Why Do Almost All Mammals Have Seven Cervical Vertebrae? Developmental Constraints, *Hox* Genes, and Cancer

FRIETSON GALIS*
Institute for Evolutionary and Ecological Sciences, University of Leiden, 2300RA Leiden, The Netherlands

ABSTRACT   Mammals have seven cervical vertebrae, a number that remains remarkably constant. I propose that the lack of variation is caused by developmental constraints: to wit, changes in *Hox* gene expression, which lead to changes in the number of cervical vertebrae, are associated with neural problems and with an increased susceptibility to early childhood cancer and stillbirths. In vertebrates, *Hox* genes are involved in the development of the skeletal axis and the nervous system, among other things. In humans and mice, *Hox* genes have been shown also to be involved in the normal and abnormal (cancer) proliferation of cell lines; several types of cancer in young children are associated with abnormalities in *Hox* gene expression and congenital anomalies. In these embryonal cancers the incidence of a cervical rib (a rib on the seventh cervical vertebra, a homeotic transformation of a cervical vertebra towards a thoracic-type vertebra) appears to be increased. The minimal estimate of the selection coefficient acting against these mutations is about 12%.

In birds and reptiles variations in the number of cervical vertebrae have frequently occurred and there is often intraspecific variability. A review of the veterinary literature shows that cancer rates appear lower in birds and reptiles than in mammals. The low susceptibility to cancer in these classes probably prevents the deleterious pleiotropic effect of neonatal cancer when changes in cervical vertebral number occur.

In mammals there is, thus, a coupling between the development of the axial skeleton and other functions (including the proliferations of cell lines). The coupling of functions is either a conserved trait that is also present in reptiles and birds, but without apparent deleterious effects, or the coupling is new to mammals due to a change in the functioning of *Hox* genes. The cost of the coupling of functions in mammals appears to be an increased risk for neural problems, neonatal cancer, stillbirths, and a constraint on the variability of cervical vertebral number. *J. Exp. Zool. (Mol. Dev. Evol.) 285:19–26, 1999.*   © 1999 Wiley-Liss, Inc.

The exceedingly low level of interspecific variation in the number of cervical vertebrae of mammals has puzzled biologists for more than 150 years. In birds, reptiles, and amphibians the number of cervical vertebrae varies considerably, and in mammals the number of vertebrae in other vertebral regions is variable as well (Lebouck, 1898; Schulz, '61). Swans' long necks have a striking 22–25 cervical vertebrae, while ducks have 16 (Woolfenden, '61), and swifts 13 (Starck, '79). Giraffes and dromedaries, however, have only seven vertebrae (Fig. 1), as do the Dugong (Fig. 2) and whales with their short necks (Starck, '79). There are only three genera with an exceptional number of cervical vertebrae, manatees (*Trichechus*) and sloths (*Bradypus* and *Choloepus*). Thus, there seems to be an evolutionary constraint towards the development of variability in the cervical region in mammals.

Intraspecific variations in the number of cervical vertebrae in mammals are extremely rare, whereas intraspecific variations in the number of more caudal vertebrae are common, especially of the lumbar, sacral, and coccygeal regions (e.g., Lebouck, 1898; Schulz, '61). However, one variation of cervical vertebrae does occur infrequently: cervical ribs. A cervical rib is on the seventh cervical vertebra, is a partially or wholly homeotic transformation of the seventh cervical vertebra into the first thoracic vertebra and, thus, reduces the number of cervical vertebrae (and increases

*Correspondence to: Frietson Gailis, Institute for Evolutionary and Ecological Sciences, University of Leiden, PO Box 9516, 2300RA Leiden, The Netherlands. E-mail: Galis@rulsfb.leidenuniv.nl
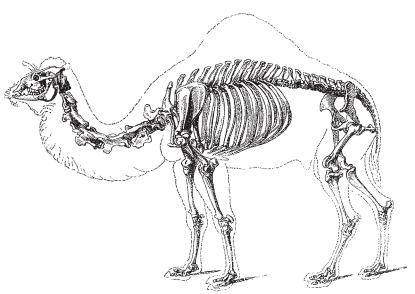
Fig. 1.   Skeleton of a dromedary (*Camelus dromedarius*). Note the large cervical vertebrae. From Owen (1866).

the number of thoracic vertebrae). Further study of this naturally occurring variation seems relevant with respect to the evolutionary constraint on cervical vertebral number, and more specifically, to the study of the selective factors against this variation.

