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ORIGINAL PAPER

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Journal Article

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Original Paper

Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus

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Abstract

Neanderthals disappeared sometime between 30,000 and 24,000 years ago. Until recently, Neanderthals were understood to have been predominantly meat-eaters; however, a growing body of evidence suggests their diet also included plants. We present the results of a study, in which sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) were combined with morphological analysis of plant microfossils, to identify material entrapped in dental calculus from five Neanderthal individuals from the north Spanish site of El Sidrón. Our results provide the first molecular evidence for inhalation of wood-fire smoke and bitumen or oil shale and ingestion of a range of cooked plant foods. We also offer the first evidence for the use of medicinal plants by a Neanderthal individual. The varied use of plants that we have identified suggests that the Neanderthal occupants of El Sidrón had a sophisticated knowledge of their natural surroundings which included the ability to select and use certain plants.

Electronic supplementary material

The online version of this article (doi:[10.1007/s00114-012-0942-0](https://doi.org/10.1007/s00114-012-0942-0)) contains supplementary material, which is available to authorized users.

Introduction

Neanderthals disappeared sometime between 30,000–24,000 years ago (Finlayson et al. [2006](#)), and little is known in detail about the way in which they lived. Large numbers of animal bones found in association with Neanderthal artefacts led to the assumption that they were predominantly meat-eaters (Burke [2000](#)). This view has been reinforced by stable isotope analyses as the $\delta^{15}\text{N}$ values of Neanderthal bone collagen are consistent with a meat-rich diet (Richards and Trinkaus [2009](#)). Most isotope studies are from temperate Europe since few sites in warmer regions have sufficient collagen surviving for analysis, but a $\delta^{15}\text{N}$ value recently obtained from El Sidrón, northern Spain, is consistent with earlier findings (Wood et al. [2012](#)).

The similarity of isotopic results across both open and forested environments has led to the suggestion that dietary rigidity may have put Neanderthals at a disadvantage in competition with modern humans (Bocherens [2009](#)); however, there is an increasing range of evidence for greater dietary breadth. The recent identification of the *TAS2R38* bitter taste perception gene in a Neanderthal individual from El Sidrón (Lalueza-Fox et al. [2009](#)) is an indicator of an ability to include plants in the diet as bitterness can warn of toxins (Miller [2011](#)). The survival of actual plant remains within Neanderthal sites is rare; however, evidence for edible grass seeds at Amud Cave, Israel (Madella et al. [2002](#)), charred legumes at Kebara Cave, Israel (Lev et al. [2005](#)), and charred nuts at Gorham's Cave, Gibraltar (Barton [2000](#)) suggest these were eaten. Starch granules found embedded in dental calculus from one Neanderthal individual from the site of Shanidar in Iraq and two individuals from Spy in Belgium (Henry et al. [2011](#)) also suggests a plant component in the diet. Furthermore, based on a comparison with the use of similar items from ethnographic contexts, Sandgathe and Hayden ([2003](#)) suggest that pointed artefacts of bone and wood found on several Neanderthal sites may have been used to obtain edible inner bark.

Although a buccal molar microwear study has suggested a largely carnivorous diet for the Neanderthals of Gorham's Cave (Lalueza and Pérez-Pérez [1993](#)), a broader survey argues for a mixed diet of animals and plants, with a possible focus on more fibrous plants such as roots and bulbs in cooler periods (Pérez-Pérez et al. [2003](#)). A comparison of occlusal molar microwear patterns including one sample from El Sidrón (SDR-005) suggests a higher consumption of plants in more wooded environments (El Zaatari et al. [2011](#)).

From a broader evolutionary perspective, human saliva contains a substantially higher number of *AMY1* copy variants of the enzyme α -amylase than most other higher primates. The primary role of α -amylase is to break down starch molecules into accessible sugars. As the main constituent of cereal grains, plant storage roots and tubers, many nuts and some inner bark, starch offers the most direct dietary source of glucose, the essential provider of metabolic energy. It has been suggested that the increase in *AMY1* copy variants may have developed as a result of a dietary shift to starchy tubers by early hominins (Perry et al. [2007](#)).

One outcome of an increase in sugar ingestion is the development of dental plaque, formed by the activity of bacteria which are energised by sugars. If dental plaque is not removed, it can calcify within 2 weeks, although accumulation rates are individually variable (Lieverse [1999](#)). Dental calculus has a rough surface that provides an ideal location for further microbial activity, leading to increased plaque development in which the calculus appears to accumulate in layers (Hardy et al. [2012](#)). Calculus is found on both the labial and lingual sides of the tooth and can occur above or below the gum, where it is known as supra- or sub-gingival calculus, respectively. Dental calculus adheres strongly to the tooth, it has been found on the teeth of hominids dating to 1.8 million years (Blumenschine et al. [2003](#)). Most of the research on archaeological samples of dental calculus has focused upon the extraction and identification of plant microfossils (Piperno and Dillehay [2008](#); Hardy et al. [2009](#); [2012](#); Charlier et al. [2010](#); Henry et al. [2011](#)), but a recent study successfully

extracted sufficient protein for stable carbon and nitrogen isotope analysis (Scott and Poulson [2012](#)).

In this study, we present new results obtained from the dental calculus of five Neanderthal individuals using sequential thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) and pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS). The combination of these techniques enables the identification of both free/unbound and bound/polymeric organic components (Buckley et al. [1999](#)). By using these methods in conjunction with the extraction and analysis of plant microfossils, we have found chemical evidence consistent with wood-fire smoke, a range of cooked starchy foods, two plants known today for their medicinal qualities, and bitumen or oil shale entrapped within the dental calculus. Yet within the same calculus, chemical evidence for lipids/proteins from meat was low to absent.

