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SPY CAVE

125 years of multidisciplinary research
at the Betche aux Rotches
(Jemeppe-sur-Sambre, Province of Namur, Belgium)

Edited by Hélène ROUGIER & Patrick SEMAL

Volume 1

2013

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BIOGEOCHEMISTRY

Patrick SEMAL, Anne HAUZEUR & H el ene ROUGIER
(Coordinators)

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CHAPTER XVII

STABLE ISOTOPES

Hervé BOCHERENS, Mietje GERMONPRÉ, Michel TOUSSAINT & Patrick SEMAL

Abstract

Carbon and nitrogen isotopic abundances of fossil bone collagen reflect those of the average diet, and they can be preserved for tens of thousands of years under favourable conditions. Spy Neandertals offer a favourable case for the use of the isotopic approach to reconstruct their diet and ecology. The collagen preservation in Late Pleistocene Belgium caves is extremely good. The new radiocarbon dates obtained on Neandertal and faunal specimens from Spy allow relevant comparison between contemporaneous organisms, from Spy or nearby caves, such as Scladina and Goyet. Comparisons of prey selection patterns based on isotopic results were made between Spy Neandertals and animal predators, such as hyaenas and cave lions. Prey selection of Spy Neandertals was oriented on megafauna, such as woolly mammoths and woolly rhinoceros, while hyaenas were more opportunistic and cave lions more oriented on reindeer. The results obtained on Spy Neandertals were compared with those obtained on other Belgian Neandertals (Scladina, Engis), other European Neandertals (Saint-Césaire, Les Pradelles), and with those of early Holocene hunter-gatherers from Belgium. The focus of late Neandertals from Spy on megafauna was also visible on the late Neandertal from Saint-Césaire. The carbon and nitrogen isotopic signatures of Spy Neandertals are not found in any analysed Holocene modern humans from Belgium.

INTRODUCTION

As the carbon and nitrogen isotope signatures of a vertebrate tissue reflect directly those of the dietary components used for its synthesis and collagen, the predominant protein in bone, can be preserved for tens of thousands of years, stable isotopes analyses have been successfully used to reconstruct the diet of Neandertals (e.g. Bocherens *et al.*, 1991, 1999, 2001, 2005; Fizet *et al.*, 1995; Bocherens, 2009, 2011). However, such investigations need to meet strict criteria for reliability, in terms of chemical composition, comparison with a valid trophic context and stage of ontogenic development (Bocherens *et al.*, 2005; Bocherens & Drucker, 2006; Bocherens, 2011).

The Belgian sites offer a very good potential for collagen preservation, as illustrated by successful isotopic studies on fossil collagen on samples from the Holocene to the last interglacial, around 120,000 years ago (Bocherens *et al.*, 1997, 1999, 2001, 2007). Therefore, the Spy Neandertals appear as very good candidates for an application of the isotopic approach for palaeodietary reconstruction.

MATERIAL

Since the animal and human remains from Spy come from ancient excavations and lack good stratigraphical record, only radiocarbon dated samples were selected for carbon and nitrogen stable isotope investigations. In order to minimise sample destruction, especially for rare specimens, portions of the collagen extracted for radiocarbon dating at the Centre for Isotope Research in Groningen were used for stable isotope measurements.

The collagen from Spy radiocarbon dated in Groningen and used in the present study corresponds to 5 human specimens dated between 4,300 and 4,900 ¹⁴C years BP, 3 human specimens dated between around 33,000 and 37,000 ¹⁴C years BP, 4 faunal specimens (reindeer *Rangifer tarandus*, woolly rhinoceros *Coelodonta antiquitatis*, cave bear *Ursus spelaeus*, and woolly mammoth *Mammuthus primigenius*) dated between 34,000 and 45,000 ¹⁴C years BP, one bone retouchoir, and one fragment of a bone spearpoint (Semal *et al.*, 2009, this volume: chapter XVI; Table 1).

The dietary interpretation of carbon and nitrogen isotopic data obtained on ancient humans requires comparison with contemporaneous herbivorous and carnivorous faunal specimens in order to avoid confusion between nitrogen isotopic variations due to trophic level and to changes in environmental parameters (e.g. Drucker & Bocherens, 2004). The dated faunal material from Spy is too limited to provide such a valid comparison dataset. Fortunately, two major sites have yielded abundant faunal material of similar age to Spy at a relatively short distance: Scladina and Goyet caves, from which additional samples were selected (Figure 1).

The Scladina and the Goyet caves are located in the Condroz, a region south of the Sambre and Meuse valleys in Belgium. The

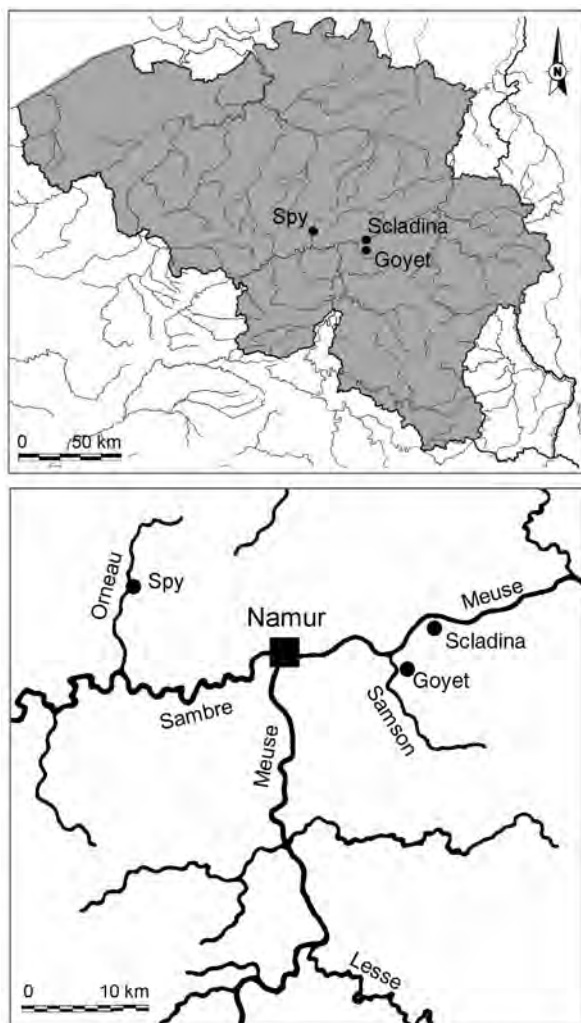


Figure 1. Location map of the studied sites.

