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Ecological niche of Neanderthals from Spy Cave revealed by nitrogen isotopes of individual amino acids in collagen

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ABSTRACT

This study provides a refined view on the diet and ecological niche of Neanderthals. The traditional view is that Neanderthals obtained most of their dietary protein from terrestrial animals, especially from large herbivores that roamed the open landscapes. Evidence based on the conventional carbon and nitrogen isotopic composition of bulk collagen has supported this view, although recent findings based on plant remains in the tooth calculus, microwear analyses, and small game and marine animal remains from archaeological sites have raised some questions regarding this assumption. However, the lack of a protein source other than meat in the Neanderthal diet may be due to methodological difficulties in defining the isotopic composition of plants. Based on the nitrogen isotopic composition of glutamic acid and phenylalanine in collagen for Neanderthals from Spy Cave (Belgium), we show that i) there was an inter-individual dietary heterogeneity even within one archaeological site that has not been evident in bulk collagen isotopic compositions, ii) they occupied an ecological niche different from those of hyenas, and iii) they could rely on plants for up to ~20% of their protein source. These results are consistent with the evidence found of plant consumption by the Spy Neanderthals, suggesting a broader subsistence strategy than previously considered.

1. Introduction

Dietary subsistence is a major component of the biology of a human population. Neanderthals became extinct around 40,000 years ago (Higham et al., 2014), and their subsistence strategies have attracted a lot of scientific attention since this information is crucial to evaluating their cognitive abilities and the possible reasons for their extinction. Several hypotheses regarding the demise of the Neanderthals at the time when early anatomically modern humans entered Europe involve dietary differences between the two types of hominins (Bocherens and Drucker, 2006; Froehle and Churchill, 2009; Hoffecker, 2009). Numerous studies, based on different approaches such as zooarchaeology and tooth wear patterns, have provided convincing evidence for a Neanderthal diet comprised largely of meat from large terrestrial herbivores (Lalueza Fox and Pérez-Pérez, 1993; Gardeisen, 1999; Boyle, 2000; Ready, 2010; Gaudzinski, 2014; Fiorenza et al., 2015). In this context, the first isotopic study of Neanderthal bone collagen published over two decades ago showing an isotopic composition close to those of animal predators such as wolves and hyenas was not surprising (Bocherens et al., 1991). Since then, many Neanderthal specimens have been analysed, and in all cases where collagen was well-preserved, a similar pattern was found (Fizet et al., 1995; Bocherens et al., 1999, 2005, 2013; Richards et al., 2008; Richards and Trinkaus, 2009). On the other hand, recent findings of plant remains trapped in a tooth calculus testify to the consumption of plants by Neanderthals (Henry et al., 2011, 2014; Hardy et al., 2012). Studies of dental microwear support a more diverse diet in some Neanderthal groups, which has been linked with increasing tree-cover (El Zaatari et al., 2011). However, as none of the palaeodietary approaches is able to accurately quantify the proportions of meat or

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plants eaten, the question remains as to how much plant material the Neanderthals consumed.

Recently an isotopic approach that provides accurate determination of trophic positions was developed for use in ecological and palaeoecological studies: nitrogen isotope analysis of individual amino acids. This approach represents a powerful tool for estimating animal trophic positions (TP: 1 = primary producer, 2 = primary consumer, 3 = secondary consumer, and so on) in aquatic (McClelland and Montoya, 2002; Chikaraishi et al., 2007, 2009; Popp et al., 2007), terrestrial (Chikaraishi et al., 2010, 2011), and complex ecosystems (Ishikawa et al., 2014). This method is based on the fact that the $\delta^{15}N$ of phenylalanine ($\delta^{15}N_{Phe}$) in organisms shows a small shift of 0.4 ± 0.4‰ from prey to consumer, and the $\delta^{15}N$ of glutamic acid ($\delta^{15}N_{Glu}$) shows a large prey–predator shift of 8.0 ± 1.1‰, reflecting the trophic position of the consumers in their respective ecosystems (McClelland and Montoya, 2002; Chikaraishi et al., 2009, 2014). An important point here is that $\delta^{15}N$ of phenylalanine reflects that of the nitrogen source of the ecosystems, therefore the primary producers (i.e., plants in most cases for terrestrial ecosystems; McClelland et al., 2003; Chikaraishi et al., 2011). Using the equation below based on $\delta^{15}N$ of glutamic acid and phenylalanine, it is possible to estimate the TP of animals in terrestrial ecosystems (Chikaraishi et al., 2011):

$$TP = (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta)/7.6 + 1 \quad \text{(Eq. 1)}$$

where TP, $\delta^{15}N_{Glu}$, $\delta^{15}N_{Phe}$, and β indicate trophic position in an ecosystem, $\delta^{15}N$ of glutamic acid, $\delta^{15}N$ of phenylalanine, and isotopic difference between $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ value) in primary producers respectively. The β value for terrestrial plants was estimated to be −8.4‰ (Chikaraishi et al., 2010, 2011). However, to date, only ancient humans from the Holocene have been studied using this approach, and most studies took place in temperate humid ecosystems. In these studies, marine protein consumptions (Styring et al., 2010; Naito et al., 2010a, b), the relative proportions of plant protein vs meat protein in diets (Naito et al., 2013a), and freshwater resource consumption (Naito et al., 2013b) were evaluated in South Africa, Japan, and France.

