Dental calculus reveals Mesolithic foragers in the Balkans consumed domesticated plant foods

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Researchers agree that domesticated plants were introduced into southeast Europe from southwest Asia as a part of a Neolithic "package," which included domesticated animals and artifacts typical of farming communities. It is commonly believed that this package reached inland areas of the Balkans by ∼6200 calibrated (cal.) BC or later. Our analysis of the starch record entrapped in dental calculus of Mesolithic human teeth at the site of Vlasac in the Danube Gorges of the central Balkans provides direct evidence that already by ∼6600 cal. BC, if not earlier, Late Mesolithic foragers of this region consumed domestic cereals, such as *Triticum monococcum*, *Triticum dicoccum*, and *Hordeum distichon*, which were also the main crops found among Early Neolithic communities of southeast Europe. We infer that "exotic" Neolithic domesticated plants were introduced to southern Europe independently almost half a millennium earlier than previously thought, through networks that enabled exchanges between inland Mesolithic foragers and early farming groups found along the Aegean coast of Turkey.

Mesolithic foragers | starch analysis | domesticated cereals | forager/farmer interaction | human dental calculus

It is commonly assumed that the spread into Europe of domesticated plants, cereals, and pulses (1, 2) was part of a Neolithic "package," which included domesticated animals and artifacts typical of farming communities (e.g., ceramics, polished stone axes). Researchers agree that such a package was introduced into southeast Europe from southwest Asia and reached inland areas of the Balkans by ∼6200 calibrated (cal.) BC or later. Genetic (3) and isotope data (4) have revealed that demographic movements brought these Neolithic novelties from the Near East into Europe through the process by which scarce Mesolithic foragers were either quickly replaced or assimilated into Neolithic lifeways. Isotope analyses have also suggested that Mesolithic diets were largely based on terrestrial, marine, or riverine protein-rich resources (5–7) with scanty evidence for the consumption of plants (8). Here we show that Late Mesolithic foragers of the central Balkans consumed domesticated specimens of wheat and barley (namely grass species of Triticeae tribe) at least from ∼6600 cal. BC, almost half a millennium earlier than previously thought. We found that starch granules entrapped in dental calculus of Mesolithic (∼6600–5900 cal. BC) human teeth at the site of Vlasac (the Danube Gorges) correspond to those found in the teeth of Neolithic (∼5900–5700 cal. BC) individuals at the neighboring site of Lepenski Vir. Our results show that these starch granules originate from the consumption of main domestic crops found among Early Neolithic communities of southeast Europe (9)—that is, *Triticum monococcum* (einkorn wheat), *Triticum dicoccum* (emmer wheat), and/or *Hordeum distichon* (barley). As genetic and botanical studies have ruled out European ancestry for domestic wheat/barley and confirmed the Near East as the source of these crops (2, 10, 11), our assay provides the earliest evidence that domesticated plants were transmitted independently from other components of the Neolithic package through existing networks that enabled exchanges between inland Mesolithic foragers and early farming groups from ∼6600 cal. BC onwards.

The Sites

The Danube Gorges area is split by the River Danube between the territories of present-day Romania and Serbia (Fig. 1). In this region, more than 20 sites with Mesolithic deposits were discovered in the mid-1960s and yielded unprecedented data for other areas with known Mesolithic presence in Europe (12–15).

The site of Vlasac is situated in the Lady Whirlpool's Gorge of the Danube on the Serbian side of the river (Fig. 1). The first excavations at the site were carried out in 1970–1971 as part of a rescue project (16). New excavations at Vlasac began in 2006, and the investigations of the site are ongoing (17). The resumed work at Vlasac has covered an area of 326 m^2 (Fig. S1) and takes place upslope from the excavation area investigated in 1970–1971. Radiocarbon dates from both old and new excavations suggest that the site was more or less continuously occupied from the Early Mesolithic, from ∼9500 cal. BC, but the intensity of occupation increases from the mid-eighth millennium BC (18, 19). The Late Mesolithic occupation/use of the site covers the period between ∼7400 and ∼6200 cal. BC. New research at Vlasac has indicated that the site was continuously used throughout the period of the Mesolithic–Neolithic transition—that is, ∼6200–5900 cal. BC. Finally, there is also evidence for the use of this site in the course of the regional Early/Middle Neolithic (∼6000/5950–5500 cal. BC). During the Early Neolithic phase, the first chaff-tempered ceramic finds appeared at Vlasac (17, 18). Late Mesolithic domestic features, such as trapezoidal dwellings and numerous and overlapping rectangular stone-lined hearths, testify to the fact that this was a relatively large and likely sedentary complex forager settlement (16, 18). The total number of formal burials at Vlasac excavated in

