

## The Genomic History of Southeastern Europe

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*Supplementary Information*

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- 2. Diversity and phenotypically informative markers in hunter-gatherer populations (pages 51-55)**
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## Supplementary Note 1: Archaeological and osteological context of newly reported individuals.

This supplement provides archaeological details for individuals that have genome-wide ancient DNA data reported for the first time in this study. They are organized first by present-day country of origin, and then by site. We also provide a brief note describing the Danube Gorges region and approximate chronologies for the Balkan Peninsula and Ukraine.

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## Austria

### Kleinhadersdorf Flur Marchleiten (2 individuals)

The graveyard at Kleinhadersdorf Flur Marchleiten, in Lower Austria, represents the largest burial site of the Early Linear Pottery Culture in Austria. There, systematic excavations were carried out in 1931 and between 1987 and 1991 and human skeletal remains of a total of 62 individuals (buried in inhumation and cremation graves) were recovered. Burial rituals include the deposition of the dead body in a crouched position and – in some cases – the application of red ochre. New radiocarbon dates yielded dates between 5220 and 4980 calBCE. It is striking that the frequency of unspecific skeletal stress indicators seems to be lower in the Kleinhadersdorf than the Schletz/Asparn group.<sup>1</sup>

- 15068 / grave 40 (Inventory no. NHM 25.929)

Male, ca. 25-35 years; well-equipped grave; fragmented cranium and mandible, 29 teeth, portions of the long bones (mostly shafts), a few small fragments of ribs, scapulae, and hand bones; lower extremities poorly preserved. Diagnostic findings: porous hyperostosis at the occipital bone, linear enamel hypoplastic lesions, caries, the two maxillary third molars are microdontic.

- 15069 / grave 55 (Inventory no. NHM 25.936)

Female, ca. 30-50 years; fragmented cranial and facial bones, 33 teeth, fragments of the postcranium belonging to clavicles, ribs, scapulae, pelvic bones, all long bones (with destroyed epiphyses), phalanges. Diagnostic findings: porous hyperostosis at the occipital bone, oval impression at the right parietal tuber (atrophy?), linear enamel hypoplastic lesions.

### Asparn (6 individuals)

The Early Neolithic (Linear Pottery Culture, final phase of “Notenkopfkeramik”) site of Asparn/Schletz, Lower Austria, is a settlement enclosed by an oval and trapezoid ditch system, interpreted as fortification constructions. Archaeological investigations between 1983 and 2005 revealed human skeletal remains of more than ~80 individuals at the base of the external ditch of the oval enclosure. Radiocarbon dating provided dates between 5210 and 4950 calBCE. The remains were found in atypical postures, mostly incomplete, and exhibit both peri- and post-mortem changes. All individuals where skull remains are preserved (n=33) are characterized by traumatic lesions in form of bending or bursting fractures. The atypical situation of the remains, the lack of young females, the injuries, and the post-mortem alterations by animal gnawing suggests that the entire population of this early farming settlement was extinguished – probably in the context of a European-wide crisis.<sup>2</sup>

- 15204 / Ind. 14/2

Juvenile, ~18-20 years; cranial fragments, isolated upper and lower jaw, 22 permanent teeth; portions of the postcranial remains (pelvic bone, right clavicle, left humerus shaft, left femur and right tibia and fibula, isolated metatarsal bone). Diagnostic findings: porotic hyperostosis at the parietal bones, porous palate, linear enamel hypoplastic lesions, persisting frontal suture, peri-mortem fractures and bite marks at pelvic and long bones.

- 15206 / Ind. 23

Infant ~1.5 years (18±6 month); represented by a few remains of the cranium and the mandible, 6 teeth; fragments of the postcranium (right clavicle, both humeri, fragments of radiuses and ulnas, remains of the hand skeleton, vertebral bodies and arches, fragments of



the pelvic bones, femurs and the left fibula). Diagnostic findings: porous structures at the external cranial layer and peri-mortal skull fractures.

- I5070 / Ind. 58

Infant ~4 years represented by several cranial fragments, isolated upper jaw and mandible, 15 deciduous teeth, portions of the postcranial skeleton (right and left clavicle, right femur, tibia and fibula); diagnostic findings: cribra orbitalia, vitamin C deficiency symptoms, peri-mortal cranial fractures and arrowhead injury.

- I5205 / Ind. 52

Infant ~5 years represented by cranial fragments, isolated upper jaw, 9 deciduous teeth, axis, fragments of the pelvis; diagnostic findings: slightly developed cribra orbitalia, peri-mortal cranial fractures.

- I5207 / Ind. 22

Infant ~ 6 years represented by cranial and facial bones, 20 deciduous teeth and 4 permanent tooth crowns of the first molars; diagnostic findings: punctiform enamel hypoplasias at the canines, slight cribra orbitalia, periostitic reactions at the mandible, peri-mortal cranial fractures.

- I5208 / Ind. 12

Infant ~4 years represented by cranial and facial bones (left parietal and occipital bones, fragments of frontal bone, upper and lower jaw, 14 deciduous teeth and 3 permanent tooth germs); fragments of postcranial remains (right clavicle, incomplete first and second cervical vertebrae, pelvic fragments); diagnostic findings: newly bone structures at the right sigmoid sinus, cribra orbitalia, porous alveolar margins and the palate, peri-mortal cranial fractures.

## Bulgaria

### Beli Breyag (2 individuals)

The Beli Breyag site (Radnevo region, South-East Bulgaria) is part of an EBA barrow necropolis consisting of at least 5 barrows. Barrow 5 was investigated in 2015.<sup>3</sup> Part of it had been destroyed before the excavations. Three features were discovered in the remaining section; feature 1 – interpreted as a symbolic grave, features 2/1 and 2/2 – two graves placed one above the other and feature 3 – double grave containing the two sampled individuals. The primary feature is 2/2 and the rest of the graves are secondary.

- Bul6 / Barrow 5, Structure 3, Individual 1  
Male 35-45 years, ~63kg. Osteochondrosis in the lumbar vertebrae of the spine.

- Bul8 / Barrow 5, Structure 3, Individual 2  
Male 50-55 yrs. ~67kg.

### Dzhulyunitsa (8 individuals)

The Early Neolithic settlement of Dzhulyunitsa-Smardesh is located in north central Bulgaria, near the slanting northern slopes of the Pre-Balkan, where it is conterminal with the Danubian plain. This area is part of the Middle Yantra river valley which belongs to the Lower Danube catchment. The site is situated on the first unflooded terrace, in a field called Smardesh, at an altitude of between 70 and 77 m. It is 4 km south of the current location of the Yantra and 2-3 km west of its tributaries - the rivers Stara and Zlatarishka. The Early Neolithic site occupies

approximately 10 hectares, decreasing in its final phase to ~0.5 ha.<sup>4,5</sup> Dzhulyunitsa-Smardesh has been excavated from 2001 up to present, revealing that the terrace was inhabited through all periods. Graves were unearthed dating to the Late Iron Age, Early Bronze Age, Late Chalcolithic and Early Neolithic. The Late Chalcolithic graves probably belong to a necropolis situated SE of the Chalcolithic settlement.

- I2509 / G2 (Bronze Age, grave 1)

Adult female. It is located in trench 1, at the bottom of a contemporaneous ditch. The skeleton is not in an anatomical position, its bones scattered over an area of approximately 4 m<sup>2</sup>.

- I2520 / G5: (Bronze Age, grave 5)

Sub-adult male. The rectangular grave has been damaged by a later pit as only the upper half of the skeleton is preserved. The individual is in flexed position on the right side, the head orientated to the East facing North. The grave goods include a necklace of *Spondylus* beads, a silver hair-ring and 2 ceramic vessels.

- I2510 / No1 (Chalcolithic, grave 2 (1))

Sub-adult male. Excavated from trench 8, exactly underneath the top level (0.30 m), it is disrupted by an Early Bronze age pit. Only the head, several bones of both hands and the upper ribs are preserved.

- I2519 / G4: (Chalcolithic, grave 4)

Juvenile female. This grave was found in trench 17, in an occupational layer (at the depth of 1.15 m). The body was laid in a rectangular pit, orientated SE-NW with its head in the SE direction facing NE.

- I0704 / DZHU7 (Neolithic, grave 3 (1))

Female aged between 10-13 years. She was found inside a pit-house, close to the hearth. The body was laid in a flexed position on the right side. Her head is leaning on the shoulder and slightly bent to the chest. The skeleton is orientated SE-NW, the facial bones to NE. She was lying on her right side; her left leg stretched on her right arm, the left arm was on her belly. Her left and right mandibular canine teeth exhibited linear enamel hypoplasia. This lesion is often associated with disease and/or poor nutrition. A moderate level of calculus formation was observed.

- I0706 / Dzhu10 (Neolithic, grave 6)

A disturbed grave structure was uncovered in trench 19, marked as 7 (6). It was excavated at the depth of 1.45 m and belongs to the Dzhulyunitsa II occupational level. The structure had 3 succeeding layers. The first one contained a compact cluster of stones, ceramic sherds and animal bones. The next revealed a concentration of animal and human bones, while the last layer included a ceramic vessel, several animal bones and a human mandible. Scattered skull and long bone fragments of a middle- to old-aged adult were found. Genetically confirmed to be male.

- I2521 / No 7 (Neolithic, grave 7 (8))

A juvenile male was unearthed in trench 23, at a depth of 1.85 m in the sterile ground. This trench contains a human skull with a missing mandible and 2 phalanges. The head is laid on the right side, facing SE. The grave was probably damaged by a pit dug during the final stages of the Neolithic.

- I5769 / No 8 (Iron Age, grave 9)

Sub-adult female. This Iron Age burial was found in an oval pit in sq. 3611. The skeleton in flexed position and is turned to the east. Orientation of the body is SE-NW, with the head to SE. The grave inventory consists of ornamental beads and 21 metal (probably copper) ornaments smaller than 5 mm.

**Ivanovo (1 individual)**

This tell is relatively small - 6.12 m high and 80 by 77 m in diameter. It is dated to the Copper Age.

- I2431 / 41

Male. The sample is a tooth taken from a mandible found in the central part of the settlement - square L11 at depth of 1,73 m - horizon V associated with pottery of the Polyanitsa III style.

**Krepost (1 individual)**

This structure, dated to the Early Neolithic Period, is dug into the ground to a maximum depth of 1.10 m. The pit has an irregular plan with an area of roughly 50 m<sup>2</sup>. Numerous ceramic remains were found within the pit; most are dated to the Karanovo II period.

- I0679\_d / kre1. Fragmentary remains. Female, estimated age 35-45 years based on dental wear patterns. Initial analysis suggested possible contamination in this sample, so we restricted all analysis to reads that showed ancient DNA damage (i.e. C->T in the last base of the read).

**Malak Preslavets (10 individuals)**

Genetic data were recovered from 10 skeletons from Malak Preslavets.

Sample ID.	Skeletal code	Burial	Anthropological interpretation			Genetic sex
			Element	Age	Sex	
I0700	MP5 / MP8	13	L femur	Adult	M	M
I1108	MP1	4	Tibia	Juvenile		M
I1109	MP10	15	L humerus	Adolescent, 14+		F
I1113	MP3	7	L femur	Adolescent, c. 20		F
I1295	MP13	18	L tibia	Adult	M?	M
I1296	MP11	16	R tibia	Adult	F?	M
I1297	MP17	D10	R humerus	Adult?		F
I2215	MP9	14	L maxilla	Child, 4-5		?
I2216	MP15	C09.A	L femur	Adult	F?	F
I3879	MP6	10	Tibia	Child		M

**Table S1.1:** Summary of genetic data from Malak Preslavets

The site of Malak Preslavets lies on the Northeast shore of Lake Malak Preslavets, less than 200 meters from the right bank of the River Danube. It was partially excavated in 1985–1986, the unexcavated portion of the site having since become submerged.<sup>6</sup> The pottery and bone artifact assemblages, for the most part, are characteristic of the Criş culture, although some ceramic vessel types of the ‘Middle’ Neolithic Dudeşti culture were also identified.

Nineteen human burials were found on the edge of the settlement, and included both adults and children.<sup>7</sup> The dominant burial position was crouched or flexed on the right or left side, which is characteristic of the Early-Middle Neolithic in the Lower Danube Valley and across much of Southeast Europe.

Published accounts of the excavation provide very little information about the subsistence base of the Neolithic occupation. There is mention of carbonized seeds from cultivated and wild plants (recovered by flotation), mainly from the settlement area. A few animal bones and “large amounts of shells” of freshwater mussels were also found in some of the burials.<sup>6</sup> Presumably, faunal remains were also recovered from the settlement area, but few data (other than information about the presence of bone tools) are available.

- I0700 / MP5,MP8 burial 13

Articulated lower extremities of an adult male from a disturbed grave. Judging by their position, it seems that it was a flexed burial on the right side. A cattle bone was discovered next to the feet; it is, however, not clear whether it was related to the burial.

- I1108 / MP1 burial 4

This is a fully preserved primary inhumation of a juvenile buried crouched on the right side, with head to west. Freshwater mussel shells were recovered from the fill.

I1108 / MP1 and I0700 / MP5,MP8 8 are first-degree relatives, most likely brothers (since they share mitochondrial and Y chromosome haplogroups) or, possibly, a father and son.

- I1109 / MP10 burial 15

Bones of the upper extremities of a young female. They were partially articulated which seems to suggest that this was a disturbed primary inhumation.

- I1113 / MP3 burial 7

A twenty-year-old female buried crouched on the left side, with head to south/southwest. Legs pulled up, hands in front of the face.

- I1295 / MP13 burial 18

Skull and separate bones of the extremities and the body of an adult male (30-35 yrs).

- I1296 / MP11 burial 16

Left tibia and fibula of an adult male; possibly disturbed inhumation.

- I1297 / MP17 burial D10

- I2215 / MP9 burial 6,14

This context was interpreted by the excavator as two separate burials and therefore labeled with two different numbers. It is actually a double secondary burial containing the skulls of two four/five-year-old children that could have originally been standing upright but one of them had later fallen laterally. One of the skulls was missing the mandible and the left half of the maxilla. It was laid upon a cattle bone; a cattle skull was lying between both child skulls. The fill contained some clamshells.

- I2216 / MP15 burial C09.A

- I3879 / MP6 burial 10

Long bones of an eight-year-old boy found immediately to the north (next to the feet) of burial 7.

### Dating of Malak Preslavets individuals

Human remains from Mesolithic and Neolithic sites located along the Lower Danube frequently produce <sup>14</sup>C dates that are anomalously old because of a “freshwater reservoir effect” (FRE), linked to the inclusion of fish and other aquatic resources in diet. A FRE of up to 540 yr has been recorded in Mesolithic humans from sites in the Iron Gates reach of the

Danube Valley.<sup>8</sup> Research in other European river systems has shown that freshwater reservoirs can vary over time as well as within river catchments.<sup>9</sup> Accurate <sup>14</sup>C dating of human bone therefore requires knowing the order of magnitude of the local reservoir effect. The likelihood of a FRE at Malak Preslavets is indicated by its proximity to the considerable fish resources of the Danube, the presence of broken harpoon heads among the archaeological remains from the site, and the association of freshwater mussel shells with some of the burials. Research to establish the magnitude of the FRE in this part of the Danube is in progress. Pending the outcome, our best estimate of the date of the burials at Malak Preslavets is *c.* 5800–5400 cal BCE, based on the “developed” character of the Criş culture ceramic assemblage from the site<sup>10</sup> and the presence of a few vessel forms reminiscent of the Middle Neolithic Dudeşti culture.<sup>6</sup>



**Figure S1.1:** Five of the Malak Preslavets burials sampled. Clockwise from top right: Plan of the site, burials 15, 4, 7, 6/14, 13. Photos courtesy of the National Archaeological Institute, Sofia, Bulgaria.

#### Mednikarovo (1 individual)

The Mednikarovo necropolis (Radnevo region, South-East Bulgaria) consists of 6 EBA barrows excavated in 1992–1997. Barrow No.2 had dimensions of 28.0 x 26.30 m, 1.2 m high. The primary grave (No. 1) contained a supine inhumation with flexed legs, arms alongside the body, with red ochre over and around the skull.<sup>11</sup>

- Bul4 / Mednikarovo, Barrow 2 grave 1  
Adult male.

#### Merichleri – Kairyaka Necropolis (2 individuals)

Merichleri village (Haskovo province) was known historically for the discovery of Greek and Roman tombs. Recently, seven graves from the Early and Middle Bronze Age period were discovered in excavations carried out in a tumulus (burial mound I, excavated in 2012, <http://fix.haskovomuseum.com>). The site is on a hill (altitude 210 m) on the west bank of the Maritsa River, 3 km south from Merichler. The mound is 2 m high, 32 m in diameter, spans three separate periods, and contains seven burials.



- I2163 / Merich 2 (Individual 5)

Adult male, found on the periphery of the second heap, buried in a shallow pit. Positioned with the head to North, legs bent at the knees and holding a small cup in its right arm.

- I2165 / Merich 4 (Individual 6)

Adult male at the center of the first heap just below Individual 4 (a child, buried with the head to west and legs bent at the knees). These two individuals are buried in a small pit, under the level of the ancient terrain. Individual 6 is buried with the head to the East and legs bent at the knees. Near its right arm were found a small askoi and a stone ball. Traces of red ochre were found on the skull.