Materials and methods

El Sidrón Cave is located in Asturias in Northern Spain, 15–20 km from the modern coastline. Approximately 2,000 skeletal remains representing at least 13 Neanderthal individuals have been found, aged between 47,300 and 50,600 BP (de Torres et al. [2010](#); Wood et al. [2012](#); Rosas [2012](#)). The total dental sample comprises 184 specimens, belonging to a minimum of 13 individuals both adult and juvenile. All the El Sidrón individuals have dental calculus. Deposits range from small (grade 1–2, [Hillson [2001](#)]), particularly in the case of the juveniles, to medium (grade 3) and large (grade 4) for the adults (Rosas et al. [2006](#)), suggesting that the calculus was cumulative and covers an extended period of the individuals' lives. Over 76 % (39 teeth) of the premolars and molars also have sub-vertical grooves; the cause of these is unclear but may be linked to erosion by particles ingested with food (Estalrich et al. [2011](#)).

Ten samples of dental calculus from five Neanderthal individuals were analyzed in this study. Each individual is identified by its overall age status (adult, adolescent or juvenile) followed by a number (1, 2, 3, etc.). Each dental calculus sample is identified by the letters SD or SDR, followed by a unique identifying code (Rosas et al. [2006](#)). Four samples are from the Neanderthal individual catalogued as Adult 2 (SD-1427m upper right M¹, SD-1427b upper left M³, SD-1427c upper left M², SDR-007c lower left P²), two samples from Adult 3 (SD-1217e lower left M¹, SD-1218a lower left M²), one sample from Adult 4 (SD-1604 upper left M²), two samples from Adult 5 (SD-1327i lower left M², SD-1327 h lower left M¹), and one sample from Juvenile 1 (SD-1716 lower right I²) (Table [1](#)). The variable number of samples from each individual is due to the differential presence of calculus.

Table 1 Dental calculus material sampled

Individual	Sample number	Mitochondrial lineage (Lalueza-Fox et al. 2011)	Tooth	Calculus location	SEM	TD-GC-MS and Py-GC-MS	Micro-fossils
Adult 2 Young male	SD 1427m	A	Upper right M ¹	Unknown (dislodged during cleaning)			X
Adult 2 Young male	SD 1427b	A	Upper left M ³	Unknown (dislodged during			X

Individual	Sample number	Mitochondrial lineage (Lalueza-Fox et al. 2011)	Tooth	Calculus location	SEM	TD-GC-MS and Py-GC-MS	Micro-fossils
				cleaning)			
Adult 2 Young male	SD 1427c	A	Upper left M ²	Lingual supra gingival	X		X
Adult 2 Young male	SDR 007c	A	Lower left P ²	Lingual supra gingival	X	X	X
Adult 3 Female	SD 1217e	B	Lower left M ¹	Removed from mould	X	X	X
Adult 3 Female	SD 1218a	B	Lower left M ²	Removed from mould			X
Adult 4 Young adult female	SD 1604	C	Upper left M ²	Lingual supra/sub gingival (mixed)		X	
Adult 5 Female	SD 1327i	A	Lower left M ²	Removed from mould			X
Adult 5 Female	SD 1327h	A	Lower left M ¹	Lingual supra gingival			X
Juvenile 1 5–6 years	SD 1716	C	Lower right I ²	Labial supra gingival			X

Although the sample size varied, no sample was larger than 2 × 1 mm. Three of the largest samples were split into two (Adult 2 SDR-007c, Adult 2 SD-1427c, Adult 4 SD-1604), and in one case (Adult 3 SD-1217e) three parts for multiple analysis. In order to record bacteria, scanning electron microscopy, using 0.5–1 mm² samples was conducted on two samples (Adult 2 SD-1427c, Adult 3-1217e), while TD-GC-MS and Py-GC-MS was conducted on three samples (Adult 2 SDR-007c, Adult 3 SD-1217e, Adult 4 SD-1604). A minimum size of 0.7 mg was required to conduct TD-GC-

MS and Py-GC-MS. Extraction and optical microscopy of plant microfossils was conducted on all samples.

Samples were collected on two separate occasions. The first batch of samples were retrieved in December 2009; this included two samples of dental calculus which were accidentally dislodged during cleaning of the mandibles (Adult 2 SD-1427m, Adult 2 SD-1427b,) and a further three samples (Adult 3 SD-1218a, Adult 3 SD-1217e, Adult 5 SD-1327i), which had adhered to the moulds made of the mandibles. In this case, the relevant parts of the moulds were cut out, placed in closed centrifuge tubes, and sonicated until the material dislodged (~6 min.). The samples which were dislodged during cleaning have only been used for extraction of plant microfossils and do not contribute to the detailed results. A further five samples (Adult 2 SDR-007c, Adult 2 SD-1427c, Adult 4 SD-1604, Adult 5 SD-1327h, Juvenile 1 SD-1716) were removed from the teeth in May 2010. These samples were prised off with a scalpel, dropped onto aluminium foil then retrieved immediately using tweezers and placed into Eppendorf tubes. All this work was undertaken in the El Sidrón laboratory at the Museum of Natural History, Madrid, except the sonication which took place in BioArCh, University of York. Under ideal circumstances, soil samples adjacent to the calculus would also be available for analysis.

Scanning electron microscopy (JSM-6490) of two samples (SD-1427c, SD-1217e) coated with 15–20 nm gold-palladium was undertaken at 20,000-fold magnification. Three samples of dental calculus from three separate individuals (Adult 2 SD-1427c, Adult 3 SD-1217e, Adult 4 SD-1604) were analyzed by TD-GC-MS and Py-GC-MS using a CDS Pyroprobe 1000. Samples were thermally desorbed at 310 °C for 10 s in open split mode at 30ml/min. Initially, the GC was held at 35 °C for 5 min, then temperature was programmed from 40–340 °C at 6 °C per minute and held at final temperature for 15 min, total 65 min. The run was repeated with the same sample being pyrolysed at 610 °C for 10 s. Four control samples of dental calculus including a modern (Hoy) sheep and an (Alsatian) dog, and two samples from the Victorian cemetery of St Barnabas, London were used for comparative purposes to assist in the interpretation of the El Sidrón material (ESM_1.pdf). Plant microfossils extraction was conducted on all samples according to a standard method (Hardy et al. 2009) in which samples were first rinsed in 0.6 M HCl for 5 min to remove adhered surface carbonates. They were then dried and coarsely ground. The resulting powder was suspended in 1.5 ml of 0.6 M HCl, vortexed every 15 min for 1 h, then centrifuged at room temperature at 13,000 rpm for 15 min. Samples were viewed on an Olympus IX 71 inverted microscope. Imaging was conducted using a Colour View camera and Cell D imaging system.

