Microfossils in calculus demonstrate consumption of plants and cooked foods in Neanderthal diets (Shanidar III, Iraq; Spy I and II, Belgium)

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The nature and causes of the disappearance of Neanderthals and their apparent replacement by modern humans are subjects of considerable debate. Many researchers have proposed biologically or technologically mediated dietary differences between the two groups as one of the fundamental causes of Neanderthal disappearance. Some scenarios have focused on the apparent lack of plant foods in Neanderthal diets. Here we report direct evidence for Neanderthal consumption of a variety of plant foods, in the form of phytoliths and starch grains recovered from dental calculus of Neanderthal skeletons from Shanidar Cave, Iraq, and Spy Cave, Belgium. Some of the plants are typical of recent modern human diets, including date palms (Phoenix spp.), legumes, and grass seeds (Triticeae), whereas others are known to be edible but are not heavily used today. Many of the grass seed stachtes showed damage that is a distinctive marker of cooking. Our results indicate that in both warm eastern Mediterranean and cold northwestern European climates, and across their latitudinal range, Neanderthals made use of the diverse plant foods available in their local environment and transformed them into more easily digestible foodstuffs in part through cooking them, suggesting an overall sophistication in Neanderthal dietary regimes.

The temporal and geographic overlap between modern humans and Neanderthals has led many anthropologists to look for differences between the groups that might explain the disappearance of the latter. Recently, researchers applied the framework and methods of dietary ecology to further explore this question and used available dietary data to propose models for Neanderthal and modern human interaction. Several authors argue that Neanderthals were unable to acquire as many calories from the same environments as were modern humans (1–4) and were thus easily outcompeted. These views are supported by data suggesting that Neanderthals focused primarily on large game (10), marine resources (11), plant foods (12–17), and for the most part eschewed foods more common in modern human diets, such as marine resources, small, hard-to-catch animals, and protein-rich plant foods like nuts (5–7).

Other evidence suggests that Neanderthal foraging patterns were much like those of modern humans (8, 9), including small game (10), marine resources (11), plant foods (12–17), similar use of fire (18), some cooking, and other food processing (10, 13), and that these behaviors may have extended back in time to the Middle Pleistocene (19). These dietary behaviors are not only important in terms of energy they provide, but also can suggest sophistication in other areas of Neanderthal behavior. For example, the inclusion of plant foods can significantly structure the mobility patterns and gendered division of labor in recent (20–22) and presumably past hunters and gatherers (7, 9). Cooking also represents a significant shift in human behavior, by improving the nutritional quality of plant foods and potentially altering the social organization of human groups (23, 24). Assessing the characteristics of Neanderthal plant food preparation and consumption can help us to address whether dietary breadth may have played a role in Neanderthal replacement and could also substantially inform our understanding of behavioral and cognitive comparisons between Neanderthals and modern humans. However, due to vagaries of preservation and insufficient attention to plant remains, data on plant consumption are rare or completely lacking from most Neanderthal occupations.

The existing evidence for Neanderthal plant consumption is richest for Near Eastern sites. Macro- and microbotanical studies suggest that Levantine Neanderthals made use of several kinds of plants, including date palms, grasses, legumes, acorns, and pistachios (12–16). However, these remains, derived from sediments, are sometimes fragmentary and not always unequivocally linked to diet. Furthermore, grass seed bract phytoliths, which commonly occurred in the phytolith records, could not be identified at taxonomic levels below the family. Although hearths and burned materials have implied the controlled use of fire in many Neanderthal occupations (12, 13), direct evidence for preparing plant foods via cooking is lacking.

In contrast to the Near Eastern record, there are very few data on the potential importance of plants in the diets of northwestern, “classic” European Neanderthals. Spanish Neanderthals made use of several kinds of plants, including grasses, as bedding (25). Pollen records have suggested that the plant communities of Northern Europe varied significantly depending on the glacial cycle (26). Even during cold cycles, however, certain calorie-dense plant foods like grass seeds (Poaceae) and cattails (Typha) would have been available (26, 27). Analysis of residues on stone tools has suggested some use of plants and wood by European Neanderthals (28), although it is not clear what species were used and if they were eaten. This overall sparse and inconclusive record of Neanderthal use of plants limits our interpretations of their diet and related behavior.