### Ohoden (1 individual)

Excavations at the Early Neolithic settlement of Ohoden-Valoga (province of Vratsa) were begun in 2002 under the Vratsa Regional Museum of History and continue today. The settlement is situated on the alluvial terrace of a small stream. Next to remains of dwelling structures, five human burials have been unearthed, among them adults of both sexes, one sub-adult and two children.<sup>12</sup>

- I1298 / OH-00 (grave 5)

A female infant ca. 1 year old ( $\pm 4$  months; dental age estimation).

### Sabrano (1 individual)

A small part of the site of Sabrano (Nova Zagora region, South-East Bulgaria) was investigated in 2009 during rescue excavations related to the “Trakia” motorway construction (Site 12). This revealed Late Neolithic (late 6<sup>th</sup> – early 5<sup>th</sup> millennium BCE) pits, an EBA inhumation grave and an EBA ditch as well as 1<sup>st</sup> millennium BCE pits. The EBA grave contained 4 individuals buried in extended position (two adult – 1A and 1B, and two infants – 1C and 1D). The grave inventory consisted of 7 vessels.<sup>13,14</sup> We thank the directors of the excavations - Dr. Anelia Bozkova and Dr. Zhivko Uzunov – for kindly giving us access to this sample.

- Bul10 Sabrano, Grave 1, Individual 1d

Infant ~7 yrs. Linear enamel hypoplasia on the upper left incisor (i2) between 1 and 2 years.

### Samovodene (1 individual)

Excavations at Samovodene Tell, Yantra river basin (Veliko Tarnovo province) were carried out in the 1970’s and 80’s by Peter Stanev. The archaeological deposits in which human skeletons were found date to the transition from Early to Late Neolithic.<sup>14</sup>

- I2526 / S2a

Adult female.

### Smyadovo (6 individuals)

The cemetery is associated with a Copper Age tell located 200 m to the Southeast. 32 burials have been excavated containing 37 individuals.

- I2175 / 10 Burial 20A
- I2176 / 12 Burial 20B

Burials 20A and 20B were found in the same grave-pit, which contained five individuals in total. Four of them (skeletons 20A-D) were laid extended on their backs, next to each other, East-West oriented with heads to the East (81-110°). The fifth skeleton (20E) was laid in the western part of the grave pit; it was also East-West oriented, but its head pointed to the West (278°). The bones of their lower limbs were found disarticulated in the southwestern part of the burial pit. The four individuals were buried in couples, i.e. two by two, with each pair laid hugging and facing each other: skeletons A-C belonged to individuals about 25 years old, D about 30 years old. The deceased were relatively tall: A, B and C measured 1.71, 1.68, and 1.80 m respectively. A and B are both male. Grave goods include ceramic bowls, silver hair-rings (one per individual), a silver and *Dentalium* shell necklace, as well as flint and bronze artefacts, and lumps of ochre and red sandstone.

- I2181 / 21 Burial 29

Skeleton in flexed position on the left side. Orientated to the East - 110°. The arms were bent, with the palms in front of the face, hips close to the chest and shins were touching the hips. The length of the skeleton *in situ* is 0.76 m, the femur is 0.45 m long. Anthropological determination: male ~25 years old. Grave good included a flint artefact, two ceramic vessels and beads of serpentine, bone and *Spondylus*.

- I2423 / 23 Burial 28

Skeleton in flexed position on the right side. Orientated to the West - 280°. The length of the skeleton *in situ* is 0.74 m to the pelvis; the femur is 0.43 m long. The arms are bent at the elbows, in front of the face. The legs are bent at a sharp angle. In front of the skull are the phalanges of the hands. Anthropological determination was male ~25-30 years, but the individual is genetically female. The long bones are solid with strong prehensile muscle relief. Grave good include flint and bone artefacts, and 2 ceramic vessels.

- I2424 / 26 Burial 31

Skeleton in flexed position on the left side. Orientated to the East - 100°. Hands are strongly bent at the elbows and adjoined to the chest; the left femur is almost at a right angle to the torso; shins are bent at the knees at about 30°. The length of the skeleton *in situ* is 1.01 m and the femur is 0.41 m long. Anthropological determination; male ~25-30 years, but the individual is genetically female. Long bones are massive, with well-developed muscular prehensile relief. Grave goods included a bone tool, *Spondylus* beads, 7 ceramic vessels and some grain.

- I2430 / 40 Burial 28

Skeleton in flexed position on the left side. Orientated to the Southeast - 130°, the facial bones are facing southwest. Bones are highly fragmented. Lower and upper limbs are strongly bent. Phalanges of the right hand are behind the occipital. Anthropological determination; male ~25 years. Length of the skeleton is 0.81 m; length of the pelvis 0.84 m, humerus length - 0.30 m and the femur - 0.41 m. Fragments of the long bones are very heavy, with thick *compacta* and very strong relief of the prehensile sites. Grave goods include a stone axe, a copper wedge, 3 ceramic vessels and 2 *Spondylus* beads.

### Sushina (3 individuals)

The cemetery is associated with a Copper Age tell located 100-120 m East. Prolonged drought in 2007 lowered the level of the water behind the Ticha Dam more than 10 m, exposing what is believed to be the periphery of the cemetery. Eleven graves have been excavated (5 children and 6 adults), all of which contain pieces of red ochre in addition to the

grave goods. A specific feature is the stone 'lining' under the bodies - either slabs or scattered pebbles.

- I2425 / 28 Grave No. 1

Crouched on the left side, orientation is to the East (85°); the facial bones are turned to the South. Anthropological determination; male ~20-25 years. The length of the skeleton is 1.07 m, the femur is 0.40 m. Poorly preserved: the skull is heavily fragmented, the ribs and the mandible are missing. The hands are strongly folded on the chest and the fingers are in front of the mouth. Legs are bent at the knees. Grave goods include a ceramic vessel.

- I2426 / 29 Grave No. 2

Crouched on the left side, orientation is to the North (355°); the facial bones are turned to the East. Anthropological determination; male ~20-25 years. The length is 1.23 m, the length of the femur is 0.44 m. The hands are folded, the palms under the head. The legs are bent at the knees. There are post-mortem trepanations on the skull. Grave good include a flint tool and an antler hoe.

- I2427 / 32 Grave No. 11

Crouched on the left side, orientation is to the East (110°). Anthropological determination; female ~20-25 years. The length is 1.10 m, the length of the femur is 0.36 m. The hands are folded in front of the chest, the fingers are in front of the face. The legs are strongly bent at the knees and tightly packed to the pelvis. Grave good include flint and bone tools.



**Figure S1.2:** Graves of the three individuals reported here.

### Varna I (5 individuals)

This Copper Age cemetery is situated at the western Black Sea coast in the western industrial zone of the Bulgarian harbor city on a slope some 200 m North of Varna Lake. The site was discovered by chance in 1972 when a cable trench was dug in an empty area between two factories. 7500m<sup>2</sup> was subsequently excavated by the Regional Historical Museum of Varna under the direction of Ivan Ivanov well into the 1990s<sup>15,16</sup>. Culturally, the burial site belongs to the Late Chalcolithic Varna group of the Karanovo VI–Gumelnița–Kodžadermen complex (KGK VI) which extended from the Danube delta to the northern edge of the Rhodope Mountains in the mid- and late 5<sup>th</sup> millennium BCE. Thorough archaeologically modeled radiocarbon analysis dates the timespan of burials to 4600 to 4300 cal BCE.<sup>17,18</sup> A small group of three graves at another cemetery – Varna II located some few kilometers to the West of Varna I – is considered to be the immediate predecessor<sup>19</sup>. Varna I is significant because of the copious grave goods and its early date. Together with a few exceptional finds in settlements and the grave group of Varna II, it constitutes the earliest known burials with gold objects and heavy copper tools in the world. The unequal distribution of the objects among



the 329 burials, symbolic graves and depositions at Varna I suggest that some Copper Age communities on the Western Black Sea coastline were already highly socially differentiated.



**Figure S1.3:** Graves of the five individuals reported here. Clockwise from left; graves 43, 117, 127, 44 and 158. Photos courtesy of the Varna Regional Museum of History.

- ANI152 / VAR43

Supine inhumation of a mature-senile male (50-65 years). The grave is extraordinarily richly furnished with seven heavy copper implements, more than 1000 single gold items, jewelry made out of the shells of *Spondylus* and imported minerals, and highly sophisticated flint tools. The social interpretation of VAR43 is mainly based on the rich grave goods and only in small part based on the so far reported anthropological data. Muscle marks on the skeleton show that the bones were exposed to great physical stress until shortly before his death. The strong muscles of his lower arm even suggest continuous work. The bones from VAR43 show arthritis on the cervical spine, the hands, and the feet. The left hip and especially the left knee were also affected. A squatting facet on the left tibia of the individual can be seen as an indication for preferentially sitting in squatting position, which hints at a working position. The presence of calculus on the teeth points to a diet containing protein. However, there is no evidence of cavities or so-called enamel hypoplasia, which is typically seen as an indicator of stress during childhood development and could point to periods of malnutrition. This implies a continuously good diet and could in itself be taken as a sign of higher social status of the individual. Due to its outstandingly rich inventory, the grave shows strong interactions in a social network analysis with many others in the course of the chronological development of the cemetery. Within this network the strongest relations are given to some symbolic graves at the very end of the development of Varna I, which is confirmed by positioning of VAR43 into the 6<sup>th</sup> phase of the cemetery in the correspondence analysis.

- ANI153 / VAR44

Partially destroyed probably supine burial of a young male (13+ years). No grave goods reported. Because of the missing inventory, the relative chronological position of the grave could not be determined.

- ANI159-ANI181 / VAR117-I

In Ivanov's field catalogue, one single individual is listed buried in a crouched position to the left with the thorax turned towards the bottom of the grave. Later investigation of the field documentation and the skeletal material attributed to this grave in the museum of Varna yielded bones of two individuals: one expected to be of an adult male (35-55 yrs.) and probably a younger woman (20+ yrs.). The genetic data comes from a male, and therefore likely the first individual. The reported grave goods include several ceramic vessels, jewelry made of *Spondylus* and mineral beads, an antler tool, as well as a fragment of a small copper lamella and a fragmented flint tool. According to statistical analysis of its grave goods, the grave falls into the 3<sup>rd</sup> phase of the cemetery.

- ANI160 / VAR127

Supine inhumation of an adult male (25-35 years). The grave goods are comparatively poor with only one flint blade and a stone adze. Even though poorly furnished statistical analysis puts the grave as well into the 3<sup>rd</sup> phase of the cemetery.

- ANI163 / VAR158

Burial of a female child (5.5-6.5 years) in crouched position on its right side. The grave is comparatively richly furnished with ceramic vessels and large amounts of jewelry made of *Spondylus* and beads of various minerals. This grave is typical of the earliest burials in the course of the development of Varna I and falls into its 1<sup>st</sup> phase.

### Yabalkovo (2 individuals)

Yabalkovo is a Neolithic settlement on the right bank of the Maritsa river valley of Upper Thrace in southeastern Bulgaria and is one of the largest prehistoric sites of the eastern Balkans. Inhabited at the turn of the 7th to the 6th millennium, it is characterized by its considerable surface area of more than two hectares and by a system of deep ditches surrounding the settlement. Yabalkovo's material culture closely fits with the early Karanavo tradition.

During the excavations running from 2004 to 2011 the remains of 9 individuals including 7 adults and 2 children were unearthed in the Early Neolithic layers of the settlement. The Yabalkovo burials can be interpreted as belonging to the category of primary interments.<sup>20</sup> Both burials number 2 and 4 had similar positions in the upper layers, analogous in composition. The stratigraphic and contextual analyses indicate that they are contemporary and that the burials immediately follow the destruction of the first EN settlement.

- I0698 / Yaba2

This grave was found in Pit 3, sq. I<sub>37</sub>, Sector North, which was dug in burnt occupation debris. The bones were darkened probably through the effect of the ashy pit fill, but showed no traces of burning. The skeleton of an adult male was unearthed in the upper layer consisting of compact burnt daub pieces. It was in contracted position, orientated NE-SW. His estimated age at death was between 33–45 years. No finds are associated with the skeleton.

- I2529 / Yaba4

The burial was unearthed in the central part of ditch B1. There is no indication of specially prepared burial facility or any inventory. Skeleton number 4 was from a middle-aged male (confirmed genetically) buried in an unusual position (Fig. S1.2). He was lying on his back oriented W-E with the legs stretched in an almost right angle to the right and the arms bent along either side of his head with the hands clenched to fists. The legs and feet rested one on the other implying that they were originally bound together. This was one of the two well-preserved (male) skeletons discovered so far in the settlement. The unusual body posture



demonstrates that this was not a regular burial. The left side of his frontal bone exhibited a very serious injury resulting in the detachment of a part of the cranium. This was a sharply edged cut mark, probably made with an axe or a somewhat similar object. The cut mark fits very well with the working edge of middle-sized axes from the settlement. Whatever may have happened to bring about this injury, the corpse of this man was dumped in the ditch and left without the usual burial rituals.



**Figure S1.4:** *Yabalkovo burial 4*

#### Yunatsite (2 individuals)

Tell Yunatsite is located in the Thracian Plain, 8 km West of the town of Pazardzhik. The mound itself is situated on a lower river terrace and is 12 m high and 100-110 m in diameter. Archaeological excavation began with a first trench in 1939, continued in 1976 (Archaeological Institute with Museum, Sofia) and revealed Medieval, Roman and Iron Age finds overlaying a thick Early Bronze Age (EBA) layer. The latter with up to 17 building levels dating to the EBA phases I-III was excavated between 1983-1990 and continued until 2011 in collaboration with Russian and Greek teams to reveal the underlying Chalcolithic settlement. The Chalcolithic houses were found underneath a layer of accumulated fine soils, which indicate a longer break in the occupation of the site (up to 1250 years based on radiocarbon dating) between the Late Chalcolithic and the EBA. The skeletons we studied were found in the house debris of the last Chalcolithic building level (LC I), which had been set on fire and destroyed, which suggests that these individuals had died violent deaths. Pottery from this layer is associated with the Krivodol culture, and that of the preceding layer is typical for phase III of Karanovo VI. We studied nine individuals in total, for which we report haplogroups from complete mitochondrial genomes, but only two individuals passed the quality control criteria for subsequent 1240k capture of autosomal SNPs. These two individuals were also radiocarbon-dated, and their dates match those of other finds from the same Chalcolithic layer.<sup>21</sup>

- I0781 / ACAD15601A; Mitochondrial haplogroup K1a9'10'13'14'15'16'26  
Skeleton 78: female, mature [40-50 years]

- I0785 / ACAD 15610A; Mitochondrial haplogroup H7  
Skeleton 99: female, senile [*~ 70 years*]

Seven individuals without genome-wide data but including mitochondrial haplotypes:

- I0779 / ACAD15595A; Mitochondrial haplogroup HV6'17  
Skeleton 24: male, mature [*45-55 years*].
- I0780 / ACAD15597A; Mitochondrial haplogroup W1  
Skeleton 68: female, adult [*25-35 years*]
- I0782 / ACAD15602A; Mitochondrial haplogroup K1a9'10'13'14'15'16'26  
Skeleton 83: female, adult [*~25 years*]
- I0783 / ACAD 15604A; Mitochondrial haplogroup H\*  
Skeleton 87: male, mature [*40-50 years*].
- I0784 / ACAD 15607A; Mitochondrial haplogroup U8b1b1  
Skeleton 96: male, mature [*50-60 years*].
- I0787 / ACAD 15612A; Mitochondrial haplogroup W5  
Skeleton 103: male, adult [*20-30 years*]
- I0788 / ACAD 15613A; Mitochondrial haplogroup H5  
Skeleton 106: male? adult / mature?

## Croatia

### Beli Manastir-Popova Zemlja (4 individuals)

The site is located approximately 2 km West of the town of Beli Manastir in Osijek-Baranja County in eastern Croatia. The rescue excavations took place in 2014 and 2015 and covered a surface of approximately 37,000 square meters. Two main cultural layers were identified at the site: a prehistoric layer consisting of several Neolithic and Chalcolithic strata, and a Roman layer in which two rectangular brick furnaces were unearthed.

The prehistoric layers of interest are dated to the early and middle Neolithic periods (Starčevo and Sopot cultures) in which the remains of a large settlement (28 dwelling pits in total, each over 100 square meters large) and 39 inhumation burials were found. A total of 21 of the prehistoric burials were found within the dwelling pits - they were located at the bottom of the pits or at the top of their backfills. The rest of the burials were found at the bottom of waste pits or at the bottom of a large canal at the eastern side of the settlement. Most of the Neolithic burials from the site were found in a contracted position on either left or right side with different orientations. In several cases, one or more ceramic vessels were placed by the head of the deceased.

- I3499 / LP13.4=GEN72 Grave number 17  
A 25-30 year old male, found in S-N orientation with the head oriented to the South. The body was in a contracted position lying on its belly / left side. The skeleton exhibits a well-healed ante-mortem depression fracture on the posterior part of his left parietal bone. He also has mild, healed ectocranial porosity on the parietals and occipital bone, healed cribra orbitalia on his superior orbits, and mild, healed periostitis on the right tibia and fibula.