Significance

The starch record entrapped in dental calculus of Mesolithic human teeth from the site of Vlasac in the central Balkans provides direct evidence that complex Late Mesolithic foragers of this region consumed domesticated cereal grains. Our results challenge the established view of the Neolithization in Europe that domestic cereals were introduced to the Balkans around ∼6200 calibrated (cal.) BC as a part of a "package" that also included domesticated animals and artifacts, which accompanied the arrival of Neolithic communities. We infer that Neolithic domesticated plants were transmitted independently from the rest of Neolithic novelties from ∼6600 cal. BC onwards, reaching inland foragers deep in the Balkan hinterland through established social networks that linked forager and farmer groups.

Author contributions: E.C. designed research; E.C. and A.R. performed research; M.E. performed anthropological analysis of dental remains from the sites presented in the paper; D.B. excavated the site of Vlasac from 2006–2009 and provided chronological framework; E.C. and A.R. analyzed data; and E.C., A.R., M.E., and D.B. wrote the paper.

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Fig. 1. Map of southeast Europe showing the locations of Vlasac, Lepenski Vir, and the other sites mentioned in the text.

1970–1971 comprises 87 graves, containing 164 individuals (20). Another 17 burials were excavated in 2006–2009, with the minimum number of 16 individuals. Numerous ground stones were also found at the site (17) (Fig. S2).

The site of Lepenski Vir is located on the right bank of the Danube River, ~3 km upstream from Vlasac. A surface of 2,500 m^2 and 3.5 m-thick cultural layers were excavated at this site from 1965 to 1970 (14, 15). Radiocarbon measurements indicate three main phases of human occupation: (*a*) Early/Middle Mesolithic occupation starting in the second half of the tenth millennium BC and continuing until around 7300 cal. BC (Proto-Lepenski Vir phase); (*b*) Mesolithic–Neolithic transition phase Lepenski Vir I–II from ~6150–5950 cal. BC; and (*c*) Early Neolithic Lepenski Vir III phase from ~5950–5700 cal. BC (13, 21–23) (Fig. S3). At the site, the transition period is broadly contemporaneous with the arrival of farming groups in the central Balkans between ~6200 and ~6000 cal. BC. During this phase, no evidence for farming or stock-herding has been identified to date (22), although different strands of evidence document contact/interaction with early farming groups (4, 13). The site furnished evidence of cultural complexity with numerous dwelling structures with trapezoidal floors and central stone-lined rectangular hearths, sculpted sandstone boulders, ornamented and plane pestles and mortars, and other stone and osseous artifacts and ornaments. A total of 134 graves containing the remains of 190 individuals were also reported (20).

Archaeobotanical investigations were not conducted at the time Vlasac and Lepenski Vir were excavated. New fieldwork at Vlasac included for the first time flotation and analysis of macrobotanical remains (17). On the Romanian side of the Danube, Schela Cladovei is the only other site of the same Mesolithic–Neolithic duration that has yielded macrobotanical remains (24). Available wood charcoals, macrobotanical remains, as well as regional palynological data provide limited information about ecological zones around the sites and wild plant resources potentially available to the Late Mesolithic population. The beginning of the Holocene in the Balkans is marked by a shift to warmer and more humid conditions, which led to the expansion of mixed deciduous forests composed of temperate arboreal species such as *Quercus*, *Ulmus*, *Tilia*, *Carpinus*, and *Corylus* (25–28). Forest undergrowth taxa such as *Cornus*, *Cotinus*, *Corylus*, *Sambucus*, and *Rosaceae* suggest the existence of patches of open woodland around the Danube Gorges sites (25). There are also indications of the presence of temporarily (and permanently) wet areas and flooded land close to the settlements. Riparian vegetation has been an important resource for the Late Mesolithic and for Early Neolithic groups as both wood and fruit of *Ulmus* and *Salix*/*Populus* were collected at sites along the Lower Danube region in association with seed/fruit of *Hyosciamus*, *Physalis*, *Vitis*, and *Trapa* (25). In addition, analyzed pollen found in coprolites from upper Mesolithic horizons at Vlasac documented the presence of Gramineae grasses around the site as well as the consumption of domesticated Cerealia (29).

