriptive Catalogue of the Osteological Series Contained in the Museum of the Royal College of Surgeons of England* by Richard Owen (1853) and were plotted onto the most reliable mammalian phylogenetic tree based on recent molecular studies. In addition to the number of cervical vertebrae that is almost fixed to 7, we found that the number of thoracolumbar vertebrae tends to be 19 in many groups of mammals. Since fidelity of the number of thoracolumbar vertebrae was also completely maintained in Monotremata and Marsupialia, we presumed that thoracolumbar vertebral number as well as cervical vertebral number might have been fixed in the primitive mammalian lineage. On the basis of primitive vertebral formulae, we could clarify the polarity of evolution and identify several deviations from the primitive states during the mammalian evolution. The changes in the vertebral formulae in eutherian mammals seem to be lineage-specific, such that most species in Carnivora have 20 instead of 19 thoracolumbar vertebrae. Because such lineage-specific vertebral formulae contrast with the estimated distribution pattern on the assumption of evolution only through the selective pressure, we concluded that developmental constraints played an important role in the evolution of mammalian vertebral formulae. *J. Exp. Zool.* 304B:91–106, 2005. © 2005 Wiley-Liss, Inc.

INTRODUCTION

Evolution is most clearly perceived as changes in morphological patterns through phylogeny, and the changes are often hierarchically distributed among animals, conferring the hierarchy of taxonomy (see Hall, '94). For example, minor and rapid changes shared by a small number of species tend to be associated with microevolution or distinction of genera and species, whereas the major and slow changes in global patterns of the animal body are shared by a larger number of species and are likely to be associated with the differences between higher taxa. These changes are hierarchical: the minor changes can be established on a higher level of changes. The mammalian heterodonty, for example, could arise only after the appearance of undifferentiated dentition on the jaw, the derivative of the mandibular arch, which was once established as the rostralmost element of the pharyngeal arches in ancestral vertebrates (Romer and Parsons, '77; Hildebrand and Goslow, 2001; Kardong, 2002). Likewise, Prum ('99) proposed a hierarchical

model regarding the origin and evolution of feathers based on developmental pattern of bird feathers. Such a hierarchical series of changes is isomorphic to the nested patterns of synapomorphies that define monophyletic groups that are also nested along the phylogenetic tree.

Importantly, morphological stasis over a long period also helps us perceive the taxonomic hierarchy; that is, there are patterns unchanged through evolutionary radiations, and these patterns tend to be used as key characters to classify higher taxa. For example, all the land vertebrates are "tetrapods" except for a few (such as snakes) that lost their limbs secondarily after the diversification from the tetrapod ancestor (Romer and Parsons, '77; Colbert et al., 2001). Here an important question arises, as Raff ('96) has asked: why are

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there neither “hexapods” nor “octapods” but only tetrapods among land vertebrates—why are certain patterns conserved very rigidly through evolution?

As has been discussed and reviewed by many researchers, there are two possible answers to this question: stabilizing selection and developmental constraints (Maynard-Smith et al., '85; Alberch, '89; Arthur and Farrow, '99). In the stabilizing selection view, it is assumed that, in addition to tetrapods, hexapods and octapods may have been produced during evolution. However, the nontetrapod patterns were eliminated because they were less adaptive than tetrapods, resulting in all extant land vertebrates' being tetrapods. Thus, in this theory, various changes are assumed at the time of radiation and secondarily screened by selective pressures, leaving only a few surviving variants. In contrast, in the developmental constraint view, the production of phenotypic variations is assumed to be limited by certain unknown developmental restrictions: hexapod or octapod animals are supposed to be rarely produced because of limitations in the possible developmental patterns.

Although both of these concepts are important in evolutionary studies, few reports analyze developmental constraints. One example for developmental constraints involves the number of mammalian cervical vertebrae. The mammalian vertebral column consists of morphologically differentiated groups of vertebrae: cervical, thoracic, lumbar, sacral, and caudal (Gaunt, '94; Burke et al., '95) (Fig. 1A). The number of mammalian cervical vertebrae is almost constant at seven, irrespective of the neck length of different species—giraffes with long necks and whales with short necks both possess seven cervical vertebrae (Fig. 1), as has described in several classic textbooks (Owen, 1853, 1866; Remane, '36). In contrast, birds and reptiles often have variable numbers of cervical vertebrae (Owen, 1853, 1866; Remane, '36). This suggests that the number of mammalian cervical vertebrae has been almost fixed by certain mammalian-specific developmental constraints (Galis, '99b). Thus, the evolution of mammalian vertebral formulae may serve as a valuable model for studying the evolution of developmental constraints.

Specification of the vertebral column is developmentally regulated by the *Hox* code, a nested expression pattern of *Hox* genes along the anterior–posterior axis of the embryo. Genetic manipulation of the *Hox* genes can cause “morphological transformation” (Rosenberg, 1875; Goodrich, '30) of the vertebral column in the mouse (Carrasco

and Lopez, '94; Maconochie et al., '96). It has also been reported that differences in vertebral specification among animals correlate with differences in expression patterns of the *Hox* genes (Gaunt, '94; Burke et al., '95; Galis, '99a; Cohn and Tickle, '99). For example, in both mouse and chicken, the anterior expression boundary of *HoxC6* falls at the same morphological boundary between the cervical and thoracic region, even though the positions of the boundary lie at different axial levels in terms of the segmental number of somites: the seventh vertebra is the last cervical one in the mouse, whereas the 14th is the last cervical vertebra in the chicken. Thus, the homology of vertebrae (including the occipital bone) originates from the homology of *Hox* genes expressed at each level. This rule also applies to other nonmodel species of vertebrates (Burke et al., '95).

Apparently, the mechanism of somite segmentation also plays a crucial role in the development of vertebrae. It has been reported that disruption of some segmentation genes, such as *Delta 1* and *Wnt-3a*, leads to homeotic transformations of vertebrae (Ikeya and Takada, 2001; Cordes et al., 2004). Importantly, however, such transformations are always accompanied by changes in *Hox* gene expression patterns, which are tightly linked with somitogenesis per se: *Hox* gene expression is also cyclical as are other segmentation genes (Zakany et al., 2001).

DEVELOPMENTAL CONSTRAINTS AND THEIR ANALYSES

Developmental constraints are defined as biases on the production of phenotypic variants or limitations on phenotypic variability caused by the structure, character, composition, or dynamics of the developmental system (Maynard-Smith et al., '85). Although developmental constraints apparently play an important role in evolution, less attention has been paid to the concept than to selection theory. This might be due in part to the conceptual ambiguity as well as the lack of established methods to analyze them (Maynard-Smith et al., '85; Schwenk, '95; Arthur, '97; Schwenk and Wagner, 2003). Although some researchers counted only biases that originally limit the production of phenotypic variants during ontogeny as “real” developmental constraints (Gould, '89), from the currently available evidence it is difficult to clarify the underlying mechanism of evolutionary stasis and distinguish the “real constraints” from the effect of internal selection.

Richardson and Chipman (2003) mentioned that there is no clear line between constraints and selection and that, in many instances, developmental constraints result from selection acting at the embryonic stages. For example, behind the panmammalian constraint on the number of cervical vertebrae, a hypothetical mechanism has been suggested: Galis ('99b) has pointed out that cervical ribs would be associated with neurological disorders leading to thoracic outlet syndrome and that the increased appearance of cervical transformations in children with embryonal cancers. With this evidence, she has suggested that changes in the cervical vertebral number may be coupled with the neuronal problem and an increased risk for neonatal cancer because of the pleiotropic

function of the *Hox* gene. These might work against the survival of the individuals with cervical ribs as direct and indirect selections. Her conclusion is that the increased costs might have prevented the different numbers of cervical vertebrae being fixed in a population, thus establishing the mammalian-specific developmental constraint.

