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## **Analysis of $^{13}\text{C}$ and $^{15}\text{N}$ isotopes from Eurasian Quaternary fossils: Insights in diet, climate and ecology**

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# CHAPTER 5

## **STABLE ISOTOPES OF THE SIBERIAN UNICORN**



The stable isotope research in this chapter describes the stable isotope research published in the following articles:

Kosintsev, P. Mitchell, K. Devière, T. van der Plicht, J., Kuitems, M., Petrova, E., Tikhonov, A., Higham, T., Comeskey, D., Turney, C., Cooper, A., van Kolfschoten, T., Stuart, A., Lister, A., 2019. Evolution and extinction of the giant rhinoceros *Elasmotherium sibiricum* sheds light on late Quaternary megafaunal extinctions. *Nature Ecology & Evolution* **3** (1), 31-38.

Kuitems, M., van der Plicht, J., van Kolfschoten, T., 2019. *Elasmotherium sibiricum*: de uitgestorven Siberische Eenhoorn – uitkomsten van opzienbarend onderzoek. *Cranium* **36** (1), 34-43.

## 5.1 Introduction

A rather unknown inhabitant of Mammoth Steppe was *Elasmotherium sibiricum*, the so-called ‘Siberian unicorn’. The Siberian unicorn was a giant rhinoceros that lived in Eastern Europe and Central Asia. The Siberian unicorn, which appeared in the Early Pleistocene, was the youngest representative of the 40-million-year-old Elasmotherinae branch. With its extinction, the last representative of the once successful subfamily disappeared. The *Elasmotherium* has been used in Eastern Europe as a guiding ‘chronospecies’ for a specific biostratigraphic unit (Schvyreva, 2015). Based on the fossil data, the extinction was generally assumed to have happened during the Middle Pleistocene around 200,000 years ago (Zhegallo *et al.*, 2005).

The co-occurrence of a few remains of *E. sibiricum* and Late Pleistocene species within the same fossil assemblages, suggested that their existence may have extended to the Late Pleistocene. However, the fossil assemblages consist often of remains that originate from multiple stratigraphic layers. For example, at the site Pyshma near Yekatarinburg in the Southern Urals, it was assumed that *E. sibiricum* fossils represented older material that had ended up in a mixed fossil assemblage. A sample for dating was nevertheless taken. The result was surprising; the age of the fossil turned out to be within  $^{14}\text{C}$  range and to be as old as the other dated remains from the same site (that is, GrA-53424;  $33380 \pm 205$   $^{14}\text{C}$  yr BP). This was the incentive to date more *Elasmotherium* fossils from that region, and led to an intensive international research cooperation.

Recently, a paper on this extinct unicorn was published in Nature Ecology and Evolution (Kosintsev *et al.*, 2019), revealing (1) that the *E. sibiricum* went extinct much later than previously thought, (2) that the Elasmotherinae and Rhinocerotinae lineages split already  $\sim 40$  million years ago, and (3) that this animal had a distinct diet. The latter was discovered by stable isotope analysis of *Elasmotherium* fossils, carried out by this thesis’ author, in combination with analysis of the species’ characteristic morphology.

This chapter focusses on the stable isotope investigation. First, some general background information on the species is provided and the results of the genetic and dating studies are summarised.

### 5.1.1 The Siberian unicorn

*E. sibiricum* is an odd-toed ungulate (order Perissodactyla) belonging to the rhinoceros family, the Rhinocerotidae; a group of about 250 described, mostly extinct, species that were very diverse and successful in the past. With a length of approximately 4.5 meters, a withers height of over 2 meters, and a weight of  $\sim 3.5$  tonnes, *E. sibiricum* was a true giant. However, it was not the largest rhinoceros that lived in Eurasia during the Pleistocene. Its predecessor, *E. caucasicum* was with a length of 5.0 - 5.2 meters, a withers height of about 2.4 meters and a weight of about 5 tonnes even larger (Zhegallo *et al.*, 2005; Schvyreva, 2015).

The skeleton of the Siberian unicorn (Fig. 5.1) is in many ways similar to that of



**Figure 5.1** Skeleton of an *Elasmotherium sibiricum* specimen in the Stavropol regional museum (Schvyreva, 2016).

other rhinos, but it has a number of clear diagnostic features. For instance, bones of the front and rear legs are relatively slender. This is seen as an evolutionary adaptation whereby the animal, despite its weight, was able to move quickly and thus travel large distances (Schvyreva, 2014; 2015; 2016).

