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Dental calculus indicates widespread plant use within the Neanderthal dietary niche

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Abstract

The ecology of Neanderthals is a pressing question in the study of hominin evolution. Diet appears to have played a prominent role in their adaptation to Eurasia. Isotope and zooarchaeological studies indicate that Neanderthals ate large quantities of meat, and that there was little variation in their diet across Eurasia. However, we have only a fragmentary picture of their dietary ecology and how it may have varied among habitats, because we lack detailed information about their use of plants and other foods. To address the problem, we examined the plant microremains in Neanderthal dental calculus from five archaeological sites from the northern Balkans, and the western, central and eastern Mediterranean. The recovered microremains revealed the consumption of a variety of non-animal foods, including starchy plants. Using a modelling approach, we explored the relationships

among the diversity of microremains with chronological, climatological and ecological variation. We find no evidence that plant use is confined to the southernmost areas of Neanderthal geographic distribution. Although Neanderthals were predominately big game hunters, evidence of diet from dental calculus indicates that plant exploitation was a widespread and deeply rooted subsistence strategy. Given the limited dietary variation across Neanderthal range in time and space in both plant and animal food exploitation, we argue that vegetal consumption was part of a generally static dietary niche.

5.1 Introduction

Neanderthals occupied a variety of environments drastically different from those where hominins first evolved, earning them a special position in evolutionary history. The ability of this hominin to settle in environments as diverse as the Mediterranean margin and steppe as cold as present-day Arctic tundra implies that Neanderthals were successful at adapting to new conditions. In particular, their diets must have been flexible enough to allow them to thrive in these varied environments. However, some researchers have linked the displacement of Neanderthals at the end of the Middle Palaeolithic to narrower diets than those from Upper Palaeolithic peoples (Hockett and Haws, 2003, 2009; O'Connell, 2006). In this view, Neanderthal subsistence was reliant on a more restricted range of staples than that of modern humans, giving them a competitive disadvantage against Upper Palaeolithic peoples.

Dietary breadth models, borrowed from the framework of behavioural ecology, have provided a means to dissect Palaeolithic dietary adaptations. These models are predicated on the idea that foragers will select the foods that provide the most nutritional benefit at the lowest costs, within the constraints imposed by the environment. The costs and benefits of food are predominantly measured in calories (Winterhalder and Smith, 2000), or other currencies such as macro or micronutrients (Rothman et al., 2006). When the return rates for preferred foods decrease, due to climate change or hunting pressure caused by a population increase, then more food types are added to the diet. A broadening diet is therefore not an adoption of an improved diet; just a response to scarcity of preferred food types.

Neanderthals are often interpreted as narrow spectrum foragers (Kuhn and Stiner, 2006; O'Connell, 2006; Stiner and Kuhn, 2009; Stiner, 2013). Models of Middle

Palaeolithic dietary ecology suggest that they hunted predominantly medium and large prime-age fauna with only infrequent use of small mammals, and aquatic resources and plant foods (Hockett and Haws, 2005). Nitrogen stable isotope ratios indicate that they were at the top of the terrestrial food web and obtained most of their total dietary protein from animal sources, (Richards et al., 2000; Lee-Thorp and Sponheimer, 2006; Richards and Trinkaus, 2009; Salazar-García et al., 2013; Wißing et al., 2015). Some zooarchaeologists argue that this diet was stable over time, with little evidence of a chronological trend towards more diverse resource use (Stiner et al., 2000; Stiner, 2013). Surviving tool repertoires show scant evidence for the investment in specialised technology for collecting plants, fish, birds and small mammals (Kuhn and Stiner, 2006; O'Connell, 2006; Henry et al., 2014), indicating an unchanging and narrow dietary niche. A low diversification in food choice and high consumption of large and medium-sized game matches evidence from site density and their genetic history that imply sparse and, dispersed populations of Neanderthals that did not deplete high-ranked prey items (Stiner, 1999; Stiner and Munro, 2002; Macdonald et al., 2009; Verpoorte, 2009; Castellano et al., 2014).

This view of rigid Neanderthal diets is complicated by recent studies suggesting evidence for variation in diets. Prey selected by Neanderthals varies throughout their range, often along ecological gradients. In southern regions, there is evidence for the consumption of hard-to-catch, low-ranked small game (Stiner, 1994; Blasco and Fernández Peris, 2009; Stiner and Kuhn, 2009; Hardy et al., 2013; Salazar-García et al., 2013; Fiorenza, 2015). In southern Iberia and western Italy, there is also zooarchaeological evidence of a contribution of marine resources (Stiner, 1994; Stringer et al., 2008; Zilhão et al., 2010). A preponderance of low-ranked small game including shellfish and tortoise (*Testudo* spp.) is also known from sites in Greece, Italy, Spain and Israel (Stiner, 1994; Cortés-Sánchez et al., 2011; Blasco and Fernández Peris, 2012; Sanchis, 2012; Harvati et al., 2013). A study of tortoise remains at Nahal Meged showed a decrease in size due to hunting pressure and climate, beginning in the late Middle Palaeolithic, suggesting that Neanderthals were collecting these foods at significant enough rates to reduce their body size (Stiner et al., 2000). In Cova del Bolomor, tortoises, rabbits and birds appear to have been foraged during MIS 6 (Blasco and Fernández Peris, 2009; Salazar-García et al., 2013). In the warm MIS 5e interglacial, a greater proportion of small game is observed at several northern European sites despite the apparent continued dependence on large game (Gaudzinski-Windheuser and Roebroeks, 2011).

The current debate between a rigid, narrow diet and a more variable range of diets continues because most of our dietary evidence is fragmentary. Large parts of diet are poorly known, especially plant foods. Recent foragers in northern environments provide a poor reference for Pleistocene foragers, in part because the treeless biomes of the Pleistocene have no analogue in the modern era (Stewart, 2005). The biomass of Pleistocene grasslands far exceeded the biomass of present day Eurasian tundra, providing a greater number of available animals for Neanderthals. We know less about the productivity of plant foods in this ecological zone (Verpoorte, 2009), but energy-rich plants were available on the steppe-tundra and throughout western Eurasia (Sandgathe and Hayden, 2003; Hardy, 2010; Pryor et al., 2013; Power et al., 2016).

Relatively little evidence of plant use in this context is available. Most isotopic profiles conducted so far have been produced from collagen, and thus reveal little information on the consumed macronutrients other than proteins that could have been obtained from vegetable resources. Macrobotanical remains that survive in a number of archaeological sites alleviate the gap in the scholarship, but surviving traces of plant use have limited interpretative power due to taphonomic bias (Weiss et al., 2004). The most comprehensive studies of dietary variability that incorporate plant foods stem from indirect lines of evidence, in particular dental wear studies. Macro- and microwear studies of dental surfaces have revealed that Neanderthals predominantly consumed meat, with a possible increased use of plant foods in the southern wooded parts of their range (El Zaatari et al., 2011; Fiorenza et al., 2011). Microwear of Neanderthals who inhabited cold-steppe environments resembled that of recent historic Fuegians who inhabited Patagonian cold wet scrublands (Grine, 1986; Fiorenza et al., 2011). However, dental wear is silent on the number and types of plants consumed, or if low- ranked foods were consumed, meaning these studies create an incomplete picture of dietary ecology in different environments.

Neanderthals appear to have broader diets in southern regions possibly due to ecological variation (Stiner, 1999, 2001; Fiorenza, 2015). Factors other than ecological variation, such as demographic pressure and available technology, can determine the proportion of food classes consumed by foragers (Kelly, 1995). One way to examine the relative influence of these factors is to assess the extent that eco-geography accounted for variation in food acquisition. Resource choice is a product of the demands of the individual foraging society, and an increasing population requires additional energy capture from its territory. Increased energy capture can be achieved by more intensive use of costlier resources, often with technological

specialisation; this model is termed 'broad spectrum foraging'. Flannery (1969) envisaged that broad spectrum foraging emerged first at the end of the Pleistocene, laying a foundation for domestication. Broad spectrum foraging is now thought to have emerged in intervals that occurred throughout the Upper Palaeolithic in Eurasia and earlier in Africa (McBrearty and Brooks, 2000). The first appearance of this pattern has been proposed in the southern Levant and Europe by about 45-30 ka (Stiner, 1999; Revedin et al., 2010) and North China by the Late Glacial Maximum (Liu et al., 2013).

