

Processing of wild cereal grains in the Upper Palaeolithic revealed by starch grain analysis

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Barley (*Hordeum vulgare* L.) and wheat (*Triticum monococcum* L. and *Triticum turgidum* L.) were among the principal 'founder crops' of southwest Asian agriculture¹. Two issues that were central to the cultural transition from foraging to food production are poorly understood. They are the dates at which human groups began to routinely exploit wild varieties of wheat and barley, and when foragers first utilized technologies to pound and grind the hard, fibrous seeds of these and other plants to turn them into easily digestible foodstuffs. Here we report the earliest direct evidence for human processing of grass seeds, including barley and possibly wheat, in the form of starch grains recovered from a ground stone artefact from the Upper Palaeolithic site of Ohalo II in Israel. Associated evidence for an oven-like hearth was also found at this site, suggesting that dough made from grain flour was baked. Our data indicate that routine processing of a selected group of wild cereals, combined with effective methods of cooking ground seeds, were practiced at least 12,000 years before their domestication in southwest Asia.

Ohalo II is located on the southwestern shore of the Sea of Galilee at an elevation of 212 m below sea level. In most years the site is submerged in 2–3 m of water. Ohalo II was exposed in 1989 and 1999, following a dramatic drop in the water level of the lake; today the site covers an area of about 2,000 m². Three seasons of excavations took place during each of the low-level events before it was submerged again. The work revealed the remains of a camp, including six brush huts built from branches and leaves, hearths and a human grave^{2,3}. All the features were well-preserved, having been found *in situ* in the waterlogged sediments. A series of 40 ¹⁴C determinations on charred plant remains dates the occupation to around 19,500 ¹⁴C yr BP (about 23,500 to 22,500 calendar years ago) placing the site within the Upper Palaeolithic period (locally also termed the Early Epipalaeolithic)⁴.

Exceptional preservation of organic material in the fine lake sediments enabled the retrieval of rich faunal (mammals such as gazelles, birds, rodents, fish and molluscs) and plant assemblages (more than 30 economic species), including numerous charred grains of wild barley (*Hordeum spontaneum* Koch) and emmer wheat (*Triticum dicoccoides* (Ascherson and Graebner) Aaronson) and other grass species (for example, *Bromus pseudobrachystachys* Hornug and *Bromus tigridis* Boiss and Noe)^{5,6}. These remains represent the earliest evidence for considerable use of grasses in the human diet.

Hut 1 at Ohalo II is the oldest and best preserved among the huts recovered to date, and three successive floors and wall bases were identified and described. A large, flat basalt stone was set on a patch of sand on the second floor supported by small pebbles, much like an anvil or a work surface (Fig. 1). Discrete concentrations of food and medicinal plant seeds were distributed on the floor around the stone. To ascertain whether the Hut I stone was used to grind the plants, and specifically to establish which taxa were processed, we

carried out starch grain studies on this artefact. To recover starch grains from the stone, we used a protocol shown to be successful with large stone artefacts in other areas of the world^{7,8} (see Methods). We developed a key for starch grain identifications on the basis of a large modern reference collection that included many grasses, other economic plants and weeds native to the study region. Plant taxa that were previously identified at Ohalo II^{5,6} were given close attention (see Methods). We found that the genera *Hordeum*, *Triticum* and *Aegilops* (hereafter, the AHT taxa group) are separable from other grasses native to the study region on the basis of morphological and size criteria. Furthermore, specific grain types as well as grain population signatures can be used to identify the presence and probable absence or low frequency of each of these genera in archaeological starch assemblages (Fig. 2a–g and Table 1a) (see also Supplementary Figs 1–7 for detailed descriptions and illustrations of modern starch grains).