To better explore Neanderthal use of plant foods, we analyzed the plant microfossils (starch grains and phytoliths) trapped in dental calculus of Neanderthal individuals. Dental calculus has been repeatedly shown to be a reliable source of plant microfossils for dietary reconstruction (29–35). The precipitation of minerals onto the surface of the teeth traps and preserves many components of the oral environment, including bacteria and food particles. The resulting dental calculus is heavily mineralized, survives well in archaeological contexts (36), and is easily recognizable on fossil teeth. Our recent study showed that calculus deposits on teeth are mineralologically similar to enamel, although with clear organic deposits, and are easily distinguishable from adherent sediments (37). Microfossils recovered from calculus

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are therefore a direct record of the plants an individual put in his mouth, which may include some nondietary plants, but should overwhelmingly consist of plant foods. Here we present results from dental calculus of two northern European Neanderthals, Spy I and Spy II from Spy Cave in Belgium, and one Near Eastern Neanderthal, Shanidar III, from Shanidar Cave in Iraq (for more information on each site and the fossil remains studied, see Materials and Methods).

**Results**

We collected and studied small calculus samples from seven teeth: three from Shanidar III (~46 ka) and two each from Spy I and II (~36 ka). Plant microfossils were recovered from all calculus samples, although the amount varied significantly between teeth even within individuals (Tables 1 and 2). The analysis below combines data from all teeth for each individual.

**Shanidar III, Shanidar Cave, Iraq.** We examined the calculus from three teeth from this individual: a left upper canine (tooth 3), a right third upper molar (tooth 4), and a left lower second incisor (tooth 5) (Fig. S1). A total of 73 starch grains were recovered from the three teeth. Ten of these starch grains exhibited features that allowed us to identify them as grass seed starches from the Triticeae tribe, including circular to subcircular shape in plane view, lenticular cross-section, central hilum usually marked with a thin line, and distinctive dimples and lamellae. Starch grains from this tribe, which includes the wild relatives of wheat, barley, and rye, have been identified in a variety of archaeological settings (40, 41). Our cooking experiments performed on a variety of grains and legumes showed that Triticeae starch grains undergo several changes when cooked before becoming completely gelatinized and unrecognizable (38). When only partially damaged, the starches can still be confidently assigned to the Triticeae. The overall pattern of damage to the starch grains matches most closely with that caused by heating in the presence of water, such as during baking or boiling, rather than “dryer” forms of cooking like parching or popping (38). The finding of cooked Triticeae starches on the Shanidar teeth reinforces evidence from other studies (13) that suggest that Near Eastern Neanderthals cooked plant foods.

Eleven of the remaining unidentified starches could be assigned to three main types. These three types and nine other unique grains have diagnostic features and probably derive from several different taxa (Fig. 2). They should become identifiable as the reference collections are expanded. One of the starches in the “other” category is likely from a legume, on the basis of its characteristic ovoid shape, distinctive longitudinal cleft fissure, and lamellae (Fig. 2 G and H). Starch grains such as these appear in *Pisum* spp. and *Cicer* spp. but the large diagnostic grains that would conclusively identify these genera were not observed in our sample. *Lens* and several species of *Vicia* can be ruled out, but there are other Near Eastern edible legumes that were unable to sample and the unknown starches may belong to one of these taxa. Three of the other starches have characteristics