The above argument also favors the idea of structural constraints. For example, a highly complicated pattern whose components are structurally interrelated with each other is hard to modify because a small change in one element is apt to destroy the whole system. Likewise, an animal group classified by its specific body plan may possess its own structural integrity that has

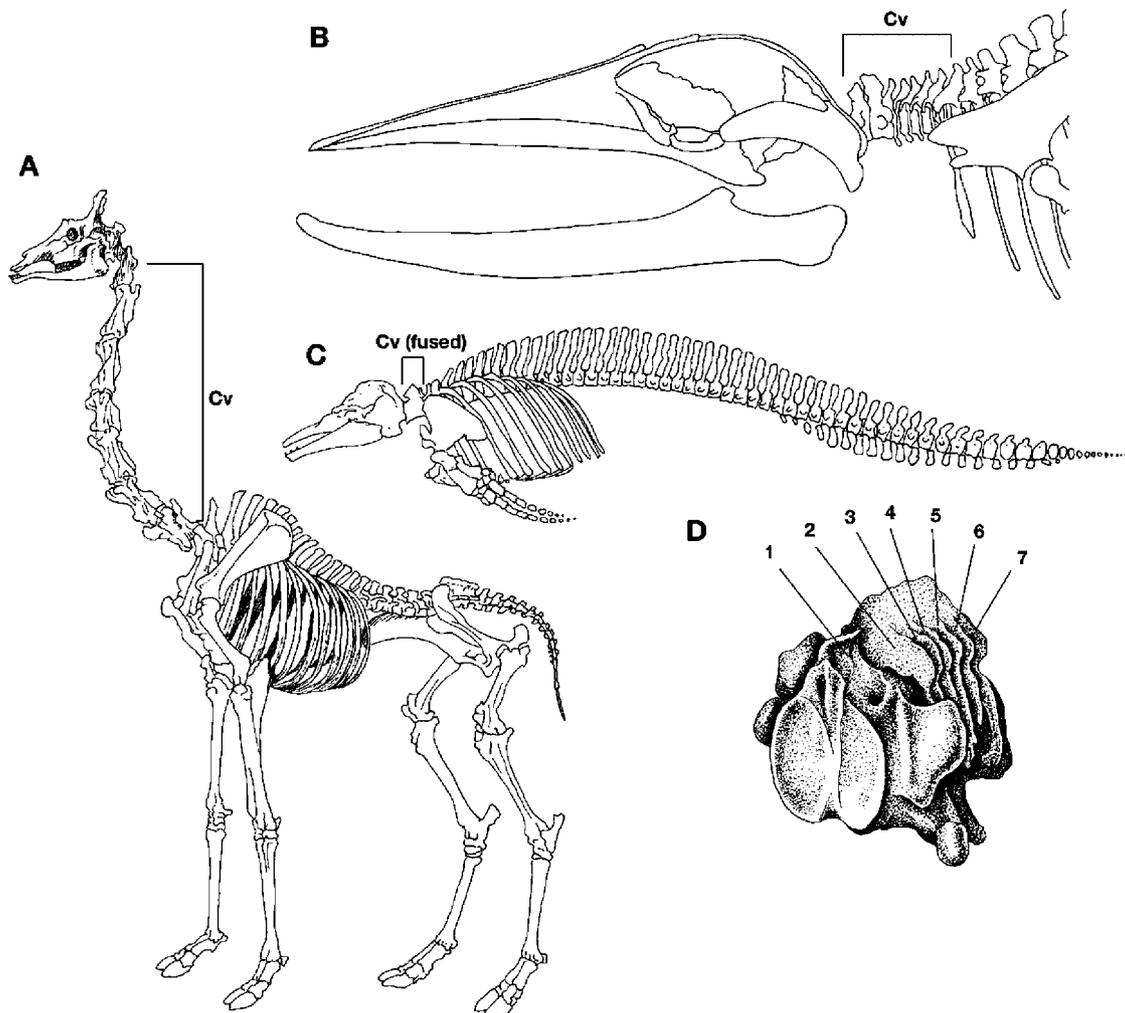


Fig. 1. Skeletons of giraffe and whales. (A) Skeleton of giraffe (*Giraffa camelopardalis*). Long neck of the giraffe consists of seven cervical vertebrae (Cv). Redrawn from Owen (1866). (B) Skeleton of a balaenopterid whale. Seven separate cervical vertebrae (Cv) are seen. Redrawn from Matthews ('68). (C) Skeleton of grampus (*Grampus griseus*). Cervical vertebrae are secondarily fused with each other to form a single bone. Redrawn from Howell ('30). (D) Magnification of the fused cervical vertebrae in the bowhead whale, *Balaena mysticetus*. Redrawn from Owen (1866). (1-7) Numbers of cervical vertebrae.

been conserved in a specific developmental context. In this article, we do not distinguish “real” constraints from those resulting from internal selection but define developmental constraints as any kinds of bias that act during embryogenesis, including the lack of variations because of a developmental limitation or the elimination of variant embryos by internal selection against them, thus leading to a group-specific evolutionary future (Spurway, '49).

One method of detecting the presence of constraints is through phylogenetic approaches (McKittrick, '93; Arthur and Farrow, '99). In this method, the distribution of both traits and possible selective pressure are plotted onto a phylogenetic tree to test the null hypothesis that the phylogenetic distribution of a trait is determined by selection. Some studies have used this approach to show the presence of constraints (Wagner and Müller, 2002; Richardson and Chipman, 2003). This approach is based on the assumption that developmental constraints should affect the relative frequency of transformations (Richardson and Chipman, 2003). In this study, we have used a similar approach to analyze the evolutionary pattern of mammalian vertebral formulae along the phylogenetic pathways and to analyze whether hierarchical pattern can be identified also in the evolution of the mammalian vertebral formulae. Instead of plotting the selective pressure onto the tree, we deduced the possible distribution patterns of vertebral formulae based on the evolution of other amniotes as well as the reported results of artificial selection experiments. Then, to analyze the contribution of developmental constraints in mammalian vertebral column evolution, we tested whether the observed evolutionary pattern could be brought about only through the effect of selective pressure, or not, by comparing the observed distribution patterns with the estimated possible patterns.

A prerequisite for this approach is a reliable phylogeny of the groups of interest. Traditionally, phylogenetic relationships among mammals have been inferred by morphology and paleontology. Novacek ('92) reviewed these studies and represented a morphology-based mammalian phylogenetic tree. Since then, molecular phylogenetic analyses have been carried out enthusiastically, and recently relationships among a majority of mammalian orders have been fairly settled (Janke et al., '96, '97; Cao et al., 2000; Murphy et al., 2001; Killian et al., 2001; Lin et al., 2002) although there are still some controversies between the

different approaches and thus some relationships still remain unclarified (Allard et al., '99; Cao et al., 2000). From these data, we obtained the phylogenetic tree of mammals that is currently the most reliable. We used this in analyzing the evolutionary pattern of the mammalian vertebral formulae.

EVOLUTION OF THE MAMMALIAN VERTEBRAL FORMULA

Primitive vertebral formula for mammals

We collected vertebral formula data for mammals (Table 1) from the *Descriptive Catalogue of the Osteological Series Contained in the Museum of the Royal College of Surgeons of England* by Richard Owen (1853). This catalogue contains data of 133 species from 15 orders of mammals and serves as a rich resource with which to survey the phylogenetic changes in developmental constraints. It should be noted that the catalogue includes only a limited data for individual variations within species: a certain amount of variation has been reported within some mammalian species based on more comprehensive analyses (Sawin, '37; Aimi, '94; Pilbeam, 2004), whereas in this catalogue, intraspecies variations were found only in 6 out of the 26 species from which more than two samples were examined (variations in caudal vertebral numbers are not counted). As an example of problematic cases in this catalogue, gorillas generally have 13 thoracic and 4 lumbar vertebrae (Pilbeam, 2004) instead of 12 thoracic and 5 lumbar vertebrae as Owen (1853) has described. In the latter case, however, the sum of thoracolumbar vertebrae remained 17, as generally found in the subspecies *Gorilla gorilla gorilla* (Pilbeam, 2004; see below). It may have been that the caudalmost ribs were lost in that particular specimen. Needless to say, further collection of vertebral formulae data will be desired for future study. Nevertheless, for several selected sample species, data were comparable to the known standard vertebral formulae elucidated by more recent studies (Sawin, '37; Aimi, '94; Burke et al., '95; Pilbeam, 2004). As will be shown below, numbers of thoracolumbar domain tended to be constant at the levels of families and orders. Thus the formulae described in this catalogue appeared trustworthy, to a certain extent, for the purpose of the present study, especially to gain a glimpse at the patterns of formulae changes on the mammalian phylogeny, which could be associated with the establishment of order-specific morphological plans.