A total of 150 starch grains were recovered from the grinding stone. A hundred and twenty-seven of these were identified as grass seed starches. Seventy-eight out of those 127 grains are diagnostic of the AHT taxa group on the basis of their lenticular shapes and sizes (Table 1b and Fig. 3a). A genus-specific identification of starch grains from *Hordeum* is possible because they commonly (about 50% of all the lenticular forms present in the starch population) have well-defined lamellae that are differentiable from lamellae in *Aegilops*; *Triticum* grains have no such demonstrable lamellae (Fig. 2a–d and Supplementary Figs 1–3). On the basis of this we identified 23 grains from *Hordeum* on the stone (Fig. 3b, c). Although the size characteristics of the *Hordeum* grains with lamellae are most similar to modern *Hordeum spontaneum* (Table 1a, b), distinguishing between *H. spontaneum*, *Hordeum glaucum* and *Hordeum bulbosum* is not possible at this time.

Hordeum marinum, the carbonized seeds of which were present in appreciable quantities around the stone (a total of 505 seeds) can, however, be ruled out from representation in the starch grain residues from the stone because starches from this species have much smaller sizes and a characteristic morphology (Supplementary Fig. 4). We found no grains with the crater-like depressions that commonly occur in populations of starches from *Triticum* and may be diagnostic of that genus (Fig. 2a). Similarly, whereas many grains from modern *Aegilops* spp. have central swellings and/or prominent fissures together with distinctive lamellae (Fig. 2c, d), no grains of that type were observed. In view of the large sample size of grains recovered from the stone, and the reasonable expectation that if *Triticum* or *Aegilops* had been processed with any frequency then grains characteristic of those genera just described should have been



Figure 1 The stone implement analysed from Ohalo II.

recovered, the majority of the grains on the stone are probably from *Hordeum*.

The 49 other Poaceae grains retrieved from the stone are compound grains without characteristic features, and can belong to any number of genera in the family, including the AHT group (Fig. 3d). Importantly, however, we could find no evidence that grass seeds from genera outside the AHT group, or other non-grass species that were commonly to moderately present as carbonized remains from the hut floor, had been processed. Starch grains from these species possess markedly different shape and surface features when compared with those recovered from the grinding stone (see Table 1a, Methods and Supplementary Figs 5–7). No starches characteristic of roots and tubers were present in the tool residues. Hence, the stone seems to have been a speciality, cereal-processing implement.

A special alignment of burned stones covered by ash at Ohalo II suggests the presence of a hearth-like structure used as a simple oven; if this assumption is correct it would represent the earliest such food-roasting strategies documented. A few metres southwest of Hut 1, 13 natural and worked stones were arranged together to form a paved circle (Supplementary Fig. 8). Above and between the stones was a thin layer of dark material rich in charcoal and ash but

with few carbonized fruits and grains, mostly from *H. spontaneum*. Other hearths at the site were not lined by or paved with any stones. They were visible instead as simple, round or oval ash deposits, usually 30–50 cm across and several centimetres thick. We believe that the stone-paved feature was used for baking food. The persistence and use of similar kinds of simple ovens by recent and modern nomads and hunter-gatherers provides an ethnographic analogy for the Ohalo II example. Groups in southwest Asia, Sahara and Australia prepare rough dough from grass and other ground seeds by mixing flour and water in a container or even on a piece of cloth, and then placing the dough on top of a layer of heated stones from which the embers have been removed. After the dough is spread on the stones, the embers are put back to cover the dough^{9–11}.

The beginning of baking was probably an important step forward in human nutrition. For example, the glycemic index reflects how rapidly glucose from carbohydrates enters the blood after ingestion, and it is influenced considerably by methods of food preparation that involve particle size reduction and mixing of starches with water, and by the duration and manner of cooking^{12,13}. Ingesting ground and baked barley and wheat products would have had the effect of increasing the glycemic index (and thus the amount of available dietary energy) by between 56% and 72%, compared with ingesting uncooked or even cooked whole-grained forms of these plants (Supplementary Table 1). Although no baked product was preserved at Ohalo II, the starch grain data together with the fact that few carbonized grains were found in the oven feature suggest that what was roasted included dough prepared from wild cereals.