Materials

Nine individuals recently excavated at the site of Vlasac were analyzed with microfossils preserved in the dental calculus of seven individuals (Fig. 2 *A*–*E*). Based on ^{14}C accelerator mass spectrometry (AMS) dates, some directly dating the analyzed burials, five of these seven individuals are dated to the Late Mesolithic (~6600–6450 cal. BC), and two individuals are dated to the Mesolithic–Neolithic transition phase (~6200–5900 cal. BC) (19) (Figs. S4 and S5 and Table 1 and Table S1). Samples were also taken from the dental calculus of three crouched female burials (individuals 8, 20, and 32a) (Fig. 2*F* and Fig. S6) from the site of Lepenski Vir. Direct ^{14}C AMS measurements on two of these three individuals put them in the Early Neolithic timespan (~5900–5700 cal. BC) (Table 1 and Table S1). All three individuals are gracile and nonlocal based on their strontium isotope ratios (4) and likely originated in the first farming communities. Results from calculus analysis were integrated with the study of dentoalveolar pathologies (caries and periapical lesions) on permanent teeth.

Results

Four different typologies of starch granules were positively identified in dental calculus from Vlasac and Lepenski Vir and assigned to four important tribes of the Poaceae (grass family) and the Fabaceae (legume family), all well-described in literature (30) (Table 1). Some of the starch granules were found to be in an extraordinary state of preservation, especially considering the age of the samples, likely due to the low level of processing as a result of inhalation or accidental ingestion during the processing of starchy foods (see *SI Results*).

Type I. The first type of starch granules found possess a strong "bimodal distribution" of simple granules characteristic of most grasses of the Triticeae tribe (barley and wheat tribe). Such distribution is very frequent in our samples and involves the presence of both large granules oval to subround in 2D shape, lenticular in 3D, with central hilum and few lamellae (known as A-type) and small almost spherical granules with a central hilum (≤10 μm, known as B-type) (30) (Table 1). Exceptionally, archaeological grains are still lodged close together, indicating a small amount of processing (*SI Results*). Bimodal distribution shows a very

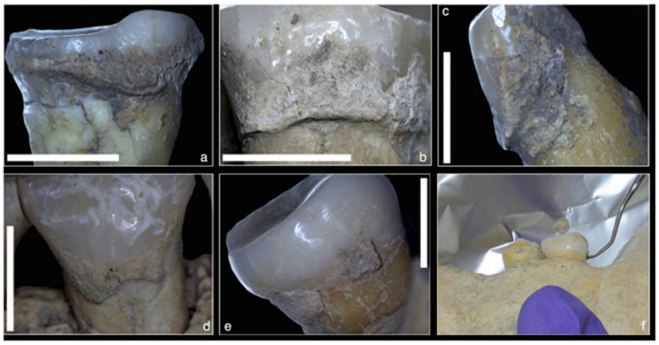

Fig. 2. Calculus preserved on teeth from Vlasac and Lepenski Vir: (*A*) Vlasac U64. × 11; (*B*) Vlasac U24. × 30; (*C*) Vlasac U44; (*D*) Vlasac H232; (*E*) Vlasac H53; (*F*) sampling Lepenski Vir individual 32a. (Scale bar, 1 cm.)