Results

Table 2 summarizes the material observed and identified from the dental calculus samples.

Table 2 Results of scanning electron microscopy, TD-GC-MS and Py-GC-MS and microfossil extraction from dental calculus samples

Sample no.	SEM analysis	MS Markers	Microfossils	Interpretation
Adult 2				
SD-1427m	NA	NA	9 starch	Ate starchy food
SD-1427b	NA	NA	>20 starch 1 phytolith	Ate starchy food

Sample no.	SEM analysis	MS Markers	Microfossils	Interpretation
SD-1427c	Filamentous and coccoidal.	NA	8 starch	Ate starchy food
SDR-007c	NA	HC, FAME, C, PAH, Ho, Ph Pr	20 starch	Ate several different cooked starchy plants. Inhaled woody smoke. No evidence for protein, Evidence of contact with oil shale/bitumen.
Adult 3.				
SD-1217e	Filamentous and coccoidal.	HC (trace)	8 starch	Ate cooked starchy food
SD-1218a	NA	NA	7 starch	Ate starchy food
Adult 4				
SD-1604	NA	HC, C, PhOH, PA, PAH, Az, Co, Ac*	0 starch	Ate a range of cooked carbohydrates. Azulenes and coumarins consistent with yarrow and camomile. Inhaled wood smoke and/or ate smoked food. Proteinmarkers. No evidence of lipids from animal meat. Traces of moulding material.
Adult 5				
SD-1327i	NA	NA	5 starch	Ate starchy food
SD-1327 h	NA	NA	8 starch	Ate starchy food
Juvenile 1				
SD-1716			4 starch	Ate starchy food

Key to abbreviations: *HC* hydrocarbon, *FAME* fatty acid methyl ester, *C* = 2-cyclopenten-1-one derivatives, *PAH* polynuclear aromatic hydrocarbons, *Ho* hopanes, *Ph* phytane, *Pr* pristane, *PhOH* phenols, *PA* phenolic acids, *Az* azulenes, *Co* coumarins, *Ac** acrylates *modern contamination (for details on chemical compounds, see ESM_4 pdf)

Adult 2—SDR-007c (lower left P²)

The thermal desorption-GC-MS (TIC) (Fig. 1 inset) is dominated by a series of *n*-alkanes (carbon

numbers C22 to C35), suggesting a higher plant source (Eglinton et al. [1962](#)), most probably derived from plant waxes in the original food consumed. Also identified were a series of hopanes (carbon numbers C29 to C33), indicative of an oil shale or bitumen and corroborated by the presence of the isoprenoid hydrocarbon biomarkers phytane and pristane (Williams and Douglas [1986](#); Connan [1999](#)). The pyrolysis-GC-MS (TIC) (Fig. [1](#)) was dominated by C4 to C30 *n*-1-alkenes and *n*-alkanes, typical of unsaturated and saturated fat/oil-derived acyl lipids and higher plant waxes (Buckley et al. [1999](#)). Thermally derived carbohydrate markers (2-methyl-2-cyclopenten-1-one and 2,3-dimethyl-2-cyclopenten-1-one) were also identified (McCobb et al. [2001](#)). The absence of these compounds in the TD profile provides molecular evidence for a polymeric carbohydrate source, consistent with a starchy plant. The presence of 20 starch granules in this sample confirms this finding and demonstrates the ingestion of starchy material. Although it is not possible to identify the exact plant source of these starch granules solely on the basis of their morphology (Wilson et al. [2010](#)), their small size and angular nature suggests a seed source rather than a tuber; granules that grow in a compact environment such as a seed case tend to develop angularly, while granules growing in a less-restricted environment such as a tuber are more likely to have smooth edges. The presence of the fatty acid methyl esters, methyl palmitate, and methyl stearate can suggest methylation of the fat/oil triglycerides at high temperature, i.e., cooking. Although fatty acid methyl esters have been previously observed as minor components of fungal spores and bacteria (Laseter et al. [1968](#); Maudinas and Villoutreix [1977](#)), their molecular composition is very different. In contrast, these methyl esters would be expected to be the main fatty acid methyl esters formed from the heating of lipids, i.e., fats. The additional presence of the main combustion markers, fluoranthene, and pyrene, along with smaller amounts of fluorene and phenanthrene, strongly supports the evidence for cooking/smoke inhalation in this sample (Ré-Poppi and Santiago-Silva [2002](#); Varlet et al. [2006](#)). It is notable and highly relevant that the relative abundances of these combustion markers are entirely consistent with those found in wood smoke (Rogge et al. [1998](#); Seng et al. [2007](#)). No free bacterially derived components such as branched acyl lipids and hydrogenated steroids were observed, nor any bacterially derived hydrocarbons originating from the branched fatty acid acyl groups (significant components of bacterial triglycerides) were observed in the TD/Py-GC-MS of this sample (Shorland [1962](#)). This suggests that the components in the dental calculus derive from the diet, yet notably, there were no diagnostic protein markers or steroidal compounds indicative of meat ingestion.