If they are going to serve as adequate dietary staples, grasses, other seeds and underground plant organs usually must be pounded and ground to separate and remove indigestible fibrous substances and toxins, reduce the size of food particles, and concentrate nutritious, high-energy starchy reserves¹⁴. The point at which technological improvements such as these began to be employed in plant food preparation, and which taxa and their specific structures (for example, roots, tubers, seeds and nuts) were first processed in this way are old and important questions. In southwest Asia, Europe and northern Africa, ground stone implements of various types, identifiable as possible plant grinders or pounders (for example, hammerstones grinding slabs, mortars and pestles), make their first appearance during the Upper Palaeolithic between around 45,000 and 18,000 BP, and they were manufactured by fully-modern humans^{15,16}. However, because once-associated macroscopic plant remains have rarely survived along with these artefacts and because

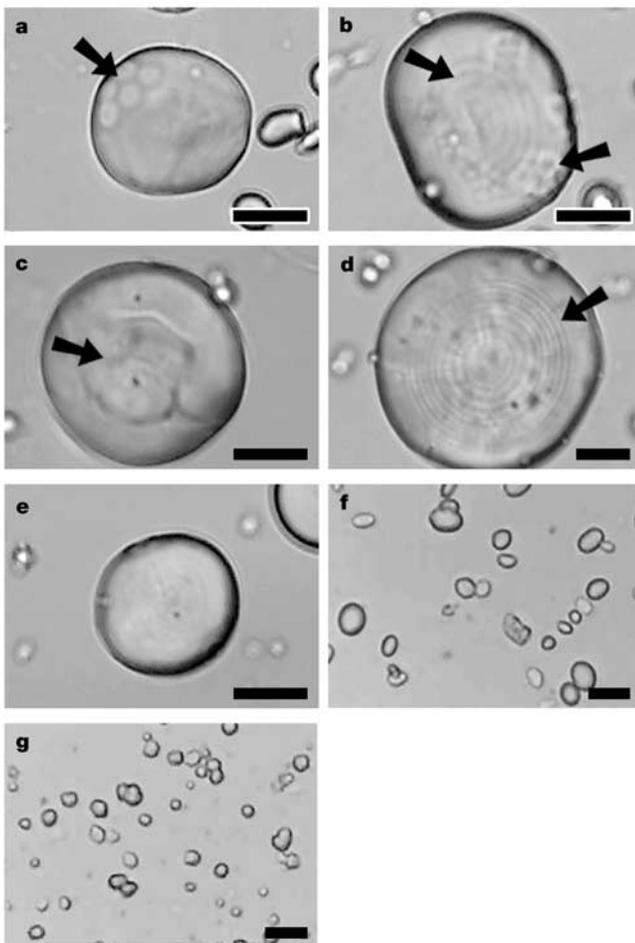


Figure 2 Characteristic starch grains from seeds of various modern cereals and other grasses. **a**, *T. dicoccoides*. The arrow on the left points to distinctive crater-like depressions. **b**, *H. spontaneum*. The arrow on the left points to lamellae; arrow on the right points to *Hordeum*-type surface depressions. **c**, *Aegilops geniculata*. The arrow points to the central protuberance with the pleat. **d**, *Aegilops peregrina*. The arrow points to the characteristic lamellae present in this species. **e**, *H. glaucum*. **f**, *B. pseudobrachystachys*. **g**, *Piptatherum holciiforme*. Scale bars, 10 μ m.