PATHOLOGIES ASSOCIATED WITH CERVICAL RIBS

Consideration of the pathologies in humans that are associated with cervical ribs reveals two types, thoracic outlet syndrome (TOS) and early childhood cancer. TOS involves pressure on the nerves of the brachial plexus and on the subclavian artery, sometimes leading to severe degenerative symptoms in the arm (Fig. 3; Makhoul and Machleder, '92; Roos, '96). Often surgery is performed to relieve symp-

toms. Research on this syndrome has revealed that cervical ribs are invariably associated with changes in the brachial plexus (a different contribution of motor and sensory nerves to the brachial plexus) and other structural abnormalities (Makhoul and Machleder, '92; Roos, '96). The correlation of symptoms must be due to mutual influences of the notochord, neural tube, neural crest, and somites at the time of somite formation (Gossler and Hrabe de Angelis, '98).

Early childhood cancer is a considerably more serious pathology. Childhood cancers tend to result from aberrant developmental processes and are generally embryonal in origin. They are associated with a high incidence of congenital abnormalities. This association is assumed to be caused by a common underlying genetic abnormality
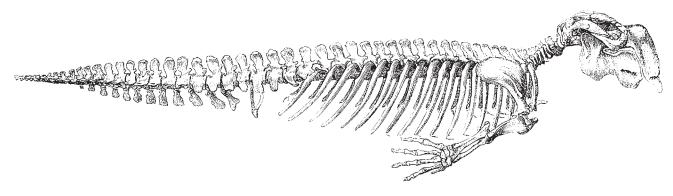

Fig. 2.   Skeleton of a dugong (*Dugong dugon*). Note the small cervical vertebrae. From Owen (1866).
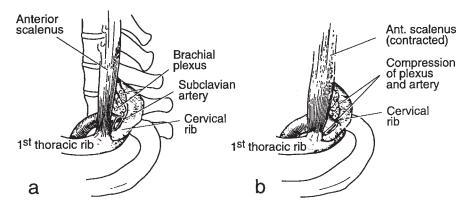
Fig. 3. Illustration of the thoracic outlet in a person with a cervical rib showing how arteries and axons are compressed when the m. anterior scalaenus contracts. The cervical rib is incomplete and fused with the first thoracic rib. (Reproduced by permission from Adson AW. 1947. Surgical treatment for symptoms produced by cervical ribs and the scalenus anticus muscle. Surg Gynecol Obstet 85:687–700.)

(Schumacher et al., '92; Anbazhagan and Raman, '97). A high incidence of vertebral anomalies, especially cervical ribs, was found in a study specifically devoted to finding vertebral anomalies, of 750 children with embryonal cancers (Schumacher et al., '92). An incidence of around 25% cervical ribs was found for the following embryonal cancers: neuroblastoma, brain tumour (astrocytoma and medulloblastoma), acute lymphoblastic and myeloid leukemia, soft tissue sarcoma, Wilms' tumour and Ewing sarcoma (Table 1). This finding confirms the observations by Adson and Coffey ('27), who found that cervical ribs are sometimes discovered in children because of the presence of a tumor in the neck. In addition, a high correlation between malformations of ribs (without further specification) and cancer of all types was found in a large study on childhood cancers (Narod et al., '97).

In agreement with the hypothesis of a common genetic abnormality underlying both early childhood cancer and cervical ribs is the observation that the relation between congenital anomalies and cancer is stronger in infants than in older children (Brodeur, '95; Breslow et al., '96; Gurney et al., '96). Many infants with cancer demonstrate unique epidemiologic, clinical, and genetic characteristics compared with cancers that occur in older children. Some of the early onset cases are familial cases, which are rare and generally characterized not only by an early onset, but also by a worse prognosis (Brodeur, '95; Breslow et al., '96; Gurney et al., '96). This phenomenon is explained by Knudson's ('84) model for embryonal childhood cancers in which two (or only a few) mutational events occur before the onset of cancer. In familial cases one of these mutations has occurred in the germ line and is transmissible to the offspring. The germ-line mutation has been identified for familial retinoblastoma (reviewed in Brodeur, '95). The timing of mutational events should influence the incidence and type of congenital anomaly and these differences in timing can, thus, explain that not all cases of childhood cancer have congenital defects and that the anomalies are variable.