- I3498 / LP13.3=GEN71 Grave number 15

A partially preserved skeleton of an older adult (50-60 years) male in a NE-SW with the head oriented to the east. The skeleton was lying on its back with its right arm pointing away from the body. The skeleton exhibits mild, healed ectocranial porosity on the parietals and occipital bone, mild, healed periostitis on the right femur, tibia and fibula, mild osteoarthritis on the left temporomandibular joint and severe osteoarthritis on the third and fourth cervical vertebrae as well as a pronounced squatting facet on his distal right tibia.

- I4167 / GEN\_69 Grave number 7

An 18-20 year old male in a NE-SW orientation with the head oriented to the north. The skeleton was in a contracted position lying on its left side. A small biconical vessel was placed above the head, and several pottery fragments were placed on each side of the body.

- I4168 / GEN\_70 Grave number 14

A 45-50 year old female in a SE-NW with the head oriented to the east. The skeleton was in a contracted position lying on its back. The skeleton exhibits 3 antemortem fractures, all of which are well-healed compression fractures on the: 9<sup>th</sup> and 12<sup>th</sup> thoracic, and on the 3<sup>rd</sup> lumbar vertebrae. She also has healed cribra orbitalia on her superior orbits and mild bilateral osteoarthritis on her knees.

### Jazinka Cave (1 individual)

Jazinka Cave is located within the boundaries of the Krka National Park, on the left bank of the Krka River in Šibenik-Knin County in southern Croatia. The cave is situated at the top of the canyon at a height of 216 m above sea level. Members of the local caving club first drew the attention to the cave in 2006 when they recorded it in detail. It is in the form of a horizontal tunnel with the total length of about 42 m. The cave was excavated in 2008 and 2009 by archaeologists from the museums in Drniš and Šibenik. The front part of the cave was used as a dwelling area (temporary or permanent) where numerous fragments of pottery and animal bones were found. Conversely, the rear part of the cave was used as a burial place where numerous fragments of scattered human bones were discovered in a thick layer of mud. Beside the human remains, numerous Bronze Age pottery fragments as well as several objects made of bronze such as one fibula, a spear tip, and one bronze button were also recovered. Based on the archaeological material the cemetery can be dated to the Late Bronze and/or to the beginning of the Early Iron Age.<sup>22</sup>

We report genetic data from one individual:

- I3313 / JAZ1

### Kargadur (2 individuals)

The site of Kargadur is located on the southeast shore of the Istrian peninsula, not far from the settlement of Ližnjan. It was discovered in 2000 during an archaeological survey of southern Istria and has evidence of human activity from the Early Neolithic, Middle Neolithic and the Copper Age. A test archaeological excavation was conducted in 2002 and the wealth of collected finds initiated a systematic investigation conducted by the Archaeological Museum of Istria between 2005 and 2007.



The investigation identified features including pits, drystone structures, accumulations of pebbles and graves, and yielded large quantities of pottery, flint, bone tools, and animal and plant remains. Particularly noteworthy are the finds of hooks, obsidian, rhyton cult vessels and pendants fabricated from marine shells and snails. Two burials were identified in Trench 5 in the course of the 2007 excavations. The first is only partially preserved, while the second is a dual burial of an adult female and an unrelated male child. We report genetic data from this dual burial. The deceased were buried in the crouched position, facing one another. The positioning of the bones indicates that the adult was laid in the grave first, and the child next to her. The adult was placed lying on the right side, in the crouched position, while the child was placed lying on the left side, with its head resting on the left knee of the adult. No goods were found in Grave 2, but a number of Early Neolithic potsherds, flint and bones were found in the sediment backfill of the grave.



**Figure S1.5:** Kargadur double burial 2

- I5071 / Grave 2A  
Female, 30-40 years of age. Mild healed ectocranial porosity is present on both parietal bones and the occipital bone while mild osteophytosis is present on L3 and L5.
- I5072 / Grave 2B  
Subadult male, 2-4 years of age. Mild healed cribra orbitalia is present in both orbits, and new periosteal bone formation was registered on both femurs.

### Osijek (2 individuals)

Osijek-Hermanov vinograd is an important archaeological site in eastern Croatia dated to the Middle Neolithic Sopot culture. The first archaeological investigations of the site were

conducted at the end of the 19<sup>th</sup> century while the most recent rescue excavations due to construction of the southern Osijek bypass were conducted in 2013 by the private company Kaducej Ltd. An area of 3,200 m<sup>2</sup> was excavated, revealing several cultural layers between 1.6 and 3.2 m deep with five distinct phases of habitation. There were numerous stone, bone and pottery artefacts (over 90,100 fragments) and four monumental objects over 100 m<sup>2</sup> in size located adjacent to each other.

- I5077 / OHV-6.1 AN 6, G 1. Male, 28-35 years of age. This skeleton was found at the bottom of a massive ditch in a supine position. Mild healed cribra orbitalia is present in both orbits. Schmorls node was recorded on T8 while mild osteophytosis was recorded on T6.
- I5078 / OHV-7.1 AN 7, G 1. Male, 30-38 years of age. This individual was found lying on its left side in a flexed position. The radiocarbon date puts the use of this burial to 4730-4579 BCE (5815 ± 31 BP, SUERC-50655) that corresponds to the Middle Neolithic Sopot culture. Mild healed ectocranial porosity is present on both parietal bones and the occipital bone; antemortem oval-shaped depression fracture is present on the frontal bone. Mild osteophytosis is present on C4-6 and L3-5 while spondylosis is present on L5.

#### Radovanci (1 individual)

The Radovanci site is located in Slavonia, in continental Croatia, approximately 15 km north of the town of Požega. The site is a destroyed settlement that was in use during the Sopot and Balaton-Lasinya cultures.<sup>23</sup> Archaeological excavations were carried out in 2005 and one skeleton was recovered.

- I5079 / RAD1  
Female, aged 25-30 years. The skeleton was placed on its side, in a flexed position. Radiocarbon analysis of the skeleton yielded a date 3710-3360 calBCE.<sup>24</sup> This individual exhibits moderate active periostitis on the midshafts of both tibiae and fibulae.

#### Vela Spila (1 individual)

Vela Spila cave is located in southern Croatia, on the Island of Korčula. Excavations took place between 1986 and 2004, directed by D. Radić,<sup>25</sup> revealing five Mesolithic skeletons. We sampled “Stanko,” who was unearthed in 2004 from stratum 12. “Stanko” died as an adult<sup>26</sup> at around 7200±30 BP (VERA-2340, 6205-6000 cal BCE on two sigma level), dated based on associated material from stratigraphic layer 7 / 4.<sup>27</sup>

- I1875 / Grave 4 STANKOa  
This is a poorly preserved skeleton of a 35-45 years old female. She exhibits a well-healed ante-mortem compression fracture on her 1<sup>st</sup> lumbar vertebra that resulted in kyphosis and scoliosis, as well as bilateral mild osteoarthritis on her shoulders, and severe osteoarthritis on the head of her right hallux.

#### Veliki Vanik (2 individuals)

Veliki Vanik burial mound is located near the town of Vrgorac in Split-Dalmatia County in southern Croatia. The mound is made of rock and soil deposit with a circular base of 20 m in diameter and a relative height of 3.5 m. It was partly destroyed during the Early Modern

Period when the stone drywall was erected and some of the rock material was harvested from the site. Three Bronze Age graves - one in the shape of a stone coffin and two inhumations in plain soil - containing the remains of five individuals were explored during the rescue excavations. Radiocarbon dates and preserved artifacts (hair ornament made of coiled copper wire and fragments of pottery) date these burials to the Early/Middle Bronze Age.<sup>28</sup>

- I4331 / VV1

Poorly preserved subadult (5-7 years).

- I4332 / VV3

Well-preserved adult female (40 to 50 years). This individual exhibits an antemortem oval-shaped fracture on the frontal bone.

### Vučedol Tell (2 individuals)

The eponymous site of Vučedol, is a long-used tell settlement located six kilometers downstream from the town of Vukovar. The site was excavated in several campaigns led by the following archaeologists: Brunšmid in 1897, Schmidt in 1938, Dorn in 1965, Dimitrijević in 1978-9, Durham / Forenbaer in 1984-91.

The earliest assemblage at Vučedol found to date is identified with the Early Neolithic Starčevo culture. Other phases include the Baden, Kostolac, Vinkovci, and Belegiš complexes. The Late Copper Age Vučedol culture period of the settlement lasted between c. 2900-2400 BCE. The Vučedol tell site consists of four distinct areas, which rise 2-5 m above the loess mounds. One of the mound areas is located to the Northwest, and is named Karasović's Vineyard. Most of the site lies South of the ravine, and consists of Streim's Vineyard, Streim's Cornfield, and the higher 'acropolis' mound of Gradac.

At the Gradac "acropolis", a large house ("megaron") was discovered. The structure may have had religious significance, though it could have functioned as a metallurgy building as there was evidence of copper smelting within. The existence of the "megaron," its location on the high ground, the slightly larger houses at Gradac, the ditches between the higher ground and the rest of the village, combined with the existence of at least two rich graves, have led to the conclusion that Vučedol was a stratified society at the chiefdom level.<sup>29,30</sup>

Remains of 18 individuals were found in nine pits of the settlement. The dead were placed in middens, covered over with dirt. At Streim's Vineyard, a multiple burial was excavated, including a skeleton of a man, five women, and a child (grave 3). The remains were covered with approximately 40 cm of charcoal, suggesting that a fire might have been placed on top of the grave. The grave also contained 4670 ceramic shards (fine pottery), as well as 2951 pieces of animal bone.<sup>29,30</sup> In another grave (pit 9 / 1985), two contracted skeletons were found in an antipodal position in a double burial.

- I4175 / GEN99 Pit 9 / 1985 skeleton 1 / 1 (H)

A 15-17 year old male, found in a double burial. He exhibits a benign cortical defect on the insertion site of the pectoralis major muscle on his right proximal humerus, as well as a sharply defined lytic lesion on his distal right humerus, which is most likely the result of osteochondritis dissecans.<sup>31</sup>

- I2792 / GEN64 Grave 3 / skeleton 6 (D)

A 40-45 year old female, found in a multiple burial together with six other individuals.



### Zemunica Cave (3 individuals)

Zemunica Cave is located in karstic terrain near the village of Bisko, East of Split in the hinterlands of Dalmatia (south Croatia). Rescue excavations of the site (three trenches in total) were carried out in 2005 by archaeologists from the Department of Archaeology, Faculty of Humanities and Social Sciences, University of Zagreb.<sup>32</sup> The cave is a single chamber (16 x 18 m) cave with a north-facing entrance and an opening at the ceiling. The stratigraphic sequence runs from the Early Bronze Age to the Late Upper Paleolithic.<sup>33</sup> Human remains were found in the Neolithic, Mesolithic and Paleolithic layers, but all ten directly dated human samples, from different stratigraphic units, are dated to around 7,000 radiocarbon years BP,<sup>34</sup> which suggests that significant mixing of Paleolithic, Mesolithic and Neolithic remains occurred in the Neolithic or later.<sup>33,34</sup>

The three human bones from which we successfully obtained ancient DNA came from three different stratigraphic units. These units are attributed to the Early Neolithic based on the presence of Impresso pottery and / or stratigraphic position. The remains were fragmented and scattered which does not indicate intentional burial.

- I3433 / ZC1 (SJ78, PU380)

The left temporal bone of a sub-adult from the scattered human remains recovered from unit 78 dated to the Early Neolithic (Impresso Culture).

- I3947 / ZC2 (SJ110, PU387)

The left temporal bone of an adult from the scattered human remains recovered from unit 110 dated to the Early Neolithic (Impresso Culture)

- I3948 / ZC3 (SJ103-35)

The left temporal bone of a sub-adult from the scattered human remains recovered from unit 103 dated to the Early Neolithic (Impresso Culture).

## France

### Aven des Iboussières à Malataverne (2 individuals)

We report genome-wide data from two individuals from this Epipaleolithic site, dated to 10090-9460 BCE (10140±50 BP, GrA-43700) based on a third individual (Iboussières39) found in the same layer. Mitochondrial DNA from the two individuals has previously been published, and we refer to that study for the archaeological site summary.<sup>35</sup>

- Iboussières25-1
- Iboussières31-2

## Greece

### Diros, Alepotrypa Cave (4 individuals)

Alepotrypa Cave is located at Diros Bay, Mani, Lakonia, Greece. The cave is about 300 m long and it is situated about 20 m above sea level, in an arid and rocky limestone environment, about 50 m from the present Mediterranean shoreline. It has been excavated since 1970 by G. Papathanassopoulos and it is dated to between 6000 and 3200 BCE (Early to Final Neolithic Periods). Artifacts include a variety of pottery, lithic tools, grindstones,

copper daggers, bone needles, clay spindle whorls, personal decoration items, and figurines. Food remains consist of cultivated cereal, legume, and fruit remains, a large number of animal bones from domesticated species, and to a lesser degree wild plant and animal resources, fish and shells. Stable isotope analysis suggests a primarily agricultural diet with an emphasis on plant resources. There is also evidence of rich ritualistic expression, including massive concentrations of deliberately broken pots possibly associated with mortuary practices. The cave has also yielded a large human skeletal assemblage with a minimum number of 161 individuals, including remains of primary single or multiple burials, two ossuaries for secondary disposals, and scattered bone.<sup>36,37</sup>

- I3920 / A68

This sample is a temporal bone from the scattered bones in the interior of the cave.

- I3708 / A561

This individual is a ~10 year old child represented by a cranium from a secondary deposit from Ossuary II.

- I3709 / A236

A 20-25 year old individual, morphologically male, represented by a cranium from a secondary deposit from Ossuary II.

- I5427 / A2055

A ~4-5 year old child represented by an almost complete cranium and teeth from a secondary deposit from Ossuary I, close to the entrance of the cave.

### Franchthi Cave (1 sample)

Franchthi cave is about 120 meters long at the tip of a limestone peninsula, overlooking a now submerged coastal plain, across the bay of Koiladha in south-western Argolid. It covers an exceptionally long archaeological sequence of approximately 25–30,000 years spanning the Upper Palaeolithic to the end of the Neolithic.<sup>38</sup> The site from the Early Neolithic also includes a large open-air settlement called Paralia. It was excavated in the 1970s and '80s by Thomas Jacobsen and Indiana University and has yielded evidence for seafaring in the Paleolithic as implied by the obsidian from Melos, in the Mesolithic as implied by large tuna vertebrae and in the Neolithic as implied by finds of marble, honey flint, and andesite. Pottery, stone tools, animal and botanical remains are also abundant. The human osteological assemblage from Franchthi cave and Paralia consists of at least 68 individuals, including 3 Palaeolithic, 17 Mesolithic, 46 Neolithic, and 2 historic. Nineteen of these individuals are primary burials, 20 are associated groups of bones, probable burials, and approximately 560 more skeletal elements are scattered bones and teeth.

- I2318 / FR115

Final Neolithic sub-adult of 8 years, from square L.

## Italy

### Grotta d'Oriente (1 individual)

Grotta d'Oriente is a small coastal cave located on the island of Favignana (NW Sicily, Italy) at about 40 m above sea level. The cave was first roughly excavated in the early 1970s<sup>39,40</sup> and subsequently in 2005 by the University of Florence.<sup>41</sup> The 2005 excavations were carried out using state of the art methodology contiguously to the trench excavated in the 1970s.

During this last field season a well-detailed Paleo-Mesolithic sequence was detected; it consists of several levels attributable to short-term episodes of human frequentation. The sedimentary succession (about 2 m thick) provided cultural evidence, spanning from the Late Pleistocene to the middle Holocene, represented by 4 main phases: Late Upper Palaeolithic (Layers 7A - 7E), Early Mesolithic (Layers 6A - 6D), Late Mesolithic or Early Neolithic (Layers 5A -5C) and Bronze Age (Layers 4 - 3). These cultural phases (layers indicated by numbers) contained occupation episodes (sublayers indicated by letters) composed of structured hearths, pits, artefacts and abundant faunal remains (both terrestrial and marine)<sup>41,42</sup>. This anthropogenic sequence overlaps a deposit (Layer 8) containing only Pleistocene fauna not associated with human activities.

Three individuals were excavated at Grotta d'Oriente ; Oriente A (probably a Late Paleolithic adult male) and Oriente B (Mesolithic adult female) during the 1972 excavation<sup>40,43</sup>; and a third, Oriente C (Palaeolithic adult, probably female), during excavations in 2005.<sup>41,44</sup>

- I2158 / Oriente C

Found in the lower portion of Layer 7 containing typical local Late Upper Palaeolithic (Late Epigravettian) lithics.<sup>41,45</sup> The funeral pit opens in the sublayer 7D. Two radiocarbon dates on charcoal 12,246-11,842 BCE (12,149±65 BP, LTL-14260A) and 12,249-11,816 BCE (12,132±80 BP, LTL-873A) from sublayers 7D and 7E respectively are consistent with the archaeological context and refer the Oriente C burial to the period 12,250-11,850 BCE, when Favignana was connected with the main island.