TABLE 1. Vertebral formulae in mammals
 Vertebral formula data of 133 species from 15 mammalian orders were collected from the Descriptive Catalogue of the Osteological Series contained in the Museum of the Royal College of Surgeons of England. (Owen, 1853). Classification and scientific names for each species were updated based on Corbet and Hill ('86). The 'No.' column represents the serial number in the original catalogue for each specimen. The blank column represents lack of, or incomplete, data due to the condition of the specimen. The numbers separated by a slash mark (/) represent the vertebral formulae that differ among individuals. The cervical column is red when the number of cervical vertebrae is 7. The thoracic and lumbar columns are yellow when the sum is 19 and blue when the sum is 20.

| Order | Family | No. | Species | The number of vertebrae | | | | | |
|--------------|-------------------|---------------|-------------------------------------|----------------------------------|----------|--------|--------|----------|-------|
| | | | | Cervical | Thoracic | Lumbar | Sacral | Caudal | |
| Monotremata | Ornithorhynchidae | 1699 | <i>Ornithorhynchus anatinus</i> | 7 | 17 | 2 | | | |
| | Tachyglossidae | 1704 | <i>Tachyglossus auleatus</i> | 7 | 16 | 3 | 3 | 12 | |
| Marsupialia | Macropodidae | 1720 | Great kangaroo | | 13 | 6 | 2 | 20/22 | |
| | | 1740 | Bennett's kangaroo | 7 | 13 | 6 | 2 | 22 | |
| | | 1777 | <i>Macropus rufogriseus potaroo</i> | 7 | 13 | 6 | 2 | 24 | |
| | Vombatidae | 1792 | wombat | 7 | 15 | 4 | 4 | 10 | |
| | Phalangeridae | 1863 | <i>Vombatus ursinus</i> | 7 | 13 | 6 | 2 | 25 | |
| | Didelphidae | 1867 | <i>Phalanger sp.</i> | 7 | 13 | 6 | 1 | 17 | |
| | | 1873 | <i>Didelphis virginiana</i> | 7 | 13 | 6 | 2 | 16 | |
| | Peramelidae | Peramelidae | 1873 | <i>Perameles nasuta</i> | | 13 | 6 | 2 | |
| | | | 1880 | <i>Perameles sp.</i> | 7 | 13 | 6 | 2 | |
| | | | 1882 | <i>Myrmecobius fasciatus</i> | | 13 | 6 | 3 | 22 |
| Dasyuridae | | 1884 | <i>Antechinus flavipes</i> | 7 | 13 | 6 | 3 | 23 | |
| | | 1887 | <i>Antechinus sp.</i> | 7 | 13 | 6 | 2 | 20 | |
| Lagomorpha | | Leporidae | 1903 | <i>Dasyurus sp.</i> | 7 | 13 | 6 | 2 | 23 |
| | | | 1914 | <i>Thylacinus cynocephalus</i> | 7 | 12 | 7 | 3 | 16 |
| | | | 1949 | <i>Lepus timidus</i> | 7 | 12 | 7 | 4 | 10 |
| | | Caviidae | 1973 | <i>Oryctolagus cuniculus</i> | 7 | 13 | 6 | 2 | 8 |
| Rodentia | | Chinchillidae | 2001 | <i>Hydrochoerus hydrochaeris</i> | 7 | 13 | 6 | 4 | 5 |
| | 2008 | | <i>Cavia aperea</i> | 7 | 13 | 6 | 3 | 23 | |
| | Myocastoridae | 2039 | <i>Chinchilla lanigera</i> | 7 | 13 | 6 | 4 | 21 | |
| | | 2041 | <i>Myocastor coypus</i> | 7 | 13 | 6 | 4 | 9 | |
| | Dasyproctidae | | 2046 | <i>Agouti paca</i> | 7 | 13 | 6 | 4 | 14 |
| | | | 2075 | <i>Myoprocta acouchi</i> | 7 | 15 | 4 | 4 | 12 |
| | | | 2113 | <i>Hystrix cristata</i> | 7 | 14 | 5 | 3 | 15 |
| | | | 2157 | <i>Hystrix sp.</i> | 7 | 11 | 8 | 4 | 25 |
| | | | 2223 | <i>Castor canadensis</i> | 7 | 13 | 6 | 3 | 30/32 |
| | | | 2225-2226 | <i>Rattus rattus</i> | 7 | 12/13 | 7/6 | 3/4 | |
| | | 2238-2240 | <i>Rattus norvegicus</i> | 7 | 12 | 6/6/7 | 3/4/4 | 29/27/27 | |
| | | 2241 | <i>Mus musculus</i> | 7 | 13 | 6 | 2 | 28 | |
| Eulipotyphla | Hystricidae | 2242 | <i>Rattus fuscipes</i> | 7 | 13 | 7 | 2 | 30 | |
| | | 2243 | <i>Conilurus albipes</i> | 7 | 14 | 7 | 2 | 30 | |
| | Hydromyinae | 2246 | <i>Hydromys chrysogaster</i> | 7 | 13 | 7 | 4 | | |
| | | 2252 | <i>Bathyergus sp.</i> | 7 | 12 | 7 | 3 | 29 | |
| | Pedetidae | 2254 | <i>Pedetes capensis</i> | 7 | 12 | 7 | 3 | 23 | |
| | | 2261 | <i>Marmota marmota</i> | 7 | 12 | 7 | 3 | | |
| | Scuriidae | 2268 | <i>Marmota sibirica</i> | 7 | 12 | 7 | 4 | 21 | |
| | | 2388/2490 | <i>Sciurus vulgaris</i> | 7 | 15 | 5/6 | 4/3 | 11/14 | |
| | Erinaceidae | | <i>Erinaceus europaeus</i> | 7 | 15 | 5/6 | 4/3 | | |