Condroz landscape is characterised by steep-sided narrow valleys cutting transversally through plateaux of relatively constant altitudes, locally reaching 350 m. The plateaux are composed of a succession of long parallel ridges consisting of Devonian psammites separated by shallow, broad depressions of Carboniferous limestones (Bourguignon, 1966; De Moor & Pissart, 1992). The landscape is quite diverse ranging between open, unprotected uplands, steep cliffs and sheltered sun-exposed gorges, with the larger Meuse valley nearby. This mosaic scenery permitted during the Last Glacial a rich diversity of fossil flora and fauna (Otte, 1994).

Discovered in 1971 by potholers, the Scladina cave lies on the southern edge of the town Sclayn on the right bank of the Meuse river. The cave is situated in carbonic limestone, 20 m above the level of the surrounding depression. The material from Scladina cave considered in the present study comes from layer 1A, dated around 40,000 years ago (Bonjean, 1998), and previously published in Bocherens *et al.* (1997) (ST1). The considered species are woolly mammoth (*Mammuthus primigenius*), woolly rhinoceros (*Coelodonta antiquitatis*), horse (*Equus ferus*), large bovids (i.e. aurochs, *Bos primigenius*, or steppe bison, *Bison priscus*), giant deer (*Megaloceros giganteus*), cave hyaena (*Crocuta crocuta*), brown bear (*Ursus arctos*) and cave bear (*Ursus spelaeus*) (Bocherens *et al.*, 1997).

The Goyet cave (or “Troisième caverne” of Goyet) is situated in a limestone cliff at the confluence of two small rivers, the Samson and the Strouvia. The Samson runs downstream more or less in a south to north direction and joins the Meuse river 3 km to the northwest. The cave entrance, 15 m above the riverbed, faces southwest and overlooks the valley of the Samson. Dupont (1872) excavated here numerous remains of ice age mammals as well as a large number of Middle and Upper Palaeolithic artefacts. The Palaeolithic artefacts date from the Mousterian, Aurignacian, Gravettian, and Magdalenian (Otte & Groenen, 2001). Material from Goyet cave comes from different units inside the cave: A2, A3, and B4 (ST1). Based on a few bone radiocarbon dating results, unit A3 was considered more recent than A2 and B4, with ^{14}C ages around 27,000 years BP and around 34,000

years BP (Germonpré, 2004). However, a series of unpublished radiocarbon dates on cave bear bones from different units of Goyet cave shows that material from these units is mixed and that the ages range from 40,000 to 27,000 ^{14}C years BP without clear chronological differences (Germonpré, Bocherens & Hofreiter, unpublished). Therefore, material from these three units was selected on the basis of species and considered broadly contemporaneous to the time of deposit in Spy cave. As far as possible, specimens of adult bones were selected in order to avoid interference of suckling signals in bones of young individuals and teeth which growth occurs essentially after weaning (e.g. Bocherens *et al.*, 1994, 2005). In total, 58 bone and tooth specimens from Goyet have been selected, including the following species: woolly mammoth (*Mammuthus primigenius*), woolly rhinoceros (*Coelodonta antiquitatis*), horse (*Equus ferus*), aurochs (*Bos primigenius*), steppe bison (*Bison priscus*), reindeer

(*Rangifer tarandus*), cave hyaena (*Crocuta crocuta*), wolf (*Canis lupus*), cave lion (*Panthera leo*), brown bear (*Ursus arctos*) and cave bear (*Ursus spelaeus*) (ST1).

METHODS

Extraction of collagen from bones and teeth from Spy was performed according to Longin (1971) in Groningen in the course of direct AMS radiocarbon dating (Semal *et al.*, 2009). Once received by HB, collagen samples were freeze-dried for more convenient handling for isotopic measurements.

Extraction of collagen from bones and teeth from Goyet cave was performed according to Bocherens *et al.* (1997) at the Institute of Prehistory and Protohistory and Medieval Archaeology at the University of Tübingen. The ele-

