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Introduction

The number of cervical vertebrae in mammals is remarkably constant at seven. In other tetrapods, the number of cervical vertebrae varies considerably, and in mammals the number of vertebrae in more caudal vertebral regions is variable as well (Leboucq 1896; Narita & Kuratani 2005; Schultz 1961; Starck 1979). Only manatees (*Trichechus*, Sirenia) and sloths (*Bradypus* and *Choloepus*, Xenarthra) have an exceptional number of cervical vertebrae (Bateson 1894; Starck 1979; Varela-Lasheras et al. 2011). Despite the extreme evolutionary conservation of the number of cervical vertebrae, intraspecific variation is not uncommon in mammals. The most common variation is represented by ribs on the seventh vertebra, so-called cervical ribs, which can be considered a partial or complete homeotic transformation of a cervical into a thoracic vertebra (involving a change in the activity of *Hox* genes (Galis 1999; Li & Shiota 2000; Varela-Lasheras et al. 2011; Wéry et al. 2003)). The strong conservation of the number of cervical vertebrae implies that there must be selection against intraspecific variation of this number. Indeed, very strong selection against cervical ribs was shown to exist in humans (Furtado et al. 2011; Galis 1999; Galis et al. 2006; ten Broek et al. 2012). Approximately 90 ~~%~~ percent of individuals possessing a cervical rib die before reaching ~~the~~ reproductive age (Galis et al. 2006). The severe selection is due to the strong association of cervical ribs with multiple and major congenital abnormalities. In other mammalian species, we have also found an association with abnormalities (Varela-Lasheras et al. 2011). A cervical rib itself is relatively harmless, but its development is induced by a (genetic or environmental) disturbance of early embryogenesis (Chernoff & Rogers 2004; Galis et al. 2006; Li & Shiota 2000; Wéry et al. 2003). Such a disturbance usually has multiple effects, due to the highly interactive nature of early embryogenesis. Hence, the strong selection against cervical ribs is indirect and due to the severity of the associated medical problems (Galis et al. 2006; ten Broek et al. 2012).

Of three caudal cervical vertebrae from *Mammuthus*, a sixth (C6) and two seventh (C7), that were recently found in the North Sea, during infrastructural works for an extension of the Rotterdam Harbour (Maasvlakte 2) and donated to the Natural History Museum in Rotterdam, two possessed articulation facets for cervical ribs (C6 and one of the C7). This surprising finding aroused our interest, and we searched the extensive collection of Late Pleistocene *Mammuthus* material in the Naturalis Biodiversity Centre (Leiden) to make an estimate of the incidence of this developmental abnormality. Additionally, we determined for comparison the incidence of cervical ribs in skeletons of the most related extant species, the Asian and African elephants (*Elephas maximus* and *Loxodonta ~~Africana~~africana*).

Methods

Specimens.

We analyzed 6 sixth cervical vertebrae (C6) and 10 seventh cervical vertebrae (C7) of Late Pleistocene mammoths (*Mammuthus primigenius*), from two collections: the Natural History Museum Rotterdam (NMR, table 1) and Naturalis Biodiversity Center (Naturalis, table 1). We analysed 28 specimens of extant elephants, 21 *Elephas maximus* and 7 *Loxodonta ~~Africana~~africana*, from 5 collections: the Natural History Museum of Denmark, Copenhagen (ZMUC), Naturhistorisches Museum Wien, Vienna (NHMW), The ~~university~~ University of Vienna, the Swedish Museum of Natural History, Stockholm (NRM) and Naturalis Biodiversity Center (Naturalis). All specimens (see table 1 for collection numbers) are of Late Pleistocene age and originate from the North Sea. Two specimens (C6, inv.nr. NMR999100006627 and C7, inv. nr. NMR999100007602) were recently found during infrastructural works in the Rotterdam harbor area ("Maasvlakte 2") on the North Sea seabed and allocated to the NMR by the Rotterdam Port Authorities.

Cervical ribs.

We analyzed the C6 and C7 vertebrae for the presence or absence of articulation facets of cervical ribs. The presence of cervical ribs can be deduced from articulation facets on the anterior side of C7 (Fig. 1A and D) and, if the cervical ribs are large enough, on the posterior side of C6, as well (Fig 1B and C).

Statistical tests.

To compare the prevalence of cervical rib facets between mammoths and elephants we used Barnard's test for 2x2 tables (Barnard 1945). P-values < 0.05 were considered as significant.