TABLE 1. Continued

| Order | Family | No. | Species | The number of vertebrae | | | | | |
|------------|------------------|----------------|---------------------------------------|---------------------------------|----------|--------|--------|--------------------|----|
| | | | | Cervical | Thoracic | Lumbar | Sacral | Caudal | |
| Chiroptera | Soricidae | 2398 | <i>Sorex minutus</i> | 7 | 14 | 6 | 3 | 15 | |
| | Talpidae | 2402-2404 | <i>Talpa europaea</i> | 7 | 13 | 6 | 5 | 10 | |
| | Vespertilionidae | 2416 | <i>Vespertilio murinus</i> | 7 | 12 | 7 | 3 | | |
| | Pteropodidae | 2417 | <i>Pteropus sp.</i> | 7 | 14 | 4 | 6 | | |
| | Cetartiodactyla | Suidae | 3248 | <i>Sus scrofa</i> | 7 | 13/14 | 6/5 | 4 | |
| | | | 3361 | <i>Phacochoerus aethiopicus</i> | 7 | 13 | 5 | 2 | 23 |
| | Tayassuidae | | 3380 | <i>Tayassu pecari</i> | 7 | 14 | 6 | 5 | 6 |
| | | Hippopotamidae | 3404 | <i>Hippopotamus amphibius</i> | 7 | 15 | 4 | 6 | 13 |
| | Camelidae | | 3445 | <i>Camelus ferus</i> | 7 | 12 | 7 | 4 | |
| | | | 3482 | <i>Lama glama</i> | 7 | 12 | 7 | 4 | |
| Moschidae | | 3490 | <i>Moschus moschiferus</i> | 7 | 14 | 5 | 5 | 6 | |
| | Tragulidae | 3494 | <i>Tragulus meminna</i> ¹³ | 7 | 13 | 6 | 5 | 13 | |
| Cervidae | | 3495/3496 | <i>Tragulus javanicus</i> | 7 | 13 | 6 | 4 | 13 | |
| | | 3497 | <i>Alces alces</i> | 7 | 13 | 6 | 4 | 11 | |
| | | 3503 | <i>Rangifer tarandus</i> | 7 | 13 | 6 | 4 | 11 | |
| | | 3512-3514 | <i>Cervus dama</i> | 7 | 14 | 5 | 4/5 | 11 | |
| | | 3536/3537 | <i>Giraffa camelopardalis</i> | 7 | 13 | 6 | 4 | 11 | |
| | | 3617 | <i>Gazella dorcas</i> | 7 | 14 | 6 | 4 | 20 | |
| | | 3648 | <i>Hippotragus equinus</i> | 7 | 13 | 6 | 4 | 14 | |
| | | 3696 | <i>Tetracerus quadricornis</i> | 7 | 14 | 6 | 4 | 14 | |
| | | 3715 | <i>Capra hircus</i> | 7 | 13 | 6 | 4 | 12 | |
| | | 3736 | <i>Ovis aries</i> | 7 | 13 | 6 | 4 | 12 | |
| Giraffidae | | 3751 | <i>Ovis ammon</i> | 7 | 13 | 7 | 4 | 10 | |
| | | 3779 | <i>Connochaetes gnou</i> | 7 | 13 | 6 | 4 | 10 | |
| | | 3808 | <i>Bison bonasus</i> | 7 | 14 | 6 | 4 | 15 | |
| | | 3819 | <i>Bos taurus</i> | 7 | 14 | 5 | 4 | 17 | |
| | | 3825 | <i>Balaena sp.</i> | 7 | 13 | 6 | 5 | 21 | |
| | | 2444 | <i>Mesoplodon bidens</i> | 7 | 11 | | | | |
| | | 2479 | <i>Tursiopus truncatus</i> | 7 | 9 | | | 29 (post-thoracic) | |
| | | 2483 | <i>Delphinus delphis</i> | 7 | 13 | | | 40 (post-thoracic) | |
| | | 2489 | <i>Phocoena phocoena</i> | 7 | 15 | | | 48 (post-thoracic) | |
| | | 2509 | <i>Monodon monoceros</i> | 7 | 13 | | | 43 (post-thoracic) | |
| Primates | Monodontidae | 2521 | <i>Indri indri</i> | 7 | 12 | 9 | 4 | 9 | |
| | Indridae | 4631 | <i>Lemur sp.</i> | 7 | 12 | 7 | 3 | 21/27 | |
| | Lemuridae | 4635/4536 | <i>Callithrix jacchus</i> | 7 | 13 | 6 | 3 | 19 | |
| | Callitrichidae | 4663/4664 | <i>Saimiri sciureus</i> | 7 | 13 | 7 | 3 | 24 | |
| | Cebidae | | 4666 | <i>Cebus capucinus</i> | 7 | 13 | 6 | 3 | 23 |
| | | | 4670/4671 | <i>Cebus sp.</i> | 7 | 14 | 5 | 3 | 25 |
| | | 4672 | <i>Ateles belzebuth</i> | 7 | 14 | 4 | 3 | 31 | |
| | | 4687 | <i>Ateles paniscus</i> | 7 | 13 | 4 | 3 | 30 | |
| | | 4690 | <i>Mandrillus sphinx</i> | 7 | 12 | 6 | 4/3 | | |

| | | | | | | | | |
|----------------|-----------|---------------------------------|---------------------|---|----|---|--------------------|----|
| | 4826 | <i>Maccaca nemestrina</i> | pig-tailed macaque | 7 | 12 | 7 | 3 | 17 |
| | 4990-4992 | <i>Maccaca mulatta</i> | rhesus monkey | 7 | 12 | 7 | 3 | 15 |
| | 4996 | <i>Macaca radiata</i> | bonnet macaque | 7 | 12 | 7 | 3 | 22 |
| | 5004 | <i>Presbytis entellus</i> | Hanuman langur | 7 | 12 | 7 | 2 | 25 |
| | 5009 | <i>Cercopithecus sp.</i> | <i>guenon</i> | 7 | 12 | 7 | 1 | 28 |
| Hylobatidae | 5026 | <i>Hylobates sp.</i> | <i>gibbon</i> | 7 | 13 | 5 | 5 | 2 |
| | 5027 | <i>Hylobates lar</i> | white-handed gibbon | 7 | 13 | 5 | 5 | 2 |
| | 5031 | <i>Hylobates syndactylus</i> | siamang | 7 | 13 | 4 | | |
| Pongidae | 5050 | <i>Pongo pygmaeus</i> | orang-utan | 7 | 12 | 5 | 5 | 3 |
| | 5082-5085 | <i>Pan troglodytes</i> | chimpanzee | 7 | 13 | 4 | 6 | 2 |
| | 5187 | <i>Gorilla gorilla</i> | gorilla | 7 | 12 | 5 | 5 | |
| | | <i>Homo sapiens</i> | human | 7 | 12 | 5 | 5 | 3 |
| Carnivora | 3860 | <i>Odobenus rosmarus</i> | walrus | 7 | 14 | 6 | 3 | 9 |
| Odobenidae | 3961 | <i>Phoca groenlandica</i> | harp-seal | 7 | 15 | 5 | 4 | 8 |
| Phocidae | 3979 | <i>Thalartctos maritimus</i> | polar bear | 7 | 15 | 6 | 5 | |
| Ursidae | 4013 | <i>Ursus americanus</i> | american black bear | 7 | 14 | 6 | | |
| Procyonidae | 4051/4054 | <i>Procyon lotor</i> | raccoon | 7 | 14 | 6 | 3 | |
| | 4064 | <i>Nasua sp.</i> | <i>coati</i> | 7 | 13 | 6 | 2 | |
| | 4085 | <i>Potos flavus</i> | kinkajou | 7 | 14 | 6 | 2 | 31 |
| Mustelidae | 4091/4092 | <i>Taxidea taxus</i> | American badger | 7 | 15 | 5 | 3 | 18 |
| | 4111 | <i>Mydaus javanensis</i> | Javanese skunk | 7 | 14 | 6 | 3 | |
| | 4112 | <i>Mellivora capensis</i> | ratel | 7 | 14 | 4 | 4 | 15 |
| | 4168 | <i>Martes zibellina</i> | sable | 7 | 14 | 6 | 3 | 18 |
| | 4188/4189 | <i>Mustela erminea</i> | ermine | 7 | 14 | 6 | 2 | 18 |
| | 4193 | <i>Mustela nivalis vulgaris</i> | common weasel | 7 | 14 | 6 | 2 | 18 |
| Viverridae | 4233/4234 | <i>Lutra sp.</i> | <i>otter</i> | 7 | 14 | 6 | 3 | |
| | 4264 | <i>Viverra civetta</i> | civet | 7 | 14 | 6 | 3 | |
| | 4283 | <i>Genetta sp.</i> | <i>genette</i> | 7 | 13 | 7 | 3 | 28 |
| | 4285 | <i>Paradoxurus sp.</i> | <i>palm civet</i> | 7 | 13 | 7 | 3 | 33 |
| Herpestidae | 4306 | <i>Herpestes ichneumon</i> | Egyptian mongoose | 7 | 13 | 7 | 2 | 27 |
| | 4326 | <i>Suricata suricatta</i> | suricate | 7 | 13 | 7 | 2 | |
| Canidae | 4328 | <i>Vulpes sp.</i> | <i>fox</i> | 7 | 15 | 6 | 3 | |
| | 4364 | <i>Canis lupus</i> | Arctic wolf | 7 | 13 | 7 | 3 | 22 |
| | 4389 | <i>Canis dingo</i> | Dingo | 7 | 13 | 7 | 3 | 15 |
| Hyaenidae | 4446 | <i>Crocuta crocuta</i> | spotted hyaena | 7 | 15 | 7 | 4 | 19 |
| Felidae | 4475 | <i>Panthera leo</i> | lion | 7 | 13 | 7 | 3 | 23 |
| | 4505 | <i>Panthera tigris</i> | tiger | 7 | 13 | 7 | 3 | |
| | 4585 | <i>Panthera pardus</i> | leopard | 7 | 13 | 7 | 3 | 23 |
| | 4608 | <i>Felis catus</i> | cat | 7 | 13 | 7 | 2 | 22 |
| Perissodactyla | 2866 | <i>Tapirus indicus</i> | Malayan tapair | 7 | 18 | 5 | 6 | |
| Tapiridae | 2933 | <i>Dicerorhinus sumatrensis</i> | Sumatran rhinoceros | 7 | 19 | 3 | 4 | 22 |
| Rhinocerotidae | 3133 | <i>Equus caballus</i> | horse | 7 | 19 | 5 | 5 | 17 |
| Equidae | 3214 | <i>Equus zebra</i> | mountain zebra | 7 | 18 | 6 | 5 | 17 |
| Procaviidae | 3094-3499 | <i>Procavia capensis</i> | cape hyrax | 7 | 22 | 8 | 11 (sacral+caudal) | |
| | 3118-3123 | <i>Dendrohyrax arboreus</i> | southern tree hyrax | 7 | 21 | 7 | 14 (sacral+caudal) | |
| Hyracoidea | 2544 | <i>Dugon dugon</i> | dugong | 7 | 19 | | 37 (post-thoracic) | |
| Procaviidae | 2647 | <i>Trichechus manatus</i> | manatee | 6 | 17 | | 25 (post-thoracic) | |