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Table 1. Context, provenance, chronology, sex, and age of individuals analyzed for dental calculus from Vlasac and Lepenski Vir and summary data on the quantity of starch granules, descriptions of their features, and proposed identifications

Individual/burial no. (dating)	Sex/age	Tooth no.	Quantity	2D/3D morphology	Hilum	Lamellae	Proposed identification
Vlasac							
U 115 (LM: ∼6800–6500 cal. BC)	?/adult	28	2	Irregular triangular of oval	Central, slightly sunken	Not visible	Unidentified starch granules (see *Supporting Information*)
H232 (LM: *TPQ* OxA-20702: 6636–6476 cal. BC)	f/∼25 y	28	<5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
H317 (LM: ∼6500 cal. BC)	f/∼30 y	14	No starch				
U 326 (LM: ∼6500 cal. BC)	m/∼50 y	1, 2	>50	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
U 64.x11/H81 (LM: OxA-20762: 6639–6440 cal. BC)	m/∼40 y	20, 26, 27, 29, 30, 31	>100	Round to oval in 2D view/distinctive lenticular shape when rotated	Central with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			<5	3D polyhedral shape	Central with fissures radiating from it	Not visible	Paniceae
U 222.x18 (LM: bottom of the sequence, ∼6700 cal. BC)	?/adult	3	2	Oval in 2D view/ kidney 3D morphology	Central, slightly sunken	Present, barely visible	Fabeae
U 24.x30 (LM-M/N transition)	?/adult	32	No starch				
U 44 (LM-Meso/Neo transition: ∼6700–5900 cal. BC)	?/adult	27	<5	Oval in 2D view/ kidney 3D morphology	Central, slightly sunken	Present, barely visible	Fabeae
			<5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
H53 (M/N transition: OxA-16544: 6006–5838 cal. BC)	f/∼50 y	3, 28, 29	>5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			> 200	Large suboval lumps of starches	Central	Not visible	Avenaea
Lepenski Vir							
8 (EN: AA-58319: 5710–5520 cal. BC; OxA-25207: 5982–5760 cal. BC)	f/∼40 y	44	>5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			<5	Oval in 2D view/ kidney 3D morphology	Central, slightly sunken	Present, barely visible	Fabeae
20 (EN: ∼6000–5700 cal. BC)	f/∼40 y	48	<5	Round to oval in 2D view/distinctive lenticular shape when rotated	Central, with distinctive extinction cross	Present, concentrated around the hilum	Triticeae
			1	Irregular triangular of oval	Central, slightly sunken	Not visible	Unidentified starch granules (see *Supporting Information*)
32a (EN: OxA-5828: 6066–5727 cal. BC)	f/∼50–60 y	42, 43, 36	> 200	3D polyhedral shape	Central with fissures radiating from it	Not visible	Paniceae

high number of B-type grains, and granules are also attached to the calculus matrix (Fig. 3 *A* and *B*). Bimodal distribution is known for Triticeae tribe (e.g., *Triticum* spp., *Hordeum* spp.) (Fig. 4 and Fig. S7), but none of these species had wild progenitors in the Balkans (9, 10). Thanks to the modality of preservation, it was possible to exclude wild species of the Triticeae and Bromideae tribes (known to have bimodal starch granules) that could have been eaten at the time in the region (31, 32) on the basis of their morphology (Figs. S7 *A–H* and S8*A*) as well as most recent literature on phylogenetic evaluation of Poaceae species (33). It was also noticed that recovering good quality seeds and starch granules from some of the mentioned wild wheat species was very difficult. Furthermore, *Aegilops* spp. are absent from assemblages with analyzed macrobotanical remains found at Mesolithic and Early Neolithic sites in the central Balkans (10). Finally, none of the species of the genus *Bromus* growing in the region are bimodal (*SI Results*). Starch granules of the Triticeae tribe with morphologies remarkably similar to those found on Late Mesolithic and Mesolithic–Neolithic phase individuals from Vlasac were found in Early Neolithic individuals from Lepenski Vir (Fig. 3*C*). A further confirmation that archaeological starch granules belong to domesticated species of the Triticeae tribe is provided by the pollen record, as very large grains of Cerealia grasses were recovered in human coprolites from two Late Mesolithic sites in the Danube Gorges (Vlasac and Icoana) and were interpreted as coming from domesticated species (29, 34). At Vlasac, Gramineae pollens of Cerealia type with a diameter no larger than 38.5 μm are dominant in coprolites from lower Mesolithic horizons (1.6%). This diameter is considered the limit between spontaneous and domesticated Cerealia grasses. The quantity of small Cerealia pollens decreases in coprolites from upper Mesolithic horizons, where large Cerealia-type grains with diameters larger than 50 μm (sometimes up to 53 μm) appear (3.5%). Interestingly, in upper Mesolithic horizons, pollen grains of legumes have also been found (29).