Oriente C is a 25-35 years old female, represented by only the upper half of the skeleton, which had been disturbed by two different events: 1) a small pit opened at the top of Layer 7 which probably partially intercepted the left lower part of the skeleton; 2) the 1972 trench which cut in half the burial approximately at the height of the pelvis. The body was deposited in dorsal-lateral decubitus, oriented S (skull) –N. Despite minor post-mortem dislocations of a few bones, most anatomical connections were still intact. As other Late Epigravettian sites in Sicily and Italy<sup>46</sup>, Oriente C has few grave goods and personal ornaments. Only a pierced shell of *Cerithium* sp. (perhaps a clothing ornament) and very small lumps of red ochre, next to the skull and the femoral head, were found in the burial. In general, the anatomical features of Oriente C are close to those of Late Upper Palaeolithic populations of the Mediterranean and show strong affinity with other Palaeolithic individuals of Sicily. A palaeodietary study using stable isotope analysis highlighted an essentially terrestrial diet with low-level consumption of marine foods, similar to other Late Upper Palaeolithic individuals from Sicily and Italy.<sup>43,47</sup>

## Latvia

### Zvejnieki (17 individuals)

The site of Zvejnieki, in Northern Latvia, was occupied from the Middle Mesolithic through the Early Iron Age.<sup>48,49</sup> The craniology and odontology of Zvejnieki population were studied by Denisova<sup>50</sup> and Gravere<sup>51</sup>. The environmental history of the site was reconstructed by Eberhards<sup>52</sup> and Kalnina<sup>53</sup>. The burials and grave goods<sup>54,55</sup> as well as finds from the occupation layers, have been considered from a variety of perspectives. The faunal remains have been analyzed.<sup>56-58</sup> Human physical development<sup>59</sup>, palaeodemography<sup>60</sup>, palaeopathology<sup>61</sup>, stable isotopes<sup>62,63</sup> and the reservoir effect of Lake Burtnieks<sup>64</sup> have all been studied.

We report genetic data from 17 individuals from the Mesolithic Zvejnieki II, and Neolithic Zvejnieki I cemeteries. We also report new data from five individuals with previously reported shotgun data<sup>65</sup>, and a more complete description of the site can be found in that reference.

- I4440 / ZVEJ21  
Mesolithic, burial 197, adult male 45-50 years old, old, 6400±95 BP, (Ua-19808). Bone material fragmented. No grave goods.
- I4432 / ZVEJ10  
Mesolithic, burial 67, sub-adult, 3-5 years old. Ochre addition. Grave goods include a flint fragment with evidence of processing. Not dated.
- I4434 / ZVEJ12  
Mesolithic / Neolithic, burial 128, infant, 1-2 years old. Ochre addition. Grave goods include: a white flint knife, a flint fragment, 13 beaver bones and 92 animal tooth pendants. Not dated.
- I4553 / ZVEJ7  
Mesolithic, burial 98, infant, 2-3 years old. No grave goods. Ochre addition. Not dated
- I4439 / ZVEJ20  
Mesolithic, burial 86, sub-adult, 3-5 years old. Grave goods include 23 tooth pendants. Ochre addition. Not dated.
- I4550 / ZVEJ3  
Mesolithic, burial 52, sub-adult 2-3 years old, No grave goods. Ochre addition.
- I4551 / ZVEJ4  
Mesolithic, burial 108, sub-adult 3-5 years old, Ochre addition. Grave goods include 19 tooth pendants. Not dated.
- I4552 / ZVEJ5  
Mesolithic, burial 117, sub-adult ~1-2 years old, No grave goods. Ochre addition. Not dated.
- I4553 / ZVEJ13  
Neolithic burial 278, child, 9-14 years old from a common burial 274 – 278. Not dated, no grave goods. Burial 277 date 5545±65 BP (Ua-19810).
- I4436 / ZVEJ14  
Neolithic, burial 261, sub-adult 2-4 years old from a common burial 258-261. Not dated, no grave goods.
- I4437 / ZVEJ15  
Neolithic, burial 226, sub-adult 2-4 years old, 5345±60 BP (Ua-1984) Ochre addition. Necklace of 80 tooth pendants.
- I4438 / ZVEJ16  
Neolithic, burial 224, subadult 2-3 years old, from a common burial 220-225. Not dated, no grave goods. Burial 225: 5110±45 BP (OxA-5986), Burial 221: 5180±65 (Ua-19813) BP.
- I4441/ ZVEJ22  
Neolithic, burial 173, adult male, 40-50 years old, 5895±65 BP (Ua-19816). Ochre addition, no grave goods.

- I4554 / ZVEJ24  
Neolithic, burial 207, sub-adult, 9-11 years old from a common burial 206-209. Ocher addition. Grave goods include: double-sided harpoon, 3 flint arrowheads, a flint scraper, a flint fragment in form of knife, 12 small flint fragments, a fragment of bone picker, a fragment of amber tablet. Not dated, Burial 208: 5345±60 (Ua-19815), burial 206: 5285±50 BP (Ua-3643).
- I4595 / ZVEJ8  
Mesolithic/Neolithic, burial 99, infant 2-4 years old. Grave goods include: quartz sliver. Ochre addition. Not dated.
- I4596 / ZVEJ9  
Likely Mesolithic, burial 49, infant 1-2 years old from a common burial 48 –51. Bone material fragmented. Ochre addition. Not dated, no grave goods.
- I4630 / ZVEJ30  
Mesolithic, burial 305, adult male, 25-30 years old, 8240±70 BP (Ua-3634). Found in the Zvejnieki II site. Grave goods include: Bone spearhead with one-sided serration. Ochre addition.

We also report new 1240k capture data on the following five individuals with previously published shotgun data:<sup>65</sup>

- I4626 / ZVEJ25
- I4627 / ZVEJ26
- I4628 / ZVEJ27
- I4629 / ZVEJ28
- I4632 / ZVEJ32

## Macedonia

### Govrlevo (Cerje), Skopje (1 individual)

Govrlevo is a large prehistoric settlement located in the province of Skopje which was excavated in the early eighties by Z. Georgiev and M. Bilbija (Museum of the City of Skopje). The 4.5 m high tell settlement was inhabited from the Early to the Late Neolithic. Excavations continued between 1982-2010 in seven dig seasons. Human skeletons were extracted from layers dating to the oldest occupation<sup>66</sup>. The skeletons were osteologically analysed by Fanica Veljanovska from the Museum of Macedonia.

- I0676 / Mace7 (no 1)  
Skeleton no.1 was discovered in 2009. Male aged 25-30 years.

## Poland

### Kierzkowo (8 individuals)

The Globular Amphora Culture is characterised by the decorated globular ceramic vessels with short necks and small handles.<sup>67-69</sup> The culture was nomadic, with unstable settlement patterns. Cultivation was not entirely abandoned, but animal husbandry was the most important part of the economy—dominated by pigs and cattle, with some horses.<sup>67,70-72</sup>

The archaeological site from which we obtained data from the Globular Amphora Culture lies in the Żnin district (Kujavia-Pommerania voivodeship, Northwest Poland). It contains a megalithic barrow tomb 22 meters long West-to-East with width varying from 3 to 6 meters North-to-South. The first 10 meters of the length of the tomb was built from stone slabs and rubble, leading to a chamber to which two low small corridors led from the south. An enormous stone divided the chamber into two unequal parts. Within the chamber there were Neolithic human bones gathered into two large clusters. Some bones were also located under the large stone dividing the chamber. In the two large bone gatherings, remains were stratified into seven layers, while in other locations bones were not so deep. Human remains were fragmentary, mixed between different individuals, and in many cases mixed with animal bones,<sup>73</sup> especially those of cattle and pigs. They were present both inside the chamber, and outside at 12 to 20 meters. These bones are the subject of a separate study (in preparation). Grave goods included pottery fragments dispersed outside of the chamber at meters 15-20. About halfway between this area and the chamber, an atypical pitcher with asymmetric handles, an earthenware drum and a double-sided blade were found. In the chamber there were numerous fragments of amber (especially at meters 3 and 4), and around the middle of the chamber a pendant made of a boar's canine.

Genetic analysis shows that four of the individuals are close relatives – with a mother (5.1), father (7.6), and two sons (6.1 and 7.1 / 8.4). Three unrelated individuals (one female ~25 years old, one infant and one ~2-3 year old child) found outside the main tomb (meters 19 and 20) proved to be recent intrusive burials (two individuals dated 1650 CE – present; Beta-430716, Beta-430715) and we therefore do not report genetic data for them.

- I2405 / 8.2a  
Tibia fragment of an ~8 year old male.
- I2433 / 5.1  
Mandible fragment of a 50-60 year old female.
- I2434 / 5.3  
Skull fragment of a 20-30 year old female.
- I2435 / 6.1b  
Femur shaft fragment of a 30-50 year old male.
- I2440 / 7.6  
Right humerus fragment of an adult male.
- I2441 / 8.5  
Left pelvis of a newborn male.
- I2407 / 7.1 and 8.4  
Jaw and left femur fragment of a young male.



- I2403 / 3.4  
Left femur fragment of a 20-30 year old male.

## Romania

### Carcea (1 individual)



**Figure S1.6:** Picture of the Early Neolithic pit no. 3 (1995) from Carcea and locations of three human skulls (after Haimovici 2006, modified). Photo courtesy of Studii de Preistorie.

The Carcea-Viaduct site is situated in southwestern Romania on a terrace of Carcea Creek, in Transylvania, near Craiova city (Dolj County). Settlement remains with different domestic structures (e.g. houses, pits, hearths, etc.), individual graves, and scattered bones belonging to various prehistoric periods (Neolithic, Eneolithic, Bronze Age, Iron Age, and Roman period) were identified during several excavation campaigns between 1970 and 2005.<sup>74</sup>

The Carcea-Viaduct settlement is one of the most famous Early Neolithic sites in Romania and southeastern Europe. During the research of the Early Neolithic settlement (Starčevo-Criș culture), several scattered human bones, especially skulls and long bones, were found in the defensive ditch and pits<sup>74</sup>. The three available radiocarbon dates place the Early Neolithic occupation from that site between 5617-5230 cal BCE.<sup>75</sup>

- I2533 / ROM29

The data included in the current study came from a cranial fragment that belonged to individual 1 - an adult female (25-30 years old) discovered in pit no. 3 (1995) that had an oval shape (4.75 x 2.5 meters) and 0.50 meters in depth. The pit also contains animal bones (*Bos taurus* and *Bos primigenius*), three human skulls that belong to 2 adult females (individuals 1 and 2), and an Infant II (individual 3), and also numerous ceramic fragments, idols, flint and stone tools.<sup>76</sup>

### Coțatcu (1 individual)

The Coțatcu site is located in eastern Romania (Buzau county), about 15 km from the town of Ramnicu Sarat, on a terrace of the Coțatcu creek. The archaeological remains from the site belong to the Early Neolithic (Starčevo-Criș culture), Eneolithic (Gumelnița culture), and Bronze Age (Monteoru culture). Several archaeological excavations were carried out here between 2006 and 2010, but the research was focused on the Eneolithic habitation.<sup>77</sup>

The Early Neolithic settlement is overlapped partially by the Eneolithic tell that belong to the Gumelnița culture. The Starčevo-Criș occupation is characterized by several pits, material agglomeration in natural depressions, a few houses (affected by erosion process of the terrace), and an inhumation. The material culture includes many pottery sherds, flint tools, stone axes, figurines, and animal bones. Some Early Neolithic pits contained scattered human bones.<sup>78</sup>

- I2532 / ROM1

The sample included in the current study (lower left M1) belongs to a young female (15-18 years old) discovered in grave no. 1 from the extremity of the settlement. The individual was deposited in crouched position on her left side, oriented North-to-South, without grave goods.<sup>79</sup>



**Figure S1.7:** Picture of the Early Neolithic grave no. 1 from Cotatcu.

### Măgura Buduiasca (Teleor 3) (1 individual)

The Măgura Buduiasca site is in southern Romania, on the Teleorman River lower terrace, 10 km Northeast from the town of Alexandria (Teleorman County). Excavations began in 2001—following fieldwork that discovered evidence of different Neolithic materials, revealing a large flat settlement. These investigations confirmed the following Neolithic stratigraphy: Early Neolithic (Starčevo-Criș), Middle Neolithic (Dudești culture), and Late Neolithic (Vădastra culture), implying a time span between c. 6100 and 5200 BCE. The Neolithic occupation is overlapped in some areas by remains from later occupations (e.g. Bronze Age, Iron Age, Migration Period, and Middle Age). The Early Neolithic habitation is characterized by numerous pit-huts, pits, and hearths, but also a rich material culture that includes



potsherds, figurines, flint and stone tools, grinding stones, wood items, bone ornaments and tools, shells, animal bones and scattered human bones.<sup>80</sup> Radiocarbon dates available (n=7) place the Early Neolithic occupation from that site between 6064-5746 cal BCE.<sup>81</sup>

- I2534 / TEL1

The sample included in the current study (a cranial fragment) belongs to an adult individual discovered in a Starčevo-Criș pit labeled C48. In the same pit were found other scattered human bones (a pair of humeri and another skull fragment), and it is possible that they belong to the same individual. Potsherds, flint tools, shells, and animal bones completed the pit inventory.

### Schela Cladovei (4 individuals)

The open-air site of Schela Cladovei is situated in the ‘downstream area’ of the Iron Gates – 7 km below the Iron Gates I dam which marks the end of the Iron Gates gorge, and some 67 km downriver from Vlasac in Serbia. It occupies an alluvial flat immediately adjacent to the Danube, which was probably overtopped by the river during extreme Holocene flood events. The site was discovered in 1964 and thirteen excavation campaigns were completed between 1965 and 1991, led by the late Vasile Boroneanț. Since 1992 the ongoing excavations have been conducted as a joint Romanian-British project.<sup>82</sup>

A large series of <sup>14</sup>C dates on animal and human bones show that the Schela Cladovei site was occupied during the Late Mesolithic (7200-6300 cal BCE) and Early Neolithic (6000-5600 cal BCE), with sporadic evidence of activity during the Iron Age and Medieval periods.<sup>83</sup>

- I4607 / SCCL\_46 / 1 (compact bone from diaphysis of right femur)
- I4655 / SCCL\_50 (compact bone from diaphysis of right tibia)
- I5411 / SCCL\_12 (Molar)
- I5436 / SCCL\_43 (Molar)

These were among a large concentration of human remains (comprising 7 articulated skeletons and disarticulated remains from at least another 18 individuals) excavated between 1990 and 1992 from a trench (Area III-IV, 11 m x 2-5 m) abutting the eroding riverbank. Burials M46.1 and M50 were extended supine inhumations of adult males, with their long axes aligned more-or-less parallel with the Danube. Body position and bone collagen  $\delta^{15}\text{N}$  values of >14‰, indicative of diets based on aquatic resources, point to a Late Mesolithic age for these burials, which was confirmed by <sup>14</sup>C dating.<sup>84</sup>

### Ostrovul Corbului (3 individuals)

The site of Ostrovul Corbului, 28 km downriver from Schela Cladovei contains human remains dating from the Mesolithic to the Early Iron Age.<sup>85</sup>

A group of six primary inhumation burials (M2, M9, M24, M25, M30 and M32) were found in Sector A, at the downstream end of the island. Previous authors assigned these burials to either the Early Neolithic or the Mesolithic. AMS <sup>14</sup>C dates on four of the burials (M2, M25, M30 and M32) confirm that they all belong to the Mesolithic.<sup>86</sup>

- I4081 / OSTCOR1a+1b / ROM47

These samples (a premolar and a canine) belong to an adult male from Burial M2. The body appears to have been buried in a sitting position with the legs flexed, raised and crossed at the ankles. Apart from a fragment of red ochre, no grave goods accompanied the burial.

- I4582 / OSTCOR32

This sample (a petrous temporal bone) comes from Burial M32. The skeleton (identified as that of an adult male, around 50 years of age at death) was lying in the extended supine position, with the arms extended along the sides of the body. No associated grave goods were reported.

- I5408 / OSTCOR24

The FRE-corrected  $^{14}\text{C}$  ages of M2 and M25 fall in the Middle Mesolithic between *c.* 7715–7190 cal BCE, and that of M32 in the Late Mesolithic between 7021–6473 calBCE (7812±69 BP, freshwater reservoir correction using ORAU  $\delta^{15}\text{N}$  value for 8302±32 BP which is a weighted average of (8305±50 BP, OxA-31598), (8300±40 BP, PSU-1749), (8335±45 BP, PSU-1904).<sup>86</sup>



**Figure S1.8:** Mesolithic graves nos. 2 (left) and 25 (right) from *Ostrovul Corbului*.<sup>85</sup> Photos courtesy of the Institute of Archaeology “Vasile Pârvan”, Romanian Academy, Bucharest.