TABLE 1. Continued

| Order | Family | No. | Species | The number of vertebrae | | | | | |
|-------------|-----------------|------|--------------------------------|-------------------------|----------|--------|--------|--------|--|
| | | | | Cervical | Thoracic | Lumbar | Sacral | Caudal | |
| Proboscidea | Elephantidae | 2654 | <i>Elephas maximus</i> | 7 | 20 | 3 | 3 | 31 | |
| | Orycteropodidae | 2336 | <i>Orycteropus afer</i> | 7 | 13 | 8 | 6 | 25 | |
| Tubidentata | Dasypodidae | 2290 | <i>Dasypus sp.</i> | 7 | 11 | 3 | 9 | 16 | |
| | | 2296 | <i>Dasypus novemcinctus</i> | 7 | 10 | 5 | 8 | 16 | |
| Xenarthra | Myrmecophagidae | 2365 | <i>Tamandua sp.</i> | 7 | 17 | 2 | 5 | 37 | |
| | | 2366 | <i>Myrmecophaga tridactyla</i> | 7 | 15 | 3 | 5 | | |
| | Bradypodidae | 2367 | <i>Bradypus tridactylus</i> | 9 | 16 | 3 | 6 | 11 | |
| | | 2387 | <i>Choloepus didactylus</i> | 7 | 23 | 3 | 8 | 4 | |
| | | | Asiatic elephant | | | | | | |
| | | | aardvark | | | | | | |
| | | | armadillo | | | | | | |
| | | | nine-banded armadillo | | | | | | |
| | | | collared anteater | | | | | | |
| | | | great anteater | | | | | | |
| | | | pale-throated three-toed sloth | | | | | | |
| | | | Linne's two-toed sloth | | | | | | |

The vertebral numbers were plotted on a phylogenetic tree of mammalian orders based mainly on recent molecular phylogenetic analyses (Janke et al., '96, '97; Cao et al., 2000; Murphy et al., 2001; Killian et al., 2001; Lin et al., 2002). There is a clear tendency for the number of cervical vertebrae in mammals to be seven. Deviations from this number are identified in only two lineages: the manatee (*Trichechus manatus*, Sirenia), which has six cervical vertebrae, and the three-toed sloth (*Bradypus tridactylus*, Xenarthra), which has nine (Table 1, Fig. 2). These animals do not seem to represent the state before the fixation of the cervical number. Rather, the fixation of seven as the common number of cervical vertebrae appears to have occurred near the common ancestor of mammals, including monotremes (Fig. 2; Owen, 1853).

In contrast to the fixed number of cervical vertebrae, the numbers of thoracic, lumbar, sacral, and caudal vertebrae fluctuate among mammalian orders and families (Table 1). As functionally important traits are likely to be highly canalized to exhibit greater developmental stability (Hallgrímsson, 2003), the higher degree of variation in the postcervical vertebral number implies either that there are looser functional restrictions on these traits and thus less canalization of the developmental process or that functionally optimal numbers of these vertebrae frequently changed through evolution.

Interestingly, however, we found that the sum of thoracic and lumbar vertebrae tends to be 19 in many groups of mammals (Fig. 2). This finding is consistent with previous preliminary reports, which have implied that the number of thoracolumbar vertebrae tends to remain fairly constant within some eutherian orders (Owen, 1853; Romer and Parsons, '77). It has been reported that the earliest known eutherian mammal, *Eomania*, also had 19 thoracolumbar (13 thoracic and 6 lumbar) vertebrae (Ji et al., 2002). Furthermore, this number was completely constant in monotremes and marsupials (Table 1). In contrast, the number of trunk vertebrae in reptiles and birds, which correspond to mammalian thoracolumbar vertebrae, is highly variable among species (Owen, 1853) (data not shown). Based on the constancy not only in the eutherians but also in these two relative groups, we presumed that the number of thoracolumbar vertebrae as well as the cervical number might have already been fixed in the primitive mammalian lineage as plesiomorphic traits for mammals. On the basis of the

mammalian primitive vertebral formulae, we could clarify the polarity of evolution and identify several deviations from the primitive states during the mammalian evolution.

As compared to the relatively fixed number of thoracolumbar vertebrae, the respective numbers of thoracic and lumbar vertebrae tend to change. Some intraspecies variations seem to have re-

sulted from “developmental trade-off” between the thoracic and lumbar vertebrae to maintain the sum of them at 19, as seen in the rat (*Rattus norvegicus*, Rodentia; Table 1) and wild boar (*Sus scrofa*, Cetartiodactyla; Table 1). However, there are exceptions, such as the house mouse (*Mus musculus*, Rodentia) and the western European hedgehog (*Erinaceus europaeus*, Eulipotyphla), in