Type II. Type II of starch granules was found in the form of intact large suboval aggregates of small compound granules (Fig. 3*D*). Compared with our reference collection and literature (30), both compound granules and the aggregate are consistent in size and shape with those of the species from the Aveneae tribe (oats tribe), both wild (e.g., *Avena sterilis*, *Phalaris minor*) (Fig. S8 *B* and *C*) and domesticated (e.g., *Avena sativa*). Starch granules of the Aveneae tribe were found in the Late Mesolithic samples only, and they could have been either deliberately harvested, exchanged, or ingested as weeds of crops.

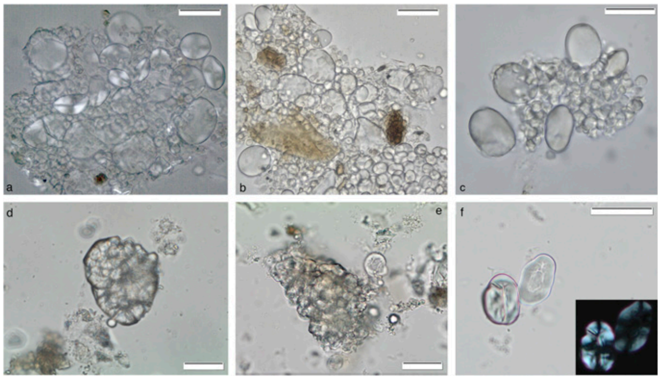

Fig. 3. Starch granules entrapped in archaeological dental calculus: (*A*) starch compound in Late Mesolithic calculus (Vlasac H232); (*B*) bimodal distribution of lenticular starch grains in calculus dated to the Late Mesolithic (Vlasac U64. × 11); (*C*) starch compound in Early Neolithic calculus from Lepenski Vir (Lepenski Vir 20); (*D*) large starch compound found in calculus (Vlasac H53) consistent with Aveneae tribe; (*E*) cluster of polyhedral starch grains with central hilum and fissures associated with the Paniceae tribe (Vlasac U64); (*F*) starch grain entrapped in Mesolithic–Neolithic calculus (Vlasac U44). Note the oval shape, kidney-like shape 3D morphology, and sunken central hilum, which are consistent with genus *Vicia* (Vlasac U44). (Scale bar, 20 μm.)

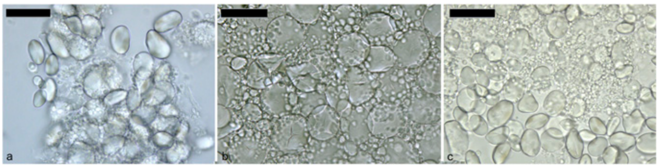

Fig. 4. Experimental starches: (*A*) *Hordeum distichon*; (*B*) *Triticum monococcum*; (*C*) *Triticum dicoccum*. (Scale bar, 20 μm.)

Type III. A third group of starch granules also retrieved in dense clusters, in one case of over 200 granules, is consistent with those of the Paniceae tribe. This type of granule has a 3D polyhedral shape, with central hilum and fissures radiating from it (Fig. 3*E*, Table 1, and Fig. S8 *F–J*). Although an overlap in size and shape exists among the smaller starch granules of the tribe (35), the large number of our starches and their overall morphologies suggest species of the genus *Setaria* spp. as potential candidates (Fig. S8 *F* and *G*). Although several species of the genus *Setaria* are diffused in the central Balkans (e.g., *Setaria viridis*, *Setaria verticillata*, etc.), a secure identification of archaeological starches needs to be taken with caution, whereas identification to tribe level is rather secure. Starch granules of the Paniceae tribe were retrieved from our samples dated to the Mesolithic, Mesolithic–Neolithic transition, and Early Neolithic phases.