Another distinctive feature is the huge skull, which was bent downward. On its forehead (the frontal bone), the Siberian unicorn had a striking dome (Fig. 5.2) that forms the basis of a large horn. The Siberian unicorn differs, also in this respect, from rhinos such as the woolly rhino where the basis of the horns is more anterior, i.e., the nasal bone. A cross section through the skull (Fig. 5.2) shows that the bump on the forehead does not consist of solid bone, but has a spongy, cellular structure similar to skulls of elephants. Traces of well-developed blood vessels, especially at the base of the dome, suggest strong blood flow. According to some researchers, this may indicate a well-developed sense of smell. The bump has a rough surface; a phenomenon that we also see in other rhinos where the horns are attached to the muzzle.

There are different opinions about the shape and the size of the horn of the Siberian unicorn; fossil remains of the horn have never been found (Zhegallo *et al.*, 2005). Also, the dentition is very specific and characteristic for the species. The teeth are reduced in terms of elements; both the upper jaw and lower jaw lack the incisors and canines as well as the anterior two premolars. The permanent teeth consist of five very high-



**Figure 5.2** Left: Cranium of an *E. sibiricum* specimen from the collection of the Zoological Museum in St. Petersburg (Russia). (Photo: Alexei Tikhonov). Right: Longitudinal section of the cranium of an *E. sibiricum* specimen (Schvyreva, 2016).



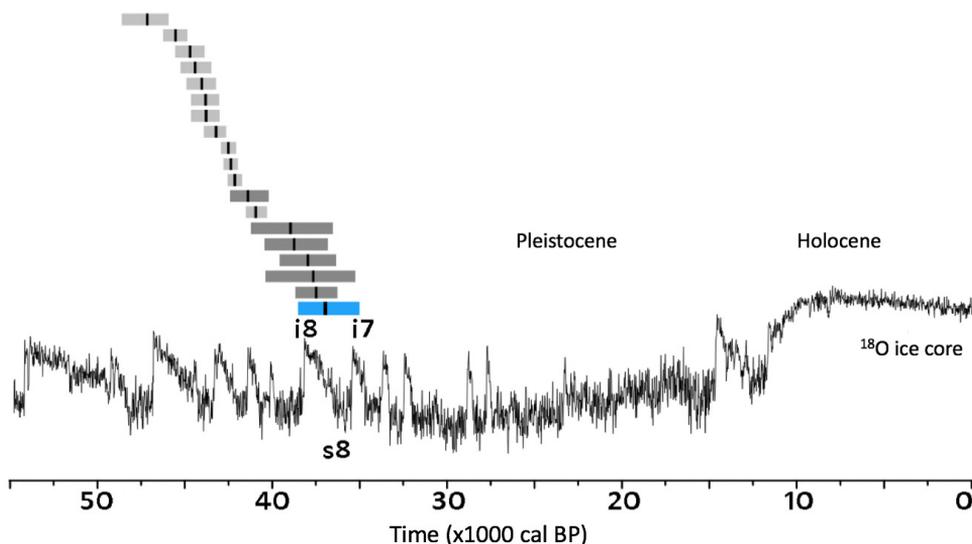
**Figure 5.3** Surface (left) and lateral view (right) of an upper M3 of *E. sibiricum* specimen from the Lower Don Region, late Middle Pleistocene. Collection Azov museum-reserve (Photos: Vadim Titov).

crown molars (2 premolars, 3 molars). The rootless, permanent growing molars have remarkably undulated enamel (Fig. 5.3).

Zhegallo *et al.* (2005) present an extensive overview of the different hypotheses on the species' habitat and diet. Inhabiting swampy areas along rivers and lakes have been suggested, but primarily the dry areas of steppes are proposed as the habitat of the Siberian unicorn (Zhegallo *et al.*, 2005; Schryreva, 2016). The characteristic hypsodont teeth of the Siberian unicorn are seen by many as an adaptation to a diet that causes exceptional rapid wear (Schryreva, 2016). The food itself can be the cause of the rapid abrasion, but also sand or grit, which comes with the food, can cause substantial part of the molar wear.