Dating sample ID	Description	Specimen	^{14}C date	$\delta^{13}\text{C}\text{-Gr}$	%C	%N	C/N	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$ (Gr-Tu)
GrA-32621	Right fibula frag. refitting with original collection	Spy 425k	4,350 ± 35 BP	-21.44	45.2	16.1	3.28	-21.6	8.7	0.16
GrA-32622	Lower right second premolar	Spy 33a	4,600 ± 35 BP	-20.66	46.1	16.3	3.30	-20.8	10.7	0.14
GrA-32625	Right parietal fragment	Spy 432a	4,635 ± 35 BP	-22.04	43.9	16.3	3.14	-21.9	9.5	-0.14
GrA-32628	Right third metacarpal	Spy 398l	4,800 ± 40 BP	-20.53	45.9	16.0	3.35	-21.2	8.8	0.67
GrA-32632	Right proximal hallucal phalanx	Spy 425n	4,835 ± 35 BP	-21.41	45.6	16.1	3.30	-21.1	8.8	-0.31
GrA-32623	Alveolar bone of upper right third molar (Spy I)	Spy 94a	35,810 +260 / -240 BP	-19.34	43.7	15.2	3.35	-19.4	11.4	0.06
GrA-32626	Upper central incisor refitting with mandible Spy 3 (Spy II)	Spy 92b	36,350 +310 / -280 BP	-19.71	47.1	16.7	3.29	-19.8	10.9	0.09
GrA-32630	Middle third manual phalanx (Spy II)	Spy 430a	33,940 +220 / -210 BP	-20.14	46.8	15.8	3.46	-20.3	10.8	0.16
GrA-21546	Right scapula fragment (Spy II?)	Spy 572a	31,810 ± 250 BP	-19.91	41.5	14.4	3.40	-19.8	11.0	-0.11
GrA-32612	Reindeer metatarsal with ochre	Spy D4 19B 121 1480	34,410 +230 / -210 BP	-18.54	45.8	16.5	3.24	-18.9	2.8	0.32
GrA-32613	Woolly rhinoceros milk molar	Spy D2 Pal Plate 4	44,350 +650 / -500 BP	-20.08	44.0	16.4	3.13	-20.1	7.2	0.07
GrA-32615	Bear incisor with ochre	Spy D1 227 9D-E	34,640 +240 / -220 BP	-18.44	45.3	16.9	3.12	-18.9	9.4	0.47
GrA-32616	Mammoth milk molar	Spy D3 19B 121 1474	42,330 +550 / -450 BP	-21.05	37.3	14.0	3.11	-21.4	11.0	0.34
GrA-32617	Bone retouchoir	Spy SP1	30,170	-22.02	49.8	15.0	3.87	-22.3	5.1	0.24
		Spy 8414	+160 / -150 BP	-22.02	50.2	15.2	3.86	-22.0	5.3	0.02
GrA-32619	Fragment of a bone flat and triangular spearpoint	Spy SP2 Spy 1954	32,830 +200 / -190 BP	-20.26	47.6	15.4	3.61	-21.1	4.7	0.81

Table 1. List of stable isotopic results obtained on the collagen extracted in Groningen for radiocarbon dating of Spy samples.

mental and isotopic measurements were performed at the Geochemical unit of the Faculty for Geosciences at the University of Tübingen, using an elemental analyser NC 2500 connected to a Thermo Quest Delta+XL mass spectrometer. The isotopic ratios are expressed using the “ δ ” (delta) value as follows: $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C})_{\text{sample}} / ({}^{13}\text{C}/{}^{12}\text{C})_{\text{reference}} - 1] \times 1000$ (‰) and $\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N})_{\text{sample}} / ({}^{15}\text{N}/{}^{14}\text{N})_{\text{reference}} - 1] \times 1000$ (‰), with the international reference being V-PDB for $\delta^{13}\text{C}$ values and atmospheric nitrogen (AIR) for $\delta^{15}\text{N}$ values. Samples were calibrated to $\delta^{13}\text{C}$ values of USGS24 and to $\delta^{15}\text{N}$ values of IAEA 305A. The reproducibility was ± 0.1 ‰ for $\delta^{13}\text{C}$ measurements and ± 0.2 ‰ for $\delta^{15}\text{N}$ measurements.

RESULTS

A complete list of chemical composition and isotopic results for the samples analysed for the first time during this study is given in Table 1 and in the supporting information (ST2, ST3). The isotopic data from Scladina cave have been taken from Bocherens *et al.* (1997).

Collagen reliability

Only extracts with %C, %N, and C/N similar to those of collagen extracted from fresh bone should be considered reliable for isotopic measurements. Several studies have shown that collagen with atomic C/N ratios lower than 2.9 or higher than 3.6 are altered or contaminated (DeNiro, 1985; Ambrose, 1990). Extracts with $2.9 \leq \text{C/N} \leq 3.6$ and %N < 5 % may also be problematic (Ambrose, 1990) and should be excluded from further palaeobiological interpretations. All the extracts of Spy specimens prepared in Groningen for radiocarbon dating fulfil the requirements for isotopic reliability, except one specimen, a bone retouchoir (GrA-32617) with a C/N of 3.9 (two measurements; Table 1). Therefore, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured for this specimen, as well as its radiocarbon age, are considered dubious and unreliable, while the isotopic data of all the other specimens are considered reliable and can be interpreted in palaeobiological terms. All the specimens from Goyet considered in this study exhibit chemical characteristics typical of collagen, with %C, %N and

C/N ranging from 36.4 to 46.1, from 13.3 to 16.2, and from 2.9 to 3.4, respectively. Therefore, they are all reliable, as well as the collagen extracted from the material from Scladina-1A published in Bocherens *et al.* (1997).

Holocene humans and Neandertals from Spy

The humans of Holocene age present carbon and nitrogen isotopic signatures clearly different from those of Neandertals, with no overlap. The $\delta^{13}\text{C}$ values range from -21.9 to -20.8 ‰ and from -20.3 to -19.4 ‰ for Holocene humans and Neandertals, respectively, while the $\delta^{15}\text{N}$ values range from 8.7 to 10.7 ‰ and from 10.8 to 11.4 ‰ for Holocene humans and Neandertals, respectively (Table 1). The $\delta^{13}\text{C}$ values of Holocene humans are clearly more negative than those of Neandertals, while the $\delta^{15}\text{N}$ values of Neandertals are more positive than those of Holocene humans from Spy.