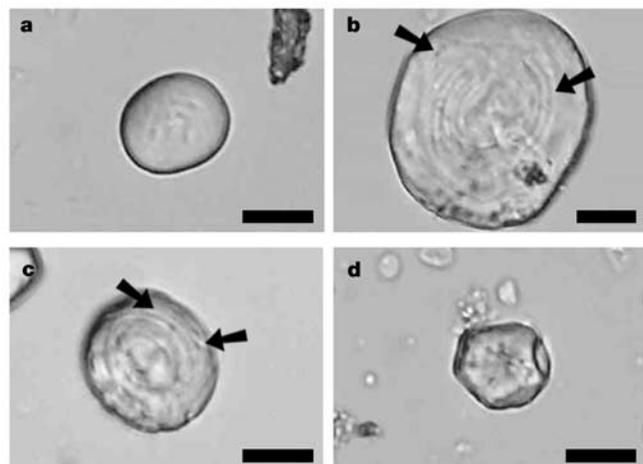


Figure 3 Starch grains recovered from the stone implement. **a**, Lenticular grain from the AHT taxa group. **b**, **c**, Lenticular grains with lamellae (right arrow in **b** and left arrow in **c**) from *Hordeum*. They also have surface depressions (other arrows) characteristic of the genus. **d**, Compound starch grain. Scale bars, 10 μ m.

Table 1 Starch grain size attributes of modern and archaeological grasses

	Mean (\pm s.d.) of length (μ m)	Range	n
(a) Analysed modern grasses from the study region			
<i>Aegilops geniculata</i> Roth	21 \pm 6.4	10–36	50
<i>Aegilops peregrina</i> (Hackel) Maire and Weiller	25 \pm 8.0	12–52	50
<i>Hordeum spontaneum</i> Koch*	20 \pm 4.7	10–26	50
<i>Hordeum bulbosum</i> L.	17 \pm 3.7	10–24	50
<i>Hordeum glaucum</i> Steudel*	18 \pm 3.9	8–24	50
<i>Hordeum marinum</i> Hudson*	10 \pm 1.8	6–14	50
<i>Triticum dicoccoides</i> (Ascherson and Graebner) Aaronsohn	17 \pm 6.1	8–30	50
<i>Hordeum bulbosum</i> with lamellae only	21 \pm 1.6	18–24	50
<i>Hordeum glaucum</i> with lamellae only	22 \pm 1.4	18–26	50
<i>Hordeum spontaneum</i> with lamellae only	28 \pm 2.9	22–40	50
<i>Alopecurus utriculatus</i> Banks and Solander	5 \pm 1.5	2–8	50
<i>Alopecurus arundinaceus</i> L.	4 \pm 0.9	2–8	50
<i>Avena barbata</i> Pott and Link	12 \pm 2.9	6–18	50
<i>Brachypodium distachyon</i> (L.) Palisot de-Beauvois	9 \pm 2.2	4–16	50
<i>Bromus pseudobrachystachys</i> Hornug*	5 \pm 1.4	4–8	50
<i>Gastidium ventricosum</i> (Gouan) Schinz and Thellung	4 \pm 1.0	2–6	50
<i>Lolium multiflorum</i> Lamarck	<6.0	–	50
<i>Lolium rigidum</i> Gaudin	<6.0	–	50
<i>Phalaris minor</i> Retzius	<2.0	–	50
<i>Phalaris paradoxa</i> L.	<4.0	–	50
<i>Puccinellia gigantea</i> (Grossheim) Grossheim	<4.0	–	50
<i>Puccinellia distans</i> (L.) Parlato	<4.0	–	50
<i>Piptatherum holciforme</i> * (Bieberstein) Roemer and Schultes	3 \pm 1.0	2–4	50
<i>Vulpia persica</i> Boissier	<2.0	–	50
(b) Archaeological Poaceae starch grains†			
All Poaceae	20 \pm 6.6	8–40	98
Lenticular grains (AHT taxa)	23 \pm 6.3	14–40	54
<i>Hordeum</i> grains with lamellae only	28 \pm 4.7	20–38	23

* Carbonized remains of these species were common around the grinding stone and on the floor of Hut 1.

† Seven of the starch grains were recovered from the 10 needle probes of small cavities in the stone. The remainder of the sample of starches, 143 grains, resulted from Step II (high energy shaking) (see Methods). Step II yielded very little sediment in association with the starch grains. These results indicate that most of the grains recovered had been lodged inside cracks and crevices on the stone, where they were protected from degradation and decay.

some of the implements contain the remains of ochre, distinguishing between plant food preparation and the grinding of mineral pigments (or even animal bone) as their primary function, together with determining which plants may have been processed, have been difficult.