As increasing high-resolution methods reevaluate the adoption of broad spectrum subsistence strategies, Middle Palaeolithic subsistence has received more attention. The actual appearance of broad spectrum diets may long predate the point at which they are currently visible in the archaeological record. Some researchers have pointed to Neanderthal charred legume assemblages from Kebara Cave (63-45 ka) and grass seed phytoliths from Amud Cave (70-55 ka), arguing that the broad spectrum economy was present already in the Late Middle Palaeolithic (Madella et al., 2002; Lev et al., 2005). Others have studied starch and phytolith microremains trapped in dental calculus, and found that Neanderthal dental calculus from sites such as Spy and Shanidar indicate the use of date palm and grass seeds in the Levant, and water lily tubers in northern Europe (Henry et al., 2011). Despite these promising insights into Neanderthal use of plants, these samples are too widespread in time and space to give reasonable coverage of potential variation in Neanderthal diets. It is noteworthy that they, these studies tell us little about the longevity of the Middle Palaeolithic dietary niche. Thus, it is unknown if Neanderthal exploitation of plant foods broadened over the hundreds of thousands of years they occupied Eurasia in response to higher populations or milder climates, similar to what is observed for the Upper Palaeolithic and recent hunter-gatherers, or if variation is only linked to different ecologies.

To explore the flexibility and stability of Middle Palaeolithic dietary breadth through environmental variation, we investigated plant consumption as recorded in dental calculus from environments with varied vegetation and winter and summer temperatures. We analysed plant microremains trapped in dental calculus from Neanderthal teeth from five archaeological sites: Vindija (Croatia), Grotta Guattari (Italy), Grotta Fossellone (Italy), Sima de las Palomas del Cabezo Gordo (Spain) and Kalamakia (Greece). These samples derive from a variety of regions and biomes across Europe: The Northern Balkans, and the western, central and eastern Mediterranean (Fig. 15). We then identified microremains to examine the variety of

consumed types or taxa. Once complete we compared this data with previously published results (Henry et al., 2014; Appendix 7.3) and finally explored if Middle Palaeolithic dietary breadth varied in different climatic and ecological conditions. We predicted that if Neanderthal diet was flexible, the number of plant types represented in the calculus should be greater in warmer, more arboreal environments. Furthermore, if their population gradually increased dietary breadth, the number of plant types represented in calculus should be higher at sites that are more recent.

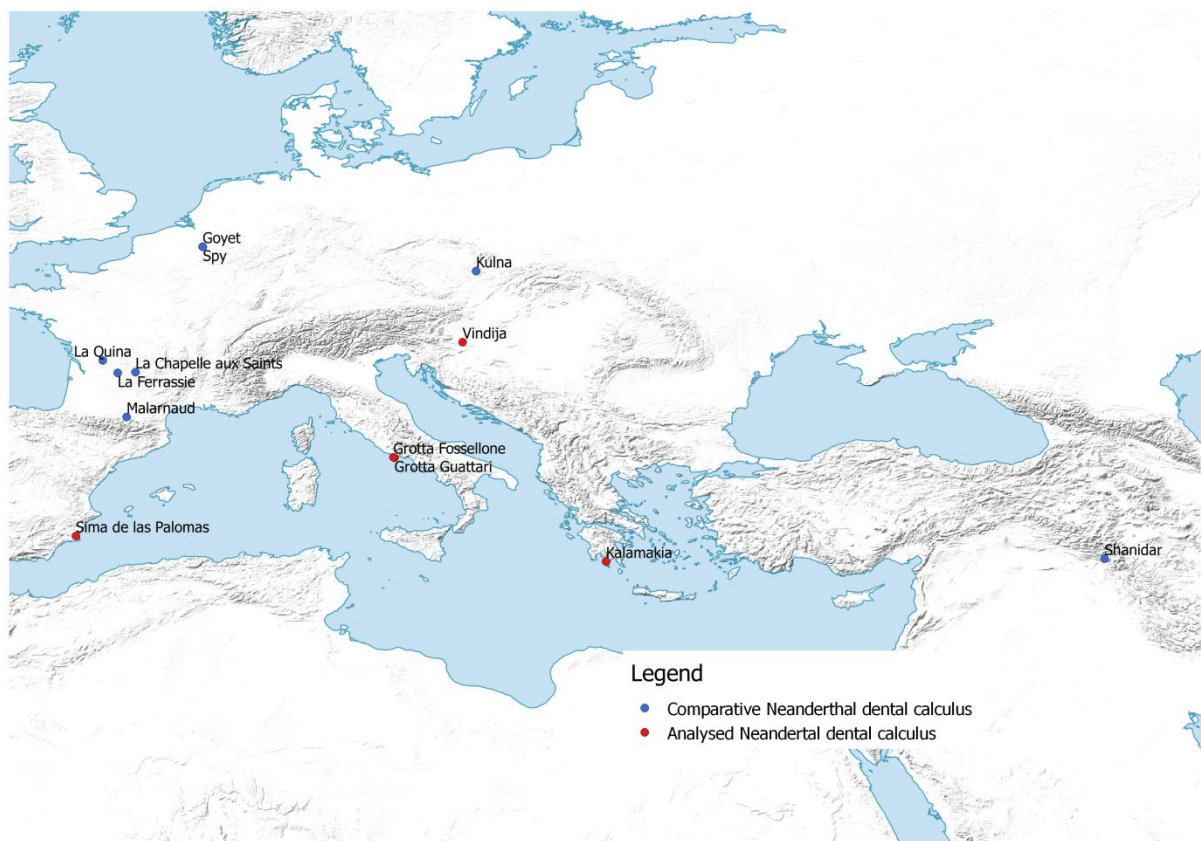


Fig. 15: Map of western Eurasia with the studied sites indicated.

5.2 Materials and Methods

5.2.1 Sites

Vindija Cave: this cave is situated on the southwest slopes of Kriznjak Peak in the Hrvatsko Zagorje region of northern Croatia (46°17'N, 16°6'E). Early exploration of the site began in 1928 with small-scale excavations. Malez and colleagues conducted large-scale archaeological excavations between 1974-1986 and 1993-1994.

These uncovered a complex of 10 m deep strata of 16 layers, with abundant palaeontological, archaeological and hominin material. A considerable number of hominin skeletal fragments was found in the cave deposits deriving from five or more individuals (Karavanić and Smith, 1998). A portion of this material was Mousterian-associated, and researchers identified the material as coming from Late Pleistocene Neanderthals due to its less pronounced archaic traits (Smith, Boyd, and Malez 1985). A radiocarbon date of >45.5 ka cal BP (Krings et al., 2000), and a U/Th date of a cave bear bone of 50.3 ka cal BP (Wild et al., 2001) have assigned layer G3 to MIS 3. Direct AMS ultrafiltration dating of hominin remains from layer G1 has assigned the most recent Neanderthals from this layer to $33,371 \pm 399$ - $35,382 \pm 2224$ ka cal BP (Higham et al., 2006). Archaeologists found Neanderthal material mostly in layers G1 and G3, but also four teeth in Layer F (of which we sampled two: 12.2 and 12.6). There was also modern human material in Layer D (MNI < 10). G3 is unambiguously Mousterian, while layers G1 and F contain some Aurignacian lithic material. However, dating and morphological evidence has firmly established the presence of Neanderthals in these layers, and cryoturbation is likely to have been responsible for bone displacement (Wolpoff et al., 1981; Higham et al., 2006; Frayer et al., 2010). Aurignacian lithic typology and early Upper Palaeolithic bone points are known in layers F and G1. The relatively low density of Aurignacian lithics, the mixing evident from contradictory dates, and the evidence of Neanderthal traits on the teeth (Frayer et al., 2010) suggest that the layer F teeth are in fact Neanderthal remains from layer G, so we feel comfortable including them also in our analyses. Excavators found red and giant deer (*Megaloceros giganteus*), elk (*Alces alces*), and aurochs (*Bos primigenius*) in layer G3, chamois (*Rupicapra* sp.), roe deer (*Capreolus capreolus*) and Merck's rhinoceros (*Stephanorhinus* sp.) in layer G1 and bison (*Bison* sp.), ibex and Merck's rhinoceros in layer F. Micromammals such as bank voles (*Myodes glareolus*) were found in layer G (Mauch Lenardić, 2014). These taxa are relatively unspecific but generally suggest continental conditions, and fauna such as roe deer and bank voles suggest at least a proportion of tree cover perhaps as parkland or riverine mosaics.