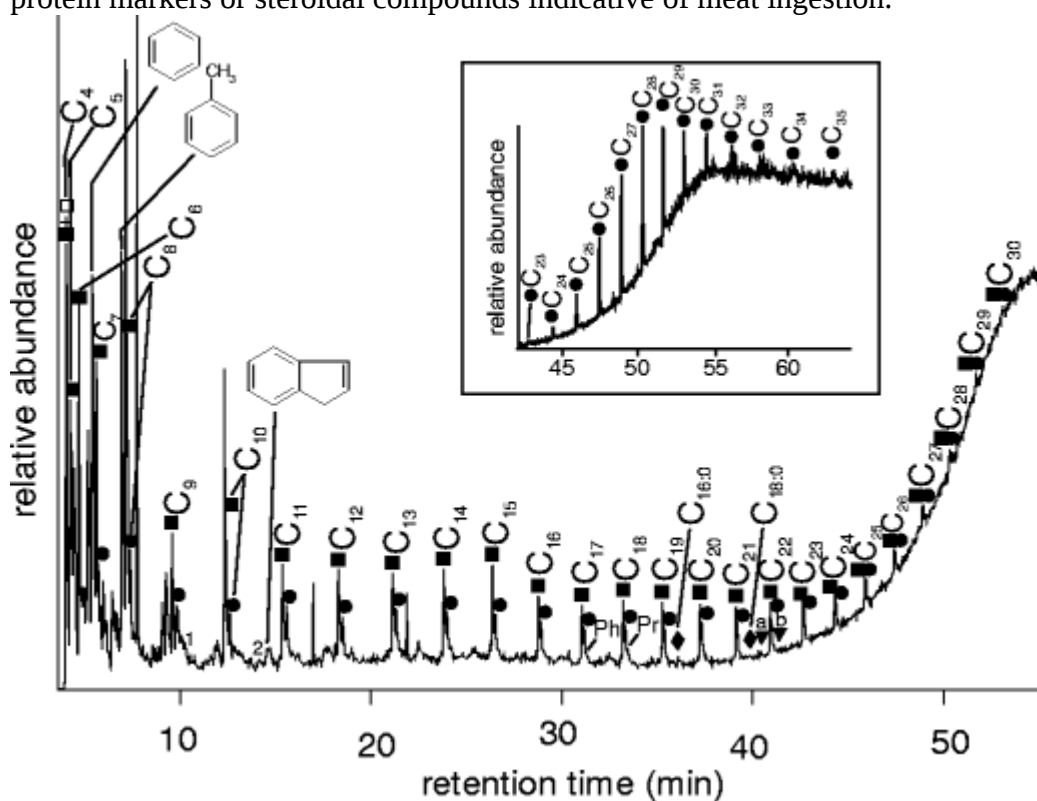


Fig. 1 Reconstructed total ion chromatogram of the pyrogram (pyrolysis profile) (610 °C for 10) of sample SDR-007c, after thermal desorption (310 °C for 10 s). Key to Fig. 1: Peak identities (x indicates carbon chain length): *filled diamonds*, Cx:0 indicates saturated fatty acid methyl esters; *filled squares*, Cx indicates alkenes; *filled circles*, Cx indicates alkanes; *inverted triangles*, *a* is fluorathene and *b* is pyrene. Ph is phytane and Pr is pristane. Peak 1 is 2-methyl-2-cyclopenten-1-one and 2 is 2,3-dimethyl-2-cyclopentene-1-one. Also shown are the structures of three aromatic compounds identified: benzene, toluene, and indene. *Inset* displays a reconstructed total ion chromatogram of the thermal desorption profile (310 °C for 10 s) of this sample. Peak identities: *filled circles*, Cx indicates alkanes

Adult 3—SD1217e (lower left M¹)

The thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS) total ion chromatogram (TIC) revealed no detectable components, indicating the absence of any free, thermally extractable lipids in this sample. The pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) TIC identified very few components, with only low molecular weight aromatic hydrocarbons and alkenes of uncertain origin detected. This absence of significant markers suggests that micro-organisms that form the calculus do not contribute significantly to the overall organic signal. All the other samples (SD-1427b, SD-1427c, SD-1427, SD-1218, SD-1716, SD-1327i) had evidence for the consumption of starchy food in the form of actual starch granules. Furthermore, the basal part of a trichome phytolith compatible with grass plants (Poaceae) (Kaplan et al. [1992](#)) was recovered in sample SD-1427b. The phytolith was broken towards the edge and the hair tip was missing. Starch is concentrated in grass seeds, and this can be accessed relatively easily by chewing, although we did not observe any of the grass-like starch granules reported by Henry et al. ([2011](#)).

Adult 4—SD1604 (upper left M²)

A range of alkyl phenols and polynuclear aromatic hydrocarbons (PAHs) suggest the ingestion of wood smoke or smoked foods by this individual (Ré-Poppi and Santiago-Silva [2002](#); Varlet et al. [2006](#)). Recent studies of modern cigarette smokers suggests that smoking increases the development of calculus (Bergström [1999](#), [2005](#)), though it is not possible at present to determine whether smoke inhalation had an effect on the quantity of dental calculus found on the Neanderthal teeth. A range of carbohydrate compounds greater than that observed in SDR-007c suggests the possibility of several different nutritional plants, with this sample being particularly notable for the presence of azulene and coumarin derivatives. The former includes azulene, dihydroazulene, 4,6,8-trimethylazulene and chamazulene (7-ethyl-1,4-dimethylazulene), and the latter 4,5,7-trimethylcoumarin, 4-methylherniarin (7-methoxy-4-methylcoumarin), and the tentatively identified scopoletin (6-methoxy-7-hydroxycoumarin). The presence of chamazulene, dihydroazulene, and 4-methylherniarin is also notable since the two former compounds, together with herniarin, occur in yarrow (*Achillea millefolium*), and chamazulene and herniarin in chamomile (*Matricaria chamomilla*). Also significant pyrolysis components of the organic material within the calculus were the thermolytically derived methyl esters of the aromatic acids 4-hydroxyphenylacetic acid and 3-hydroxyphenylacetic acid, together with their methoxy-derivatives (McCobb et al. [2001](#)). These, and similar phenolic acids, occur in a number of food sources including nuts (Senter et al. [1983](#)). Toluene and *o*-, *m*-, and *p*-xylenes in moderate abundance, while lower abundances of pyridine, 2-methyl pyridine, 3-methyl pyridine, 4-methyl pyridine and pyrrole were present; these are indicative of the consumption of protein. Notably, however, the absence of any lipid components in the dental calculus means that this individual has no evidence of fat consumption, as would be expected in a meat-based diet. Traces of the material used to make moulds of the mandibles were also found. The pyrogram displayed a number of carbohydrate markers, in addition to those detected in SDR-007c, suggesting a more complex range of carbohydrates was present. Protein markers were also present but protein–lipid condensation products (e.g., aliphatic nitriles and amides) were absent, suggesting the absence of a significant

amount of lipid in this sample. No starch granules were detected in this sample, which may simply be a product of sampling, as carbohydrate markers were found (see above). A possible indication of pyrolyzed lutein was also detected; this is a xanthophyll (yellow pigment) commonly found in green vegetables. The combined presence of the alkyl phenols and polynuclear aromatic hydrocarbons (PAHs) has been observed in wood smoke and smoked foods (Ré-Poppi and Santiago-Silva [2002](#); Varlet et al. [2006](#)), their presence in the calculus suggesting proximity to a fire, and/or the ingestion of cooked or smoked food. Other significant pyrolysis components detected are also known to occur in a number of other food sources, including nuts (Senter et al. [1983](#); McCobb et al. [2001](#)) (See ESM_2.pdf for detailed results).