Type IV. The fourth type of starch granules is consistent with the Fabaeae tribe (peas and beans) and is remarkably consistent with those found in the genus *Vicia* (vetches) for its small oval 2D shape and its kidney-shape 3D morphology and hilum sunken in a central cleft fissure (Fig. 3*F*, Table 1, and Fig. S8*J*). These were lower in number. Species of vetches can grow as weeds of crops, and sometimes are eaten raw.

Single starch granules were found in a number of samples, but these were low in number and often damaged, and it was difficult to reach a secure identification for them. These starches were considered undiagnostic, as they could belong to groups that could not be identified because they were damaged by processing and chewing.

Finally, a pollen grain, characterized by a spheroidal and pantaporate morphology, ca. 50 μm in diameter, pores between 3 and 5 μm, and spines protruding from the surface, was found embedded in the calculus matrix (Fig. 5*A*) and identified as *Malva* sp. (36). The presence of such pollen in Late Mesolithic calculus could be suggestive of the consumption of honey or its use as herbal medicine. In addition, nondietary evidence in dental calculus is consistent with a variety of animal (e.g., feather barbules) and plant remains (wood debris and plant fibers) (Figs. S9 *C* and *D* and S10), likely to be the result of exposure to dust generated by crafts. Such remains being below 20 μm are interpreted as potential respiratory irritants (37).

The analysis of dentoalveolar pathologies has shown the absence of caries and periapical lesions in examined individuals from Vlasac. However, antemortem loss of two teeth (38 and 48) has been recorded in one case (U64. × 11). Root exposure caries were found in Neolithic individual 32a from Lepenski Vir (teeth 16, 25, and 26) (Fig. 5*B*). Individuals 32a (tooth 17) and 20 (teeth 45 and 46) exhibit antemortem tooth loss. Lastly, three periapical lesions were identified in individual 8 (teeth 12, 21, and 23) from Lepenski Vir.

Discussion and Conclusions

Our evidence suggests that starch granules found in large numbers in dental calculus of both Late Mesolithic and Mesolithic–Neolithic individuals from Vlasac are consistent with tribes of

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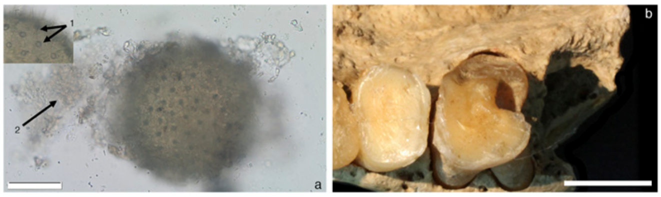

Fig. 5. (*A*) Large spheroidal and pantaporate pollen of *Malva* sp. with spines protruding from the surface (*1*, spines and pores; *2*, remains of ancient calculus) (Vlasac U64); (*B*) caries found in Neolithic individual 32a from Lepenski Vir. (Scale bar: *A*, 20 μm; *B*, 1 cm.)

plants that became key staple domestic foods with the start of the Neolithic in this region. The status of preservation of entire "groups" of starch granules suggests that their presence in the calculus matrix could be the result of inhalation or ingestion during processing rather than ingestion after cooking, a pathway of inclusion of starch granules rarely taken into consideration in other studies (see *Supporting Information*). The data indicate that the earliest domesticated seeds would have reached the Danube Gorges area already by the mid-seventh millennium BC, if not earlier—that is, at least four centuries before what is commonly believed. In Greece, at the site of Franchthi Cave (Fig. 1), three carbonized seeds of *T. dicoccum* from mixed Final Mesolithic/Initial Neolithic aceramic units were directly AMS-dated to between 6750 and 6430 cal. BC (95% confidence), suggesting the presence of domesticated emmer wheat in southern Greece likely before ∼6500 cal. BC (38), which is contemporaneous with the analyzed Mesolithic individuals in the Danube Gorges. Although the dated *T. dicoccum* seeds from Franchthi were tentatively attributed to the Neolithic occupation (38), in the light of our results one may speculate that they relate to forager rather than first farming occupation of the site. These dates are also broadly contemporaneous with the Early Neolithic occupation at Ulucak (level VI) on the Turkish side of the Aegean Sea (Fig. 1) (39). A recent series of dates from the Early Neolithic sites of Dikili Tash, Mavropigi, and Paliambela in Macedonia (Fig. 1) suggest that Neolithic communities settled in the region already by ∼6500 cal. BC (40). In the central Balkans, the earliest date for an Early Neolithic occupation comes from the site of Blagotin (Serbia) (Fig. 1), where OxA-8608 (7480 ± 55) dated a red deer antler to 6440–6230 cal. BC (95% confidence) (41).