Grotta Guattari: this site is one of a complex of caves located in Monte Circeo, a limestone massif in Lazio, Central Italy (41°14'N, 13°05'E). The site was discovered in 1939 inadvertently when surface fauna and the remains of one Neanderthal (Guattari I) in layer G0 were discovered. Later explorations found more Neanderthals, firstly in a bone scatter (Guattari II) in layer G0, and subsequently in breccia (Guattari III) at the cave entrance (Sergi, 1954). Of the three Neanderthal

Guattari II and Guattari III were sampled. The cave has seven stratigraphic layers (G0-G5), but G0 is not vertically discrete partially due to carnivore disturbance (Stiner and Kuhn, 1992). Layers G1-G5 produced lithic artefacts and were deposited rapidly, but layers G6-G7 are beach deposits that accumulated more slowly (Stiner and Kuhn, 1992). Researchers identified the hominin remains as morphologically Neanderthal with a “classic” morphotype, suggesting they date to the Late Pleistocene (Howell, 1957). Stratigraphically below the fossils are the sequence’s basal marine-influenced deposits (G7), which are thought to relate to the final high sea level event of oxygen isotope stage 5a [84-74 ka] (Martinson et al., 1987; Grün and Stringer, 1991). U-series and electron spin resonance dating of calcite encrustations on bones and mammal teeth from the stratum that produced Guattari I and II suggest a date of 60-50 ka, while Guattari III dates to the end of MIS 5, 74-60 ka (Grün and Stringer, 1991; Schwarcz and Schoeninger, 1991). Regional palynology studies indicate grasslands in cold periods and tree cover in warmer phases (Van Andel and Tzedakis, 1996; Follieri et al., 1998). A variety of fauna were found on site. Fauna such as ibex indicate mountainous open habitats, while boar (*Sus scrofa*) and roe deer, are thought to indicate tree cover or shrub. Other fauna may represent either open grasslands/parkland or mixed environments such as Merck’s rhinoceros, aurochs and mammoth (*Elephas antiquus*). Extreme cold-adapted species like reindeer (*Rangifer tarandus*) or arctic fox (*Vulpes lagopus*) are absent on coastal sites in the region, demonstrating the absence of a bitter cold environment (Kuhn, 1991).

Sima de las Palomas del Cabezo Gordo: The site is a karstic vertical cave in the Permo-Triassic marble hill of Cabezo Gordo overlooking the Mediterranean Sea, in Torre Pacheco municipality, Murcia, SE Spain (37°47'59" N, 0°53'45" W). Much fossiliferous breccia was extracted from the 18-m-deep entrance shaft by 19th-century miners and discarded as rubble both on the hillside and inside the cave. Fortunately, inside the shaft there remained untouched a column of breccia in which was found a fossil (SP1) of a Neanderthal mandible fused to both maxillae. Subsequent sieving of rubble and systematic excavations by Walker and Gibert recovered Neanderthal skeletal elements, Late Pleistocene faunal remains, and Mousterian Middle Palaeolithic artefacts (Trinkaus and Walker; Walker et al., 2008, 2010, 2011a). The main in-situ archaeological layer has been dated using U-series and radiocarbon to between roughly 56 and 34 ka (Trinkaus and Walker). Three articulated Neanderthal skeletons were found in this layer: an adult woman (SP96; Walker et al., 1999, 2011a, 2012) lying over a child (SP97) below which lay another adult (SP92). The adult woman SP96 was directly dated using U-series to 54.1 ± 7.7

ka (APSLP1) (Walker and Ortega, 2011). Several taxa are typical of the Iberian Late Pleistocene (*Equus caballus*, *Bos primigenius*, *Capra pyrenaica*, *Cervus elaphus*, *Lynx lynx*, *Oryctolagus cuniculus* and *Testudo hermanni* etc.) whereas others occur that rapidly became extinct at the close of the early Late Pleistocene (*Panthera pardus*, *Crocota crocuta*, *Stephanorhinus* sp., *Hippopotamus amphibius*, *Hystrix javanica*). Pollen from the uppermost sediments indicates presence of pines and moisture-dependent deciduous woodland (which is absent in the region today), and thermophylls characteristic of southeastern Iberian and North Africa that do not regenerate after frost (Carrión et al., 2003). Neanderthal teeth with carious lesions have been identified (Walker et al., 2011b). Teeth sampled for dental calculus come from excavated sediments except for one (SP50) recovered from the hillside rubble.

Kalamakia: this Middle Palaeolithic site is a cave on the western coast of the Mani peninsula in the Peloponnese in southern Greece (36°40'43.3"N 22°21'59.3"E). Archaeologists excavated Kalamakia from 1993 until 2006 (Harvati et al., 2009, 2013). Chronologists have dated basal deposits with U/Th radiometric dating to the MIS 5c transgression (109 ± 14/-13 ka; De Lumley et al., 1994). Two of the five units produced substantial Middle Palaeolithic remains (Units III and IV). Excavation concentrated on Unit IV due to hard breccia in Unit III. Seventeen occupation levels were identified in the sedimentary deposits of Unit IV. In addition to fauna and Mousterian lithics, ten hominin teeth, crania and postcranial elements with diagnostic Neanderthal morphology were found, comprising of at least eight individuals, three of which we sampled for dental calculus (KAL 3, 5 and 8). Unit IV's youngest archaeological level has been dated to >39 ka (Harvati et al., 2013), placing KAL 5 and KAL 8 between MIS 5a (74 ka) and 39 ka. Excavators uncovered KAL 3 in Unit III, which overlies 5c beach rock and was truncated by sea transgressions in MIS 5a. Evidence of other truncations from sea transgressions from local caves implies that KAL 3 dates to the MIS 5b (Darlas, 2012). Faunal and palynological studies reveal that prevailing climatic local conditions were mild. Fallow deer (*Dama dama*) is particularly common in the assemblages, followed by ibex, wild boar, red deer, tortoise and some modified seashell. Maquis shrubland and Mediterranean pre-steppic forest species covered the peninsula (Lebreton et al., 2008). Extensive avian remains reveal evidence of tree cover in a predominantly open warm/temperate environment (Roger and Darlas, 2008).

From each site we collected a variety of control samples, including sediments from the sites, dust on the skeletal material, and samples of the material in which the

remains were stored (Appendix table 15, 16, 17, 18, 19). We also tried to sample dental calculus from the teeth of herbivorous and carnivorous fauna as an additional control and to explore if Neanderthals, like carnivores, consumed the stomach contents of herbivores (Buck and Stringer, 2014). Unfortunately, we were only able to access faunal material from Vindija and Sima de las Palomas del Cabezo Gordo. These samples included wolf (*Canis lupus*), which is mostly carnivorous but also known to consume some plant material; an unspecified feline (c.f. *Panthera*), which is nearly strictly carnivorous (Bocherens et al., 2011); and cave bear (*Ursus spelaeus*), which had a plant rich diet (Pacher and Stuart, 2009). In addition to the 30 Neanderthal calculus samples from the five sites that we processed for this study, we also included data from a variety of other northern European, Levantine, and southern European sites (Appendix 7.3.1) (Salazar-García et al., 2013; Henry et al., 2014).

In summary, our five sites represent a variety of environmental contexts. They range from more open temperate environment at Vindija to more Mediterranean mosaic woodland at Sima de las Palomas del Cabezo Gordo, and from cooler at Vindija to warmer at Kalamakia. This range reflects the bulk of environments Neanderthals occupied. We did not try to evenly represent different age classes or sexes, as often this information is not available.

5.2.2 Dental calculus and control sampling

Neanderthal teeth from each site were examined for deposits of dental calculus situated on the tooth surface in a cleaned lab of the institution where each specimen is curated. Deposits of dental calculus were common on teeth examined, but it was not present on all specimens. We documented the dental calculus deposits with photography before sampling. We then collected 14 samples of dental calculus from the Vindija Neanderthal teeth (levels F, G1 and G3), five from the Grotta Guattari teeth (levels G0), two from the Grotta Fossellone teeth (level 4), six from Sima de las Palomas del Cabezo Gordo teeth (Upper Cutting level 2 and I), and three from the Kalamakia teeth (Unit III and Lower IV) (Table 9). Many of the sampled teeth had a visible band of hard supragingival dental calculus, except the Iberian teeth that were encrusted in calcium carbonate. In these samples, we therefore took 'deep' and 'shallow' samples. "Shallow" samples were closer to the surface and likely to represent the sediment while "deep" ones were more likely calculus.