### Table 1. Counts and descriptions of plant microfossils recovered from the dental calculus from Shanidar III

<table>
<thead>
<tr>
<th>Tooth no.</th>
<th>Type 1 Triticeae (cf. <em>Hordeum</em>)</th>
<th>Type 1 cooked</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
<th>Other cooked</th>
<th>Dmg/Enc</th>
<th>Phoenix spp. (date palm) phytolith</th>
<th>Other phytolith</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shanidar III tooth 3 LuC</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Shanidar III tooth 4 RM²</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Shanidar III tooth 5 Lb</td>
<td>2</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>14</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Totals per type</td>
<td>10</td>
<td>15</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>9</td>
<td>16</td>
<td>12</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

Type 1: Medium, circular to oval to D shaped, lenticular when turned, center usually dimpled/marked hilum, usually with clear lamellae visible under normal light but always visible at least under polarized light, cross arms are occasionally faded, line down center of grain when turned, sometimes dimpled. Type 2: Small, decorated oval with distinct projections, center hilum, clean cross. Type 3: Small, oval to circular, mostly central dimpled hilum occasionally with a fissure that crosses the grain entirely, too small for other features. Type 4: Small, subangular/rectangular, center dimpled hilum, clean cross. Other: A variety of unique/unusual forms that do not fit into the other types. Dmg/Enc: A variety of starches that may belong to one of the named types but are too damaged (cracked, broken) or encrusted (covered in calculus material) to be confidently identified.
similar to known plant underground storage organ “USO” starches (Fig. 2 I and J), but no Middle Eastern USOs were included in our reference collection. The final 28 starches were severely damaged and retained few diagnostic characteristics. However, the damage to 16 of these is characteristic of cooking (38). In total, starches with damage that is consistent with cooking represent 42% of the starch assemblage (Table 1), but we expect that the actual proportion of cooked foods within the diet of this individual was probably much higher. Our cooking experiments have shown that within those samples cooked for long periods (≥30 min), only a very small proportion of the starch grains remain undamaged or only partially undamaged, which would reduce the number of identifiable starches preserved in calculus (38).

Phytoliths were less numerous than the starch grains, but they document the consumption of another plant that would continue to have significant importance in later, modern human plant economies. Sixteen of the 20 phytoliths are small, decorated spheres or subspherical shapes from the fruits of the date palm (*Phoenix spp.*)) (Fig. 3). It does not appear possible to differentiate among the species of *Phoenix;* thus the identification must remain at the genus level. Nevertheless, the fruits of almost every species of date palm are edible (42) and would have been a nutrient-rich addition to the diet. Only two species of palms are native to the region, with date palm being the single edible one. This evidence, plus the morphological characteristics of the palm phytoliths from the teeth, matching in all respects the fruit of the date palm, strongly suggests that dates were consumed. The other four phytoliths are similar to those from tree fruit phytoliths but could not be confidently identified.

### Spy I and Spy II, Spy Cave, Belgium.

We sampled two teeth from both individuals (Fig. S6): From Spy I, we sampled the lower left fourth premolar from fragment 12A (11H) and the lower right first molar from fragment 12B (580c). From Spy II, we sampled the lower right fourth premolar from fragment 3 (577i) and the upper right third molar from fragment 2 (578i). The numbering reflects the most recent categorization by the Institute Royal des Sciences Naturelles de Belgique, which houses the fossils.

Both individuals from Spy had one tooth that contained many microfossils and one tooth that did not have as many (Table 2). Over 60 of the 136 starches had a unique, characteristic morphology, with a large ovoid shape, an eccentric hilum with a distinctive crack, distinct lamellae, and a polarization cross with smoothly curving “arms” (Spy I RM 580c had a large clump of calculus with up to 30 starches embedded, most of which were probably from this type but were too encrusted to identify with confidence). These attributes suggest that the starches are from a USO (Fig. 4 A and B). These starches share many similarities with the starches from the USOs of water lilies, but do not match those from either of the common local water lily species (*Nymphaea alba* and *Nuphar lutea*) (Fig. 5). There are several other species of water lily that are native to the study area, but we have not acquired USO samples from them.
Table 2. Counts and descriptions of plant microfossils recovered from the calculus from the teeth of Spy I and Spy II