### Urziceni (2 individuals)

The Urziceni-Vama site is situated in northwestern Romania on a terrace of the Pârâul Negru creek, in Satu Mare county. On the occasion of the construction of the Urziceni Duane and of a Duty-Free shop, rescue excavations discovered several graves belonging to the Bodrogkeresztúr culture (Middle Eneolithic – second half of the 5<sup>th</sup> millennium BC), which form part of a necropolis. So far, 68 graves have been excavated. The graves have rectangular or oval-oblong or irregular pits and contain skeletons deposited in crouched position, on the right or the left side, and oriented East-to-West. Over 75% of the graves contained inventory that was particularly rich (e.g. gold and copper items, many ceramic pots, obsidian and flint tools).<sup>87</sup>

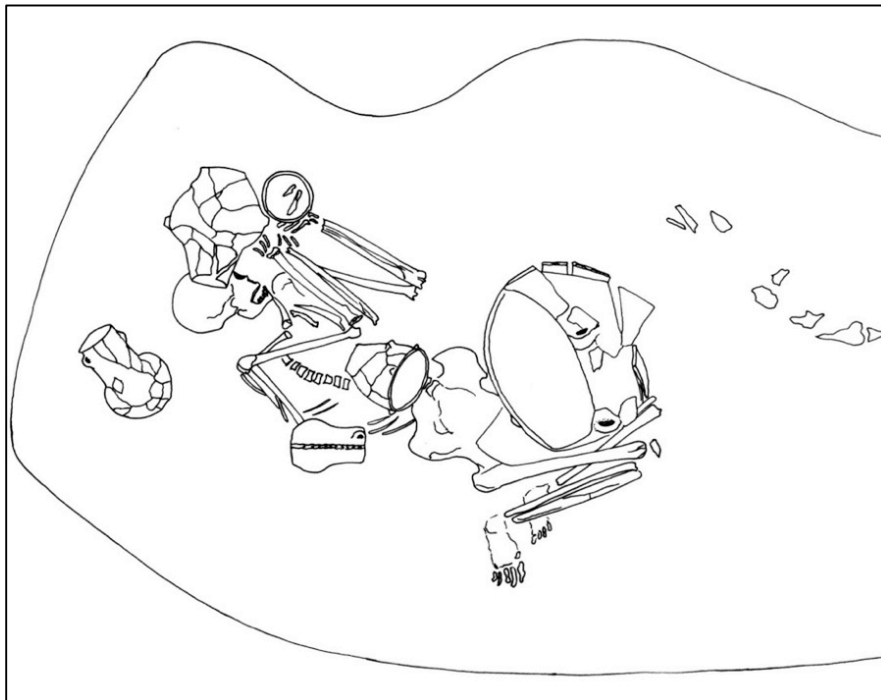
- I4088 / URZI16

The sample included in the current study belongs to an adult individual discovered in grave no. 16 from 2003. The individual was deposited in crouched position on its left side, oriented East-to-West, and accompanied by six Bodrogkeresztúr pots as funeral inventory.<sup>88</sup>

- I4089 / URZI48

The sample included in the current study belongs to an adult individual discovered in grave no. 48 from 2014. The individual was also deposited in crouched position on its left side,

oriented East-to-West, and accompanied by several Bodrogkeresztúr pots as funeral inventory.



**Figure S1.9:** *Layout of Bodrogkeresztúr grave no. 16 from Urziceni-Vama.*

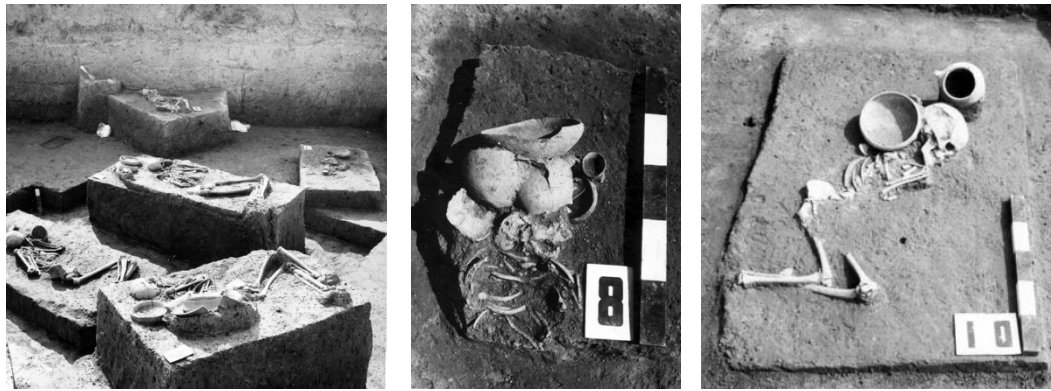
## Serbia

### Gomolava (3 individuals)

On the banks of the Sava river in southern Vojvodina, the site of Gomolava has been occupied for around 5 millennia, with five distinct periods of occupation from the late Neolithic to the early Middle Ages.<sup>89</sup> It is situated on a loess plateau overlooking alluvial valleys. Although known since the beginning of the 20<sup>th</sup> century, intense excavations of this site were performed from 1953 onwards.

The samples included here all date from the late Neolithic Vinča occupation of Gomolava. This site is one of only 2 known cemeteries of this cultural group in this region.<sup>90,91</sup> Demographic studies suggest that the Vinča burials are not representative of the average Vinča population, as there is an overwhelming prevalence of males amongst both adult and subadult individuals.<sup>92,93</sup>

- I0633 / NG11 / burial 3 (1975)  
A 5-6 year old male.
- I0634 / NG19 / burial 8  
A 9-12 month old male.
- I1131 / NG21 / burial 10  
A 5-6 year old male.



**Figure S1.10:** Late Neolithic burials at Gomolava. (center, burial 8; right, burial 10). Photos courtesy of Jelka Petrović.

### Hajdučka Vodenica (6 individuals)

The Mesolithic-Neolithic site of Hajdučka Vodenica is situated downstream from the Lower Gorge known as Kazan (“Cauldron” in Romanian and Serbian) in the Danube Gorges area, on the right (Serbian) bank of the river. The site was investigated in 1967–9 by B. Jovanović who examined an area of 630 m<sup>2</sup> along the river bank below 70 m a.s.l. which was subsequently submerged beneath the reservoir created by the Iron Gates I dam. At Hajdučka Vodenica there are two distinct areas of the site. In the first, southwestern area, rectangular stone-lined hearths were found with several superposed levels of stone constructions and associated with several burials among which are the two individuals analyzed here: Burials 31 and 33. The second, central area of the site consisted of a burial “chamber” where 29 burials were placed in extended supine positions primarily parallel with the Danube (with the exception of Burials 9 and 12, which were perpendicular to the Danube) and associated with a rectangular stone-lined hearth, named “sacrificial” hearth area, which was surrounded by a packed red burnt earth flooring.<sup>94-96</sup> The analyzed individual that was, after osteological analysis, marked as Burial 19-20(1) belongs to one of several primary burials that were buried close to each other in this zone along with Burial 21 found buried still deeper inside the slope. A total of six published AMS <sup>14</sup>C dates on human remains from Hajdučka Vodenica range (after FRE correction) between c. 7100–5800 calBCE, covering the period of the Late Mesolithic and the Mesolithic-Neolithic transition phase.

We report genetic data from six individuals:

- I4914 / HJDK\_19-20(1)
- I4915 / HJDK\_21
- I4916 / HJDK\_31
- I4917 / HJDK\_33
- I5401 / HJDK\_8
- I5402 / HJDK\_15(1)

### Lepenski Vir (4 individuals)

Lepenski Vir is one of the best-known archaeological sites in Europe. Situated c. 5 km downstream from Padina in the upper part of the Iron Gates Gorge, the site was discovered in the 1960s during archaeological surveys in advance of construction of the Iron Gates I dam. The most abundant archaeological remains from Lepenski Vir belong to the Mesolithic and Early Neolithic periods, although there are also traces of occupation dated to the Chalcolithic, Bronze Age, Iron Age, Roman and Medieval periods. Excavations between 1965–70 led by

Dragoslav Srejšović examined an area of *c.* 2500 m<sup>2</sup> where an unprecedented array of archaeological features and artefacts relating to repeated use of the site over thousands of years was found. These included the remains of around 70 buildings with trapezoidal bases and (often) furnished with lime plaster floors and stone bordered hearths, over 200 burials, and exceptional numbers of stone and bone artworks and body ornaments. A revised chronological framework<sup>97</sup> proposed by Dušan Borić recognizes three main phases of Stone Age occupation of the site: Early–Middle Mesolithic, *c.* 9500–7300 cal BC (‘Proto-Lepenski Vir’), Mesolithic-Neolithic Transition, *c.* 6150–5950 cal BC (‘Lepenski Vir I–II’), Early/Middle Neolithic, 5950–5500 cal BC (‘Lepenski Vir III’). No evidence of a Late Mesolithic (7300–6200 cal BC) occupation has been identified at Lepenski Vir, but the phase is well represented among the burials from Padina, Vlasac, Hajdučka Vodenica, Schela Cladovei and Ostrovul Corbului.

We report genetic data from four individuals:

- I4665 / LEPI\_54E

This sample (a fragment of compact bone from the diaphysis of the left femur) comes from Burial 54e, one of five individuals (labelled a-e) that constituted ‘Burial 54’ and who were buried within the confines of a stratigraphically older building (65/XXXV) (Fig. S1.12). The skeleton (tentatively identified as that of a young adult female, around 20 years of age at death) was lying in the extended supine position, with the arms extended along the sides of the body. The orientation of the burial was parallel to the Danube with the head downriver. A direct AMS <sup>14</sup>C measurement of 7474±35 BP (OxA-25210) (6210–5925 calBCE) dates this individual to Borić’s Mesolithic-Neolithic transition (‘Lepenski Vir I–II’) phase.<sup>98</sup> This dating is supported by the δ<sup>15</sup>N value of +13.0‰, which is lower than the Mesolithic average and suggests a mixed terrestrial/aquatic diet, and the association with the skeleton of a bracelet of disk-shaped limestone beads, which are technologically characteristic of Neolithic modes of manufacturing and aesthetics.<sup>97</sup> <sup>87</sup>Sr/<sup>86</sup>Sr analysis of tooth enamel suggests the individual was an immigrant whose childhood years were spent outside the Iron Gates region.<sup>99</sup>

- I4666 / LEPI\_61

This sample (a fragment of compact bone from the diaphysis of the right femur) comes from Burial 61. This extended supine inhumation burial of a male child (~8 years) was found below the floor of trapezoidal building 40 (Fig. S1.11). The burial was oriented parallel to the Danube with the head downriver. It is possible that burial was placed here before the construction of the building floor as no burial pit at the floor level was identified.<sup>97</sup> An AMS <sup>14</sup>C measurement of 7670±35 BP (OxA-25211) (6225–5910 calBCE) (Bonsall et al. 2015) dates the burial to Borić’s Mesolithic-Neolithic transition (‘Lepenski Vir I–II’) phase, although the δ<sup>15</sup>N value of +16.1‰ suggests a typical Mesolithic diet in which fish was the main source of protein.

- I5405 / LEPI\_17

The sample comes from lower left PM1 of a ~10-year-old female possibly placed in a crouched position on the lateral left side although only a damaged skull and torso were found. The burial was oriented NW-SE. The burial was found in the same multiple burial location with individuals marked as burials 13 to 16, all placed in extended positions and parallel to the Danube with the head downriver, i.e. oriented S-N. The direct date obtained on one of these inhumations, Burial 14, is 7226±72 BP (OxA-25090) (6236–5985 calBCE) and dates this individual to Borić’s Mesolithic-Neolithic transition (‘Lepenski Vir I–II’) phase.<sup>97</sup> On the other hand, Burial 17 is directly dated to 7007±48 BP (AA-58320) (5836–5632 calBCE) and seems to belong to the Early/Middle Neolithic phase of occupation at the site (Lepenski Vir III). This dating is supported by the δ<sup>15</sup>N value of +10.9‰, which suggests a predominantly terrestrial diet.<sup>87</sup> <sup>87</sup>Sr/<sup>86</sup>Sr analysis of tooth enamel suggests the individual was an immigrant whose early years were spent outside the Iron Gates region,<sup>98</sup>



- I5407 / LEPI\_126

The sample comes from lower incisor of a disarticulated mandible that was possibly structurally deposited on a large rock and was found beneath the limestone floor of building 54 at Lepenski Vir. Due to the stratigraphic position of this mandible the assumed date is Early to Late Mesolithic, i.e. it belongs to the Proto-Lepenski Vir phase.<sup>97</sup>

### Padina (13 individuals)



**Figure S1.11:** Burials from Padina. Clockwise from top left, 12, 4, 15 and 16, 18, 6a. Photos courtesy of the Institute of Archaeology, Belgrade.

The Mesolithic-Neolithic site of Padina - Gospođin Vir (Serbian: “Lady’s Whirlpool”) is situated at the upstream entrance to the Lady’s Whirlpool Gorge of the Danube Gorges area, on the right (Serbian) bank of the river. The site was investigated in 1968–1970 by B. Jovanović who examined three connected coves marked as sectors I (675 m<sup>2</sup>), II (650 m<sup>2</sup>), and III (1100 m<sup>2</sup>) along the bank of the Danube below 70 m a.s.l. – which was subsequently submerged beneath the reservoir created by the Iron Gates I dam. Excavations produced 33 burials of primarily Early to Late Mesolithic date, comprising primary inhumations, primary disturbed burials and secondary inhumations. However, three individuals (burials 4, 5 and 5a) found in a group burial at sector I are dated to the Mesolithic-Neolithic transition phase at the

end of the 7<sup>th</sup> millennium BCE.<sup>94-96</sup> A total of 13 published AMS <sup>14</sup>C dates on human remains from Padina range (after FRE correction) between ~9200–5800 cal BCE.<sup>95,99</sup>

The samples analyzed in the current study come from burials found at each of the three investigated sectors. Burials 9, 12, 14, 16a, 17, 18b, 22, 24, and 26 all come from Sector III and were found on the slope at the rear of the site away from the river and, based on their stratigraphic positions and some of the associated AMS radiocarbon dates, can all be dated to the Early/Middle Mesolithic. Burial position could not be established for burial 9, which was found in the vicinity of more than two millennia later trapezoidal building structure 17. Close to a linear stone construction built in several levels were found burials 12, an extended supine inhumation; 14, an extended supine inhumation; 16a, seated with crossed lower limbs; 17, partly disturbed; 18b, some sort of seated position; 22, partly disturbed; and 26 as an extended supine inhumation. Burial 24, an extended supine inhumation, was found farther to the south from the burial concentration found around the stone construction. At Sector II, Burials 6, a seated inhumation, and 30, a crouched inhumation, were found. A possibly Early/Middle Mesolithic date can be assumed for both burials in the absence of radiocarbon dates. At Sector I, Burials 4 and 5 were found one on top of the other with Burial 4 placed over Burial 5. Burial 4 was found as an extended supine inhumation and Burial 5 was a crouched inhumation and both of these burials can on the basis of radiocarbon dates be assigned to the Mesolithic-Neolithic transition phase in the last century of the 7<sup>th</sup> and the first century of the 6<sup>th</sup> millennium BCE. <sup>14</sup>C dates given in Supplementary Table 1 have a FRE correction. We report genetic data from 13 individuals:

- I5241 / PADN\_24
- I5232 / PADN\_4
- I5233 / PADN\_5
- I5234 / PADN\_6
- I5235 / PADN\_9
- I5236 / PADN\_12
- I5237 / PADN\_14
- I5238 / PADN\_16a
- I5239 / PADN\_17
- I5240 / PADN\_2
- I5242 / PADN\_26
- I5244 / PADN\_18b
- I5409 / PADN\_25

#### Saraorci-Jezava (1 individual)

- I4918 / SAJE  
Burial 1, chalcolithic

#### Vlasac (17 individuals)

The Mesolithic-Neolithic site of Vlasac is situated in the upper part of the Danube Gorges, on the right (Serbian) bank of the river 3 km downstream from Lepenski Vir. The site was investigated in 1970–1 by D. Srejović and Z. Letica who examined an area of 640 m<sup>2</sup> along the river bank below 70 m a.s.l., which was subsequently submerged beneath the reservoir created by the Iron Gates I dam. Further excavations were undertaken between 2006–9 by Dušan Borić, who examined a further 326 m<sup>2</sup> upslope of the area excavated in 1970–1. The two series of excavations produced over a hundred burials of primarily or exclusively Mesolithic date, comprising primary inhumations and secondary inhumations and cremations.<sup>101,102</sup> A total of 14 published AMS <sup>14</sup>C dates on human remains from Vlasac range (after FRE correction) between *c.* 9300–6000 cal BCE.





**Figure S1.12:** Four reported Vlasac individuals. Upper L-R, 34, 36, 51A. Lower, H53. Upper photos courtesy of the Archaeological Collection of the Faculty of Philosophy, Belgrade

The samples analyzed in the current study come from both the 1970–1 and 2006–2009 excavations. Burial 17 was a seated burial with crossed lower limbs and, on the basis of a direct AMS radiocarbon date, can be dated to the Middle Mesolithic at the end of the 9<sup>th</sup> and the beginning of the 8<sup>th</sup> millennia BCE. Burial 16 was a disarticulated skull found close to Burial 17 and by association could also date to the same Middle Mesolithic time span. Burial 51B was a secondary inhumation, comprising a pile of disarticulated bones. Burial 45 was a disturbed (extended supine?) inhumation burial of which only a small number of bones were found *in situ* (skull, clavicle, right humerus and a number of vertebrae). The skull was found resting on a large stone. Around the skull were found a number of cyprinid teeth (possibly ornamental appliqués originally attached to some form of headgear). Behind the stone supporting the skull was found a pile of cremated human bones and charred cyprinid teeth, designated Burial 45a, although these may represent secondary treatment and disposal of bones exhumed from Burial 45. Adult male Burial 6 was found as an extended supine inhumation and the torso of this individual was covered by ochre while a neonate burial marked as 6a was found on the right shoulder of this inhumation. The disarticulated remains of likely primary disturbed inhumation Burial 9 were found in a natural rocky depression, encircled by large rocks. Burial 80A was an extended supine inhumation of which only lower limbs were preserved *in situ* due to the damage caused by the interment of another extended supine inhumation Burial 80. All these burials can be dated to the duration of the regional Late Mesolithic.