THE ROLE OF *HOX* GENES IN PATTERNING OF THE SKELETAL AXIS AND IN CELL PROLIFERATION

Hox genes play an important role in the patterning of the axial skeleton in all vertebrate classes (Krumlauf, '94). *Hox* gene mutants display

TABLE 1. *The incidence of a cervical rib in children with embryonal cancers*[1]

Type of childhood cancer	Number of cases	Incidence of a cervical rib
Neuroblastoma	88	33%
Brain tumour	234	27.4%
Leukemia	227	26.8%
Soft tissue sarcoma	98	24.5%
Wilms tumour	68	23.5%
Ewing sarcoma	35	17.1%

[1]Data from Schumacher et al. ('92).

abnormalities of the vertebral column. Particularly common phenotypic abnormalities in mice mutants are cervical ribs. At least four knock-out mutants of *hox* genes in mice have an increased incidence of cervical ribs (*Hoxa*-4, *Hoxd*-4, *Hoxa*-5 and *Hoxa*-6) (reviewed in Horan et al., 95). In addition, transgenic mice overexpressing *Hoxb*-7 or *Hoxb*-8 and mice mutants lacking the polycomb-group genes bmi-1 and mel-18 (involved in the regulation of *Hox* genes) display cervical ribs (McLain et al., '92; Charité et al., '94; Akasaki et al., '96; van der Lugt et al., '96). Thus, the formation of cervical ribs is a process that seems to be particularly susceptible to perturbations in *Hox* gene expression (Horan et al., '95). Most of these mutant mice have a severely impaired viability.

At the same time *Hox* genes have been shown to be involved in the proliferation of cell lines in mice and humans (e.g., Corte et al., '93; Lawrence et al., '96; Anbazhagan et al., '97). In a study in which cells of the myeloid, macrophage, erythroid, and B- and T-lymphoid lingeages were investigated for expression of homeotic genes, up to 20 different *Hox* genes were found to be activated (Kongsuwan et al., '88). Some of the genes were ubiquitously expressed, while others were restricted to particular cell lineages or lines (see also Lawrence et al., '96). When the cells were induced to differentiate, the pattern of *Hox* gene expression changed. Changes in *Hox* gene expression have been demonstrated for several types of cancer, including some childhood cancers that were found to have a high incidence of vertebral anomalies: neuroblastoma, Wilms' tumour, and leukemia (Corte et al., '93; Lawrence et al., '96; Manohar et al., '96; Anbazhagan et al., '97). The coupling between these two functions of *Hox* genes is clearly demonstrated in mice with mutations of the Polycomb- and trithorax-group genes (Pc-G and trx-G genes). The evolutionary-conserved Pc-G and trx-G genes are involved in the maintenance of expression of homeobox genes including Hom and *Hox* genes. Mice lacking or overexpressing Pc-G and trx-G genes have altered expression areas of *Hox* genes and display both vertebral anomalies (including cervical ribs and other changes in the number of cervical vertebrae) and leukemia or related cancers (Corte et al., '93; van der Lugt et al., '94; Yu et al., '95; Akasaki et al., '96; Schumacher et al., '96; Coré et al., '97). One of these genes is the trx-G gene Mll, the most commonly involved gene in infant leukemias (Pui et al., '95). Mice heterozygous for the knock-out allele of the *caudal* gene Cdx2, which is involved in the regulation of *Hox*

genes (Epstein et al., '97), also display both vertebral abnormalities and a predisposition for intestinal cancer (He et al., '97). Furthermore, rostral overexpression of *Hox*b-8 leads to cervical ribs in mice, whereas overexpression in bone marrow is associated with leukemia (Perkins and Cory, '93) and overexpression in fibroblasts with fibrosarcoma (a cancer) (Aberdam et al., '91).