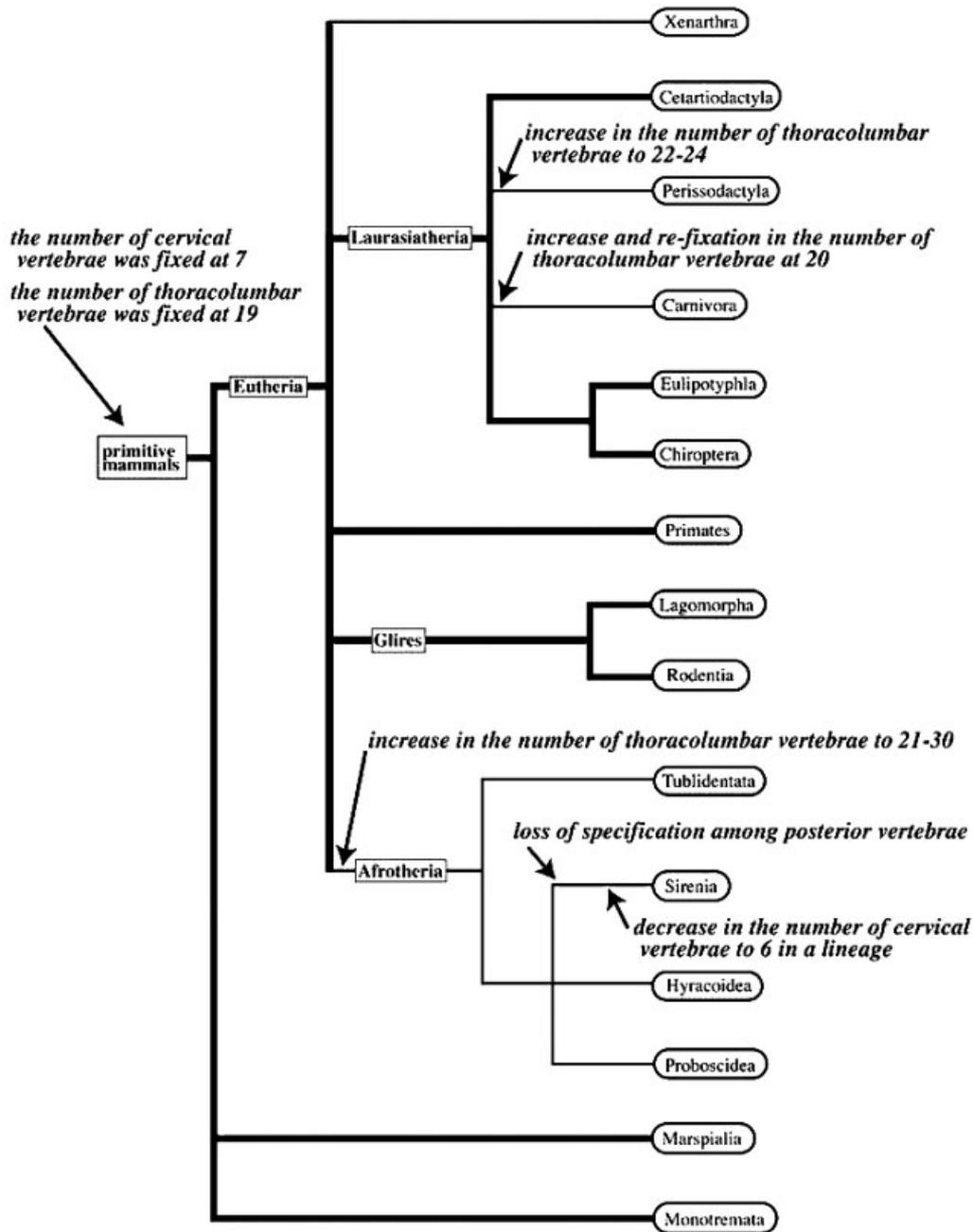


Fig. 2. Distribution of mammalian vertebral formulae along the phylogeny. The vertebral formula of each animal was plotted onto a mammalian phylogenetic tree based mainly on recent molecular analyses (Janke et al., '96, '97; Cao et al., 2000; Murphy et al., 2001; Killian et al., 2001; Lin et al., 2002). The branches with bold lines represent the lineages that retain the primitive vertebral formula, and those with thin lines represent deviation from the primitive state.

which the number of lumbar vertebrae varies in the species whereas the number of thoracic vertebrae remains constant. The sum of these two types of vertebra appears to be highly canalized, but the segmental level of transition between the two vertebral identities is not and is more likely to fluctuate in both development and evolution.

The distribution pattern of changes in the number of thoracolumbar vertebrae seems to favor the idea of developmental constraint-based stasis: the changes in the vertebral formulae in eutherian mammals do not seem to occur in each species randomly but are limited to certain taxa, allowing us to locate the evolutionary events at specific segments or branches in the phylogenetic tree (Fig. 2). In turn, there appear to be lineage-specific developmental constraints that cannot easily be overcome, as members of the group radiate with the same basic body plan (which does not necessarily include the vertebral formula *per se*) shared by the group. We further discuss the contribution of developmental constraints in mammalian vertebral formulae in “Selection or constraints?” below.

Deviation from the primitive formula

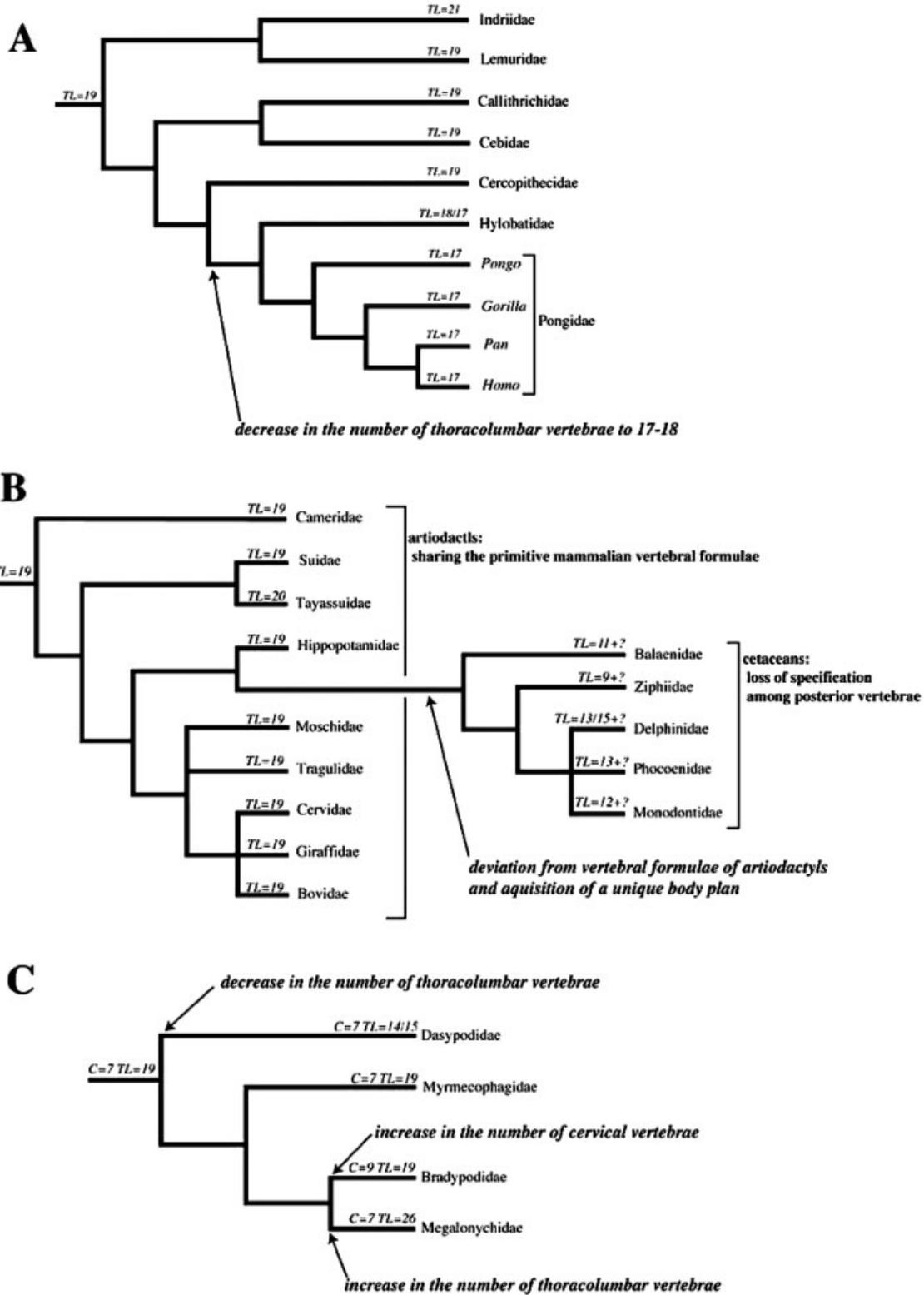
One group that shows deviation from the primitive state is Carnivora. In this group, most of the species that we analyzed possess 20 instead of 19 vertebrae in the thoracolumbar region (Table 1). We predict that the common ancestor of carnivores acquired a new developmental program to specify the vertebral column, and this has been maintained during the radiation of Carnivora (Fig. 2). In contrast, in Primates, a secondary decrease in the number of thoracolumbar vertebrae to 17 or 18 can be detected in the hominoid lineage (Hylobatidae + Pongidae) (Fig. 3A).