### 5.1.2 Genetic data and age

During the Miocene (~ 23-5 million years ago), the Rhinocerotidae dominated the large mammalian fauna of Africa, Eurasia and North America (Kosintsev *et al.*, 2019). They are subdivided into two subfamilies: 1) the Rhinocerotinae, to which the five recently living species belong as well as the Pleistocene species of rhinoceros that lived in Western Europe (for example the steppe rhino *Stephanorhinus hemitoechus* and the woolly rhino *Coelodonta antiquitatis*), and 2) the extinct Elasmotherinae, with a geographical range limited to Eastern Europe and Central Asia, and a number of geographically



**Figure 5.4** Calibrated  $^{14}\text{C}$  dates of *E. sibiricum*, compared to the chronology and temperature of Greenland ice. The blue area marks the extinction event, as determined from modelled  $^{14}\text{C}$  dates. Calibration and modelling were performed using respectively IntCal13 and the function Single Phase model in OxCal version 4.3.2 (Bronk Ramsey, 2009; Reimer *et al.*, 2013). From Kosintsev *et al.* (2019).

isolated areas in Mongolia and China. Based on the fossil record, it was assumed that the split between the two subfamilies took place very early, during the Eocene (around 56-34 million years ago). The analysis of the fossil DNA in the recently examined bones has confirmed the earlier assumption with a calculated genetic divergence of about 47.4 million years ago (Kosintsev *et al.*, 2019).

Radiocarbon dates clearly show that *E. sibiricum* got extinct much later than previously thought. The species still lived during the late Quaternary. There are no finds of *Elasmotherium* known from an LGM (or younger) context. The extinction of the Siberian unicorn can therefore be seen as belonging to the ‘Late Quaternary megafaunal extinctions’ during the Weichselian (Stuart, 2015).

In Fig. 5.4 the dates are compared with  $^{18}\text{O}$  data from Greenland ice cores (Rasmussen *et al.*, 2014). The relative concentration of the oxygen isotope  $^{18}\text{O}$  ( $\delta^{18}\text{O}$ ) in ice is a proxy for temperature and climate; more positive values correspond to relatively warm periods, more negative to colder periods. Holocene and Pleistocene are clearly distinguishable and indicated in the figure. The Pleistocene is characterized by climate fluctuations called the Greenland Stadials and Interstadials. The extinction of the *Elasmotherium* took place during an era of extreme climate fluctuations, known as Marine Isotope Stage (MIS) 3, as can be observed in deep sea core record and in Greenland ice cores as the alternation of interstadials and stadials (Fig. 5.4; interstadials GI-7, GI-8 and stadial GS-8). It is striking that the extinction of *Elasmotherium* roughly coincides with GS-8 (Fig. 5.4) and also with a period in which extreme ‘ice rafting’ occurs in the North Atlantic, a period known as the Heinrich Event H4 (Bassis *et al.*, 2017).

## 5.2 Material and Methods

A total of 25 fossils from various locations throughout the Siberian unicorn’s distribution area were sampled (Table 5.1, Fig. 5.5). Sample preparation and measurement of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values that are discussed in this chapter (Table 5.2) was carried out at Groningen, CIO, following procedures as described in Chapter 2 (section 2.4).

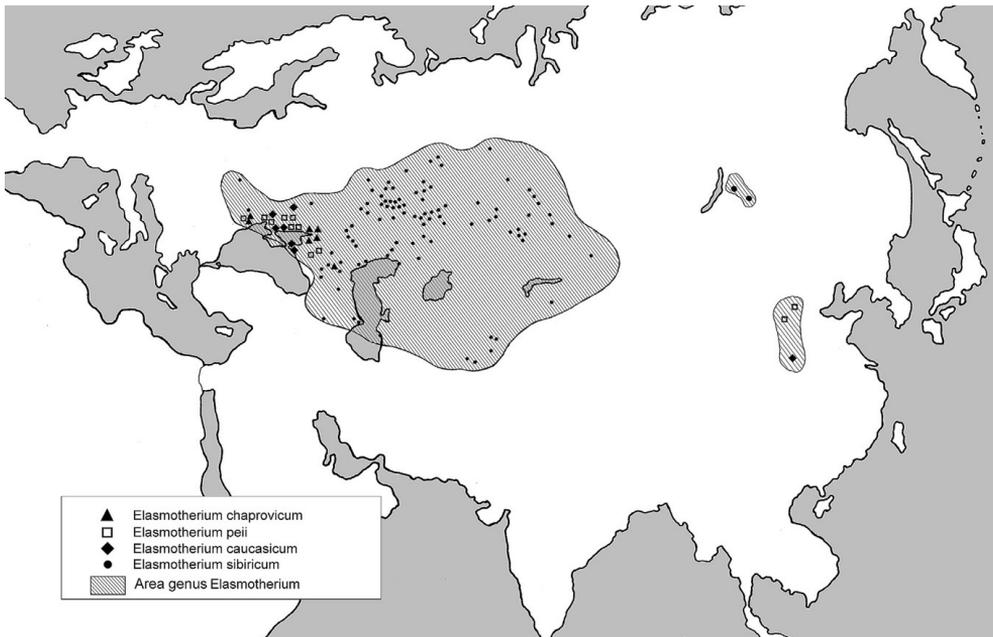
$^{14}\text{C}$  dating was performed at Groningen and Oxford. The youngest samples according to the original Groningen data set were subsequently also dated in Oxford. In case  $^{14}\text{C}$  results of a fossil were different (within the mutual measurement error) for Groningen and Oxford, the specific fossil was dated again by hydroxyproline (or HYP). In this method, developed at Oxford to improve dating of potentially contaminated samples, the amino acid hydroxyproline is extracted from the collagen. This method was chosen because hydroxyproline is unique to mammals, thus minimises contamination. In Table 5.2, the stable isotope values (measured at Groningen) are shown in combination with what was considered the best current date for each specimen.