It remains difficult to evaluate the importance of carbohydrate foods in the overall subsistence of Late Mesolithic foragers at Vlasac (25), as it is likely that fish was an important staple resource (5). However, antemortem tooth loss documented in some individuals could indicate a deteriorated dental health status due to specific dietary habits (e.g., glucose/fructose consumption derived from plants and honey). The absence of caries during the Mesolithic at Vlasac does not contradict the evidence about the consumption of plants rich in fermentable carbohydrates and could directly be related to high calculus rates documented among Mesolithic individuals at Vlasac, as calculus forms during the process of mineralization whereas caries develops through the process of tooth demineralization (42). The similarities between starch grains present in the calculus of Mesolithic individuals from Vlasac and Early Neolithic individuals from Lepenski Vir provide evidence that Mesolithic foragers of the Danube Gorges must have had acquired domestic grains before farming practices became rooted in the Balkans more permanently ∼6200/6100 cal. BC. This could be similar to the process that led to the spread of domesticated maize through trade networks among forager communities of North America before evidence of its cultivation (43, 44). It has also been argued that domesticated wheat reached Mesolithic Britain already by 6000 cal. BC—that is, 2,000 y before full-scale agricultural practices were adopted (45). In the central Balkans, foragers' familiarity with Cerealia grasses from ∼6500 cal. BC, if not earlier—that is, at least 400 y before their full cultivation in this regional context—might have eased the later quick adoption of agricultural practices. The existence in this region of large sedentary settlements of complex foragers along with numerous grinding stone tools (Fig. S2) is in agreement with our results that processing of plant foods, including domesticate species, might have been common already in the Late Mesolithic. This significantly challenges our hitherto understanding of pre-Neolithic adaptations in southeast Europe and suggests that elements of the "Neolithic package" were transmitted piecemeal and at different times using established networks of interaction between foragers and farmers.

Methods

Calculus sampling took place on aluminum foil with starch-free gloves worn at all times. The average weight for calculus samples was 0.983 g. Once removed, samples were sealed into sterile Eppendorf tubes. In the laboratory, samples underwent specifically developed decontamination procedures (46) (*SI Methods*). Extraction and mounting of the microfossils entombed in the calculus matrix involved a weak solution 0.06 N of HCl (*SI Methods*). A solution of 50:50 glycerol and ultrapure water was used to allow for the rotation of microfossils. Examination of microfossils was carried out using Zeiss and Olympus compound polarized microscopes (100–630×) at the University of York and a Leica DM2500 polarized microscope (100–1,000×) at the University of Cambridge. Starch granules were identified on the basis of their 3D morphology, presence, and shapes of features (lamellae, hilum, bumps, and depressions of their surface), characteristics of the extinction cross under polarized light microscopy. A large collection of microremains from modern plants native to the central Balkans and the Mediterranean region collected at the Botanical Garden of Belgrade and stored at the University of Cambridge was used as an experimental reference. A collection of plants of north European, Mediterranean, and north African origin as well as nondietary items (e.g., plants used for crafts and occupational dust such as wood and pottery) hosted by the University of York was also consulted and successfully used before in other studies (35, 46). Fresh and dry botanical samples were ground in agate mortar using distilled water. Different modalities of grinding were applied to record level of damage and/or change in the size of granules. Ground plant material has also been left in water to understand the swelling process. Our reference collection also comprised experimentally processed seeds (e.g., boiled, ground, chewed) (*SI Materials*). Criteria of identification that are current standards in the field of modern and ancient starch granules research (47) were followed.

Caries and periapical lesions have been recorded macroscopically and using a magnifying lens. Visibility location and size of caries were described following the methodology proposed by Hillson (42). Both caries and periapical lesions were recorded at the individual tooth level (42, 48) and the Fédération Dentaire Internationale system was used for tooth numbering (49).

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