Thus, in mammals *Hox* genes are involved in patterning of the skeletal axis and in the proliferation of cell lines (among other functions) and aberrations in the regulation of *Hox* genes may lead to abnormalities in both these functions.

Selection against cervical ribs

The occurrence of cervical ribs in various mammalian species and the particularly frequent occurrence of cervical ribs in experimental mice mutants indicate that there is not a lack of genetic variation for this phenotype. Thus, there must be strong stabilizing selection against the establishment of this trait. The correlated incidence of cervical ribs and childhood cancer presents a strong case for apparent selection against cervical ribs due to deleterious pleiotropic effects. This correlation is strengthened by the mice mutants that not only display variations in cervical vertebral number, but also have cancer and a much reduced fitness (Akasaki et al., '96; van der Lugt et al., '96; Schumacher et al., '96; Coré et al., '97; He et al., '97). The incidence of cervical ribs in the general human population averaged over several large studies (Adson and Coffey, '27; Etter, '44; Sycamore, '44; Crimm, '52; Menárguez Carretero and Campo Muñoz, '67) is approximately 0.2% (347 cases out of 220,026; percentages varied from 0.03–0.5). The frequency of these embryonal cancers added together in the U.S. and Europe is approximately 0.1% (0.01% Wilms' tumour (15), 0.033–0.075% leukemia (Stiller and Parkin, '96), 0.014% neuroblastoma (Gurney et al., '96); braintumours 0.02–0.06%).

Assuming a chance for embryonal cancers of 0.1% and a chance for cervical ribs associated with embryonal cancers of 25% (Shumacher et al., '92) implies a 0.025% chance for children to have both a cervical rib and early childhood cancer. Assuming a frequency of cervical ribs of 0.2% in the general population *after* early childhood and an average survival of 60% for early childhood cancers (Miller et al., '95) implies that the total incidence of cervical ribs at birth is 0.21%, of which 11.9% will develop an embryonal cancer. This suggests that children with embryonal cancers have a 125-fold increased incidence of cervical ribs (25% vs. 0.2%), and that

children born with a cervical rib have an almost 120-fold chance of early childhood cancer (11.9% vs. 0.1%). Thus, neonatal cancer alone seems to present sufficient apparent selection against the establishment of cervical ribs.

In addition, the symptoms of TOS will enhance natural selection against cervical ribs by direct stabilizing selection. The seriousness of the symptoms is correlated with the amount of manual labour that is being performed. Therefore, under natural circumstances the selective disadvantage will be larger than in the sheltered present-day human environment. Adults with a rudimentary first rib (a partial transformation towards eight cervical vertebrae) often have TOS, suggesting natural selection against this variation in cervical vertebral number as well (Gelabert et al., '97).

A further selection factor against cervical ribs could be an increased chance of stillbirths. A large minority (>30%) of fetuses between 49 and 150 mm has ossification centers in the seventh cervical prevertebra (Peters, '27; Noback, '51; Meyer, '78). These ossification centers appear in the same position as those of thoracic prevertebrae's future ribs. An explanation of this phenomenon could be that the high percentage of ossification centers (cervical ribs) is related to the causes that have led to the premature death of these fetuses. Again the interactive nature of the early processes which involve *Hox* genes may present a link between cervical ribs and other abnormalities, as it is unlikely that the ossification centers themselves cause stillbirths. It is possible that the problems in the proliferation of cell lines that lead to neonatal cancer are also causally related to the stillbirths.

A COMPARISON OF MAMMALS, REPTILES AND AMPHIBIANS

The number of cervical vertebrae is variable in amphibians, reptiles, and birds, in strong contrast to mammals (in fishes no cervical vertebral region is distinguished). The selection against such variation in the number of cervical vertebrae must be considerably weaker or absent in these other vertebrate classes. In necropsy studies of zoo animals, cancer rates of birds and reptiles are low compared to mammals (Fox, '12; Ratcliffe, '33; Ippen, '59; Lombard and Witte, '59; Effron et al., '77). The low susceptibility to cancer in reptiles makes intuitive sense because of their low metabolic rate, which leads to an expectation of low oxidative DNA damage (cf. Adelman et al., '88; Perez-Campo et al., '98). The low susceptibility in birds may seem surprising given their high meta-

bolic rate (McNab, '88; Ricklefs et al., '96). However, there is evidence (from canaries and pigeons) that birds have a remarkably low free radical production and, thus, a low amount of oxidative damage (Perez-Campo, '98).