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values are compared with those of other coeval living species, specifically data from woolly rhinoceros (*C. antiquitatis*; Stuart and Lister, 2012) and saiga

**Table 5.1** Information about the *E. sibiricum* samples taken for isotope analysis.

<b>museum &amp; number</b>	<b>locality</b>	<b>skeletal element</b>	<b>latitude</b>	<b>longitude</b>
C/M 12836	Irbit	cranium	57.67	63.07
IPAE 420/111	Smelovskya	molar	53.60	58.90
NHM M7099	Saratov	molar	51.54	46.01
ZIN 36330	Hydroelectric pwr. st., Ship Canal #32, Eur. Russia	cranium		
IPAE 915/2804	Tobolsk	radius	58.00	68.00
IPAE 2388/2	Bashkiriya	cranium	54.50	56.33
ZIN 31791	Samara district	cranium	53.20	50.14
IPAE 2388/1	Bashkiriya	cranium	55.00	55.83
IPAE 17-5703/7	Samara district	femur	53.20	50.14
IPAE 5-147	Samara district	tooth	53.20	50.14
IPAE 12-001	Samara district	cranium	53.20	50.14
IPAE 10-5566	Samara district	cranium	53.20	50.14
IPAE 897/200	Borovlyanka	molar	56.80	62.87
ZIN 36331	unknown	cranium		
ZIN (26021) 3963	Luchka (Svetliy Yar), Volgograd Region	cervical vertebra	48.47	44.77
ZIN 3986	Incineration factory of bones, European Russia	cervical vertebra		
ZIN 10794	Luchka (Svetliy Yar), Volgograd Region	cranium	48.47	44.77
IPAE 2388/3	Bashkiriya	cranium	54.00	56.50
IPAE 2388/5	Bashkiriya	mandible/molar	53.17	56.00
IPAE 1871/200	Voronovka	cranium	55.45	65.33
IPAE 897/172	Borovlyanka	metacarpal	56.80	62.87
IPAE 897/123	Borovlyanka	molar	56.80	62.87
NHM M12429	Sarepta	cranium	48.52	44.51
IPAE 2388/4	Bashkiriya	cranium	53.67	56.08
IPAE 9-133	Odessa	tooth	46.47	30.73

antelope (*Saiga tatarica*; Jürgensen *et al.*, 2017). Only data from pre-LGM central Asia were used for direct comparison with *E. sibiricum*. Also, published  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data of other fossil rhinoceroses, specifically, Merck's rhinoceros (*Stephanorhinus kirchbergensis*) and indeterminate *Stephanorhinus* specimens were used for comparison with the stable isotope data of the Siberian unicorn.