Results

Articulation facets for cervical ribs on cervical vertebrae are characterized by the following combination of characteristics: (i) they have a smooth, polished-looking surface, visibly smoother than the (surrounding) cortical surface of the vertebrae; (ii) the surfaces have no vascular or nervous foramina; and (iii) the facets are bordered by a clear edge, distinguishing them from the surrounding cortex.

We found one C7 with a unilateral sinistral anterior rib facet indicating a left cervical rib (Fig. 1A). Five C7 did not have rib facets anteriorly and four could not be judged due to the absence of the relevant part of the vertebra. We found two C6 with rib facets on the posterior side indicating cervical ribs: one on the right side and one on the left side (Figs. 1B and C respectively). We found one C6 without rib facets posteriorly and three that could not be judged.

Thus, out of the nine C6 and C7 that could be evaluated, three indicate the presence of a cervical rib, i.e. an incidence of cervical rib facets of 33.3 %. We found in one of the 21 *E. maximus* a minute cervical rib facet on C7 (Fig 1d, 4.8%) and no articulation facet visible posteriorly on C6 of the same individual. None of the ~~7~~ seven *Loxodonta* ~~*Africana*~~ *africana* individuals ~~did have~~had cervical rib facets, nor were rudimentary cervical ribs found. The overall incidence of cervical ribs in the two species is, thus, 3.6%. This is significantly lower than the 33.3% in mammoths, if we only consider vertebrae that can be evaluated for cervical rib articulation facets (Barnard's exact test, $p<0.05$).

Discussion

The incidence of cervical rib facets in mammoths is extremely high (33.3%), almost ten times higher than that of extant elephants. In humans, incidences higher than 1% have only been found in hospital or isolated populations (Galis et al. 2006). Incidences of more than 25% have only been found in children with leukemia, brain tumours and neuroblastoma (Galis & Metz 2003; Merks et al. 2005; Schumacher et al. 1992) and in deceased fetuses and infants (Furtado et al. 2011; Galis et al. 2006; ten Broek et al. 2012). Along with the high incidence of cervical ribs in mammoths, the size of the articulation facets is particularly large (Figs 1A-C), substantially larger than the articulation facet found in the one *Elephas minimus* (Fig. 1D) and, pointing to substantially larger cervical ribs than usually found in humans (see (Bots et al. 2011; ten Broek et al. 2012) for examples). Size of cervical ribs is assumed to be negatively correlated with fitness (Bots et al. 2011; Jeannotte et al. 1993).

The exceptionally high incidence of large cervical ribs in our set of Late Pleistocene *Mammuthus primigenius* recovered from the North Sea can be due to two factors. Firstly, it can be due to a high rate of inbreeding in declining populations, before final extinction. A high incidence of cervical ribs (7.46%) has been observed in an isolated human population

(Palma & Carini 1990) in Sicily, in inbred pedigreed dogs (11~~,~~.4% (Breit & Kunzel 1998)) and inbred minipigs (11% at birth (Jørgensen 1998)). Generally, in inbred mammals there is an increased incidence of congenital anomalies (Cristescu et al. 2009; Räikkönen et al. 2013). Recent studies have shown that the genetic diversity was extremely low in late Pleistocene mammoth populations in Siberia (Miller et al. 2008; Nyström et al. 2012). Additionally, the increased incidence of cervical ribs may be due to harsh conditions that impact early pregnancies, ~~as~~ because diseases, famine, cold and other stressors can lead to disturbances of early organogenesis, that can result in the induction of cervical ribs (e.g. (Chernoff & Rogers 2004; Li & Shiota 2000; Sawin 1937; Steigenga et al. 2006; Wéry et al. 2003)). Harsh conditions during the late Pleistocene, a period of intensive climatic fluctuations and ecosystem instability, are plausible (Brace et al. 2012). Furthermore, bone dystrophy in mammoth calves of Northern Eurasian late Pleistocene populations is found regularly and assumed to be caused by ~~a~~ mineral deficiencies in pregnant females (Leshchinskiy 2012). Hence, a combination of inbreeding and harsh conditions may be the most likely explanation, given the extremely high incidence of cervical ribs. Our results, thus, are in agreement with inbreeding in populations in North-Western Eurasia, just as has been found for Siberian populations (Miller et al. 2008; Nyström et al. 2012). Finally, the high incidence and large size of the cervical ribs indicates a strong vulnerability, given the association of cervical ribs with diseases and congenital abnormalities in mammals. The vulnerable condition ~~probably~~ may have contributed to the eventual extinction of the woolly mammoths.

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