Fauna

The distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in faunal specimens is clearly linked to their taxonomic attribution (ST2). The whole $\delta^{13}\text{C}$ values range from -22.5 to -18.3 ‰, with the most negative values for cave bears (-22.5 to -20.8 ‰; $\delta^{13}\text{C} = -21.7 \pm 0.5$ ‰, $n = 30$), mammoths (-21.6 to -20.7 ‰; $\delta^{13}\text{C} = -21.3 \pm 0.4$ ‰, $n = 6$) and horses (-21.9 to -20.5 ‰; $\delta^{13}\text{C} = -21.3 \pm 0.5$ ‰, $n = 9$), the less negative values for lions (-18.7 to -18.5 ‰; $\delta^{13}\text{C} = -18.6 \pm 0.1$ ‰, $n = 2$), reindeer (-19.6 to -18.3 ‰; $\delta^{13}\text{C} = -18.7 \pm 0.4$ ‰, $n = 7$), wolves (-19.1 to -18.8 ‰; $\delta^{13}\text{C} = -19.0 \pm 0.1$ ‰, $n = 2$), hyaenas (-20.2 to -19.2 ‰; $\delta^{13}\text{C} = -19.6 \pm 0.3$ ‰, $n = 11$) and brown bears (-20.3 to -19.1 ‰; $\delta^{13}\text{C} = -19.8 \pm 0.4$ ‰, $n = 5$). Intermediate $\delta^{13}\text{C}$ values are exhibited by rhinoceros (-20.8 to -20.0 ‰; $\delta^{13}\text{C} = -20.4 \pm 0.3$ ‰, $n = 3$), large bovids (-20.7 to -19.9 ‰; $\delta^{13}\text{C} = -20.3 \pm 0.3$ ‰, $n = 9$) and giant deer (-20.2 ‰, $n = 1$). The $\delta^{13}\text{C}$ values are roughly distributed according to trophic level, with carnivores having the least negative values, with the exception of reindeer which present significantly more positive $\delta^{13}\text{C}$ values than the rest of the herbivores.

The whole range of $\delta^{15}\text{N}$ values is from 2.6 to 10.1 ‰, with the most positive values for

hyaenas (7.7 to 10.1 ‰; $\delta^{15}\text{N} = 9.1 \pm 0.7$ ‰, $n = 11$), lions (8.4 ‰, $n = 2$), mammoths (6.7 to 9.4 ‰; $\delta^{15}\text{N} = 8.0 \pm 0.9$ ‰, $n = 6$) and wolves (6.6 to 7.2 ‰; $\delta^{15}\text{N} = 6.9 \pm 0.3$ ‰, $n = 2$). The least positive values are exhibited by reindeer (2.6 to 4.5 ‰; $\delta^{15}\text{N} = 3.4 \pm 0.6$ ‰, $n = 7$), cave bears (2.6 to 6.0 ‰; $\delta^{15}\text{N} = 4.3 \pm 1.1$ ‰, $n = 30$), giant deer (4.5 ‰, $n = 1$) and large bovids (3.8 to 5.7 ‰; $\delta^{15}\text{N} = 4.6 \pm 0.6$ ‰, $n = 9$). Intermediate $\delta^{15}\text{N}$ values are exhibited by rhinoceros (4.3 to 5.9 ‰; $\delta^{15}\text{N} = 5.3 \pm 0.7$ ‰, $n = 3$), horses (4.8 to 7.0 ‰; $\delta^{15}\text{N} = 5.7 \pm 0.8$ ‰, $n = 9$) and brown bears (4.0 to 9.0 ‰; $\delta^{15}\text{N} = 6.5 \pm 2.1$ ‰, $n = 5$). The $\delta^{15}\text{N}$ values are roughly distributed according to trophic level, with carnivores having the most positive values, with the exception of mammoths which present significantly more positive $\delta^{15}\text{N}$ values than the rest of the herbivores, and cave bears which present $\delta^{15}\text{N}$ as low as those of typical herbivores. Brown bears exhibit a large range of $\delta^{15}\text{N}$ values, ranging from those of herbivores to those of carnivores, which could reflect a large dietary flexibility of these bears with strong individual preferences. For mammoths and rhinoceros, milk teeth from Spy exhibit $\delta^{15}\text{N}$ values 2 to 3 ‰ more positive than those of the adults from the same species, and similar to those of coeval carnivores.

DISCUSSION

Trophic position of Spy Neandertals

The three newly analysed Neandertal specimens exhibit $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values very similar to those of the specimen previously published (Bocherens *et al.*, 2001), and the four specimens altogether exhibit a narrow range of isotopic variation, from -20.3 to -19.4 ‰ and from 10.8 to 11.4 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Although collagen isotopic data are available for four Neandertal specimens, they may belong to only two individuals, the so-called Spy I and Spy II (Semal *et al.*, 2009; Rougier *et al.*, volume 2: chapter XIX). When considering together the three specimens that may belong to individual Spy II, the average isotopic values are $\delta^{13}\text{C} = -20.0 \pm 0.24$ ‰ and $\delta^{15}\text{N} = 10.9 \pm 0.07$ ‰ (Table 1). They are very close to the isotopic values of Spy I ($\delta^{13}\text{C} = -19.4$ ‰ and $\delta^{15}\text{N} = 11.4$ ‰).

This indicates that, on average, the source of proteins was similar for both Neandertal individuals studied. A similar pattern was observed at Les Pradelles, in Western France, for three Neandertal specimens aged 40 to 45 thousand years ago (Bocherens *et al.*, 2005).

Reconstructing the trophic position of Spy Neandertals within their food web requires comparison of their carbon and nitrogen isotopic signatures with those of contemporaneous herbivorous and carnivorous mammals. The first step is to check the reliability of the chosen fauna from Spy, Goyet and Scladina that we plan to use as reference for this purpose. This being done, it is possible to evaluate the proportions of different food items consumed by Spy Neandertals and compare these results with those obtained for other predators.

To check the isotopic homogeneity of the combined faunal material from Spy, Goyet and Scladina, we were able to test if statistically significant differences were present between the sites of Goyet and Scladina for species with a sufficient isotopic record in both sites, and we compared the few data from Spy faunal specimens with those obtained on the same species from Goyet and Scladina. Graphs showing the distribution of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each species from Goyet and Scladina-1A are presented as supporting data (SF1). Statistical tests (Mann-Whitney) could be performed on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of five species: hyaena, mammoth, horse, large bovids, and cave bear. All the p values are well above the 0.010 threshold that would correspond to a significant difference, except for the $\delta^{13}\text{C}$ values of horses ($p = 0.014$) and cave bears ($p = 0.003$) (ST3). In both cases, the $\delta^{13}\text{C}$ values measured on the samples from Scladina are more negative than those measured on the samples from Goyet, and the difference is 1 ‰ for horses and 0.6 ‰ for cave bears (ST3). Such a difference could be linked to local environmental differences, with more humid and closed conditions around Scladina than around Goyet cave. Although both caves are located in the valleys of tributaries from the Meuse, the orientations of these valleys are different, south to north in the case of Scladina cave and southeast to northwest in the case of Goyet. Moreover, the valley of the Samson river, where Goyet lies, is

more open than the valley of Scladina. However, both caves are not far from the plateaux. The carbon isotopic results may reflect that horses and cave bears lived closer to the respective sites in which their fossil remains were found, in valleys with different characteristics, while the other species, such as mammoths and large bovids were dwelling on the plateaux and therefore do not show any significant isotopic differences. The hyaenas would be predators of herbivores living in different areas including the plateaux, and would therefore be less affected by these local differences. In any case, the differences are slight, 1 ‰ at most, and a majority of species exhibit no significant difference.