**Figure 5.5** Geographical distribution of *E. sibiricum* and related species (Schvyreva, 2016).

### 5.3 Results and discussion

Of the 25 samples, 23 samples yielded enough collagen for  $^{14}\text{C}$  dating and stable isotope analysis. The atomic C:N ratio of these samples (3.0-3.2) were within the acceptable range, with the exception of sample IPAE 420-111, which was just outside the range and was excluded from further analyses. The *Elasmotherium*  $\delta^{13}\text{C}$  values ( $n = 22$ ) range from  $-21.5$  to  $-16.3\text{‰}$  with a mean of  $-18.1 \pm 0.3\text{‰}$ . The  $\delta^{15}\text{N}$  values ( $n = 21$ ) range from  $+6.5$  to  $+12.8\text{‰}$  with a mean of  $+9.4 \pm 0.3\text{‰}$  (Table 5.2). The differences in the stable isotope values might partly be due to geographic or temporal variation, although no trend through time or correlation with latitude was observed in the data (Fig. 5.6).

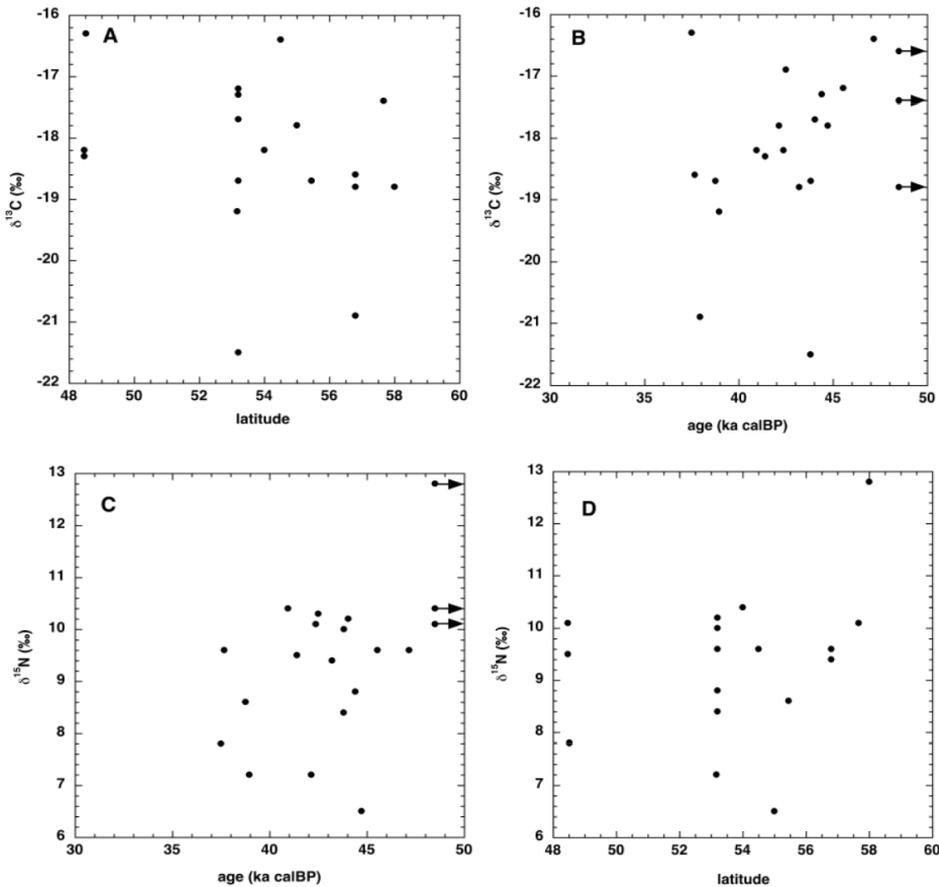
High  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, as seen in *E. sibiricum*, are observed in mammals inhabiting dry steppes or deserts (Bocherens, 2003). The *Elasmotherium*  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values clearly differ from those of other fossil Rhinocerotidae from Eurasia (Fig. 5.7). Most of the samples had higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values compared with the woolly rhinoceros (*Coelodonta antiquitatis*) from within the temporal and geographical range of the *Elasmotherium* material. Moreover, the majority of *E. sibiricum* samples were relatively higher in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than narrow-nosed and/or Merck's rhinoceroses (*Stephanorhinus hemitoechus* and *S. kirchbergensis*, respectively) from the Middle Pleistocene sites of Schöningen, Germany. Among other rhinoceros species, morphological and

**Table 5.2** Isotope measurement results for collagen (rejected collagen crossed out) from investigated samples of *E. sibiricum*.