The sampling surface was gently dry brushed with a disposable toothbrush to dislodge contaminants at the sampling locations. We then used a dental scalar to remove small areas of dental calculus onto creased weighing paper underlain by aluminium foil. The material collected in the paper was then transferred to a microcentrifuge tube. After sampling, we photographed the teeth and the remaining unsampled dental calculus. We then transported the samples to the Plant Foods lab at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA).

To minimize risk of contamination from airborne modern plant material and lab supplies (Langejans, 2011; Crowther et al., 2014; Henry, 2014), we conducted a regime of weekly laboratory cleaning. All lab work surfaces were cleaned with hot water, washed with starch-free soap and with 5 % sodium hydroxide (NaOH). To assess contamination types, we additionally performed wipe tests before and after weekly cleaning to quantify starch and other contaminants. Wipe tests retrieved settled particles of the surface area (74 x 43 cm²) of the laboratory positive-pressure laminar flow hood used for mounting.

Table 9: Neanderthal dental calculus Grotta Guattari, Grotta Fossellone, Sima de las Palomas del Cabezo Gordo and Vindija analysed. Dates are ka cal BP.

Sample	Site	Specimen	Tooth	Weight (mg)
FON1	Grotta Fossellone	Fossellone 3	LL M1	0.067
FON2	Grotta Fossellone	Fossellone 3	LL M2	0.1
GTN1	Grotta Guattari	Guattari II	RL M3	0.654
GTN2	Grotta Guattari	Guattari III	RL M1	0.871
GTN3	Grotta Guattari	Guattari III	LL I2	0.654
GTN4	Grotta Guattari	Guattari III	RL I2	0.258
GTN5	Grotta Guattari	Guattari III	LL M1	0.289
KAL_3	Kalamakia	KAL 3	UL M3	2.866
KAL_5	Kalamakia	KAL 5	UR P2	0.05
KAL_8	Kalamakia	KAL 8	UR M2	N/A
Vja-13	Vindija	12.1	UR M2	0.393
Vja-14	Vindija	12.2	LR I2	0.046
Vja-16	Vindija	12.4	UR I1	0.046
Vja-17	Vindija	12.5	UR C	0.045
Vja-18	Vindija	12.6	LL C	0.02
Vja-19	Vindija	12.7	LL I2	0.89
Vja-20	Vindija	11.39	LR C	0.446
Vja-21b	Vindija	11.39	LR M1	0.408
Vja-21a	Vindija	11.39	LR M1	0.502
Vja-24	Vindija	11.45	LL M3	0.672
Vja-26	Vindija	11.46	UL M2	0.865
Vja-51	Vindija	11.4	LL M1	0.19
Vja-54	Vindija	11.4	LL M1	0.046
Vja-55	Vindija	11.4	LL M1	0.085
SP45	Sima de las Palomas del Cabezo Gordo	SP45	LR P3	0.08
SP54	Sima de las Palomas del Cabezo Gordo	SP54	LR C	0.102
SP78a	Sima de las Palomas del Cabezo Gordo	SP78	P4	0.415
SP79	Sima de las Palomas del Cabezo Gordo	SP79	I1	N/A
SP83	Sima de las Palomas del Cabezo Gordo	SP83	LR DM2	0.09
SP84	Sima de las Palomas del Cabezo Gordo	SP84	M2	N/A

5.2.3 Sample preparation and mounting

Using standard procedures (Power et al., 2014b) each sample was weighed and transferred to microcentrifuge tubes while in a clean laminar flow hood at the Plant Food Group Laboratories at the MPI-EVA. We then ground the samples with a micropestle in a 1.5 ml Eppendorf microcentrifuge tube containing ~30 μ l of a 25 % glycerine solution to reduce sample loss due to static electricity. The samples were then centrifuged at 1691 \times g (Heraeus MEGAFUGE 16 with TX-400 Swinging Bucket

Rotors) for 10 minutes. These samples were mounted on glass slides and examined under bright field and cross-polarized light on a Zeiss Axioscope microscope at 400 × magnification.

5.2.4 Identification and classification

We photographed and described recovered microremains using the international nomenclature codes (Madella et al., 2005; ICSN, 2011). Phytoliths were classified into conventional morphotypes, while we developed types to classify other microremains based on shared morphology. Starches were classified according to shape, the presence and prominence of lamellae, hilum morphology, formation type (i.e. simple or compound), cross features, cracks and other surface features. Some types are unique to a single plant taxon, but in other cases, several types may all have originated from a single taxon, or one type may be common to several taxa. For example, several phytolith types (short-cell, bulliform and psilate) may all represent a single species of grass. When possible, we identified the types to the lowest taxonomic level possible, usually family or genus (Appendix 7.3). Many categories of plant foods that could have been important have few or no microremains. These include lipid-, sugar-, and inulin-rich plants, like olives, walnuts, and Asteraceae tap roots. Images of all microremains are deposited on the Archaeological Microremain Database of the Plant Foods in Hominin Dietary Ecology Research Group in Leipzig.

Once we classified the microremains, we calculated ratios that may provide quantitative information about the assemblage. These included Menhinick's index, a richness metric common in ecological studies, which is the ratio of the number of taxa to the square root of sample size (Magurran, 2004). We used this index to compare samples to test breadth in each assemblage. We calculated total number of unique starch and phytoliths types. We also prepared ratios that are phytolith-specific such as the monocot: dicot phytolith ratio, which may indicate contribution of grasses, sedges and other monocots versus the contribution of flowering plants; and the variable: consistent morphology (v/c) phytolith ratio, which indicates taxon.

5.2.5 Palaeotemperature reconstruction

In order to best approximate the climatic conditions of each site, we used detailed climate simulations for western Eurasia created as part of the Stage 3 Project

(Van Andel and Davies, 2003). This project quantified climatic variables during much of the range of the last glaciation from 59 up to 24 ka, and generated four regional model simulations: a MIS 3 warm climatic event, a MIS 3 cold climatic event, the extremely cold Last Glacial Maximum (LGM), and finally a modern climatic model. These simulations are also created to model conditions in other periods such as Stage 4 (e.g. Aiello and Wheeler, 1995; Wales, 2012). Unfortunately, these models cannot account for third order climate fluctuations that occurred within these phases. However, when each simulation is examined for each Neanderthal site, we see that the variation in temperatures is driven more by the latitude and longitude of the site than by the specific climatic period. Therefore, despite being somewhat coarse-grained, these models allow us to quantify much temperature variation.

These simulations of temperature can be made more ecologically relevant by calculating effective temperature, a climatic predictor that evens out yearly temperature variation. Binford used this powerful measure to explain why recent forager subsistence varies latitudinally (Bailey 1960; Binford 2001). The necessary data to calculate effective temperature was unavailable so we developed modified effective temperature (MET) to adapt effective temperature for available palaeotemperature data. This differs from effective temperature in that it uses the mean of the three warmest and three coldest months instead of the warmest and coldest month. Modified effective temperature is identical to effective temperature in all other ways. Effective temperature is based on three constants- the minimum mean temperature (18°C) that supports tropical plant communities (a 365 day growing season), the minimum temperature (10°C) at the start of the growing season at the zonal boundary of polar and boreal environments and the minimum temperature (8°C) at the beginning of the growing season (Binford 1980, 2001). Modified effective temperature (MET) is as follows

$$MET = \{18 * MST - (10 * MWT)\} / (MST - MWT + 8)$$

where

MST is mean summer temperature (June, July and August)

MWT is mean winter temperature (December, January and February)

The Stage Three Project supplied mean temperature (°C) 2 m above ground level from June through August and December through February for each climate

simulation. We matched plots of each simulation to the climatic phases covered in our sample set (Table 10, Table 11), and we collected relevant values from each simulation plot and then calculated modified effective temperature for each hominin sample (Table 11; Appendix 7.2).

Table 10: Stage 3 Project simulations used to predict average summer and winter temperatures experienced by each Neanderthal. Dates are ka cal BP. See Table 11 for the actual predicted temperatures per specimen.