The few dated faunal specimens from Spy can be compared with those from Scladina and Goyet belonging to the same species, i.e. mammoth, woolly rhinoceros, brown bear and reindeer. The $\delta^{15}\text{N}$ value of the mammoth deciduous tooth from Spy (Spy D3 19B 121 1474; Table 1) is more positive but falls within the range of $\delta^{13}\text{C}$ values of the adult mammoths from Scladina-1A and Goyet. Such a pattern is similar to the observations made by Rountrey *et al.* (2007) on juvenile mammoth tusks and probably results from the consumption of mother milk during suckling, a food resource that is one trophic level higher than the adult diet and therefore enriched by 3 to 5 ‰ in $\delta^{15}\text{N}$. The same reason probably explains the high $\delta^{15}\text{N}$ values observed for the milk molar of the woolly rhinoceros from Spy (Spy D2 Pal Plate 4) compared to the specimens from Scladina-1A and Goyet. The isotopic signatures of the bear incisor from Spy (Spy D1 227 9D-E) are consistent with the specific attribution to brown bear rather than cave bear, as its $\delta^{13}\text{C}$ values are clearly too positive to correspond to the former but well within the range of the latter. Finally, the reindeer from Spy (Spy D4 19B 121 1480) presents similar isotopic values to the specimens from Goyet. Therefore, we considered a global average for each species, taking all relevant specimens from Spy, Goyet and Scladina into account.

A mathematical mixing model based on isotopic data from Wallonia between 30 and 40,000 years ago can be used to determine the likely composition of the diet of Neandertals and

that of an animal predator such as hyaena, cave lion, and wolf. Until recently, mixing models have been developed in order to cope with a situation with two isotopic pairs, such as $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$, and no more than 3 possible dietary sources (e.g. Phillips, 2001; Phillips & Gregg, 2001; Phillips & Koch, 2002). We have here a situation with at least 5 prey species and two isotopic pairs. In order to deal with such a situation, three approaches are possible: 1) combining several prey species as one isotopic pole to reduce the number of poles to 3 (e.g. Drucker & Bocherens, 2004); 2) forcing the proportion of some of the prey to zero to take into account only 3 prey at a time, considering all the possible combinations of 3 prey alternatively, and taking the extreme values obtained for each combination; 3) using an upgraded mixing model that can cope with a situation with two isotopic pairs and more than 3 dietary sources (Phillips & Gregg, 2003; Newsome *et al.*, 2004; Bocherens *et al.*, 2005). We chose the second approach in the present case in order to yield minimum and maximum proportions of each prey that are consistent with the isotopic data.

Two modifications have been performed relative to the original approach developed by Phillips & Gregg (2003), as suggested for trophic studies based on ancient bone collagen (Bocherens *et al.*, 2005; Drucker & Henry-Gambier, 2005). Firstly, we subtracted the fractionation factors from the consumer signatures rather than adding it to the prey signatures. The aim of this modification was to enable the use of the isotopic signatures measured on the fossil material. Secondly, we used for these calculations a range of carbon and nitrogen isotopic fractionations between prey and predator collagen instead of a constant value for these fractionation factors. Previous work has shown that the $\delta^{13}\text{C}$ values of predator collagen is 0.8 to 1.3 ‰ more positive than that of its average prey, whereas the $\delta^{15}\text{N}$ values of predator collagen is 3 to 5 ‰ more positive than that of its average prey (Bocherens & Drucker, 2003; Drucker & Bocherens, 2004). This calculation yields four extreme carbon and nitrogen isotopic signatures for the prey collagen of each predator, which can be graphically depicted as a rectangle that includes all the possible isotopic signatures for the collagen of the prey.