<b>museum &amp; nr</b>	<b>laboratory nr</b>	<b><sup>14</sup>C age (yr BP)</b>	<b>σ</b>	<b>%C</b>	<b>%N</b>	<b>C:N</b>	<b>δ<sup>13</sup>C (‰)</b>	<b>δ<sup>15</sup>N (‰)</b>
C/M 12836	OxA-X-2750-15	51,100	>	48.4	17.6	3.2	-17.4	10.1
	OxA-X-2750-14	49,000	>					
IPAE 420/111	GrA-32606	<del>45,000</del>	<del>&gt;</del>	<del>35.9</del>	<del>15.2</del>	<del>2.8</del>	<del>-15.8</del>	<del>11.0</del>
NHM M7099	OxA-X-2762-57	45,700	>	45.0	16.5	3.2	-17.3	11.6
ZIN 36330	OxA-X-2750-38	50,300	2,700	32.4	11.9	3.2	-16.6	10.4
IPAE 915/2804	OxA-34900	49,200	>	42.2	16.0	3.2	-18.8	12.8
IPAE 2388/2	GrA-55451	43,900	575	45.0	16.2	3.2	-16.4	9.6
ZIN 31791	GrA-62202	42,230	370	41.8	15.6	3.1	-17.2	9.6
IPAE 2388/1	GrA-55450	41,220	445	47.8	17.2	3.2	-17.8	6.5
IPAE 17-5703/7	GrA-61035	40,860	455	42.9	15.5	3.2	-17.3	8.8
IPAE 5-147	GrA-61030	40,470	445	42.0	15.1	3.2	-17.7	10.2
IPAE 12-001	GrA-61033	40,200	445	41.3	15.0	3.2	-18.7	10.0
IPAE 10-5566	GrA-61031	40,180	445	42.2	15.4	3.2	-21.5	8.4
IPAE 897/200	GrA-53425	39,480	355	43.4	17.1	3.0	-18.8	9.4
ZIN 36331	GrA-62203	38,440	295	45.2	16.7	3.2	-16.9	10.3
ZIN (26021) 3963	GrA-62200	38,230	285	42.3	15.6	3.2	-18.2	10.1
ZIN 3986	GrA-62197	37,850	285	43.3	15.9	3.2	-17.8	7.2
ZIN 10794	OxA-X-2750-13	36,850	650	44.3	16.4	3.2	-18.3	9.5
IPAE 2388/3	GrA-55452	36,290	275	45.4	17.5	3.0	-18.2	10.4
IPAE 2388/5	OxA-X-2756-57	34,400	1,000	44.4	16.9	3.1	-19.2	7.2
IPAE 1871/200	OxA X-2677-55	34,250	700	45.4	16.6	3.2	-18.7	8.6
IPAE 897/172	OxA-X-2756-56	33,300	1,100	43.5	16.3	3.1	-18.6	9.6
IPAE 897/123	OxA X-2677-54	33,650	650	35.2			-20.9	
NHM M12429	OxA X-2677-52	33,250	500	46.4	17.0	3.2	-16.3	7.8
IPAE 2388/4	no collagen							
IPAE 9-133	no collagen							

tooth-wear data show that *S. kirchbergensis* and *S. hemitoechus* were browse- and graze-dominated mixed feeders, respectively, while *C. antiquitatis* was predominantly a grazer (Boeskorov *et al.*, 2011; Pushkina *et al.*, 2014; Rivals and Lister, 2016; Saarinen *et al.*, 2016; see also Chapter 6).

In contrast to other rhinoceroses, *E. sibiricum* δ<sup>13</sup>C and δ<sup>15</sup>N values overlap strongly with those of samples of saiga antelope (*Saiga tatarica*) from the same spatiotemporal range (Fig. 5.7). Both fossil and modern saiga antelopes are well adapted to inhabit