Our findings provide direct empirical data that grinding stones from the Upper Palaeolithic were used to process plant foods, and they constitute the earliest evidence for this activity. Our data indicate that a selected cadre of wild cereals, including barley and possibly also wheat and/or goat grass—the first two of which were subsequently domesticated between around 10,000 and 9,000 BP—received preferential emphasis for processing, and may thus have contributed significantly to the human diet as far back as around 20,000 BP. Wild cereals have the largest and densest grains of any grasses native to southwest Asia, an attribute that may have caused them not only to be attractive to early seed collectors¹⁷ but also to be favoured for intensive methods of processing once gathered. Smaller-seeded grasses may simply have been parched before consumption⁶. Furthermore, grinding the largest-grained grasses may have ameliorated the high 'handling costs' and decreases of foraging efficiency associated with the shift to broader spectrum subsistence and incorporation of intensive grass seed exploitation strategies evidenced at Ohalo II^{18–21}. This would have further encouraged close relationships between foragers and the closest wild relatives of domesticated cereals. Nutritionally effective ways of preparing foods from wild cereals, evidenced at Ohalo II, likely facilitated other forms of subsistence intensification (such as seed storage²²) that were preludes to domestication and became established in the region during the following 10,000 yr.

Our research opens up a new avenue of palaeoethnobotanical investigation in southwest Asia and other areas of the world where ground stone tool assemblages were used before the Neolithic period. Future starch grain studies have the potential to elucidate when and where grasses, other seeds and nuts, and underground plant organs first became important components of human diets and technological efforts. The functions of ground (and chipped) stone tools recovered from early archaeological sites can also be

clarified by such research. In sites of any age from southwest Asia where other types of plant remains are not well-represented, starch grain data may stand alone as definitive evidence of cereal and other plant usage. □

Methods

Extraction of starch grains

The grinding stone was examined under a stereoscopic microscope at a magnification of $\times 100$. The point of a fine needle was inserted into cracks and crevices to loosen and remove any residue. Two probes, each at five different locations on the stone, were examined in this manner (step I). The residue was mounted in water on a slide and examined with polarized and unpolarized light at a magnification of $\times 400$. The stone was then shaken in an ultrasonic bath for 5 min to completely dislodge adhering sediment and starch. Starch was then isolated by adding a heavy liquid solution of caesium chloride (CsCl) with a density of 1.8 g cm^{-3} (step II). Starch grains were mounted on slides in water and examined microscopically as in after Step I. Sediments that originally surrounded the stone *in situ* were not available for analysis. All archaeological starch grains possessed the starch-specific extinction cross when examined under cross-polarized light.

Starch grain identification

We used a modern reference collection of over 400 different species from 45 different families of plants that is housed at the Smithsonian Tropical Research Institute. Included were 21 species of the Poaceae and 13 other species from seven different families whose carbonized remains were commonly retrieved and identified at Ohalo II, including around the grinding stone that was analysed (Table 1; *Galium tricornutum* Dandy, *Malva parviflora* L. and *Atriplex* spp. were the most numerous non-Poaceae taxa identified near the stone). The numerous studies available on starch grain morphology were also consulted^{7,8,23–28}. Our starch keys and classifications emphasize attributes shown to be useful in identifying individual families, genera and species of plants, including: (1) overall grain type (simple or compound) and shape (bell-shaped, circular, lenticular or oval), (2) contour and surface features, (3) position and form of the hilum (the botanical centre of the grain) and fissure (internal cracks emanating from the hilum of some starch grains, formed when the grain begins to grow outward from the hilum and quite literally cracks), (4) number and characteristics of pressure facets present on compound grains and (5) presence or absence of demonstrable lamellae (rings formed during starch grain growth).