Starch granules

Starch granules were found in nine of the ten samples (Table [2](#), ESM_3.pdf). While there is always a potential risk of contamination, starch granules were recorded embedded in the dental calculus matrix, eliminating the possibility that these could be recent contaminants (Fig. [2a](#)). Starch is identified initially through its size, shape, and birefringence. The birefringence is caused by the ordered arrangement of the component amylose and amylopectin polymers, which produces a characteristic “Maltese Cross” interference pattern when viewed under cross-polarized light. Other materials, including water bubbles, can however produce a similar effect (Hardy et al. [2009](#)). To confirm that the material observed microscopically is indeed starch (*Bacillus licheniformis*), alpha-amylase (0.25 ml of undiluted 1, 4-alpha-D-glucan-glucanohydrolase) was dropped onto a sample of granules and incubated at 25 °C for 24 h. Positive and negative controls (modern starch with and without amylase) were also used. Since all the positive control samples and archaeological granules disappeared while the negative control sample survived, we therefore conclude that the granules were composed of starch. The different sizes and shapes present in the samples suggest the possibility of more than one plant genus. This is also consistent with the mass spectrometry results which suggest a range of carbohydrate sources.



Fig. 2 Microscopically visible material entrapped in dental calculus samples. **a** Group of starch granules still embedded in dental calculus matrix (SD 1427b). **b** Damaged starch granule (17 µm diameter) viewed under polarized light. Note the cracking around the exterior caused by damage or degradation of the granule (SD 1327i). **c** Filamentous and cocci bacteria (sample SD 1427c)

It has been argued that it is difficult to confirm that partially gelatinized starch granules are in fact cooked, as gelatinization would be a stage in the long-term disintegration of the granule (Collins and Copeland [2011](#)). However, if the starch is dry-heated (parching or popping), granules lose water and this can cause cavities (Copeland et al. [2009](#)). Edge cracking, which may be consistent with dry-heat, was observed in several of the starch granules (Fig. [2b](#)). It is not unreasonable to suggest that dry heat produces a hardening of the granule case, which can then result in a resistance to moisture adsorption (Collison [1968](#)) and may assist in long preservation.

Oral health

Fossilized bacteria, both elongated filamentous and, less commonly, coccoidal, were observed by scanning electron microscopy (Fig. [2c](#)). Both morphotypes have been identified in Neanderthal individuals from Subalyuk 2, Kebara 2, and Spy (Vandermeersch et al. [1994](#); Pap et al. [1995](#)). The

proportion of filamentous to cocci type bacteria is similar to that observed in a sample from Subalyuk 2 (N Hungary) but differs from a sample from Kebara 2 in which cocci bacteria were more common (Vandermeersch et al. [1994](#)). The presence of bacteria with different morphologies in all observed samples from El Sidrón, together with those found in the samples from different sites, suggests this is an untapped resource for information on past dental and physical health.

Discussion

Oil shale

In one sample (SDR-007c), traces of a possible oil shale or bitumen were identified by a series of hopanes and the isoprenoid hydrocarbons phytane and pristane. Bitumen is known to have been used as a hafting material by Neanderthals (Boeda et al. [2008](#)), while the nearest oil shale source to El Sidrón is located 15 km east at Llames de Parres (Kruge and Suárez-Ruiz [1991](#)). Although further biomarker work is needed to determine a specific characteristic molecular fingerprint, our findings raise the possibility that this individual may have visited Llames de Parres, or worked with bitumen collected from this site.

Food and medicine?

The principal problem in evaluating the exploitation of plants in pre-agricultural times is the lack of direct evidence; nonetheless, O'Connell et al. ([1999](#)) argue that *Homo erectus* had a high dependence on tubers. It has also been argued that the exploitation of starchy roots was a significant factor in the expansion of hominins into a savannah environment (Laden and Wrangham [2005](#)). Although these are theoretical perspectives, they correlate well with the high salivary amylase identified in modern humans in comparison with other higher primates (Perry et al. [2007](#)).

The presence of pigments and bitter-tasting appetite suppressants (dihydroazulene and chamazulene, and the coumarin, 4-methylherniarin) in the calculus of Young Adult 4—SD1604 is intriguing. One possible reason for the consumption of bitter-tasting plants with no nutritional value and containing these compounds (such as yarrow and camomile) would be for self-medication. All the higher primates have a wide and applied knowledge of the edible plants within their environments, and there is an extensive body of evidence demonstrating the complex use of medicinal plants for zoopharmacognosy by animals including all modern higher primates (e.g., Rodriguez and Wrangham [1993](#); Cousins and Huffman [2002](#); Huffman [1997](#), [2003](#); Singer et al. [2009](#); Lisonbee et al. [2003](#); Krief et al. [2005](#); Huffman and Vitazkova [2007](#)).

The starch granules and carbohydrate markers in these samples, the evidence for the azulene and coumarin compounds, the possible evidence for nuts, grasses, and possibly even green vegetables, argue for a broader use of ingested plants than is often suggested by stable isotope analysis. This view is compounded by the surprisingly low levels of protein markers (in the form of diketopiperazines, DKPs), which were lower than in control samples of modern calculus from dog and sheep (ESM_1.pdf). Though preferential degradation of residual protein was considered, the survival of sufficient levels of collagen in both bones and teeth to undertake racemization and radiocarbon dating (de Torres et al. [2010](#)) suggests that the absence of protein is a genuine indicator of low protein levels in the diet during the period over which the dental calculus accumulated.