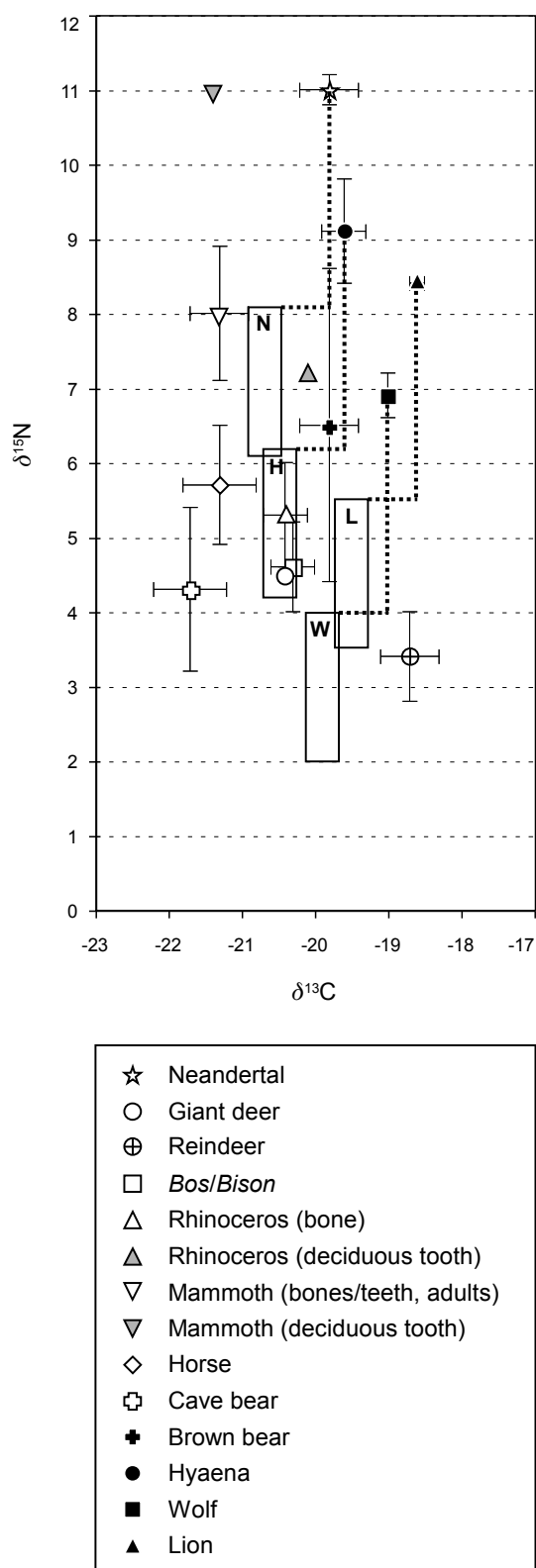


Figure 2. Average and standard-deviation $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the studied species. The rectangles represent the range of isotopic values for the average prey consumed by each predator.

Using this approach, we obtained average carbon and nitrogen isotopic signatures of the collagen of five potential prey (i.e. Bovinae [*Bos/Bison*], reindeer, horse, woolly rhinoceros, and woolly mammoth). These five isotopic signatures were compared with rectangles of carbon and nitrogen isotopic signatures representing the average isotopic signatures possible for the prey of Neandertal, and for those of hyaena, lion and wolf (Figure 2). Following Phillips & Koch (2002), the isotopic values of the collagen of three of the prey delineated a triangle delimited by the average isotopic signature of the chosen prey. In the cases when the rectangles of the isotopic signatures of the collagen of the possible prey of a predator intersect the triangle of the three chosen prey, the corresponding proportions of each prey at these intersection points were calculated using a spreadsheet available at <http://www.epa.gov/wed/pages/models.htm> (Phillips & Koch, 2002). The extreme values of proportions calculated for all combinations are reported in Figure 3. In some cases, one prey has to be present in the diet of a predator with a minimum proportion, as for instance reindeer for lion (minimum 25 %) and mammoth for Neandertal (minimum 21 %). Maximum proportions are reported for each prey in the diet of each predator and proportions in-between represent the range of proportions consistent with the measured isotopic values.

Comparison with other Belgian Neandertals

A comparison of the isotopic signatures of the Spy Neandertals with those of Neandertals from Belgium previously analysed is not directly informative, since these other specimens present too many differences with the Spy specimens.

One Neandertal specimen of similar age to the Spy individuals that yielded collagen isotopic values is Engis 2 (Bocherens *et al.*, 2001). However, this juvenile individual is possibly under the influence of breast-milk, which could explain the high $\delta^{15}\text{N}$ value measured for this specimen compared to the Spy Neandertals (Bocherens *et al.*, 2001).

One adult specimen from Scladina, initially thought to be from layer 1B (Bocherens *et al.*, 2001), is actually from layer 3 (Bocherens

et al., 2005). Therefore, the palaeodietary interpretation of its isotopic signatures is pending further investigations of contemporaneous fauna, which is in progress.

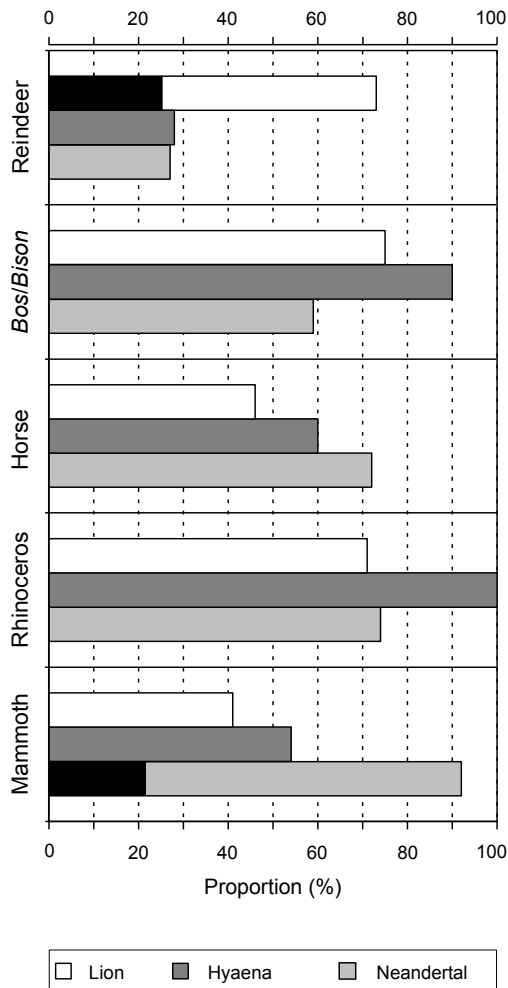


Figure 3. Range of possible proportions of each prey species in the diet of different predators.

One 11-year-old child has been recovered from layer 4A in Scladina (Toussaint *et al.*, 1998). Contrarily to the other Neandertals from Belgium, this specimen is linked to a temperate context (Bocherens *et al.*, 1999). The carbon and nitrogen isotopic signatures of this specimen point to the consumption of meat of ungulates from open environment, despite the occurrence of potential prey species such as fallow deer and horse which carbon isotopic signatures correspond to individuals living under a dense canopy.