<table>
<thead>
<tr>
<th>Tooth no.</th>
<th>Type 1 USO</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4 Andropogoneae?</th>
<th>Type 5</th>
<th>Type 6</th>
<th>Other</th>
<th>Dmg/Enc</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spy II RM3 578f</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Spy II RP4 577i</td>
<td>27</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>9</td>
<td>3</td>
<td>13</td>
<td>21</td>
<td>82</td>
</tr>
<tr>
<td>Spy I LP4 11H</td>
<td>30</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Spy I RM1 580c</td>
<td>32</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>1*</td>
<td>45*</td>
</tr>
<tr>
<td>Totals per type</td>
<td>60</td>
<td>3</td>
<td>2</td>
<td>9</td>
<td>15</td>
<td>5</td>
<td>17</td>
<td>25</td>
<td>136</td>
</tr>
</tbody>
</table>

**Type 1:** Large, oval to egg shaped to avocado shaped, ovoid in 3d, hilum very eccentric, usually at the thinner end of the oval. Hilum is marked with a fissure or dimple of varying forms: small dimple, single transverse fissure, one transverse and one longitudinal fissure, or X-shaped fissure. Lamellae are quite visible, most often at the end opposite the hilum. Occasional small surface dimples are shown. Cross arms are clear and smoothly bending across the surface of the grain. **Type 2:** Medium, clamshell shaped with eccentric, dimpled hilum near bulging long side. Cross is clean, with straight arms. **Type 3:** Two tightly compound grains, with the juncture between the two unclear. The overall shape is ovoid, whereas each grain is hemispherical. The hila are centric and unmarked. **Type 4:** Small to medium, subangular/faceted with slightly eccentric dimpled/open hilum, usually but not always marked with a deep single, Y-shaped or stellate fissure; cross arms are clean but usually bend over facets. Sometimes two grains are seen still in compounds. **Type 5:** Small, spherical to ovoid, center hilum that is sometimes marked with a dimple or a dimple and fissure. Cross arms are clean and straight across. **Type 6:** Large, very irregular, usually ovoid to subrounded, with deep lines and cracks radiating from the hilum, which is slightly off center and usually raised above the rest of the grain. Overall the starch appears to have a “volcano” shape. Other: A variety of unique/unusual forms that do not fit into the other types. Dmg/Enc: A variety of starches that may belong to one of the named types but are too damaged (cracked, broken) or encrusted (covered in calculus material) to be confidently identified.

*This sample includes a large clump of calculus with up to 30 starches embedded in it. Some of the starches appeared to be similar to type 1, but were too encrusted to be accurately identified, and have not been included in the overall counts for this sample.

Discussion

These sites record some of the extremes of the environments experienced by Neandertals, from a Mediterranean, although inland and mountainous, environment at Shanidar to a northern, although oceanic, environment at Spy. Our data show that Neandertals in both environments included a spectrum of plant foods from their diets, including grass seeds (Triticeae cf. Hordeum), dates (Phoenix), legumes (Faboideae), plant underground storage organs, and other yet-unidentified plants, and that several of the consumed plants had been cooked. The identified plant foods from Shanidar match well with the soil phytoliths and macrobotanical remains found at other Neanderthal sites in the Near East (12–16), whereas those from Spy show use of USOs as predicted for European Neandertals (27). Neandertals’ consumption of these starchy plant foods does not contradict data from isotope analysis, because nitrogen isotopes record only the consumption of meat and protein-rich plant foods (6).