The beginnings of cooking are suggested to be as far back as 1.9 Ma (Wrangham et al. [1999](#)); the presence of hearths and burnt bone on many Neanderthal sites suggests that they cooked at least some of their food. Using mass spectrometry, we have identified the ingestion of cooked carbohydrates in the calculus of two adults, one adult in particular having apparently eaten several different carbohydrate-rich foods. The evidence for cooked carbohydrates is confirmed both by the cracked/roasted starch granules observed microscopically and the molecular evidence for cooking and exposure to wood smoke or smoked food in the form of methyl esters, phenols, and polynuclear aromatic hydrocarbons (notably pyrene and fluoranthene) found in the dental calculus.

Neanderthals lived through different climatic regimes, including periods in which numerous edible plants were available for exploitation (Jones [2009](#); Hardy [2010](#); El Zaatari et al. [2011](#)). We propose that the Neanderthal occupants of El Sidrón, whose hypothesized, cannibalized remains (Rosas et al. [2006](#)) were discarded at the site, had a sophisticated knowledge of their natural surroundings, and were able to recognize both the nutritional and the medicinal value of certain plants. Although the extent of their botanical knowledge and their ability to self-medicate must of course remain open to speculation, the fact that higher primates have some understanding of the flora within their environment, and the extensive evidence for self-medication within the animal kingdom, would surely make it surprising if the Neanderthals did not also share such knowledge.

We believe that our findings offer the first direct molecular evidence for the ingestion of carbonized food and the inhalation of smoke by a Neanderthal individual. We also offer the first measurable molecular evidence that dental calculus is a trap for ingested material, the starch granules reported from El Sidrón representing the oldest granules ever to be confirmed using a biochemical test. Our approach to the study of this material, combining analytical chemistry with morphological observation, offers the opportunity to maximize the biographical detail to be gained for ancient human populations.

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Electronic supplementary material

Below is the link to the electronic supplementary material.

[ESM 1 \(PDF 690 kb\)](#)

[ESM 2 \(PDF 24 kb\)](#)

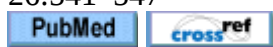
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[ESM 4 \(PDF 139 kb\)](#)

References

Barton RNE (2000) Mousterian hearths and shellfish: late Neanderthal activities in Gibraltar. In: Stringer CB, Barton RNE, Finlayson JC (eds) Neanderthals on the edge: 150th anniversary conference of the Forbes' Quarry discovery, Gibraltar. Oxbow Books, Oxford, pp 211–220

Bergström J (1999) Tobacco smoking and supragingival dental calculus. *J Clin Periodontol* 26:541–547



Bergström J (2005) Tobacco smoking and subgingival dental calculus. *J Clin Periodontol* 32:81–88



Blumenschine RJ, Peters CR, Masao FT, Clarke RJ, Deino AL, Hay RL, Swisher CC, Stanistreet IG, Ashley GM, McHenry LJ, Sikes NE, van der Merwe NJ, Tactikos JC, Cushing AE, Deocampo D, Njau JK, Ebert JI (2003) Late Pliocene homo and hominid land use from Western Olduvai Gorge, Tanzania. *Science* 299:1217–1221



Bocherens H (2009) Neanderthal dietary habits: review of the isotopic evidence. In: Hublin JJ and Richards MP (eds.) *The Evolution of Hominin Diets: Integrating Approaches to the Study of Palaeolithic Subsistence*. Springer, <http://www.springer.com/social+sciences/archaeology+%26+anthropology/book/978-1-4020-9698-3>, pp 241–250

Boeda E, Bonilauri S, Connan J, Jarvie D, Mercier N, Tobey M, Valladas H, Al-Sakhel H, Muhesen S (2008) Middle Palaeolithic bitumen use at Umm el Tiel around 70,000 BP. *Antiquity* 82:853–861

Buckley SA, Stott AW, Evershed RP (1999) Studies of organic residues from ancient Egyptian mummies using high temperature-gas chromatography-mass spectrometry and sequential thermal desorption-gas chromatography-mass spectrometry and pyrolysis-gas chromatography-mass spectrometry. *Analyst* 124:443–452



Burke A (2000) Hunting in the Middle Palaeolithic. *Int J Osteoarchaeol* 10:281–285



Charlier P, Huynh-Charlier I, Munoz O, Billard M, Brun L, Grandmaison GLD (2010) The microscopic (optical and SEM) examination of dental calculus deposits (DCD). Potential interest in forensic anthropology of a bio-archaeological method. *Legal Medicine* 12:163–171



Collins MJ, Copeland L (2011) Ancient starch: cooked or just old? *Proc Natl Acad Sci USA* 108(22):E145



Collison R (1968) Starch retrogradation. In: Radley JA (ed) *Starch and its derivatives*. Chapman and Hall Ltd, London, pp 168–193

Connan J (1999) Use and trade of bitumen in antiquity and prehistory: molecular archaeology reveals secrets of past civilisations. *Phil Trans Royal Soc B (Biological Sciences)* 354:33–50



Copeland L, Blazek J, Salman H, Tang CM (2009) Form and functionality of starch. *Food Hydrocolloid* 23:1527–1534



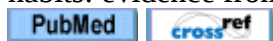
Cousins D, Huffman MA (2002) Medicinal properties in the diet of gorillas: an ethnopharmacological evaluation. *African Study Monographs* 23:65–89

de Torres T, Ortiz JE, Grün R, Eggins S, Valladas H, Mercier N, Tisnérat-Laborde N, Julià R, Soler V, Martínez E, Sánchez-Moral S, Cañaveras JC, Lario J, Lalueza-Fox C, Badal E, Rosas A, Santamaría D, de la Rasilla M, Fortea J (2010) Dating of the hominid (*Homo neanderthalensis*) remains accumulation from El Sidrón Cave (Piloña, Asturias, North Spain): an example of multi-methodological approach to the dating of Upper Pleistocene sites. *Archaeometry* 52:680–705