Interval	Phase	Simulation model used	Date
MIS 5e	Eemian Interglacial	Modern	130-117
MIS 5d	Early Glacial Stadial Phase	Warm	117-105
MIS 5c	Early Glacial Interstadial Phase	Warm	105-95
MIS 5b	Early Glacial Stadial Phase	Warm	94-85
MIS 5a	Early Glacial Warm Phase	Warm	85-74
MIS 4	Transitional Phase	Warm	74-66
MIS 4	First Glacial Maximum	Last Glacial Maximum	66-59
MIS 3	Stable Warm Phase	Warm	59-44
MIS 3	Transitional Phase	Warm	44-37
MIS 3	Early Cold Phase	Cold	37-27
MIS 2	Last Glacial Maximum	Last Glacial Maximum	27-16

Table 11: Palaeoenvironment reconstructions for each specimen used in this study. Tree cover: O=open, C=closed, M=mixed. P=publication, 1=this study, 2=Henry et al., 2014, 3=Salazar-García et al., 2013. Dates are ka cal BP.

Specimen	Site	Date	Tree cover	Palaeotemperature		MET	P
				Dec- Feb	June- Aug		
Fossello 3	Grotta Fossellone	70	O	-6	16	11.6	1
Guatt II	Grotta Guattari	55	O	-4	16	11.71	1
Guatt III	Grotta Guattari	67	O	-6	16	11.6	1
KAL 3	Kalamakia	91	O	4	20	13.33	1
KAL 5	Kalamakia	63	O	4	20	13.33	1
KAL 8	Kalamakia	63	O	4	20	13.33	1
12.1	Vindija	34.3	O	-8	20	12.22	1
12.2	Vindija	34.3	O	-8	20	12.22	1
12.4	Vindija	34.3	O	-8	20	12.22	1
12.5	Vindija	34.3	O	-8	20	12.22	1
12.6	Vindija	34.3	O	-8	20	12.22	1
12.7	Vindija	34.3	O	-8	20	12.22	1
11.39	Vindija	45.5	O	-8	20	12.22	1
11.45	Vindija	45.5	O	-8	20	12.22	1
11.46	Vindija	45.5	O	-8	20	12.22	1
11.4	Vindija	45.5	O	-8	20	12.22	1
SP45	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	1
SP50	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	3
SP53	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	3
SP54	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	1
SP58	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	1
SP60	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	3
SP68	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	3
SP74	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	3
SP78	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	1
SP79	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	1
SP83	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	1
SP84	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	1
SP88	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	3
SP100	Sima de las Palomas del Cabezo Gordo	50	C	4	16	12.4	3
Kůlna 1	Kůlna	45	O	-8	16	11.5	2
GoyetVII	Goyet	40.5	O	-8	12	11.5	2
Chapel 1	La Chapelle-aux-Saints	57	O	-4	12	10.57	2
Malarn 1	Malarnaud	75	M	-4	12	10.57	2
LFI	La Ferrassie	39	M	0	12	10.8	2
LFII	La Ferrassie	39	M	0	12	10.8	2
Quina V	La Quina	64	M	-4	12	10.68	2

5.2.6 Palaeoenvironmental reconstruction

In contrast to temperature, we assessed tree cover using all published data on the habitats that existed at each site. We used investigations of macromammals, micromammals and pollen that record palaeovegetation at different scales from local and regional studies to classify each environment. Based on the prevalence of tree cover we assigned each sample as coming from open, mixed or closed habitats (Table 11).

5.2.7 Statistical analysis

To explore the relationships among environment, trends in foraging breadth, and microremains found in our samples and those from previous studies (Salazar-García et al., 2013; Henry et al., 2014) we fitted an Observational random effect Poisson model with likelihood ratio tests, using the `glmer` function of the R package `lme4` (Bates et al., 2013). We chose this Observational model because it is appropriate for count data, like ours, which is not normally distributed, and instead is skewed towards zero. We only included those samples that had been weighed prior to processing, and for which the recovered microremains were assigned to specific types. If any dental calculus samples produced no microremains, they were included as zero values. Our full model included modified effective temperature, the chronological time period in which the age of the Neanderthal lived and tree cover (open, mixed or closed) as fixed effects. It also included the weight of each dental calculus sample as model offset to factor in significant differences of sample dental calculus. We prepared the data by z-transforming age and modified effective temperature. The site and analyst were treated as random intercept terms. The weight of the dental calculus sample in mg was included as an offset. An id was assigned to each observation, and this was also included as a random intercept, thus removing overdispersion ($\chi^2=30.62$, $df=44$, dispersion parameter=0.696). To test the significance of the full model, it was compared with a null model excluding fixed effects of modified effective temperature, age of each fossil specimen and tree cover. Variance inflation factors (VIF) were derived to assess collinearity, from a standard linear model minus random effects and offsets. Variance inflation factors indicated collinearity to not be an issue (largest VIF=1.27). We tested model stability by excluding levels of random effect one by one from the data set, running the full model and comparing the results with those from the original model that suggest no highly influential cases. To allow for the possibility of mixing between layers F, G1

and G3 in Vindija Cave we built an identical model except that samples from F and G1 derive from G3. We performed similar checks on this alternative model as the previous model. We removed overdispersion on this model ($\chi^2=32.90$, $df=0.89$, dispersion parameter=0.748) and ensured VIF was not an issue (largest VIF=1.371).

5.3 Results

5.3.1 Contamination controls

Vindija Cave: We collected some samples of faunal calculus, as well as adhesives used to hold Vindija tooth 11.39 (Appendix 7.3.4). Bear, wolf and felid samples from Vindija yielded 81 plant microremains, but these were disproportionately more common on bear than wolf samples, consistent with the expected diets of these species (Pacher and Stuart, 2009).

38 phytoliths were found in bear and wolf samples. Multicellular polyhedrons were abundant in one bear sample, reflecting rich consumption of dicot fruit and leaves. Multicellular polyhedrons are rare or absent in most wolf samples except Vja-12-31. We found a small number of dicot phytoliths in a few other wolf samples. Present-day wolves consume plant matter, and plants may comprise up to 40 % of their food intake in certain seasons (Meriggi et al., 1991). European wolves especially favour fruit, but wolves may also consume plants in stomach contents or intentionally consume grass to smooth digestion or ease parasites (Murie, 1944; Stahler et al., 2006).

Starches were particularly uncommon in these fauna samples. Most were small nondiagnostic types. A Triticeae grass aggregate was found in a wolf control sample (Vja-30). Total numbers of starches found and the number of starches per milligram were lower than in Neanderthal samples (Appendix 7.3.4). Furthermore, the faunal samples appear to share similar starch types (1-7 types), while the Neanderthal calculus had more varied starches (1-15 types). Two control samples of mandible adhesive revealed 56 contaminant starches but nearly all of these were heavily damaged potato starch. These starch are morphological distinct from those in the Neanderthal dental calculus samples (Appendix table 15, Appendix table 16, Appendix table 17, Appendix table 18, Appendix table 19).

Grotta Guattari and Grotta Fossellone: We took a variety of control samples, though not all preferred control types (e.g. faunal teeth) were available. Most controls were samples of adhesives used to bond bone, or washes of distilled water

taken from the surfaces of the sampled mandibles. These contamination assays produced no or few microremains, and where microremains were found they showed a narrow range of types (Fig. 16; Appendix 7.3.4). We found that these samples contained few types of starch, and contaminating grains appeared distinctly fresh and usually occurred as starch aggregates unlike more damaged and isolated starch in dental calculus samples. A Triticeae grass seed starch aggregate was found in controls 2e and Fon3. None of this type of aggregates were found in hominin samples.

Sima de las Palomas del Cabezo Gordo: In addition to controls (non-worked stone from archaeological strata, carnivore dental calculus and packing cotton) published in Salazar-García et al., 2013, we sampled carnivore dental calculus and sediment found attached to hominin teeth. One sediment sample produced a single isolated subspherical starch. These results show a very low rate of background starch and phytoliths.

Kalamakia: We did not have access to any contamination control materials for Kalamakia.