Deviation from the primitive formula can also be identified in a sublineage of Cetartiodactyla (Fig. 3B). Recent phylogenetic studies have resolved the relationships between cetaceans and

artiodactyls by analyzing insertion patterns of short and long interspersed elements (Nikaido et al., '99), and it is now generally accepted that cetaceans are deeply nested within the artiodactyls. Despite the phylogenetic position of cetaceans, their vertebral formulae deviate from the primitive formula shared by all the artiodactyls: cetaceans have lost specification of the vertebral column at the post-thoracic level. A similar loss of specification in posterior vertebrae is also encountered in sirenians. These deviations from the primitive formula may be related to the fact that these two groups are aquatic and possess body plans unique to these taxa. It is interesting that the pinnipeds (walrus and seals) retain the Carnivora-specific formula, although these animals have also adapted to an aquatic niche. The difference seems to be related to the fact that the pelvic girdle is still attached to the vertebral column in pinnipeds but is detached in the other two groups. The lack of evidence of convergence between the vertebral columns of pinnipeds and those of cetaceans and sirenians might oppose the selection concept and supports the contribution of developmental constraints in the mammalian vertebral column evolution.

An outstanding increase in the number of thoracolumbar vertebrae is found in Afrotheria. This superordinal clade was suggested by recent molecular phylogenetic analyses (Stanhope et al., '98; Cao et al., 2000; Murphy et al., 2001) and includes six mammalian orders: Hyracoidea (hyraxes), Sirenia (dugongs and manatees), Proboscidea (elephants), Tubulidentata (aardvarks), Afrosoricida (golden moles and tenrecs), and Macrocelidea (elephant shrews). We analyzed the vertebral formulae of the first four orders. Tubulidentata, which is thought to have diverged from other afrotherians first, has a slightly increased number of thoracolumbar vertebrae (21). However, the other orders show a dramatic increase in thoracolumbar vertebrae, and the cape hyrax (Hyracoidea) has 30 thoracolumbar

Fig. 3. Deviation from the primitive mammalian vertebral formula. C and TL represent the number of cervical and thoracolumbar vertebrae, respectively. (A) Evolution of vertebral formulae in Primates. Primate phylogenetic relationship was based on Purvis ('95) and Shoshani et al. ('96). Note that the number of thoracolumbar vertebrae was decreased specifically in hominoid lineage (Hylobatidae + Pongidae). (B) Evolution of vertebral formulae in Cetartiodactyla. Phylogenetic relationships among cetaceans and between artiodactyls and cetaceans were based on SINE (short interspersed element) and LINE (long interspersed element) insertion patterns (Nikaido et al., '99) as well as molecular phylogenetic analyses (Cao et al., 2000; Murphy et al., 2001; Lin et al., 2002). Internal relationships among ruminants were based on Beintema et al. (2003). Note that, although cetaceans are deeply nested within artiodactyls, the vertebral formula of the cetacean deviates from those of artiodactyls and constitutes a unique body plan. (C) Evolution of vertebral formulae in Xenarthra. Phylogenetic relationships among xenarthrans were based on Delsuc et al. (2002). Note that vertebral formulae of this group have secondarily deviated from the primitive state in many directions.



vertebrae. The exact number of thoracolumbar vertebrae in sirenians could not be counted because of the loss of specification in the posterior

vertebral column. Nevertheless, comparison with related animals permits us to speculate that the number of sirenian thoracolumbar vertebrae

would be much larger than 19, judging from the remarkable increase in their thoracic vertebrae (17 in manatee and 19 in dugong). Similarly, an increase in the number of thoracolumbar vertebrae can be identified in perissodactyls (22–24). However, these are likely to be independent evolutionary events, because Perissodactyla belongs to Laurasiatheria, which is distantly related to the lineages in Afrotheria.

In Xenarthra, the number of thoracolumbar vertebrae tends to vary (Fig. 3C). Armadillos (Dasypodidae), for example, have a reduced number of thoracolumbar vertebrae (14–15), whereas the two-toed sloth (Megalonychidae) retains as many as 26 vertebrae in the same region. Furthermore, in the three-toed sloth (Bradypodidae), the number of cervical vertebrae has increased to nine. Such a wide variation in the xenarthran vertebral formulae implies that the primitive mammalian vertebral formulae might have been canceled specifically in this monophyletic group, suggesting a possibility that release from the primitive formula also takes place group-specifically. An analogous situation can be found in lineages of birds and reptiles other than turtles (turtles constantly possess eight cervical and ten thoracic vertebrae).

This study also showed that rodents maintained the primitive feature of mammalian vertebral formulae, in which the numbers of cervical and thoracolumbar vertebrae are fixed at 7 and 19, respectively (Table 1, Fig. 2), although they are thought to be a highly derived lineage among mammals. Therefore, the phylogenetic position of a group is not always consistent with, or correlated with, the primitiveness of its body plan (known as mosaic evolution). The latter rather depends on the changes brought into its developmental program.

DEVELOPMENTAL CONSTRAINTS AND THE VERTEBRAL FORMULA

Selection or constraints?

Could the lineage-specific fixation and changes in vertebral numbers be explained through the taxon- or lineage-specific selections that would lead to the invariable number of certain vertebrae? This possibility deserves to be tested. In the following discussion, we define “group-specific selection” as the selective pressure that may be caused by a common morphological feature or habitat of the group and leads the group to the

changes in axial morphological traits, such as the relative length of the neck. If the group-specific vertebral formula has arisen through group-specific selection, would the changes in axial morphological traits always correlate directly with the changes in vertebral formulae or not correlate with the formulae at all?

In nonmammalian amniotes, such a correlation can be encountered in some cases, as is typically seen in the evolution of plesiosaurs (Fig. 4A–C). This group (superfamily, Plesiosauroidea) seems to have originated from nothosaurs in the middle Triassic, which had necks with about 17 cervical vertebrae (Fig. 4A) (Carroll and Gaskill, '85). The primitive plesiosaurs in the late Jurassic acquired longer necks by an increase of cervical vertebrae up to 30 (Fig. 4B) (Andrews, '10; Brown, '81), and more specialized species, such as elasmosaurid in the late Cretaceous, had extremely long necks with as many as 70 cervical vertebrae (Fig. 4C) (de Saint-Seine, '55; Ginsburg, '70). Thus, in this group, the elongation of the neck synchronizes with an increased number of cervical vertebrae; the external morphology is in parallel with the changes in vertebral formulae.

However, a change in axial morphological traits does not necessarily associate with changes in the vertebral formulae in other situations, such as artificial selection. For example, Rutledge et al. ('74) analyzed the effect of experimental selection for increased tail length using an inbred strain of mice. After seven generations of selection, the researchers produced two different lines of mice with longer tails than the nonselected lines. Although the two lines did not differ in tail length, they did differ in the anatomy of elongated tails: in one line, the number of caudal vertebrae had increased, whereas in the other, the number of caudal vertebrae remained the same as in the nonselected lines but the length of each caudal vertebra had increased. Thus, the developmental bases for increased tail length clearly differ between these two lines. The result suggests that the genome can potentially respond to the same selective pressure by at least two different pathways of development.

It is very likely that such potential plasticity in developmental mechanism also played important roles in phylogenetic evolution. Similarly to the two different selection responses toward tail elongation, another pattern of neck evolution is found in the Mesozoic aquatic reptiles. For example, the necks of *Tanystropheus* from the early to middle Triassic (Fig. 4D) had elongated by