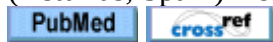
Eglinton G, Hamilton RJ, Raphael RA, Gonzalez AG (1962) Hydrocarbon constituents of the wax coatings of plant leaves: a taxonomic survey. *Nature* 193:739–742



El Zaatari S, Grine FE, Ungar PS, Hublin JJ (2011) Ecogeographic variation in Neandertal dietary habits: evidence from occlusal molar microwear texture analysis. *J Hum Evol* 61:411–424



Estalrich A, Rosas A, García-Vargas S, García-Tabernero A, Santamaría D, de la Rasilla M (2011) Brief communication: subvertical grooves on interproximal wear facets from the El Sidrón (Asturias, Spain) Neanderthal dental sample. *Am J Phys Anthropol* 144:154–161



Finlayson C, Pacheco FG, Rodríguez-Vidal J, Fa DA, Gutierrez López JM, Pérez AS, Finlayson G, Allue E, Baena Preysler J, Cáceres I, Carrión JS, Fernández Jalvo Y, Gleed-Owen CP, Jimenez Espejo FJ, López P, López Sáez JA, Riquelme Cantal JA, Sánchez Marco A, Giles Guzman FK, Fuentes N, Valarino CV, Villalpando A, Stringer CB, Martinez Ruiz F, Sakamoto T (2006) Late survival of Neanderthals at the southernmost extreme of Europe. *Nature* 443:850–853



Hardy BL (2010) Climatic variability and plant food distribution in Pleistocene Europe: implications for Neanderthal diet and subsistence. *Quat Sci Rev* 29:662–679



Hardy K, Blakeney T, Copeland L, Kirkham J, Wrangham R, Collins M (2009) Starch granules, dental calculus and new perspectives on ancient diet. *J Archaeol Sci* 36:248–255



Hardy K, van de Locht R, Wilson J, Tugay O (2013) Starch granules and complex carbohydrates at Çatalhöyük. In: Hodder I (ed) *Humans and landscapes of Çatalhöyük: reports from the 2000-2008 seasons*. Los Angeles: Cotsen; Ankara: British Institute of Archaeology at Ankara

Henry AG, Brooks AS, Piperno DR (2011) Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium). *Proc Natl Acad Sci USA* 108:486–491



Hillson S (2001) Recording dental caries in archaeological human remains. *Int J Osteoarchaeol* 11:249–289



Huffman MA (1997) Current evidence for self-medication in primates: a multidisciplinary perspective. *Yearb Phys Anthropol* 40:171–200



Huffman MA (2003) Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proc Nutr Soc* 62:371–381



Huffman MA, Vitazkova SK (2007) Primates, plants, and parasites: the evolution of animal self-medication and ethnomedicine. In Elisabetsky E, Etkin NL (eds.) *Ethnopharmacology*, e-book <http://www.eolss.net>, Eolss Publishers, Oxford

Jones M (2009) Moving North: Archaeobotanical Evidence for Plant Diet in Middle and Upper Paleolithic Europe. In Hublin JJ and Richards MP (eds.) *The Evolution of Hominin Diets: Integrating Approaches to the Study of Palaeolithic Subsistence*. Springer, <http://www.springer.com/social+sciences/archaeology+%26+anthropology/book/978-1-4020-9698-3>, pp 171–180

Kaplan L, Smith MB, Sneddon LA (1992) Cereal grain phytoliths of southwest Asia and Europe. In Rapp, G Jr, Mulholland SC (eds) *Phytolith systematics—emerging issues*. *Advances in Archaeological Museum Science Vol.1* Plenum Press, New York, pp149–174

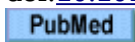
Krief S, Hladik CM, Haxaire C (2005) Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. *J Ethnopharmacol* 101:1–15



Kruege MK, Suárez-Ruiz NI (1991) Organic geochemistry and petrography of Spanish oil shales. *Fuel* 70:1298–1302



Laden G, Wrangham R (2005) The rise of the hominids as an adaptive shift in fallback foods: plant underground storage organs (USOs) and australopith origins. *J Hum Evol* 49:482–498. doi:[10.1016/j.jhevol.2005.05.007](https://doi.org/10.1016/j.jhevol.2005.05.007)



Lalueza C, Pérez-Pérez A (1993) The diet of the Neanderthal child Gibraltar 2 (Devil's Tower) through the study of the vestibular striation pattern. *J Hum Evol* 24:29–41



Lalueza-Fox C, Gigli E, de la Rasilla M, Fordea J, Rosas A (2009) Bitter taste perception in Neanderthals through the analysis of the *TAS2R38* gene. *Biol Letters* 5:809–811



Lalueza-Fox C, Rosas A, Estalrich A, Gigli E, Campos PF, GarcíaTabernerero A, García-Vargas S, Sánchez-Quinto F, Ramírez O, Civit S, Bastir M, Huguet R, Santamaría D, Gilbert MPT, Willerslev

E, de la Rasilla M (2011) Genetic evidence for patrilocal mating behaviour among Neandertal groups. *Proc Natl Acad Sci* 108:250–253



Laseter JL, Weete J, Weber DJ (1968) Alkanes, fatty acid methyl esters, and free fatty acids in surface wax of *Ustilago maydis*. *Phytochemistry* 7:1177–1181



Lev E, Kislev M, Bar Yosef O (2005) Mousterian vegetal food in Kebara Cave, Mt. Carmel. *J Arch Sci* 32:475–484



Lieverse AR (1999) Diet and the aetiology of dental calculus. *Int J Osteoarchaeol* 9:219–232



Lisonbee LD, Villalba JJ, Provenza FD, Hall JO (2003) Tannins and self-medication: implications for sustainable parasite control in herbivores. *P Nutr Soc* 62:361–370. doi:[10.1079/PNS2003243](https://doi.org/10.1079/PNS2003243)



Madella M, Jones MK, Goldberg P, Goren Y, Hovers E (2002) The exploitation of plant resources by Neanderthals in Amud Cave (Israel): the evidence from Phytolith studies. *J Arch Sci* 29:703–719