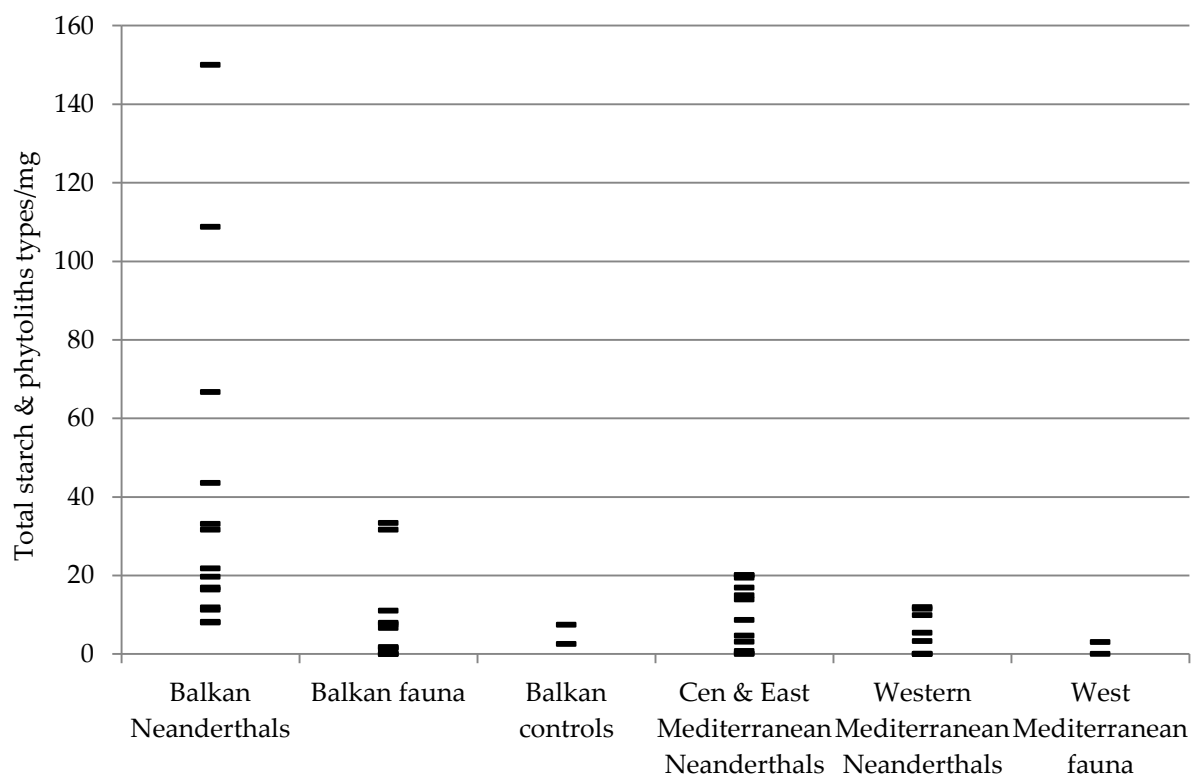


Fig. 16: Starch and phytoliths from Neanderthal calculus, fauna calculus and controls show that Neanderthal dental calculus samples show a distinct signal indicating they reflect hominin diet.

5.3.2 Dental calculus microremain assemblages and dietary breadth

Vindija Cave: We collected calculus from six isolated teeth and five *in situ* teeth (Table 9). Isolated teeth included a right second molar, a lower second incisor, upper first incisor, upper canine, lower canine, and lower second incisor. Our sample of *in situ* teeth included a lower canine, a lower third molar, an upper second molar, and a lower first molar. Microremains were recovered in all Neanderthal dental calculus samples but there was major variation in the numbers and classes present. The plant microremain assemblages found on the Vindija samples is considerably more diverse than what was reported in the previous studies of Neanderthal calculus (Hardy et al., 2012; Henry et al., 2012, 2014; Salazar-García et al., 2013).

229 microremains were found in Vindija dental calculus samples, including 87 starch microremains (Appendix 7.3.4). 15 starches displayed a lenticular cross-section, circular or subcircular plane view, a hilum exhibiting a thin line, and distinctive surface dimples and lamellae, clearly representing starches from Triticeae grass seeds (Fig. 5). Although grass leaf microremains may arise from non-edible resources such as bedding, this seems unlikely to be the case for grass seeds.

Two of the starches are likely to derive from a legume based on their characteristics: circular, oval, ovoid shape, the presence of lamellae, and the characteristic longitudinal cleft fissure. We have observed these traits in peas (*Pisum* sp.), vetches (*Vicia* sp.), and sweet peas/vetchlings (*Lathyrus* sp.). Three other starches (Fig. 17) displayed the size, highly faceted surface and polyhedral shape consistent with those of starches from hard endosperm (Eliasson and Larsson, 1993). Plants that produce this starch morphology include nuts, hard seeds, seeds from grasses not in the Triticeae tribe, and seeds of sedges like *Schoenoplectus*. Two starches from underground storage organs were evident from large elongated shapes and highly eccentric polarisation crosses. None of these legume, hard endosperm, or underground storage organ starches had specific enough morphological characteristics to identify them to a lower taxonomic category. The remaining starches fall into nine groupings, probably reflecting several taxa, but due to starch damage, redundant types and a limited reference collection, they cannot be identified. Five starch types also found in Neanderthal samples were also found in cave bear samples, but these were nondiagnostic types.

We recovered 91 phytoliths from the Vindija dental calculus samples (Appendix 7.3.4). Thirty-two of the 91 were long cell morphotypes, which are

common in the leaves of monocots. However, because monocots produce more phytoliths than dicots per gramme (Tsartsidou et al., 2007), they are more visible in the archaeological record. Phytolith production between the two categories varies from 80:1 to 20:1 (Tsartsidou et al., 2007). Ratios of monocot to dicot in our sample of Vindija Neanderthal dental calculus vary from 5:1 to 0.67:1, which suggests an abundance of dicot types such as fruits, nuts and leaves rather than grasses and sedges.

Twenty-five spores were also found, representing approximately five types of fungus. However, these are nondiagnostic and could represent mushroom-bearing higher fungi or lower fungi such as moulds. Pollen was rare and only one Betulaceae pollen was found. Ten unsilicified plant tissue fragments were recovered, two reflecting grass and one an unspecific monocot, but others were indeterminate.

Grotta Guattari and Grotta Fossellone: We examined the calculus from the right lower third molar of Grotta Guattari II and the lower first molars (right and left) and lower second incisors (right and left) of Grotta Guattari III. Calculus samples from the five teeth from Grotta Guattari produced high numbers of microremains and high levels of diversity per mg. A total of 151 microremains were found in the dental calculus of the five teeth (Appendix 7.3.4).

Starch grains were found on four of the five teeth and totalled to 69 grains. Six starches found in an amyloplast cell were elongate ovoid in plane-view and oval in cross-section, with an eccentric polarisation cross, all characteristics matching *Lilium* type starches (Fig. 3). One starch clearly represented a Triticeae grass seed starch. Further evidence of grass use is evident from intact grass leaf tissue found in one sample. The other detected starches represented five unknown types.

Thirty-nine phytoliths were recovered, 31 of which originated in monocot tissue and eight from dicot plants. Nine short cell rondel phytoliths were identified. One phytolith was a multicellular epidermal jigsaw morphotype, indicating dicot leafy or fruit matter. We also note the presence of a tracheid vessel, which is another dicot marker.

Other microremains were numerous. Ten spores were observed, some of which exhibited features that enabled us to identify them as coming from bracken (*Pteridium* sp.). We also noted the presence of spores from *Nigrospora* sp. We also identified other spores such as fusiform spores, indicative of boletoid fungi. Many bolete fungi are edible and widely consumed, while *Nigrospora* is a diverse genus of

fungi that are mostly agents of decay. Five pollen grains were found including two Betulaceae pollens. In total 14 other cellular plant tissue fragments were noted, including vascular bundles reflecting plants that entered the mouth. Also recovered were a number of stellate hairs and a pennate diatom.