Comparison with other Neandertals from Europe and beyond

Most of the Neandertal specimens relevant for the comparison of their isotopic signatures with those from Spy come from French sites. Some Neandertals from other areas have been analysed for their stable isotopic signatures but these specimens either present problematic conservation or lack relevant comparison data. The isotopic data published with the radiocarbon dates for the Neandertals from Vindija (Richards *et al.*, 2000) were recently proved to be unreliable due to collagen preservation problems (Higham *et al.*, 2006) and even the newly measured specimen from this site cannot be directly compared with contemporaneous fauna from the same area, which prevents a detailed dietary reconstruction.

Carbon and nitrogen isotopic data have been recently published for a fossil human from Siberia with mitochondrial DNA characteristic of Neandertals (Krause *et al.*, 2007). Unfortunately, three replicates on one single humerus yielded very different radiocarbon ages (from $29,900 \pm 500$ to $37,800 \pm 450$ BP) and the $\delta^{15}\text{N}$ values obtained are up to 2.5 ‰ different, which is beyond the intraindividual variability. In addition, no isotopic data from coeval fauna are available so far. For these reasons, these data cannot be interpreted in palaeodietary terms at the moment.

One French site that provided isotopic data on Neandertal collagen with that of contemporaneous fauna is Saint-Césaire (Charente-Maritime: Bocherens *et al.*, 2005). In this study, the Neandertal specimen exhibited a $\delta^{15}\text{N}$ value higher than that of an animal predator, the hyaena, and the application of mixing models led to the conclusion that this Neandertal consumed high amounts of meat from mammoth and woolly rhinoceros. An alternative explanation for the more positive $\delta^{15}\text{N}$ values in Neandertals than in hyaenas is the consumption of freshwater resources by the humans (Drucker & Bocherens, 2004; Balter & Simon, 2006), and this suggestion could also be made for the Neandertals from Spy. Such a hypothesis is not supported by any archaeological evidence for exploitation of fish in sites occupied by Neandertals, but taphonomic

biases and differential alteration of fish remains could account for this absence. More significant is the very tight clustering of the isotopic values of Neandertal specimens from a given site, as at Spy. This is totally unlike the isotopic pattern exhibited by freshwater fish consumers from a given site, as exemplified by the very large range of $\delta^{13}\text{C}$ values measured on fishbones from French and German sites (Drucker & Bocherens, 2004; Bösl *et al.*, 2006). Moreover, the trace-element data presented to support fish consumption by Neandertals by Balter & Simon (2006) are affected by diagenesis and lack faunal comparison data, which makes them unreliable for dietary reconstructions (Lee-Thorp & Sponheimer, 2006). For all these reasons, it is very unlikely that fish were significantly involved in the diet of the studied Neandertals.

The remaining reliable isotopic data obtained from Neandertal collagen are from Les Pradelles (Marillac-le-Franc, France: Fizet *et al.*, 1995; Bocherens *et al.*, 2005) and Les Rochers-de-Villeneuve (Lussac-les-Châteaux, France: Beauval *et al.*, 2006). Although the specimen from the latter site is isolated, its proximity to Les Pradelles makes it provisionally possible to compare its isotopic data with those of the Neandertals and the fauna from this site. Here again, the Neandertal isotopic data are very similar and present more positive $\delta^{15}\text{N}$ values than the contemporaneous animal predators. In this case, no mammoth remains were found in the site, and the high $\delta^{15}\text{N}$ values could relate to the consumption of large bovids, probably bison (Bocherens, 2011).

It is interesting to point that the Neandertals from Spy exhibit a similar isotopic and palaeodietary pattern than the Neandertal from Saint-Césaire, all being among the youngest Neandertals in Western Europe, dating from around 32 to 36,000 years ago (Mercier *et al.*, 1991; Semal *et al.*, 2009). The high level of megafauna consumption could be a feature of late Neandertals in Western Europe, but evidence for rhinoceros and proboscidean hunting and/or scavenging has also been reported for much older European hominids (e.g. Scott, 1980; Thieme & Veil, 1985; Conard & Niven, 2001; Moncel, 2001; Piperno & Tagliacozzo, 2001; Wenban-Smith *et al.*, 2006).

It is to be reminded that, in all these isotopic studies based on bone collagen, the dietary information is limited to the protein fraction of the diet. Therefore, it does not preclude significant consumption of other foodstuff poor in nitrogen, such as plant material (see Henry, volume 2: chapter XXV-3). Therefore, the dietary conclusions do not yield the picture of purely carnivorous Neandertals, but allow the determination of the most important prey species, and also indicate that proteins from plant food were in too low quantities to provide significant signal to the studied Neandertals.

Comparison with Holocene Belgian hunter-gatherers and early agriculturalists

The human specimens from Spy of Holocene age present a chronological range corresponding to the middle to late Neolithic and can be compared to the large isotopic dataset published for the Meuse basin for this period (Bocherens *et al.*, 2007). The isotopic data from the Spy specimens (Table 1) are very similar to those obtained on specimens from other sites in Belgium, and suggest a similar subsistence strategy.

Interestingly, when the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Spy Neandertals are plotted together with all the Holocene modern humans from Belgium (Bocherens *et al.*, 2007 and this study), Neandertals exhibit the highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, although some modern humans can reach the same range for either carbon or nitrogen, but do not overlap when both isotopic signatures are considered together. The ancient Mesolithic humans present $\delta^{13}\text{C}$ values similar to those of Spy Neandertals indicating similar open vegetation in both cases, but the $\delta^{15}\text{N}$ values of Mesolithic specimens are lower than those of Neandertals, probably due to lower $\delta^{15}\text{N}$ values of the plants at the beginning of the foodweb. Some middle Neolithic specimens present $\delta^{15}\text{N}$ values as high as or higher than those of Neandertals, but their $\delta^{13}\text{C}$ values are lower. This is probably linked to the consumption of freshwater resources by these middle Neolithic humans (Bocherens *et al.*, 2007). Among the middle Neolithic specimens from Spy, one seems to fit this pattern (Spy 33a), while the others, mostly immatures, probably did not consume high proportions of freshwater resources (Figure 4).