Maudinas B, Villoutreix J (1977) Fatty acid methyl esters in photosynthetic bacteria. *Phytochemistry* 16:1299–1300



McCobb LME, Briggs DEG, Evershed RP, Hall AR, Hall RA (2001) Preservation of fossil seeds from a 10th Century AD Cess Pit at Coppergate, York. *J Arch Sci* 28:929–940



Miller G (2011) Sweet here, salty there: evidence for a taste map in the Mammalian Brain. *Science* 333(6047):1213



O'Connell JF, Hawkes K, Blurton Jones NG (1999) Grandmothering and the evolution of *Homo erectus*. *J Hum Evol* 36:461–485



Pap I, Tillier AM, Arensburg B, Weiner SM (1995) First scanning electron microscope analysis of dental calculus from European Neanderthals: Subalyuk, (Middle Palaeolithic, Hungary). Preliminary report. *B Soc Ant Paris* 7:69–72



Pérez-Pérez A, Espurz V, Bermúdez de Castro JM, de Lumley MA, Turbón D (2003) Non-occlusal dental microwear variability in a sample of Middle and Late Pleistocene human populations from Europe and the Near East. *J Hum Evol* 44:497–513



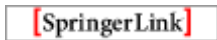
Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, Werner J, Villanea FA, Mountain JL, Misra R, Carter NP, Lee C, Stone AC (2007) Diet and the evolution of human amylase gene copy number variation. *Nat Genet* 39:1256–1260



Piperno DR, Dillehay TD (2008) Starch grains on human teeth reveal early broad crop diet in northern Peru. *Proc Natl Acad Sci USA* 105:19622–19627



Ré-Poppi N, Santiago-Silva MR (2002) Identification of polycyclic aromatic hydrocarbons and methoxylated phenols in wood smoke emitted during production of charcoal. *Chromatographia* 55:475–481



Richards MP, Trinkaus E (2009) Isotopic evidence for the diets of European Neanderthals and early modern humans. *Proc Natl Acad Sci* 106:16034–16039



Rodriguez E, Wrangham RW (1993) Zoopharmacognosy: the use of medicinal plants by animals. In: Downum KR, Romeo JT, Stafford H (eds) *Recent advances in phytochemistry*, vol. 27: phytochemical potential of tropic plants, vol 27. Plenum, New York, pp 89–105

Rogge WF, Hildemann LM, Mazurek MA, Cass GR, Simoneit BRT (1998) Sources of fine organic aerosol 9. Pine, oak, and synthetic log combustion in residential fireplaces. *Environ Sci Technol* 32:13–22



Rosas A, Martínez-Maza C, Bastir M, García-Tabernero A, Lalueza-Fox C, Huguet R, Ortiz JE, Julià R, Soler V, de Torres T, Martínez E, Cañaveras JC, Sánchez-Moral S, Cueva S, Lario J, Santamaría D, de la Rasilla M, Fordea J (2006) Paleobiology and comparative morphology of a late Neandertal sample from El Sidrón, Asturias, Spain. *Proc Natl Acad Sci* 103:19266–19271



Rosas A, Estalrich A, García-Tabernero A, Bastir M, García-Vargas S, Sánchez-Meseguer A, Huguet R, Lalueza-Fox C, Peña-Melián A, Kranjoti E, Santamaría D, Rasilla de la M, Fordea J. (2012) Les Néandertaliens d'El Sidrón (Asturies, Espagne). Actualisation d'un nouvel échantillon. *L'Anthropologie* 116:57–76

Sandgathe DM, Hayden B (2003) Did Neanderthals eat inner bark? *Antiquity* 77:709–718

Scott GR, Poulson SR (2012) Stable carbon and nitrogen isotopes of human dental calculus: a

potentially new non-destructive proxy for paleodietary analysis. *J Archaeol Sci* 39:1338–1393



Seng TH, Tahir NM, Abas MR (2007) Aliphatic and PAHs emissions from open burning of selected tropical woods. *Malaysian J Anal Sci* 11:36–41

Senter SD, Horvat RJ, Forbus WR (1983) Comparative GLC-MS analysis of phenolic acids of selected tree nuts. *J Food Sci* 48:798–799



Shorland FB (1962) The comparative aspects of fatty acid occurrence and distribution. In: Florkin M, Mason HS (eds) *Comparative biochemistry Vol 3*. Academic, New York, pp 1–102

Singer MS, Mace KC, Bernays EA (2009) Self-medication as adaptive plasticity: increased ingestion of plant toxins by parasitized caterpillars. *PLoS One* 4(3):e4796



Vandermeersch B, Arensburg B, Tillier AM, Rak Y, Weiner S, Spiers M, Aspillaga . (1994). Middle Palaeolithic dental bacteria from Kebara, Israël. *C R Acad Sci Paris* 319:727–731

Varlet V, Knockaert C, Prost C, Serot T (2006) Comparison of odor-active volatile compounds of fresh and smoked salmon. *J Agric Food Chem* 54:3391–3401



Williams PFV, Douglas AG (1986) Organic Geochemistry of British Kimmeridge clay 2. Acyclic isoprenoid alkanes in Kimmeridge shale oils. *Fuel* 65:1728–1734



Wilson J, Hardy K, Allen R, Copeland L, Wrangham R, Collins M (2010) Automated classification of starch granules using supervised pattern recognition of morphological properties. *J Archaeol Sci* 37:594–604



Wood RE, Higham TFG, de Torres T, Tisnerát-Laborde N, Vallardas H, Ortiz JE, Lalueza-Foz C, Sánchez-Moral S, Cañaveras JC, Rosas A, Santamaría D, de la Rasilla M (2012) A new date for the Neanderthals from El Sidrón Cave (Asturias, Northern Spain). *Archaeometry*. doi:[10.1111/j.1475-4754.2012.00671.x](https://doi.org/10.1111/j.1475-4754.2012.00671.x)

Wrangham RW, Holland Jones J, Laden G, Pilbeam D, Conklin-Brittain NL (1999) The raw and the stolen. Cooking and the ecology of human origins. *Curr Anthropol* 40:567–594



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