We note that, as at other Neanderthal sites, there is no evidence of intensification (e.g., stone artifacts specialized for use as grinding implements or storage features) in the use of certain foods as seen in later, Upper Paleolithic (43, 44), Epipaleolithic, and Natufian preagricultural modern human groups. However, there is clear evidence of cooking in the recovered starch grains, and furthermore, several of the identified plant foods would have required moderate to high levels of preparation, including husking the grass seeds and harvesting the submerged USOs of water lilies. These lines of evidence indicate Neanderthals were investing their time and labor in preparing plant foods in ways that increased their edibility and nutritional quality (24, 45). It should also be noted that date palms and possibly other unidentified plants have different harvest seasons than barley and legumes, a factor that may suggest that the Shanidar Neanderthals practiced seasonal rounds of collecting and scheduled returns to harvest areas. Overall, these data suggest that Neanderthals were capable of complex food-gathering behaviors that included both hunting of large game animals and the harvesting and processing of plant foods.

The timing of two major hominin dietary adaptations, cooking of plant foods and an expansion in dietary breadth or “broad spectrum revolution,” which led to the incorporation of a diversity of plant foods such as grass and other seeds that are nutritionally rich but relatively costly to exploit, has been of

Fig. 5. Starch grains from modern water lilies under brightfield and cross-polarized light. Each box is 50 μm on a side. (A and B) Nymphaea alba. (C and D) Nuphar lutea.
central interest in anthropology (1, 2, 46, 47). Our evidence indicates that both adaptations had already taken place by the Late Middle Paleolithic, and thus the exploitation of this range of plant species was not a new strategy developed by early modern humans during the Upper Paleolithic or by later modern human groups that subsequently became the first farmers. Further plant microfossil research will continue to address the substantial data lacunae on dietary diversification during the Middle and Upper Paleolithic caused by inadequate floral records, and also better document trends that may have existed in Neandertal diets through time and space. Our studies extend the known record of starch survival in dental calculus into the Middle Paleolithic and indicate that starch grain analysis will enable reconstructions of diet in a range of fossil specimens.

Materials and Methods

Site and Sample Descriptions. Shanidar Cave is located in the Zagros Mountains of northwestern Iraq (44° 13' E, 36° 50' N). It was excavated between 1952 and 1957 by Ralph Solecki and colleagues (48–55). Four main archaeological layers were described in the nearly 14 m of sediments removed (48), and Mousterian artifacts and Neandertals’ remains were found in the lowest layer (layer D) (48, 56). A date of 44,000 B.P. was acquired using a bulk sample radiocarbon analysis on sediments at the top of this 10.0-m-thick layer; the lower age is unknown. Palynological samples indicated a warm climate and the presence of a variety of edible plants in the site’s vicinity, including date palms (Phoenix dactylifera), walnuts (Juglans), chestnuts (Castanea), oaks (Quercus), relatives of chicory and lettuce, and relatives of culinary herbs (49, 57–59). The Shanidar III fossil remains were found at 5.4 m below datum and 1–1.4 m below the sloping surface of level D, in the northeast corner of square B9 and near to it, but slightly below the remains of Shanidar I (48). The teeth were not immediately recognized during excavation and were found later in bone bags from that same square and level. On the basis of a radiocarbon date of 44,000 B.P. near Shanidar I, Shanidar III was thought to be slightly older, possibly as old as 50,000 B.P. (48).

Spy Cave is located in the commune of Jemeppe-sur-Sambre, province of Namur, Belgium, along the left bank of the Orneau River (50° 28' N, 4° 40' E). De Puydt and Lohest uncovered the Neanderthal remains in 1885. They recognized several layers in the site (the number varied between four and six depending on the publication and), but most recent studies revealed that the remains of a child and up to five adults were lumped together (65). The two fragmentary skulls and mandibles retained the names Spy I and Spy II. Previously unrecognized human teeth that refitted with Spy I and II were found in faunal bags, and these were directly dated using accelerator mass spectrometry radiocarbon to ~36 kya (63).

Fig. 6. Additional starch grains recovered from the calculus of Spy I and Spy II. The Upper image of each pair shows the starch under brightfield light, and the Lower image shows it under cross-polarized light. Each box is 50 μm on a side. (A and B) Type 4, starch possibly from the Andropogoneae grass tribe. (C and D) Type 5. (E and F) Type 6, unusual starch grain.

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