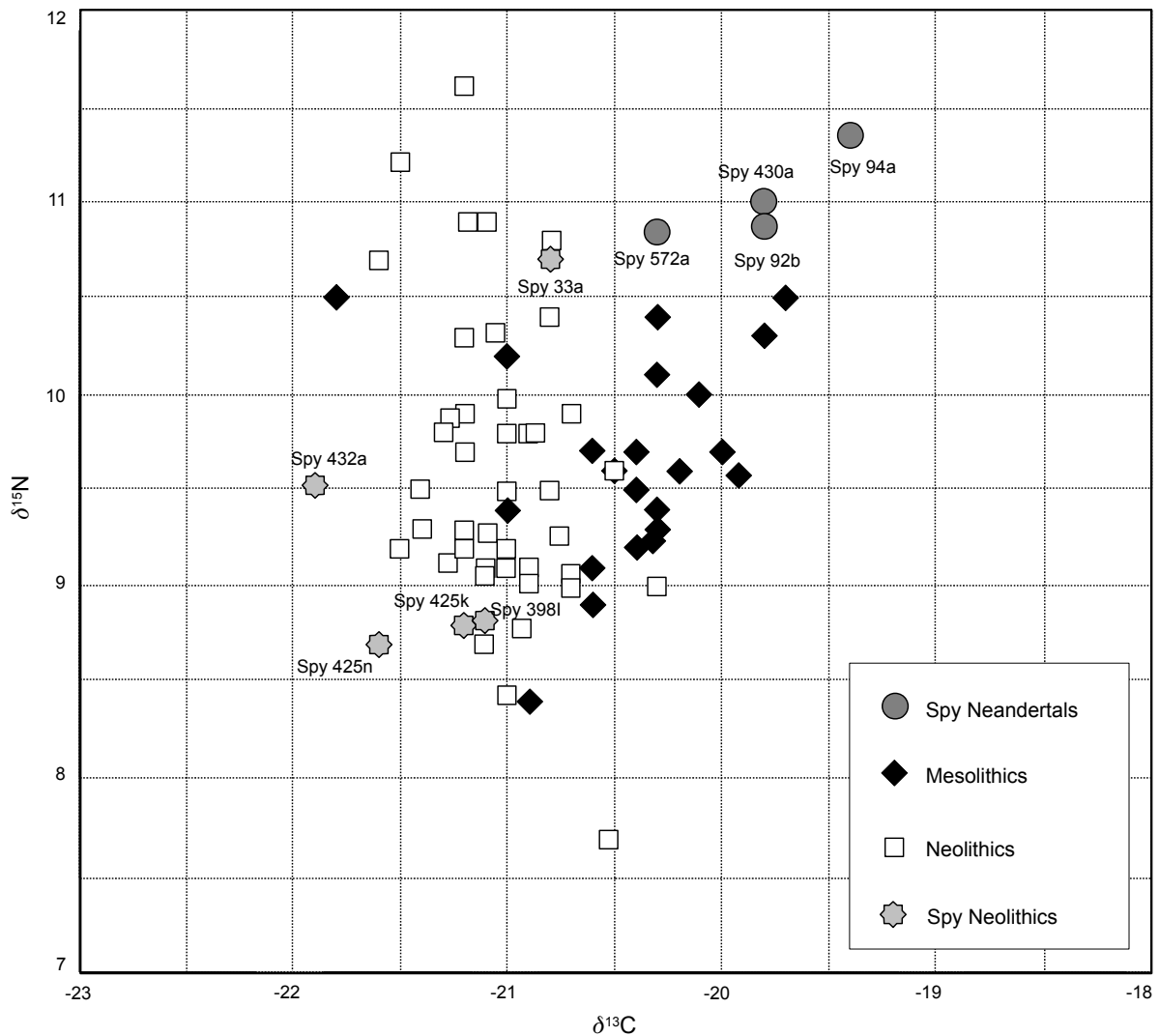


Figure 4. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Spy Neandertal collagen compared to those of Holocene humans from Belgium.

Note on the specific attribution of some artefacts

The radiocarbon dated bone spear point (Spy SP2 Spy 1954; GrA-32619) could well have been produced from a cave bear bone, as suggested by its $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Ethnographic evidence indicates that bear bones were used to make bone tools. For the Netsilik Inuit of Canada bone was an essential element in their technology. They were experts in adapting the qualities of bone to the specific use of the tool. The most important characteristics were size, hardness and elasticity. Although caribou antler was the basic raw material, bear bone was the hardest and points made of bear bone were used for the hunting of musk ox and bear (Balıkcı, 1970). Also the Mon-

taignais of Quebec used bear bones to make bone tools (Vanstone, 1974). Moreover, bone spear points from the same period from Potocka zijalka cave in Slovenia have also been attributed to cave bear, based on ancient DNA analysis (Hofreiter & Pacher, 2004).

Although the isotopic values of the spear clearly fall within the variation observed for cave bear bone collagen and outside the range observed for reindeer bone, we cannot completely exclude that such values could be obtained on reindeer antler which bones fall within some isotopic values observed at Spy (see example in Nelson & Møhl, 2003). This would be more in agreement with the tentative typolo-

gical interpretation (Flas *et al.*, this volume: chapter XII). More work on the isotopic signatures of reindeer antlers would certainly improve the reliability of identification of the origin of artefacts made of mammal skeletal parts.

CONCLUSION

The present study allows the reconstruction of origin of the protein part of the diet of two late Neandertals from Spy. The isotopic results point to a protein source based on terrestrial herbivores, with a focus on the meat of very large species, such as the woolly mammoth and possibly woolly rhinoceros. This pattern is in contrast with that documented for animal predators such as cave hyaenas, cave lions and wolves, but similar to the pattern observed in the roughly contemporaneous Neandertal from Saint-Césaire. This possibly

indicates that this dietary orientation was spread among late Neandertals in Western Europe. In the present case, the carbon and nitrogen isotopic signatures of the Spy Neandertal specimens are not found in any early Holocene modern humans of Mesolithic or Neolithic culture.

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