Three of the burials sampled for this study had previously been radiocarbon dated.<sup>103,104</sup> Burial 51A has an AMS <sup>14</sup>C date of 8760±110 BP (OxA-5822) which, after applying a FRE correction, calibrates to 7595–7080 cal BCE, Burial 83 has an AMS <sup>14</sup>C date of 8200±90 BP (OxA-5826) which, after FRE correction, calibrates to 7030–6460 cal BCE, while Burial 45 has an AMS date of 8117±62 BP which, after FRE correction, calibrates to 6680–6375 cal BCE. Based on these results, Burial 51A belongs to the end of the Middle Mesolithic or beginning of the Late Mesolithic in the Iron Gates, while Burials 45 and 83 can be assigned to the Late Mesolithic.

From the 2006–9 excavations, Burials U21, H53, U62, U69 and U64 all come from a multiple burial with a vertical stratification of burial remains found in Trench 3/2006. U21 is a disarticulated child skull found in a secondary burial position on top of the burial sequence and was possibly removed from a primary burial containing the remains of a child of the same age found laid atop of primary burial H63 within a stack of burials at this location. H53 is the last primary burial that caps the sequence of burials and was placed in extended supine position parallel with the Danube with the head pointing to the upstream direction of the river. U62 and U69 were two neonate burials found one on top of the other interred through the remains of a primary disturbed headless burial H63 belonging to an adult female. These two neonates as well as other burials in this sequence apart from the last burial, individual marked as H53, were placed in extended supine positions parallel with the Danube with the head pointing to the downstream direction of the river. The analyzed disarticulated remains marked as U64 were found in the burial fill of primary burial H63 and probably come from partially preserved primary disturbed adult male burial H81, which were disturbed by the interment of H63. If this assumption about the connection between the disarticulated remains in the infill of H63 and the undisturbed remains of H81 were true, U64 dates to the Late Mesolithic of the mid-6<sup>th</sup> millennium BCE based on a direct AMS date for H81 and are several centuries older than the upper part of the burial sequences with burials U21, H53, H63, U62 and U69, which all can be dated to the Mesolithic-Neolithic transition period at the end of the 7<sup>th</sup> millennium BCE (Borić and Griffiths 2015). Burial H232 was found in the same trench as the previously described burial sequence but as a single burial one meter to the south of the burial sequence and was placed directly on top of a cremation pit containing human remains, charcoal, and burnt artifacts (Borić et al. 2009; 2014). Burials H267, H317, and H327 were found in Trenches 3/2007 and 1/2008 as single burials to the west of the described burial sequence all three placed as extended supine inhumations parallel with the Danube and with the head pointing in the downstream direction.

We report genetic data from 17 individuals:

**1970-1 excavations: (C. Bonsall, D. Borić)**

- I4660 / VLSC\_51B
- I4870 / VLSC\_45
- S5772.E1.L1 / VLSC\_16
- S5773.E1.L1 / VLSC\_17
- S5771.E1.L1 / VLSC\_6
- I4871 / VLSC\_80A
- I4872 / VLSC\_9

**2006-9 excavations (D. Borić)**

- I4874 / VLSC\_H232
- I4875 / VLSC\_H267
- I4876 / VLSC\_H317
- I4877 / VLSC\_H327
- I4873 / VLSC\_H53

- I4878 / VLSC\_U21
- I4880 / VLSC\_U62
- I4881 / VLSC\_U64
- I4882 / VLSC\_U69

#### Unknown

- I4657 / VLSC\_1G/3

## Ukraine

### Alexandria (1 individual)

An Eneolithic cemetery of the Sredny Stog II culture was excavated by D. Telegin in 1955-1957 near the village of Alexandria, Kupyansk district, Kharkov region on the left bank of the river Oskol.<sup>105</sup> A total of 33 individuals were recovered.<sup>106</sup> Based on craniometric analysis (I. Potekhina 1999) it was suggested that the Eneolithic inhabitants of Alexandria were not homogeneous and resulted from admixture of local Neolithic hunter-gatherers and early farmers, possibly Trypillian groups.<sup>107</sup> We report genetic data from one individual:

- I6561

### Dereivka I (23 individuals)

This is the largest known Neolithic cemetery of the Mariupol type, containing 173 burials. It was excavated by D. Telegin in 1961-1967,<sup>108</sup> and anthropologically characterized by G.Zinevich in 1967 and I. Potekhina in 1978.<sup>109,110</sup> It is located on the right bank of the Omelnik tributary of the Dnieper River, near the village of Dereivka, Onufriivsky district, in the Kirovograd region,<sup>111</sup> in the southern part of the middle Dnieper, at the boundary between the forest-steppe and the steppe zones. It contains both single and multiple burials, most of which are in extended supine position. According to craniometric analysis, the Dereivka I population consists of two components, one of which was similar to previous hunter-gatherers of the same region while another is more closely related to individuals from the northern forest zone.<sup>112</sup>

The main collection of skeletal remains from Dereivka is housed in the Anthropology Archives of the Institute of Archaeology of the National Academy of Sciences of Ukraine in Kiev (Inventory #196-449). The samples analyzed here are from dental material from the main collection in Kiev that came to the Institute of Ethnology and Anthropology of the Russian Academy of Sciences in Moscow for odontological study and that were accessioned there.

We report genetic data from 23 individuals:

- I3717 / Grave 42
- I3718 / Grave 73
- I3719 / Grave 102
- I4110 / Grave 9
- I 4111 / Grave 123
- I 4112 / Grave 1
- I 4114 / Grave 103
- I5875 / Grave 53

- I5876 / Grave 142
- I5881 / Grave 20
- I5882 / Grave 94
- I 5883 / Grave 39
- I5884 / Grave 68
- I 5885 / Grave 84
- I 5886 / Grave 12
- I 5889 / Grave 109
- I 5890 / Grave 87
- I 5891 / Grave 18
- I 5892 / Grave 33
- I 5893 / Grave 93
- I 5878 / Grave 41
- I 5879 / Grave 78
- I 5888 / Grave 27

### Ilyatka (3 individuals)

The site is located in the Stara Synjava district of Khmelnytskyi region (Ukraine) on the right bank of the Iqua river ~1 km away from Ilyatka village. The site is located on the western slope of the Kohaniv valley, 200 m from the river bank. It was found accidentally by local workers, and became the subject of an emergency excavation in 2011. Three limestone plates were placed tightly together on top of the grave. Under the plates one group of mixed bones and two separates burials were located. All skeletons form one burial complex.

The group burial consists of the remains of 5 individuals, two of which we report genetic data from (Individuals 1 and 2, ILK001 and ILK002). The bones in this grave were arranged artificially. Smaller bones were placed on the ground layer, skulls were placed on top of them – three in a row from south to north, and two perpendicular to it. Long bones and pelvic bones were placed above. All bones formed a four-angular plateau, 1.55 x 0.95 m. In the grave two pairs of horseshoe shaped horn buckles and linear ornaments were found.<sup>113</sup>

East of the main burial group, a sixth skeleton was placed with its head directed to the South. Under the skull a limestone pillow was found. Near it were fragments of an ornamented black pot and a fragment of a polished non-ornamented amphora of brown-red color, indicating that the complex belonged to the Globular Amphora Culture (GAC). Finally, on the other side of the pit, under the western stone plate, separated from the group burial with 2 stones, was a female skeleton (Individual 7, I ILK003), for which we also report genetic data.

- ILK001
- ILK002

Individuals 1 and 2 are adult males based on anthropological and genetic analysis, aged ~45-55 and ~40-50 years. The radiocarbon dates from the two skeletons of the main burial group suggest that they were deposited at the same time.

- ILK003

Female aged 20-30 years. She was lying on her back, in a hyper-flexed position, characteristic of the podolian group of the GAC, following a North-South axis (head facing the North), with a stone pillow under the skull. No grave goods were found.

### Ozera (1 individual)

A sample from Ozera, Kurgan 18, grave 14 which was excavated by L. Chernykh in 1999. It is located 4 km southeast of Ordzhonikidze, Dnepropetrovsk region. In the burial mound, 22 graves were investigated, including two Eneolithic graves, and six of the Yamna (Pit Grave) culture (Nos. 10, 12, 14, 15, 19, 22), with the other burials belonging to later cultures of the Bronze Age.

- I1917 / OAE-99

The poorly preserved skeleton of a young woman (20-25 years old). She was buried in crouched position on her right side, with her head to the northeast. The left arm is bent at the elbow, the right arm, apparently straightened—the elbow was missing—was directed towards the knees. The skeleton is colored with brown ochre, particularly intensely on the skull. There was no inventory.

### Shevchenko (2 individuals)

Seven Yamna (Pit Grave) culture burials were discovered by L. Chernykh in 2003 when excavating the burial mound (kurgan) #28.<sup>114</sup> It is located on the terrace of the left bank of the Solena River, 500 m east of the small village of Shevchenko, a suburb of the city of Ordzhonikidze (renamed Pokrov in 2017), Dnepropetrovsk region. A total of 17 burials were found in this burial mound. In addition to the seven burials of the Yamnaya culture (Nos. 6, 9, 12 -16), seven burials belonged to the Catacomb culture, one to the Middle Bronze Age, one to the pre-Scythian period, and one to the Sarmatian. We report genetic data from two individuals:

- I2105 / Yamna 4

Kurgan 28, grave 6 contained a poorly preserved skeleton of a 45-55 years old female. The pit was covered with two stone slabs, including one anthropomorphic stele in the form of an elongated pentagon. The skeleton lay on its right side, with the head to the NW. The legs were bent almost at right angles to the spine, tibia bones were pressed against the femoral, heels near the pelvis. Arms bent at the elbows. On the skull there were traces of dark red ochre.

- I3141 / Yamna 5

Kurgan 28, grave 12 contained a poorly preserved skeleton of a 35-45 years old female. The skeleton was lying on its back, with the head to the SE. Legs are bent with knees up, arms extended and slightly withdrawn from the body. From the cervical vertebrae along the right humerus lay a necklace made of bone beads, terminated at the elbow with a mallet-shaped pin. At the level of the elbow bones, near the pelvis, there were remains of the newborn child. Near the left foot were two hoofs (sheep-goat), at the bottom of the pit there were traces of multi-layered plant litter.

### Vasil'evka (5 individuals)

#### Vasil'evka 2

The Vasil'evka 2 burial ground located near Vasil'evka village (Dnepropetrovsk region) was excavated by A. Stolyar in 1953. The cemetery was initially assumed to be Neolithic because of the burial rituals<sup>115</sup> but was later attributed to the Late Mesolithic based on radiocarbon dating.<sup>116</sup> Our data contains an even earlier date for one the samples: 7446-7058 calBCE (8190±60 BP, Poz-81129) for I1734, confirming the latter interpretation.

The pattern of the burials is different than both Mesolithic and Neolithic burial grounds of the region. While the Neolithic Mariupol and Vovnigi cemeteries are characterized by collective burials where deceased were placed in long trenches, in Vasil'evka 2 individuals were buried in a single or double grave pits arranged in lines. These consisted up to five burials, possibly reflecting the family structure of the Vasil'evka 2 society. In total the burial ground includes 27 single and double grave pits.

All skeletons in Vasil'evka 2 hold supine position with the arms stretched out along the body, which is very characteristic of Neolithic, but not Mesolithic, burials of the Azov-Dnieper area. Some skeletons were covered with ochre. The use of old graves for repository burials has also been reported. The inventory of burials consists mostly of horn bracelets with geometrical ornaments, and pharyngeal teeth of the fish *Rutilus frisii* which inhabits most of the rivers of the Black and Azov sea areas of Ukraine. In one grave a crafted tortoiseshell was found.<sup>115</sup> One male skull from burial 10 has traces of intravital trepanation with obliterated edges. Craniometric studies indicated that Vasil'evka 2 people resulted from admixture of local Mesolithic populations similar to Vasil'evka 1 and 3 and groups that migrated to the area from more Northern forest-steppe area of present-day Ukraine.<sup>117</sup>

- I1736 / 6285-11
- I1734 / 6285-14

### Vasil'evka 3

We report genetic data from three individuals from the Neolithic Vasil'evka 3 cemetery. We also report 1240 capture data from one individual with previously reported shotgun data<sup>65</sup>, and a more complete description of the cemetery can be found in that reference.

The human remains from Vasil'evka 3 were originally studied by Konduktorova (1973) and Gokhman (1966). Based on craniometric analysis, it was suggested that Mesolithic groups of the Ukrainian steppe had origins in the local Upper Paleolithic groups who in turn were biologically related to the Middle Paleolithic populations. In later periods, cranial characteristics similar to Vasil'evka 3 are observed for populations of the Yamna culture. It was also argued that the population of Vovnigi 2 resulted from admixture of local Mesolithic groups and migrants from Northern territories.<sup>117</sup> We report genetic data from four samples:

- I1733 / 6462-30
- I1737 / 6462-11
- I1763 / 6462-23

We also report new genome-wide 1240k capture data from the following individual with previously published shotgun data:<sup>65</sup>

- I1819 / 6462-25 / Ukraine\_HG1

### Verteba Cave (4 individuals)

Verteba cave is located in the Podillya region in modern-day western Ukraine. Verteba is one of very few Eneolithic Trypillian culture (TC) (ca. 5,400/4,900–2,700 BCE) sites to contain human remains attributed to TC and the only site discovered to date to contain remains from the pre-CII period of Trypillian chronology.<sup>118,119</sup> Human remains from the Late Bronze Age have also been found at Verteba.<sup>119</sup>

The specimens reported here were recovered during excavations at Verteba undertaken under the direction of the Borschiv Museum of Regional History and Ethnography in Borschiv, Ukraine (M. Sokhatsky, Museum and excavations Director) in 2007 and 2008. Human osteological material from Verteba is housed at the Borschiv Museum of Regional History and Ethnography in Borschiv, Ukraine.

We report genetic data from four individuals:

- I1926 / 1 V1a-H1 – V3.17.1
- I2110 / 4 V4a-H4 – V3.14.1
- I2111 / 5 V5a-H5 – V3.13.1
- I3151 / 6 V6a-H6 – V3.15.1

V3.13.1, V3.14.1, V3.15.1, and V3.17.1 are four crania found at site 7.<sup>118</sup> The crania were arranged in a group, located at the same level as the cave floor, and separated from the general cave deposits by a stone wall.<sup>120</sup> Site 7 where the crania have been discovered has been dated to the range of 3700–2700 BCE, with the peak activity at the site around 3500 BCE.<sup>118</sup>

#### Vil'nyanka (Volniensky) (12 individuals)

The Vil'nyanka Mariupol-type cemetery is situated on the left bank of the Dnieper River South of the Vilny rapids. It was excavated by D. Telegin in 1956.<sup>108</sup> Excavation artefacts are housed at the Institute of Archaeology in Kiev, Ukraine (Collection #355).<sup>121</sup> In total, 50 skeletons were recovered from this cemetery, in various degrees of preservation. The majority – 30 in total – were those of adults. All the burials were in extended supine position: arms slightly bent at the elbows, hands near the pelvis, and legs straight and close together.<sup>111</sup> The cemetery contains several grave pits, at different stratigraphic depths, and of different types indicating that this cemetery may have been in use for a long period of time. Six groups of grave pits are differentiated based on their locations and depth: A1-A6. The human remains from Vil'nyanka were first studied by T. Surnina (1961).<sup>122</sup> Analysis of the skull series showed the presence of two different craniological versions in Vil'nyanka.<sup>123</sup>

- I3714

Grave 28 was a 20-25 years old male in grave pit B1. On both sides of the skull lay four deer tooth pendants. Five similar pendants were found below the pelvis as well as two additional pendants placed on the bones of the left foot. Between the tibial bones of skeleton 28 lay a child's skull (No. 27a).

- I3715

Grave 9 is part of Grave Pit A1 and contains a 20-25 years old female. The skeleton was damaged by the digging of the grave pit for burial 19. Grave goods consisted of a deer tooth pendant placed between the ribs on the left side, and a number of fish teeth.

We also report genetic data from the following nine individuals:

- I3712 / Grave 35
- I3713 / Grave 1
- I3716 / Grave 25
- I5868 / Grave 27
- I5869 / Grave 8
- I5870 / Grave 32



- I5872 / Grave 26
- I5873 / Grave 14
- I5957 / Grave 10
- I6133 / Grave 13

### Vovnigi 2 (2 individuals)

We report genetic data from two individuals from the Vovnigi 2 Neolithic cemetery (Vovnigi village, Dnepropetrovsk region). We also report new data for one individual that has previously been reported with shotgun data,<sup>65</sup> and a more complete description of the site can be found in that reference.

- I1732 / 6204-7
- I1738 / 6204-4

We also report new genome-wide 1240k capture data from the following individual with previously published shotgun data:<sup>65</sup>

- I1378 / Ukraine\_N1

While this article was in press, we identified genetically that that I1732 is likely the mother of I1378, although we note that the radiocarbon dates for these samples are inconsistent with this identification. We therefore retained I1378 in the analysis, even though it is likely a first-degree relative.

## Appendix A: The Iron Gates / Danube Gorges

Six sites included in this study – Hajdučka Vodenica, Lepenski Vir, Ostrovul Corbului, Padina, Schela Cladovei and Vlasac– are situated in that part of the Lower Danube Valley that forms the modern political border between Romania and Serbia, known archaeologically as the *Iron Gates* or *Danube Gorges* region. Here, a large series of Mesolithic and Early Neolithic sites were discovered during archaeological surveys and rescue excavations ahead of dam construction in the 1960s and 1980s.