A considerable amount of research indicates that Poaceae seed starches are readily distinguishable from those of other families^{7,8,23–28}. We found that size alone effectively separates many wild grass species and genera of the study region from the AHT group, as mean sizes and ranges in the latter group far exceed those of other grasses studied (Table 1a). Importantly, morphological criteria also distinguish these grasses from the AHT group. AHT starches are usually simple (versus compound) grains, and circular, oval or kidney-shaped in outline, and when rotated into side view they assume a lenticular form (Fig. 2a–d and Supplementary Figs 1–4). In contrast, starch grains of other genera studied, including *Bromus* and *Piptatherum*, are either compound, flat and/or angular to

irregular in shape, and lack the features characteristic of the AHT starches (Fig. 2f, g).

It is also clear that using specific grain types together with attribute combinations in a multiple grain analysis is an effective and perhaps the most conservative means to distinguish individual genera and species. Such an approach employs all the morphological characteristics that account for the population of starches in a single species as well as their quantitative frequency tendencies, and it takes into account intra- and interspecific variation in grain attributes. For example, starch grain populations from *T. dicocoides* contain high proportions (about 40%) of grains that have distinctive, large crater-like impressions on the surface; these grains are also without lamellae (Fig. 2a). These types were not observed in either *Hordeum* or *Aegilops*, which, in turn contributed high frequencies of other types of characteristic starch grains (Fig. 2b–e and Supplementary Figs 1–4). In archaeological starch grain assemblages of sufficient sample sizes it would be possible to identify the presence and/or probable absence or low frequency of individual genera and species using these kinds of signatures.

Other families and species represented through their carbonized remains at Ohalo II were: Asteraceae (*Centaurea pallescens* Delile, *Silybum marianum* (L.) Gaertner), Chenopodiaceae (*Atriplex halimus* L., *Suaeda aegyptiaca* (Hasselquist) Zohary), Fabaceae (*Melilotus indicus* (L.) Allioni, *Pisum elatius* Marschall von Bieberstein), Malvaceae (*Malva aegyptia* L.), Potamogetonaceae (*Potamogeton pectinatus* L., *Potamogeton perfoliatus* L.), Ruppiales (*Ruppia maritima* L.) and Zygothallaceae (*Nitraria schoberi* L.). Most of these species produced oils and not starches. The taxa with starches (*Pisum*, *Potamogeton* and *Ruppia*) have grains that can be distinguished from others in our and other established reference collections (Supplementary Figs 5–7).

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Effect of trace metal availability on coccolithophorid calcification

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The deposition of atmospheric dust into the ocean has varied considerably over geological time^{1,2}. Because some of the trace metals contained in dust are essential plant nutrients which can limit phytoplankton growth in parts of the ocean, it has been suggested that variations in dust supply to the surface ocean might influence primary production^{3,4}. Whereas the role of trace metal availability in photosynthetic carbon fixation has received considerable attention, its effect on biogenic calcification is virtually unknown. The production of both particulate organic carbon and calcium carbonate (CaCO₃) drives the ocean's biological carbon pump. The ratio of particulate organic carbon to CaCO₃ export, the so-called rain ratio, is one of the factors determining CO₂ sequestration in the deep ocean. Here we investigate the influence of the essential trace metals iron and zinc on the prominent CaCO₃-producing microalga *Emiliania huxleyi*. We show that whereas at low iron concentrations growth and calcification are equally reduced, low zinc concentrations result in a de-coupling of the two processes. Despite the reduced growth rate of zinc-limited cells, CaCO₃ production rates per cell remain unaffected, thus leading to highly calcified cells. These results suggest that changes in dust deposition can affect biogenic calcification in oceanic regions characterized by trace metal limitation, with possible consequences for CO₂ partitioning between the atmosphere and the ocean.

The production of CaCO₃ in the surface ocean, its export to greater depths and its deposition in the sediment above the lysocline (the depth below which CaCO₃ dissolves) affect atmospheric CO₂ in two ways. On a timescale that is shorter than the ocean mixing time, CaCO₃ export reduces alkalinity in the surface ocean and lowers its storage capacity for atmospheric CO₂. On a timescale of thousands of years, a mechanism called carbonate compensation balances CaCO₃ burial with its supply of raw materials (riverine calcium and carbonate ions) by adjusting the depth of the lysocline⁵. This determines the deep ocean's carbonate ion concentration and