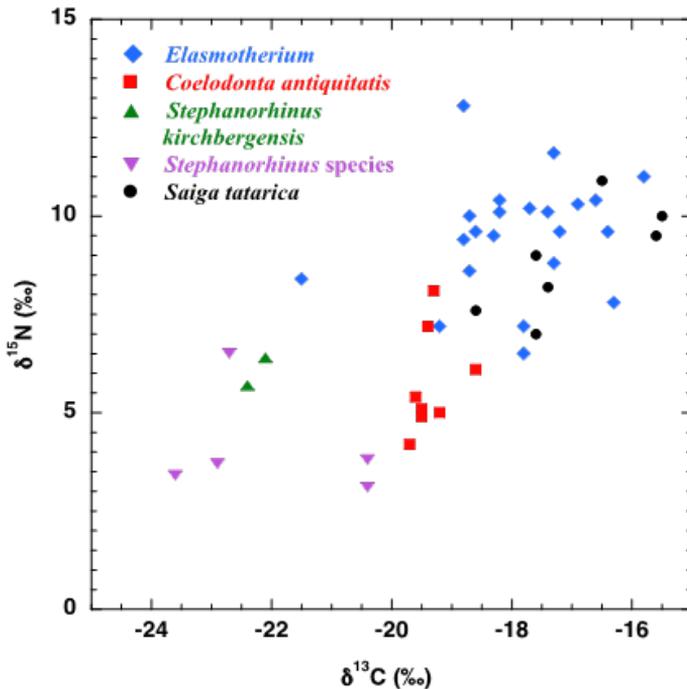


**Figure 5.6** Stable isotope values of *E. sibiricum* versus latitude and  $^{14}\text{C}$  age: (A)  $\delta^{13}\text{C}$  versus latitude of samples; (B)  $\delta^{13}\text{C}$  versus  $^{14}\text{C}$  date of samples; (C)  $\delta^{15}\text{N}$  versus  $^{14}\text{C}$  date of samples; (D)  $\delta^{15}\text{N}$  versus latitude of samples.

dry steppe. The  $\delta^{13}\text{C}$  values of modern *S. tatarica* may have been influenced by the consumption of Chenopodiaceae (C4 photosynthesizers), but the mammoth steppe flora is considered to have consisted of C3 plants only (Jürgensen *et al.*, 2017).

Another aspect that may have affected the high  $\delta^{13}\text{C}$  values of *E. sibiricum* is consumption of underground plant parts, since these tend to contain higher  $\delta^{13}\text{C}$  values than photosynthetic plant parts (Badeck *et al.*, 2005), and/or the pulling up and consumption of roots (as suggested for *E. sibiricum*; Schvyreva, 2015). It would also explain why the molars of *E. sibiricum* are extremely high-crowned and without roots, giving them unlimited growth: when eating tubers, a lot of sand comes along, which means that the animal's teeth are likely to wear down at a high rate.

The extinction of *Elasmotherium* may have been linked to its high degree of



**Figure 5.7**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of various species. *E. sibiricum* samples are compared with those of the Late Pleistocene woolly rhinoceros (*C. antiquitatis*; Stuart and Lister, 2012), Middle Pleistocene Merck's rhinoceros (*S. kirchbergensis*; Kuitems *et al.*, 2015a), indeterminate Middle Pleistocene *Stephanorhinus* specimens (*S. kirchbergensis* or narrow-nosed rhinoceros (*S. hemioechus*; Kuitems *et al.*, 2015a) and Late Pleistocene saiga antelope (*Saiga tatarica*; Jürgensen *et al.*, 2017). *Coelodonta* and *Saiga* data are restricted to the spatiotemporal extent of the *E. sibiricum* samples.

specialization, including extreme dietary adaptations (Schvyreva, 2015). Previous stable isotope studies on mammalian herbivore species from Europe have illustrated change in ecological niches during the pre-LGM (Drucker *et al.*, 2015) and the late glacial (Drucker *et al.*, 2014). Both *Coelodonta antiquitatis* and *Saiga tatarica* survived the MIS 3 extinction of *E. sibiricum*. *C. antiquitatis* with an isotopic signature implying a dietary niche consistently different from that of *E. sibiricum*, *S. tatarica* with a shift in its range of isotopic values. While *Saiga* samples from pre-LGM central Asia show  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values overlapping strongly with contemporary *Elasmotherium* (Fig. 5.7), samples from later periods (LGM to GI-1), and those from other regions, include lower  $\delta^{15}\text{N}$  values (down to +2.8‰), indicating dietary flexibility (Jürgensen *et al.*, 2017). The implication is that both *Coelodonta* and *Saiga* could cope with the environmental change that began around 38ka, but *E. sibiricum* could not. In addition to this, the persistently restricted geographical range of *Elasmotherium* (also probably linked to its specialized habitat), as well as the assumed low population size and slow reproductive rate associated with its large body size (Johnson, 2009) would have predisposed it to extinction in the face of environmental change, while the ecologically similar, but much smaller species (*S. tatarica*) survived. The extinction of *E. sibiricum* could in theory have been exacerbated by human hunting pressure. However, there is currently no record of the hunted/butchered species' remains from any archaeological site.





Schöningen, photo: T. van Kolfshoten