The Iron Gates reach of the Danube falls naturally into two contrasting physiographic zones – the 130-kilometer-long Iron Gates Gorges (sometimes referred to as the “Danube Gorges”) where the river cuts through the Carpathian Mountain range, and the 80-kilometer-long “Downstream Area” of more moderate relief where the Danube enters the Wallachian Plain. The Iron Gates I dam marks the *de facto* boundary between these two zones, while the “Downstream Area” ends at the Iron Gates II dam.

The Iron Gates region has a more-or-less continuous record of Stone Age settlement from c. 12700–5500 cal BCE, and over 400 Mesolithic and Early Neolithic burials have been recorded from 15 sites. Research involving paired AMS  $^{14}\text{C}$  dating and stable isotope analysis of human remains and associated terrestrial animal bones has shown that radiocarbon dates on human bone are often too old (i.e. older than their archaeological context, by up to 550 years) due to regular consumption of fish from the Danube<sup>124</sup>. Cook *et al.* (2009) developed a correction for this “freshwater reservoir effect” (FRE) based on the  $\delta^{15}\text{N}$  value of human bone collagen, and this FRE correction has been applied to all  $^{14}\text{C}$  dates for Mesolithic human remains from the Iron Gates reported here.<sup>8,125</sup>

## Appendix B: Chronology of the Balkan Peninsula

Period	Approx. date range
Early Neolithic	6200 - 5500 BCE
Late Neolithic	5500 - 5000 BCE
Early Chalcolithic	5000 - 4500 BCE
Late Chalcolithic	4500 - 4000 BCE
Final Chalcolithic	4000 - 3600 BCE
Early Bronze Age	3600 - 2000 BCE
Middle Bronze Age	2000 - 1500 BCE
Late Bronze Age	1500 - 1100 BCE

**Table S1.2:** Approximate dates for archaeological periods referred to in the main text

## Appendix C: Chronology of Ukraine

Period	Cultures	Approx. date range
Mesolithic	Kukrek, Grebenyky, Mariupol	10000 - 6500 BCE
Neolithic	Bug-Dniester, Sursky, Mariupol	6500 - 5500 BCE
Late Neolithic-Early Eneolithic	Mariupol, Trypillia	5500 - 4400 BCE
Middle Eneolithic	Trypillia, Sredny-Stog II	4400 - 3500 BCE
Late Eneolithic	Trypillia, Mikhailivka, Usatovo	3500 - 3200 BCE
Early Bronze Age	Yamna, Catacomb	3200 - 2300 BCE
Middle Bronze Age	Catacomb, Babino/KMK	2300 - 1700 BCE
Late Bronze Age	Srubnaya, Sabatinovskaya	1700 - 900 BCE

**Table S1.3:** Approximate dates for archaeological periods referred to in the main text.<sup>118,126,127</sup>

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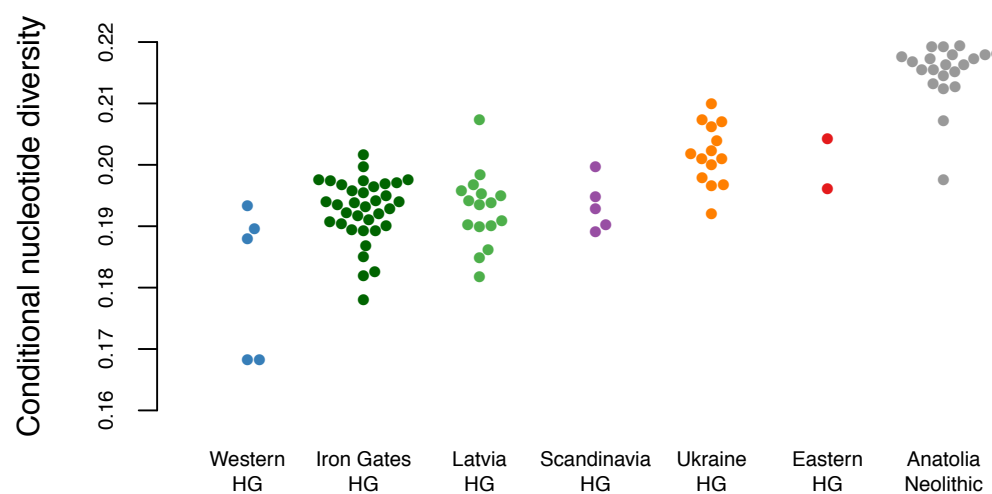
## Supplementary Note 2: Diversity and phenotypically informative markers in hunter-gatherer populations.

### Introduction

At least some Mesolithic hunter-gatherer groups had combinations of phenotypes that are unusual in present-day populations. In particular, western European hunter-gatherers (WHG) typically lacked the variants that contribute to light skin pigmentation in present-day Europeans, though the *OCA2/HERC2* variant that is the major determinant of light (including blue) eye color was common<sup>1,2</sup>. In this note, we describe the distribution of these, and other, phenotypically important markers in Mesolithic and Neolithic hunter-gatherer groups from both eastern and western Europe.

### Diversity

Mesolithic hunter-gatherers have been shown to have had lower genetic diversity than either Neolithic farmers, or present-day Europeans,<sup>3,4</sup> and we explored hunter-gatherer diversity in much richer detail using the dataset newly reported here. Specifically, we computed a version of conditional nucleotide diversity<sup>3,\*</sup> for the hunter-gatherer individuals in our dataset (Figure S2.1), which confirms that hunter-gatherers had lower diversity than NW Anatolian Neolithic farmers, but also suggests that diversity increases along the WHG-EHG cline – consistent with the suggestion of a stronger bottleneck in WHG relative to EHG.<sup>5</sup>



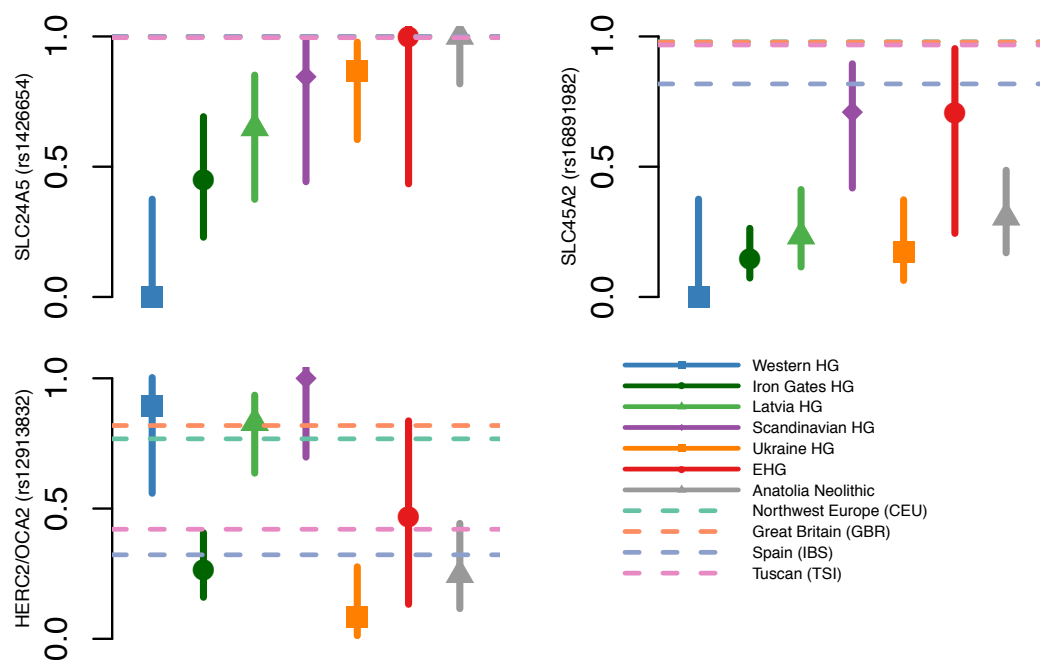
**Figure S2.1:** Conditional nucleotide diversity (CND) of hunter-gatherers ( $N=100$  individuals). We exclude individuals with fewer than 5,000 SNPs hit with coverage  $>1$ , two WHG individuals with  $CND < 0.05$ , and the individual I0013/Motala3, which has  $CND=0.21$  and may be contaminated by another Motala sample.<sup>5</sup>

\* We restrict to a set of 1240k SNPs that had been ascertained in a single Yoruba individual.

For each individual, we compute  $\frac{2}{N} \sum_{i=1}^N \left\{ \frac{n_i^2 - k_i^2 - (n_i - k_i)^2}{n_i(n_i - 1)} \right\}$  where  $N$  is the total number of SNPs covered by  $>1$  read,  $n_i$  is the number of reads and  $k_i$  is the number of non-reference reads covering SNP  $i$ . The term in brackets is the probability of sampling two different alleles if we subsampled two reads from the observed data (this is expected to be half the heterozygosity).

## Pigmentation

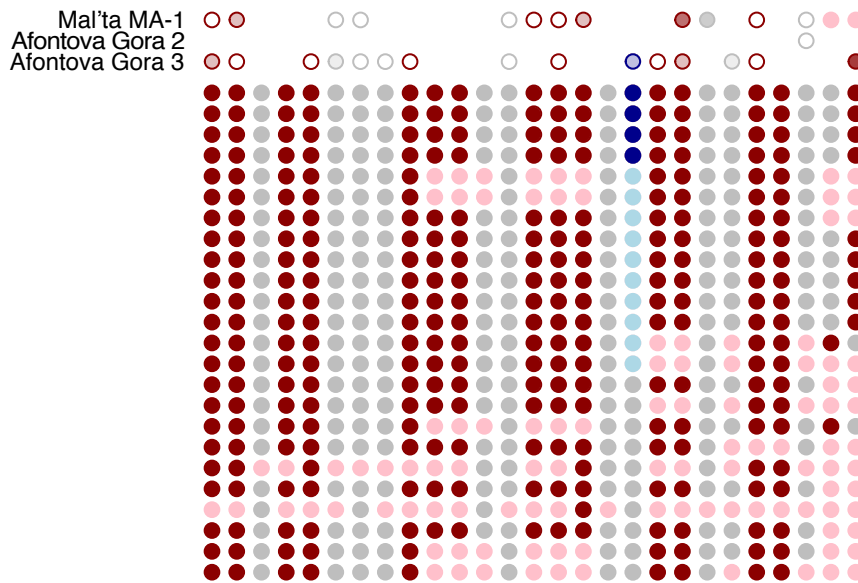
Western hunter-gatherers (WHG) had a distinctive blue-eyed, dark skin pigmentation phenotype<sup>1,2</sup> that emerged in the Mesolithic.<sup>6</sup> In contrast, we show that Mesolithic and Neolithic individuals from Ukraine, Latvia and the Iron Gates had, like Scandinavian and Eastern hunter-gatherers, intermediate to high frequencies of the derived skin pigmentation allele at *SLC24A5*. Unlike Scandinavian and Eastern hunter-gatherers, however, they have low frequency of the derived *SLC45A2* allele. The derived *OCA2/HERC2* allele associated with light (particularly blue) eye color is common in WHG, SHG, and hunter-gatherers from Latvia, but at low frequency in hunter-gatherers from Ukraine and the Iron Gates. This allele appears to be differentiated in a North-South gradient, as it is today – suggesting the possibility of long-term balancing selection due to geographic variation in selective pressure. The WHG phenotype of light eye and dark skin pigmentation<sup>1</sup> thus appears to be restricted to western Europe and is far from universal in European hunter-gatherers, with light skin pigmentation common in Northern and Eastern Europe before the appearance of agriculture.



**Figure S2.2** Maximum likelihood allele frequency estimates (points) and 95% confidence intervals (bars) for three major pigmentation alleles, in different hunter-gatherer populations ( $N=11,40,16,6,36,3$  individuals), in order of WHG-EHG-related ancestry (Anatolian Neolithic [ $N=19$  individuals]) and four present-day populations included for comparison

The derived allele of the *KITLG* SNP rs12821256 that is associated with – and likely causal for – blond hair in Europeans<sup>7,8</sup> is present in one hunter-gatherer from each of Samara, Motala and Ukraine (I0124, I0014 and I1763), as well as several later individuals with Steppe ancestry. Since the allele is found in populations with EHG but not WHG ancestry, it suggests that its origin is in the Ancient North Eurasian (ANE) population. Consistent with this, we observe that the earliest known individual with the derived allele (supported by two reads) is the ANE individual Afontova Gora 3,<sup>6</sup> which is directly dated to 16130–15749 cal BCE (14710±60 BP, MAMS-27186: a previously unpublished date that we newly report here). We cannot determine the status of rs12821256 in Afontova Gora 2 and MA-1 due to lack of sequence coverage at this SNP.<sup>9</sup>





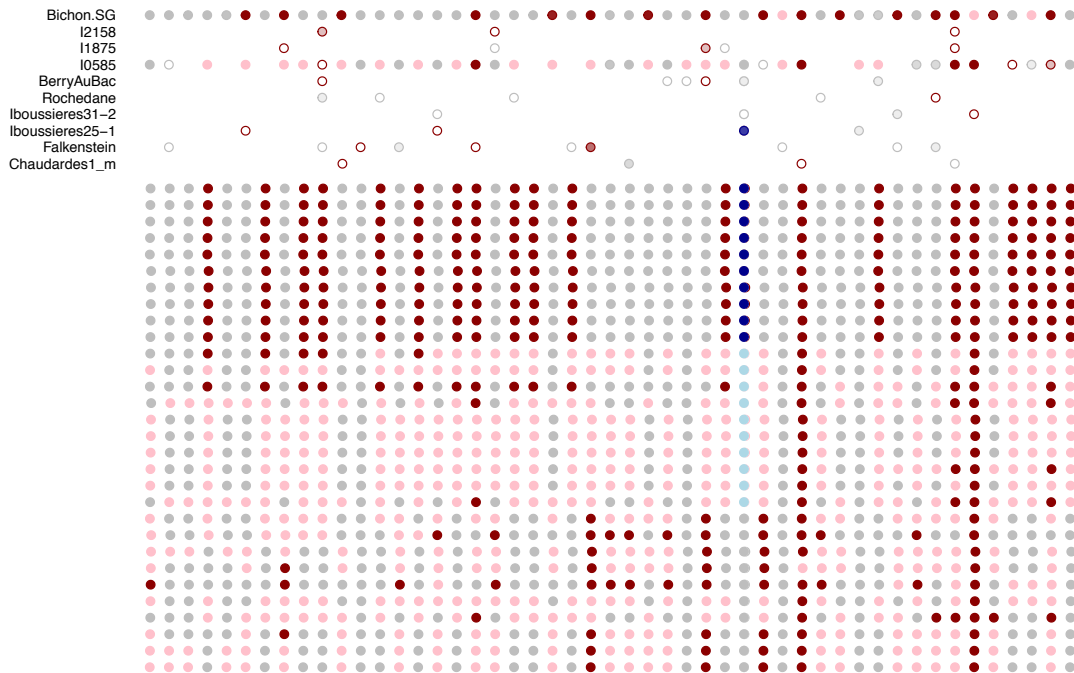
**Figure S2.3:** Haplotypes at the *KITLG* locus for ANE individuals, and 24 present-day individuals from the GBR population of the 1000 Genomes project. Each column is a SNP with the focal SNP rs12821256 in blue. Each row is an individual. Grey points indicate homozygosity for the ancestral allele, light colored heterozygosity, and dark points homozygosity for the derived allele. Ancient individuals are shaded according to how many reads there are at that site. Open circles means there is only one read, darker colors indicate more reads.

## EDAR

The derived allele of rs3827760 in *EDAR*, which is common and has been a target of strong selection in the ancestors of present-day East Asians, is present in a single copy in one Middle Neolithic individual from Latvia (I4435), consistent with previous observations of the allele in hunter-gatherers from Motala in Sweden.<sup>2</sup> This continues to support the possibility that this allele may have originated in the Ancient North Eurasians and not in ancestral East Asians.<sup>2</sup>

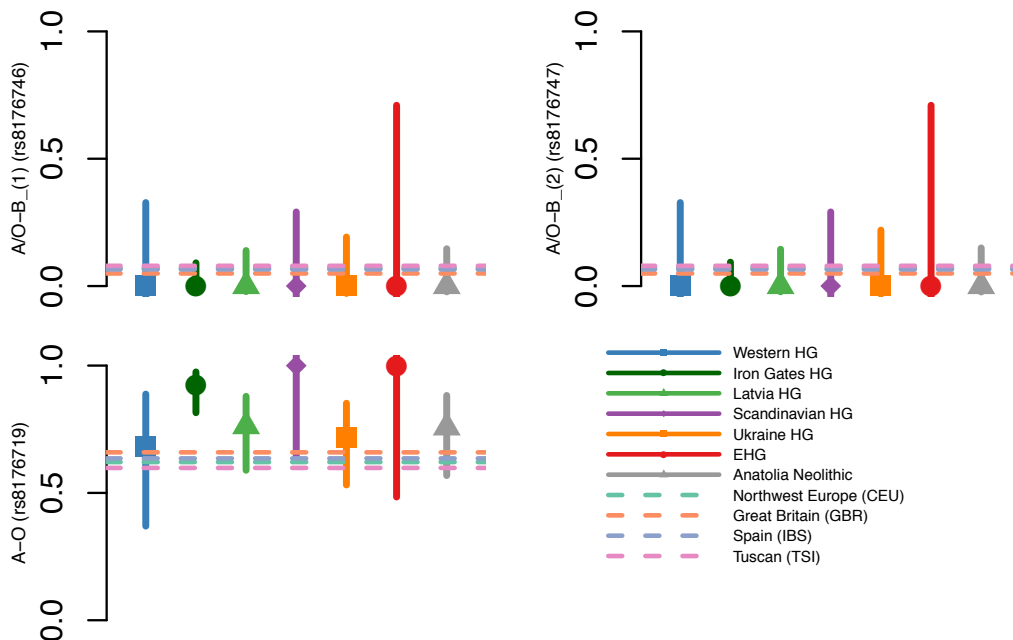
## Lactase persistence

The approximately 12,000 year old WHG individual Iboussieres-25 appears to carry the derived allele at the SNP rs4988235 that is strongly associated with lactase persistence in present-day Northern Europeans<sup>10</sup>. Four reads at this SNP all carry the derived allele, although we caution that this is a C>T SNP in a non-UDG treated sample and so might be affected by deamination, and two reads at neighboring SNPs do not support the persistence haplotype, at least in a homozygous state (Supplementary Figure S2.3). The observation of this allele, long before domestication and dairying, would be surprising, but might be consistent with observation of lactase persistence in early Neolithic populations in Iberia and Sweden<sup>11,12</sup> – observations that were themselves surprising based on the absence of persistence in large samples of Anatolian Neolithic and LBK individuals<sup>2,13</sup>. One possibility is that the allele was widely distributed at low frequencies before being strongly selected in the Bronze Age, perhaps due to the spread of dairying. We also note that the Ukrainian Eneolithic individual I6561 dated to ~4000 BCE that we report is the oldest known individual with UDG-treated genome-wide data that carried a copy the persistence allele.