In addition, cancer in birds, especially in young birds, is generally believed in the majority of cases to be induced by viruses (Effron et al., '77; Reece, '96; Misdorp and Kik, personal communication). In mammals viral cancers are estimated to occur in 15% of cases, mainly liver cancer, cervical cancer, and Hodgkin's disease in children (Pisani et al., '97). A survey of 343,600 young chickens showed that none developed a non-virally associated cancer in the first five weeks of life whereas 53 developed a virally associated cancer (Helmsley, '66). This pattern, confirmed by Reece ('96), is in striking contrast to that in human infants where in the first month of life almost all cancers are non-virally associated embryonal cancers, predominantly neuroblastoma (35%; Gurney et al.,'96).

In reptiles the viral induction of cancer has been studied much less. However, reptilian cancers seem more similar to cancers in birds than in mammals (Effron et al., '77) and the viruses that induce cancer in reptiles also seem more similar to those in birds than in mammals (Trubcheninova et al., '77). In addition, reptiles with cancer at necropsy are usually very old, and one study has shown that snakes with cancer are even older on average than snakes without cancer (Ramsay et al., '96). In amphibians the situation is even less well documented; however, the one type of cancer that is well documented, Lucké's tumour in *Rana pipiens*, is a virally induced cancer (McKinnell and Carlson, '97).

There are a few mammalian species with an aberrant number of cervical vertebrae: manatees and sloths. Sloths especially show a spectacular breakdown of the constraint on variation as the number of cervical vertebrae varies from 6 to 9 (Giffin and Gillett, '96). There is no explanation for these exceptions, but I suggest as hypothesis that the extremely low metabolic rate of manatees and sloths (e.g., McNab '88; Gallivan and Best '89; Koteja '91; Hammond and Diamond, '97) is associated with low oxidative DNA damage and, thus, with a low susceptibility to cancer (Adelman et al., '88; Shigenaga and Ames, '93). This hypothesis needs to be tested.

CONCLUSIONS

It appears, therefore, that the cause of the conservation of seven cervical vertebrae should be

sought (1) in a genetic link between early childhood cancer and stillbirths and variation in cervical vertebrae number, and (2) in the neuronal problems leading to the thoracic outlet syndrome in adults associated with cervical ribs. The involvement of *Hox* genes in the cancers that are associated with cervical ribs in mice and men points to a coupling between functions of *Hox* genes that appears to be lacking in birds, reptiles, and amphibians, or at least has no apparent consequences when cervical vertebral number is changed. There are two possible explanations for the observed coupling in mammals: (1) the coupling of functions of *Hox* genes has newly appeared in mammals due to a change in the functioning of *Hox* genes (e.g., a new function in proliferation in mammals); and (2) the coupling of functions was already present in reptiles, but hidden because of the low susceptibility to cancer. This coupling has only become detectable in mammals because of an increase in susceptibility to cancer. And this increase in cancer susceptibility can be the direct result of the increase in metabolic rate, which is associated with an increase in oxidative damage (Adelman et al., '88; Shigenaga and Ames, '93).

The increase in cancer susceptibility and the presumed increase in stillbirths are pleiotropic deleterious effects, whereas the neuronal problems are a direct consequence of the change in cervical vertebral number. The fact that the pleiotropic effect of cancer recurs for what presumably are a large number of different mutations allows us to classify these collectively as a developmental constraint. To further understand the constraint on changes in the number of cervical vertebrae that exists in virtually all mammals, a study of the function of *Hox* genes in cell proliferation and carcinogenesis in birds, reptiles, and amphibians is urgently needed. Furthermore, it should be an interesting experiment to select for complete cervical ribs in a mammalian species to see whether a healthy strain can be produced, or whether this would lead to the predicted increase in susceptibility for cancer, stillbirths, and neuronal problems.

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