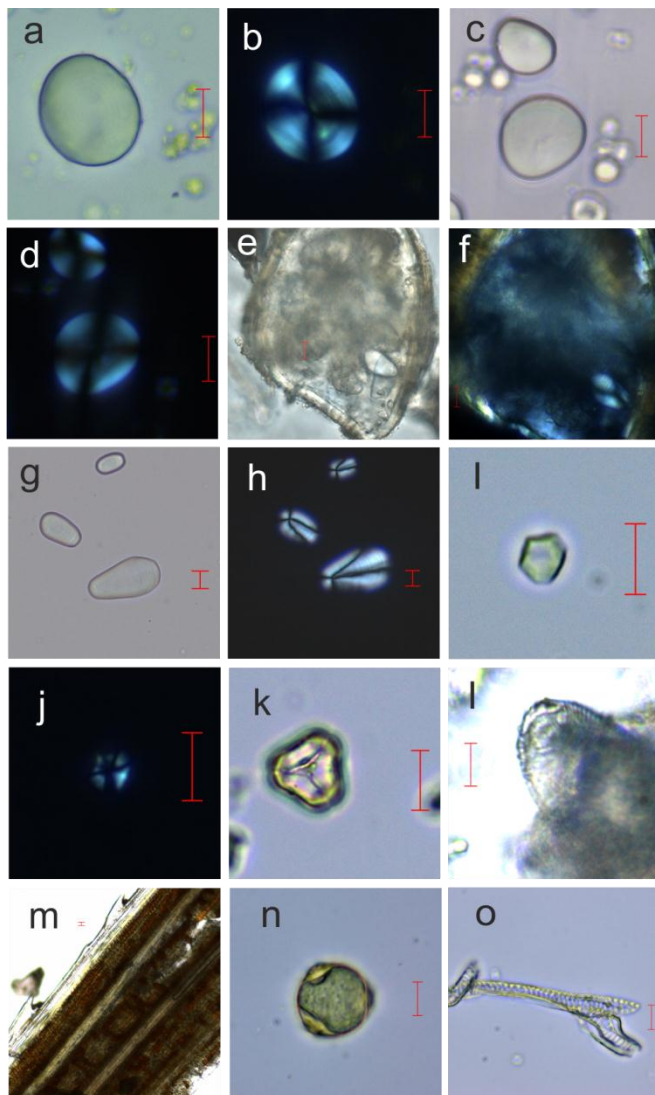


Fig. 17: Mosaic of microremains and comparative modern reference plant matter. Each scale bar represents 10 μm . (a) Starch from Vindija Neanderthal identified as Triticeae under bright field and (b) cross polarized light, (c) a reference Triticeae starch (*Triticum turgidum* sp.) under bright field and (d) cross polarized, (e) Amyloplast with several ovoid starches resembling *Lilium* bulb starches under bright field and (f) cross polarized light, (g) reference *Lilium* sp. bulb starches under bright field and (h) cross polarized light, (i) polyhedral starch under bright field and (j) cross polarized light, (k) *Pteridium* sp. spore, (l) diatom embedded in calculus, (m) fragment of grass leaf, (n) triporate Betulaceae pollen, (o) Unsilicified tracheid plant tissue.

Grotta Fossellone: We sampled dental calculus from the left lower first molar and second molar of Grotta Fossellone III. Eleven starches were found in the two

Grotta Fossellone dental calculus samples. These comprised of indeterminate starches that cannot yet be matched to reference material. Only one phytolith was found in the assemblage: a rondel phytolith from a grass. Additionally, one piece of monocot and one piece of unidentified plant tissue were found.

Sima de las Palomas del Cabezo Gordo: For this study, we sampled dental calculus from six hominin teeth, including a lower third premolar, lower canine, lower deciduous second molar, a lower fourth premolar, an upper first incisor, and a lower first molar. We found relatively few microremains in these samples, reflecting the very small amount of dental calculus in each sample. We recovered only five starches and phytoliths, and one diatom. None could be identified to plant taxon.

Kalamakia: We sampled dental calculus from three Kalamakia teeth - an upper molar (KAL 3), an upper fourth premolar (KAL 5) and an upper molar (KAL 8). Only five starch grains were found on the three teeth. Two phytoliths were also found: one from a grass and one from a non-monocotyledon plant. Sixteen possible calcium oxalate forms were found. Calcium oxalate represents consumed plant matter, but it is readily soluble and occurs in most plants, and is therefore not assignable to taxon. Lastly, we found one fragmented sponge spicule. This last microremain likely entered the mouth through accidental consumption.

5.3.3 Dietary flexibility and dietary niche stability

We predicted that if the breadth of Neanderthal plant use was driven by ecological conditions, then the number of consumed types should be influenced by temperature and tree cover. We produced a Menhinick's index comparison of all available samples, including all previously published data and the new samples from this study. Although there is no distinct trend among Neanderthals from different periods or chronologies (Fig. 18; Appendix 7.3.4), there is a possible curvilinear relationship, with the Menhinick's index /mg increasing with temperature until a peak is reached, at which point the index drops again. It is possible this pattern reflects the degradation of starches in the warmest environments (Langejans, 2010).

We condensed the list of samples to include only those with documented weights (Wt column, Appendix 7.3.4). We then used an observational random effect Poisson model to test dietary breadth patterns (described above). We find no relationship between the number of microremain types found in calculus and the

chronological age or environmental conditions of the sample, even when accounting for the effects of variation between tree cover, sites, analyst, age, and weight of the dental calculus sample (Appendix table 20). More specifically, an increase in temperature did not lead to an increase in the number of types represented in dental calculus and younger sites did not show an increase in the number of types represented in dental calculus ($\chi^2=5.148$, $df=4$, $P=0.273$; Appendix fig. 3; Appendix table 20). Even in the alternative model, which assumed bones in Vindija Cave layer G1 are older than thought and derive from G3, there was still no relationship ($\chi^2=2.683$, $df=4$, $P=0.612$; Appendix fig. 3; Appendix table 20).

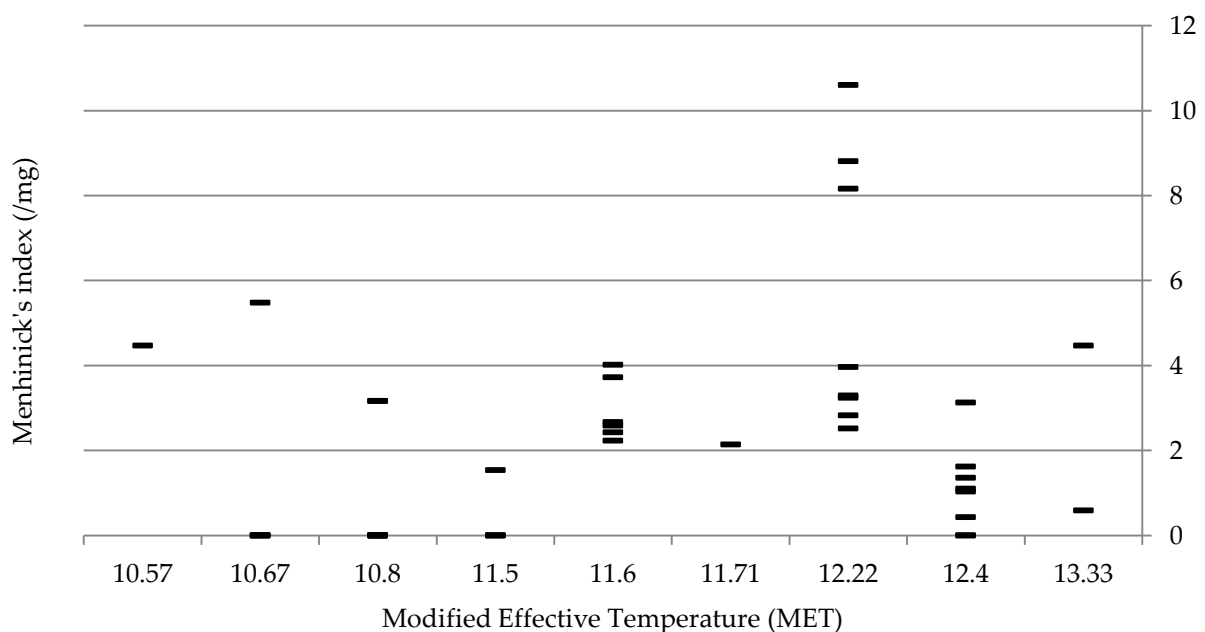


Fig. 18: A Menhinick's index of types of starch and phytolith from Neanderthal dental calculus shows that warmer climates are not associated with increased diversity. Samples are from Neanderthal remains presented in this study and Salazar-García, et al., 2013 and Henry et al., 2014. Each dash represents an individual sample.

5.4 Discussion

Microscopy revealed starch and phytoliths in most samples but many samples were highly variable. Fig. 16 and Appendix fig. 3 shows that many dental calculus samples from Grotta Fossellone, Sima de las Palomas del Cabezo Gordo and Kalamakia yielded few microremains. Previous work that established baselines with chimpanzee (Power et al., 2015b) and living human (Leonard et al., 2015) populations indicates that this stochastic pattern is normal. These studies also

emphasize that we have not recovered information on the majority of consumed plants when using this approach.