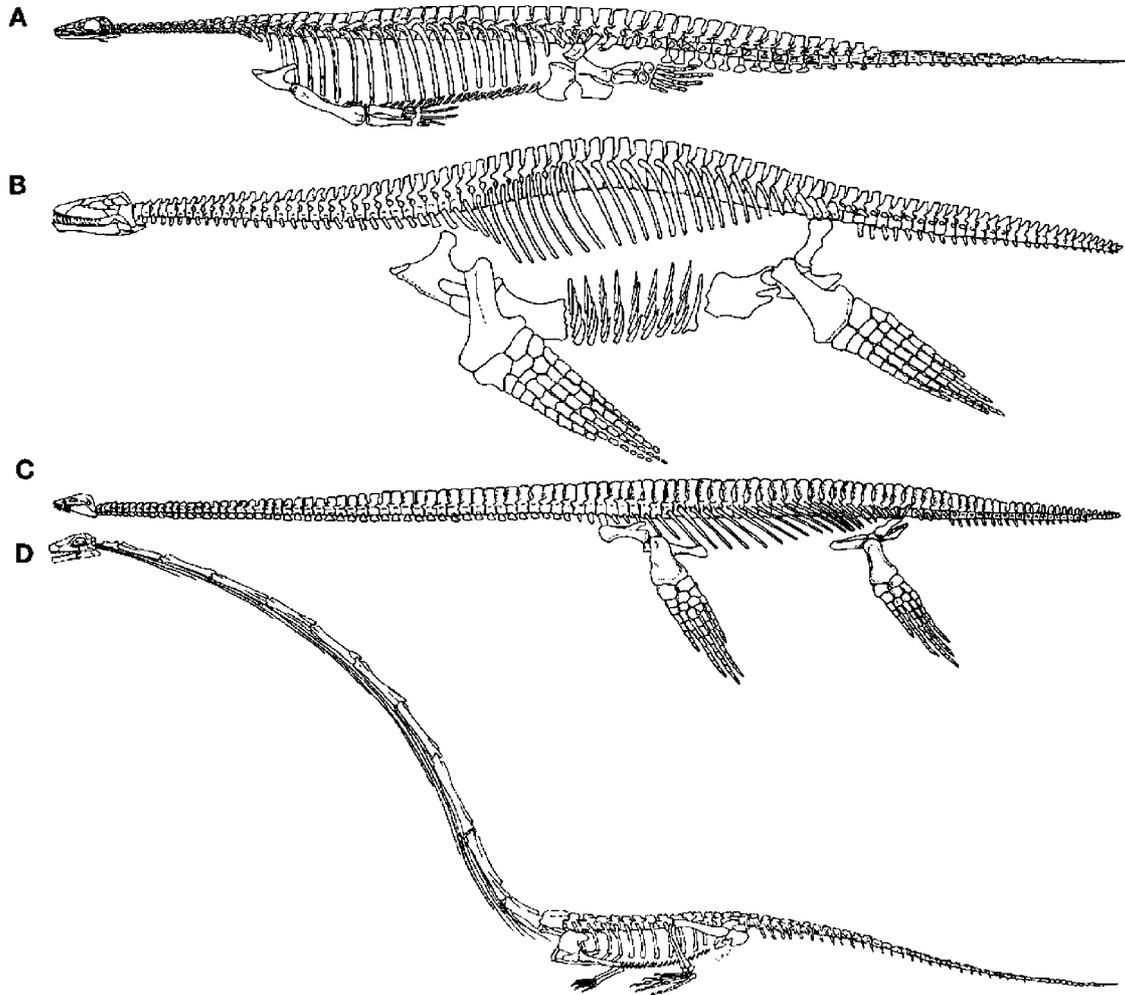


Fig. 4. Skeletons of Mesozoic aquatic reptiles. (A) Skeleton of nothosaur, *Pachypleurosaurus*. Redrawn from Carroll and Gaskill ('85). (B) Skeleton of the plesiosauroid *Cryptoclidus*. Redrawn from Andrews ('10). (C) Skeleton of elasmosaurid *Hydrothecrosaurus*. Redrawn from de Saint-Seine ('55). (D) Skeleton of *Tanystropheus*. Redrawn from Wild ('73). Note that the number of cervical vertebrae increased with neck elongation in the first three species. In *Tanystropheus*, however, the length of each cervical vertebra increased.

increasing the length of each cervical vertebra (Peyer, '31; Wild, '73); unlike in plesiosaurs, the number of cervical vertebrae remained around 10. The method of neck elongation reminds us of a presumed analogous developmental response in the emergence of the giraffe (Fig. 1A). Such different modes of changes in vertebral formulae have also been reported in a group of salamander that acquired an elongated trunk during evolution (Parra-Olea and Wake, 2001).

Overall, these evidences indicate that, during the evolution of body plans in amniotes, the developmental program can potentially respond to the same selection pressure in at least two different modes. In other words, the developmental mechanism in response to a specific selection

pressure can have a wider range of variations than the equally adaptive patterns of phenotypes required by the selection. Therefore, even though we accept the existence of "group-specific selection," we can expect that selective pressure may still create varied numbers of vertebrae and keep changing together with the axial body plans throughout the evolution of that lineage. The expected distribution pattern of vertebral formulae, which was estimated on the assumption of the null hypothesis that the evolutionary pattern of mammalian vertebral formulae is determined only by selective pressures, contrasts with the observed evolutionary pattern of the mammalian vertebral column. Therefore, it is likely that developmental constraints played

important roles in mammalian vertebral formulae evolution and gave rise to the group-specific vertebral formulae.

Evolutionary patterns of vertebral formulae in marsupials also suggest the presence of developmental constraints. As an apt illustration for convergence or parallel evolution, marsupials have radiated into diversified morphologies adapted to distinct habitats in a pattern similar to that in eutherian mammals. If the evolution of vertebral formulae tends to change under taxon-specific selection toward the taxon-specific habitat and morphology (which should be based on the same specific vertebral formulae), the constant vertebral formulae in marsupials and variable vertebral formulae in placentals could not be reconciled. Thus, the stasis in the vertebral formulae of marsupials contrasts with the expected level of variation based on the assumption of selection. It is again much more appropriate to consider that the stasis has occurred as a result of marsupial-specific (and mammalian primitive) developmental constraints that could not be overcome in the radiation of marsupial lineages.

Evolutionary events on hypothetical developmental constraints identified in the present study seem to appear hierarchically along the phylogenetic evolution (Figs. 2 and 3). However, the evolutionary changes of vertebral formulae never constitute a hierarchy by themselves. Instead, the primitive vertebral formulae are often secondarily cancelled, and a new taxon can evolve by acquiring a new body plan based on a new vertebral formula. For instance, cetaceans deviated from the primitive vertebral formulae that was shared by artiodactyls and acquired a unique body plan (Fig. 3B). We cannot view the cetacean vertebral formula as a sophisticated version of the artiodactyl formula. Therefore, evolutionary patterns of the mammalian vertebral column do not exhibit hierarchical constitution and do not imply polarity of evolution per se but are more like the description of body plans in taxonomy.

Evolution of the Hox code in mammals

It may be possible to regard the evolutionary history of developmental constraints on mammalian vertebral formulae as the trail of *Hox* code evolution in mammals since differences in vertebral specification are thought to be caused by the changes in *Hox* codes (Gaunt, '94; Burke et al., '95; Galis, '99a; Cohn and Tickle, '99). For instance, the decrease in cervical vertebral number in the

manatee may have been brought about by an anterior shift of the *HoxC6* expression boundary. Further, triple knockout of *Hox11* paralogs in the mouse results in the transformation of sacral and some caudal vertebrae into the lumbar vertebrae; i.e., complete loss of specification in the posterior vertebral column (Wellik and Capecchi, 2003). The latter mutant may be viewed as a possible phenocopy of cetaceans and sirenians in both evolutionary and developmental senses. Thus, the unique body plans of these animals may be brought about either by their own changes in *Hox11* paralogs or their regulation, or the same phenotype could have been obtained through modification of the post-*Hox* code programs in development.

Furthermore, some reports have recently suggested the possibility that divergence in *cis*-regulatory elements of *Hox* genes would have played a critical role in changes of *Hox* gene expression pattern among the animals (Belting et al., '98). With regard to mammalian vertebral formulae evolution, the next and the most important step might be to analyze comparatively the enhancer activities of some *Hox* genes that have central functions in vertebral specification, such as *HoxC6*. This may provide an opportunity to analyze the mechanism of the evolution of developmental constraints in terms of gene regulation. On the other hand, precise analysis of the intraspecies variation in *Hox* gene expression patterns may also provide clues to understanding the mechanism behind the developmental constraints on mammalian vertebral formulae. If the *Hox* expression pattern is completely constant and no variation is identified among individuals, this would imply a limitation on the production of variations, leading to the concept of a "real" developmental constraint. In contrast, if a significant level of variation among individuals is identified, this may imply internal selection that eliminates embryos with variant characters later in ontogeny because of developmental disruptions caused by abnormal *Hox* gene regulation.

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