We applied this novel isotopic approach to Neanderthal specimens from the Spy Cave, Belgium and quantitatively evaluated this group's consumption of plant and animal foods. Three Neanderthal specimens, attributed to two individuals, were analysed: SPY-94a from Spy I and SPY-92b and SPY-430a from Spy II (Supplementary Online Material (SOM) Table S1). These specimens were selected because consumption of plants was suggested by both microwear and tooth calculus micro-fossils found on Neanderthal remains at this site (El Zaatari et al., 2011; Henry et al., 2011).

2. A brief introduction to trophic position (TP) estimation

Trophic position estimates for the Neanderthals and animals in this study are based on well-founded empirical data: (i) the large ^{15}N-enrichment of glutamic acid from prey to consumer (+8.0 ± 1.1‰) and the limited ^{15}N-enrichment of phenylalanine (+0.4 ± 0.4‰) as mentioned above (McClelland and Montoya, 2002; Chikaraishi et al., 2009, 2011); and (ii) the characteristic β value ($= \delta^{15}N_{Glu} - \delta^{15}N_{Phe}$) in the primary producer among terrestrial plants are −8.4‰, while aquatic ones such as algae and cyanobacteria present a β value of +3.4‰ (Chikaraishi et al., 2007, 2010; Fig. 1).

Chikaraishi et al. (2007) hypothesised that, during metabolism, glutamic acid rapidly undergoes transamination and the C–N bond is cleaved, leading to the large enrichment in ^{15}N, while, in contrast, the dominant metabolic step of phenylalanine adds a hydroxyl group to form tyrosine and does not cleave the C–N bond, leading to the small enrichment in ^{15}N (Chikaraishi et al., 2007; SOM Fig. S1). Thus, the $\delta^{15}N$ of phenylalanine reflects that of the primary producers (e.g., plants and algae) at the base of food webs (McClelland et al., 2003; Chikaraishi et al., 2011). Though factors controlling β values in plants are not fully understood, one promising hypothesis has been proposed: an isotope fractionation during the synthesis of lignin from phenylalanine through the phenylpropanoid pathway, used by terrestrial plants for structural reinforcement, may cause elevated $\delta^{15}N_{Phe}$ values relative to those of other amino acids (Ohkouchi and Takano, 2014; Ohkouchi et al., 2015; SOM Fig. S2). Because aquatic primary producers do not utilize this pathway, they may have less elevated $\delta^{15}N_{Phe}$ values than that of terrestrial plants. Based on these observations, the following equations that estimate the trophic position of animals were proposed (Chikaraishi et al., 2009, 2011; see Fig. 1):

$$TP\ (Terrestrial) = ([\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + 8.4]/[8.0-0.4]) + 1 \text{ for terrestrial ecosystems,}$$

$$TP\ (Aqua) = ([\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4]/[8.0-0.4]) + 1 \text{ for aquatic ecosystems.}$$

It is supposed that these equations can offset the background fluctuation in $\delta^{15}N$ of ecosystems because they utilize the isotopic spacing between two amino acids from the same individual organism, providing a means that the traditional isotopic method based on bulk protein lacks to reconstruct trophic positions (Fig. 2; Naito et al., 2013b).

3. Materials and methods

3.1. Materials

The Spy Cave is located in the village of Spy in the municipality of Jemeppe-sur-Sambre (province of Namur, Belgium), about 18 m above the present level of the Orneau River, a tributary of the Sambre. It opens to the southwest in a carboniferous limestone massif below a vast plateau. In 1886, the discovery of two adult Neanderthal skeletons in the terrace sediments along with Ice Age fauna and Middle Palaeolithic artefacts was a major milestone in the history of palaeoanthropology. The important skeletal assemblage was presented in the first monograph dedicated to Neanderthal fossils (Fraipont and Lohest, 1887). A multidisciplinary research project (2004–2007, BELPO [/36/0112]) enabled the recent discovery of new (unvarnished) Neanderthal remains amongst the faunal collections. These specimens have recently been re-evaluated, and have undergone dating (Semal et al., 2009, 2013) and new isotope analysis (Bocherens et al., 2013). This new anthropological study produced a new inventory of the human remains and re-attributed the fragments to the Spy I and Spy II individuals (Rougier et al., in press). Based on this work, the three specimens analysed in the present study could be attributed to either Spy I or Spy II (SOM Table S1).

In addition to human and animal bone material from Spy Cave, we also analysed faunal remains from the nearby cave of Scladina (Sclayn, Belgium), where a contemporary rich and diverse mammal fauna has been found and already analysed for bulk collagen isotopic values (Bocherens et al., 1997, 2011, 2013; SOM Tables S2 and S3). We analysed bone and tooth collagen samples that were already extracted from those skeletal remains in previous studies (Bocherens et al., 1997, 1999, 2011, 2013). All samples have atomic C/N ratios within established criteria (2.9–3.6; DeNiro, 1985).