Our previous work with chimpanzees indicated that age influences the microremain record, with older individuals having more microremains and more plant types represented. Though we do not have precise age estimates for the individuals in this study, all of the teeth were from adults. Furthermore, we have no reason to expect that any site was biased towards individuals from one particular age class. Taken together, these data suggest that the differences among the sites do not reflect simple age differences within our sample.

By using this metric of dietary breadth we were able to show that Neanderthals in warmer environments who had better access to arboreal edible resources might not have used a broader range of plant foods than Neanderthals from colder environments, and in some cases they even show less diversity than cool climate ones. This picture could arise by methodological limitations of this approach, since it is possible that plant remains such as starches are underrepresented in samples from warmer environments due to worsened taphonomic conditions (Smith et al., 2001). However, the phytoliths follow a similar pattern in our results, despite being insensitive to temperature, suggesting that the observed pattern in our study samples is due to dietary, not post-depositional, trends. Our results on microremain diversity do not negate occlusal dental wear findings that link tree cover to plant use, as occlusal wear only approximates classes of the total diet and not its composition. The availability of Pleistocene plant foods, however, likely reflects forest type (Mediterranean or Boreal) far more than tree cover alone. Open and mixed environments have less primary biomass than closed canopy environments, but they may offer significantly more edible plant biomass, as much of the biomass in forests consists of tree trunks, and is thus unavailable to hominin consumers (Odum, 1975). Pleistocene aridity may also have encouraged plant use; amongst recent foragers at a given latitude plant consumption usually increased in more open environments, largely because aquatic animal foods are less available in these dryer habitats (Keeley, 1992).

The plants used indicate how Neanderthals sourced nutrition from their environment. We find evidence of the use of grass seeds, true lily tubers, legumes and other starchy plants that leave no taxon-attributable types. Other microremains types included pollen and spores. Spores from Guattari III suggest interaction with fungi but these spores are too rare to ascertain the presence of deliberate interaction

with fungi such as the consumption of mushrooms (Power et al., 2015a). Not all recovered microremains reflect intentionally consumed food. Recovery of Betulaceae pollen and bracken spores may highlight use of birch, or hazel and bracken, but as these particles are excellent dispersers, they probably simply reflect characteristics of the airborne suspensions and aerosols in the Pleistocene airborne environment.

Some of the types we were able to identify also tell us about Neanderthal dietary behaviour. In particular, many of the microremains come from low-ranked foods, like grass seeds and tubers (Simms, 1985). Grass seeds are widely used by recent foragers in warm and cool environments (Lothrop, 1928; Simms, 1985; Harlan, 1989; Brand-Miller and Holt, 1998). Grass seeds used at Vindija and at Guattari demonstrate an investment in a low-rank plant food in cool habitats of the northern Balkans and coastal Italy. The use of grass seeds is often linked to terminal Pleistocene Southwest Asian foragers invested in broad spectrum diets because grass seeds are usually costly to harvest and prepare for consumption (Simms, 1985). On the other hand, there is abundant evidence that groups like the Vindija Neanderthals were big game hunters and that energetic contribution from plants is not likely to have rivalled meat. Middle Palaeolithic foragers probably only used grass seed as a limited component of the broader plant diet as this resource offers limited nutritional return (Simms, 1985). This is the same pattern observed in Upper Palaeolithic human foragers of Southwest Asia where grass seed use is most prominent (Savard et al., 2006; Rosen, 2010).

Overall, there is no indication that Neanderthals gradually used a more diverse array of plants, despite some evidence of a modest increase in population from 70 ka onwards (Foley and Lahr, 2003; Van Andel and Davies, 2003; Speth and Clark, 2006). The possible absence of a chronological trend in vegetal dietary breadth agrees with the lack of a trend in their predation niche prior to 55 ka. Yet dental calculus may hint that Neanderthal vegetal dietary breadth diverged from the narrow spectrum hunting economy. While the exploitation of small, fast and hard-to-catch game necessitated a costly increase in technology, some plants can be harvested and processed without technological investment. Although this may contradict conventional expectations of glacial period foragers in Central Europe, the cold temperatures of Pleistocene Eurasia may mislead us on the ecological productivity of this region. The pattern is better explained by decoupling seed and nut use from the dietary expectations of broad spectrum diets. Low intensity use of plants outside broad spectrum diets is possible (Hockett and Haws, 2003; Revedin et

al., 2010). Although an expanding plant food niche may be a sign of demographic packing population increase, its presence need not signify a total investment in complex foraging/broad spectrum foraging if such plant exploitation was possible without costly plant harvesting and processing technology (Hockett and Haws, 2003). Non-intensive use of these plants was possible with the technology available to Neanderthals.

Neanderthals could have reduced their processing costs by making use of caches of USOs and seeds, such as rodent stores (underground winter food stores), and by choosing to harvest the plants during seasons when they were easiest to prepare. The raiding of rodent stores requires little technology, though it often requires considerable ecological knowledge (Jones, 2009). For example, Siberian peoples raided rodent stores to obtain *Lilium* tubers all year round (Ståhlberg and Svanberg, 2010, 2012), but they had to be able to discern edible tubers from toxic USOs. Neanderthals ecological knowledge may have also been useful for the consumption of grass seeds. As Neanderthals exhibit no evidence of plant processing or food storage, we propose Neanderthals collected these seeds without laborious and expensive processing costs. One of the few ways this is possible is by plucking green grain from spikelets before they ripen and harden (Rosner, 2011). Unlike ripe grain, green grain requires no grinding or pulverising and may be consumed once dehusked, which can be done by hand. Green grain starch grains are smaller than those of ripe grain but they share most morphological characteristics and are likely to be identified as Triticeae with our methodology (Evers, 1971). Green grain is a resource that is only available in a narrow window before the grain ripens into a hard dry grain (Rosner, 2011). This collection of green grain would be suggestive of precisely seasonally organized Neanderthal foraging. Unfortunately, there is insufficient data to reconstruct a seasonal round of plant food gathering, but gathering during at least the spring is evident

5.5 Conclusions

The dental calculus microremain assemblages present strong evidence of Neanderthal use of various plants as foods, and complement our understanding of Neanderthal subsistence. This suggests that plant-harvesting strategies existed alongside their hunting economy. Plant foods were likely valued for their micro- or macronutrient profiles rather than caloric energy alone. Hominin physiology limits the total dietary protein intake, impeding an absolute reliance on protein-rich foods

such as terrestrial mammals lean meats (Cordain et al., 2000; Speth, 2010; Hockett, 2012). Recent foragers have avoided the effects of protein overconsumption by incorporating other macronutrients in diet. Foragers often source animal fat as the preeminent strategy for offsetting risk of protein poisoning (Speth and Spielmann, 1983; Cordain et al., 2000). However, animal fat from a diet of terrestrial ungulates may have been insufficient. Triticeae, Fabaceae and Liliaceae offer rich sources of carbohydrates that may have offset the problems of lean protein consumption.

The incorporation of diverse plant foods including those with low- or middle-ranking returns into the human diet probably predates Neanderthal diets, has a long history in the human lineage, and is likely that such diets persisted throughout hominin evolution mediated by energetic ecological necessity and labour availability. Similarly, resource depletion-driven subsistence changes may have occurred at many points in hominin evolution. Indeed it is observed elsewhere in Homininae, in present day chimpanzees, where increases in chimpanzee populations have been linked to increased use of low ranked prey (Watts and Mitani, 2015).

Regarding Neanderthal subsistence, we find no evidence of variation throughout the final 60,000 years they occupied Eurasia. Our model also finds no indication that plant use was confined to certain parts of their range. Surprisingly we did not find that a more diverse range of types were consumed in southern areas. Although this may suggest dietary inflexibility, it could also reflect relatively unchanging strategies, stable thanks to their success. While past research has revealed unappreciated variability in Neanderthal animal food use (Stiner, 1994; Speth and Clark, 2006), as a whole animal food provision centred on hunting of large and medium-sized game and thus Neanderthals exhibit lower intensity of diverse resources than early modern counterparts in Eurasia (Richards et al., 2000, 2001). A large- and medium-sized game hunting economy supplemented with plant foods may have evolved as a specialisation strategy in response to Eurasian environments (Stiner, 2013).