**Figure S2.4:** Haplotypes at the *LCT* locus for WHG individuals (top, labeled), and 30 present-day individuals from the GBR and TSI populations, with 10 of each genotype at the focal SNP rs4988235 (in blue). Each column is a SNP, each row is an individual. A grey point indicates homozygous for the ancestral allele, light colored heterozygous, and dark colored homozygous for the derived allele. Ancient individuals are shaded according to how many reads there are at that site, so an open circle means there is only one read, and darker colors indicate more reads, and therefore more certainty about homozygous genotypes.

### ABO blood group



**Figure S2.5:** Maximum likelihood allele frequency estimates (points) and 95% confidence intervals (bars) at three markers associated with *ABO* blood type ( $N=11,40,16,6,36,3$  individuals). Top: Two SNPs associated with type B. Bottom left: Frequency of deletion associated with type O.

We estimated allele frequencies at three markers that largely determine ABO blood group.<sup>14</sup> The 1-base deletion rs8176719 that is the most common type O mutation is at high frequency in hunter-gatherers. Combining all hunter-gatherer populations, we estimate the frequency of the O allele to be 84% (95% CI: 76-89%), which implies that the frequency of the type O phenotype which requires homozygosity for the O allele was 71%. This is significantly higher than the O allele frequency in present-day Europeans (60-65% in 1000 Genomes populations, with corresponding phenotype frequency ~40%). We do not detect the B allele (rs8176746 and rs8176747) in any hunter-gatherers suggesting that all other individuals were type A. In fact, the B allele is not seen in any Neolithic populations either, and is introduced into Europe by Steppe populations who we estimate carry it at ~8% frequency. We cannot infer Rhesus blood groups in our data because we are unable to reliably call the *RHD* gene deletion that is, in European-ancestry populations, the most common molecular basis for the Rh- phenotype<sup>15,16</sup> and the SNPs that determine the C (rs676785) and E (rs609320) alleles<sup>16-18</sup> are not part of the 1240k capture array.

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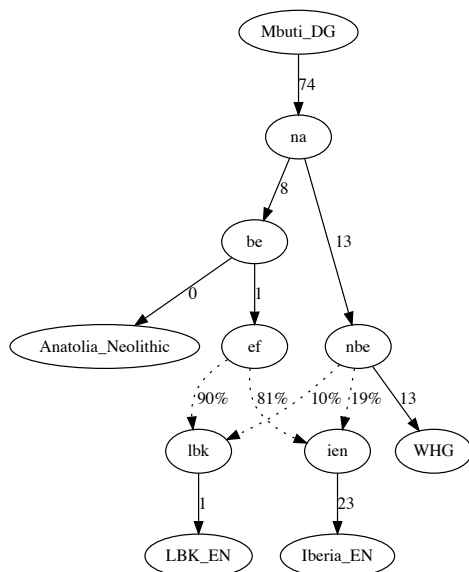
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## Supplementary Note 3: Admixture graph modeling of the relationship among Neolithic populations.

### Introduction

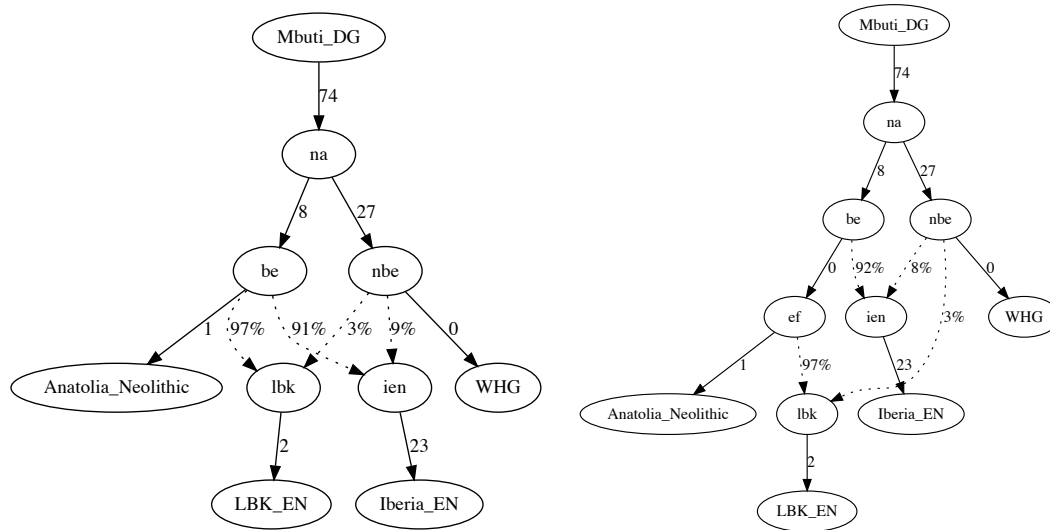
The relationship among Neolithic populations is difficult to establish because many populations have different proportions of hunter-gatherer-related admixture. This means that  $D$ -statistics, for example, are confounded. As a concrete instance of this, neither of the statistics  $D(\text{Mbuti}, \text{Iberia\_EN}, \text{Anatolia\_Neolithic}, \text{LBK\_EN})$  or  $D(\text{Mbuti}, \text{Anatolia\_Neolithic}, \text{Iberia\_EN}, \text{LBK\_EN})$  are significantly different from zero ( $Z=1.6$  and  $Z=1.2$ ), so we cannot resolve the phylogeny. However, note that Iberia\_EN has significantly more WHG-related ancestry than either LBK\_EN or Anatolia\_Neolithic ( $D(\text{Mbuti}, \text{WHG}, \text{LBK\_EN}, \text{Iberia\_EN})$ ;  $Z=3.4$ , for example). Therefore, if we are interested in learning about the relationship between the non-hunter-gatherer components of ancestry in these populations, we need to correct for the differing proportions and types of hunter-gatherer admixture. To do this, we model populations in an Admixture Graph framework, using the software *qpGraph* (<http://github.com/DReichLab/AdmixTools>). This allows us to model multiple admixture events, and to fit all the drift weights and admixture proportions jointly by matching observed  $f$ -statistics as closely as possible.

This resolves some of the issues described above. For example, by explicitly modeling the WHG-related ancestry in Iberia\_EN and LBK, we can show that they are consistent with being a clade relative to Anatolia\_Neolithic (Figure S3.1 and S1.2,  $Z=0.3$  compared to  $Z=3.1$  if there is a trifurcation). *qpGraph* requires us to specify the topology of the admixture graph and thus make explicit assumptions about the relationships between the populations in the graph. Therefore, while it is possible to reject any particular graph based on the data, it is not possible to determine whether a graph that fits is the best fit, or whether there are other graphs that fit equally well. In the rest of this note we describe a series of Admixture Graphs representing the relationship between Neolithic populations across Europe. These graphs are built manually by using a combination of prior information about admixture events and local systematic enumeration of possibilities. They represent plausible, although not necessarily unique, models for the genetic relationships among populations.



**Figure S3.1:** An Admixture Graph showing the relationship between LBK\_EN and Iberia\_EN that fits the data ( $N=53$  ancient individuals in total). Nodes representing populations with data are capitalized and other nodes are lowercase. Dashed lines represent admixture edges, with proportions. Numbers along edges represent drift lengths. We test the goodness-of-fit of this model by computing a jackknife  $Z$  score for the worst-fitting  $f$ -statistic – here  $Z=0.3$  for  $f_4(\text{Mbuti\_DG}, \text{WHG}, \text{Mbuti}, \text{LBK\_EN})$ . Specifically, we compute the expected  $f$ -statistics under the fitted model (all possible  $f_2$ ,  $f_3$ - and  $f_4$ -statistics relating the populations), compared to the observed  $f$ -statistics in the data, and use a block jackknife to obtain a standard error and  $Z$ -score.

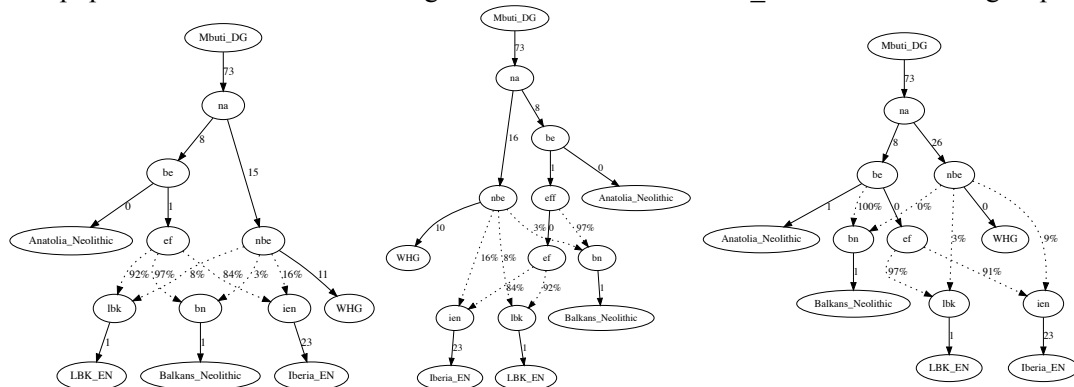




**Figure S3.2:** Systematic local searching to check alternative Admixture Graphs. Two modifications of the graph shown in Figure S3.1 that can be rejected ( $N=53$  ancient individuals in total). Left; the graph supported by naïve  $D$ -statistics where Iberia\_EN is an outgroup to Anatolia\_Neolithic and LBK\_EN rejected with  $f_4(\text{Mbuti\_DG}, \text{Iberia\_EN}, \text{Anatolia\_Neolithic}, \text{LBK\_EN}) = 4.2$ . Right; a graph where the Early Farmer ancestry in the three Neolithic populations trifurcates is rejected with  $f_4(\text{Mbuti\_DG}, \text{Iberia\_EN}, \text{Anatolia\_Neolithic}, \text{LBK\_EN}) = 4.3$ . (These two topologies actually lead to similar models, since one of the drift edges in the left model is fitted as 0, hence the same  $f_4$ -statistic rejects them both. These results tell us that, to fit the observed data, Iberia\_EN and LBK\_EN must share more alleles, leading us to the topology in Figure S3.1. We have not modeled alternative sources of hunter-gatherer ancestry, which could affect these graphs, but we know from PCA and ADMIXTURE analysis that the majority of the hunter-gatherer ancestry in Iberia\_EN and LBK\_EN is consistent with a WHG-related source.

### Balkan, Iberian and LBK Neolithic are a clade relative to Anatolia Neolithic

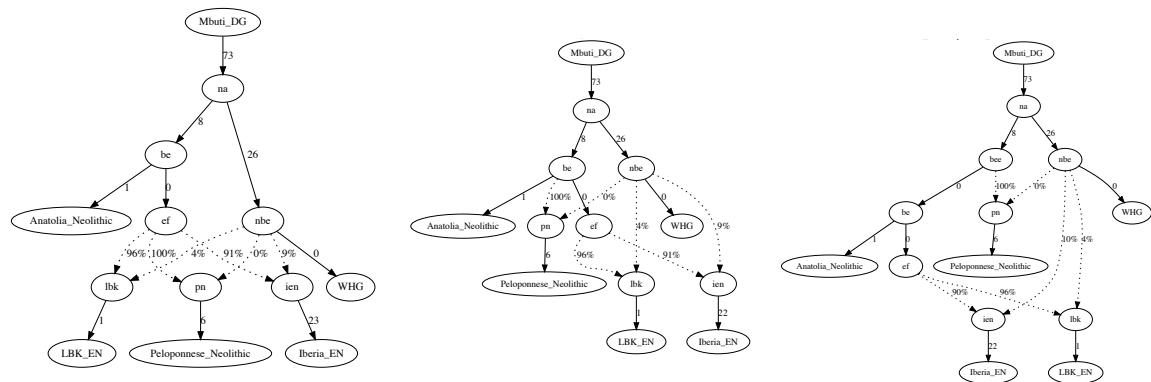
Starting from the best fitting model for LBK and Iberia\_EN (i.e. Figure S3.1), we added Balkans\_Neolithic into the model (Figure S3.3). By systematically moving the point at which the Balkan ancestry splits from the other populations we show that we reject a model where Balkan\_Neolithic shares no drift with LBK and Iberia, consistent with the model that these three populations descend from a single source that has Anatolia\_Neolithic as an outgroup.



**Figure S3.3:** Fitting Balkans\_Neolithic into the graph ( $N=77$  ancient individuals). Left to right, Z-scores are 1.7, 0.7, 4.6 so reject the graph on the right (Balkan Neolithic shares no drift with LBK\_EN and Iberia\_EN). Center: Balkan ancestry is intermediate to (LBK, Iberia) and Anatolia. Left/Right: Balkan ancestry it is moved down and up one node, respectively.

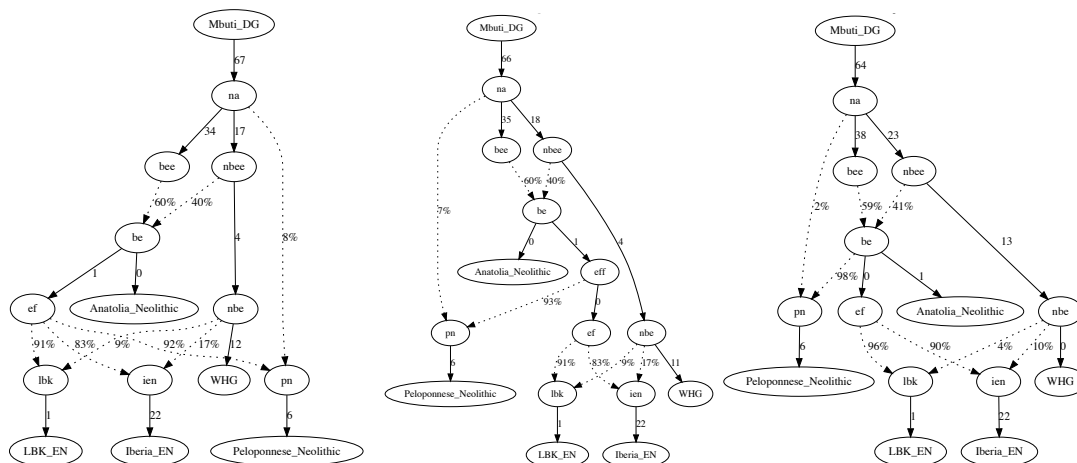
## Peloponnese Neolithic does not fit the same graph as Balkan Neolithic

We attempted to add Peloponnese\_Neolithic to the graph from Figure S3.1 in the same way as we fitted Balkans\_Neolithic (cf. Figure S3.3) but all these topologies were rejected. Therefore, we conclude that Peloponnese Neolithic is not nested within the variation of other European Neolithic populations.



**Figure S3.4:** We attempted to fit Peloponnese Neolithic in the same way as Balkan Neolithic ( $N=59$  ancient individuals). These graphs are all rejected, with  $Z=4.6$ ,  $4.2$  and  $3.8$  (left to right). In the rightmost graph, the worst  $D$ -statistic is  $D(\text{Mbuti.DG}, \text{WHG}, \text{LBK\_EN}, \text{Peloponnese\_Neolithic})$ ;  $Z=3.8$ , which is not obviously interpretable, but might suggest a basal component in Peloponnese Neolithic.

We added a component to Peloponnese Neolithic that was basal to all other populations in the graph, and estimated that 7% of Peloponnese ancestry derived from this source. Other analyses suggest that this component likely has an affinity to CHG (Supplementary Table 2).



**Figure S3.5:** Fitting Peloponnese Neolithic including a basal component, and testing different positions for the non-basal component that is shared with other Neolithic population ( $N=59$  ancient individuals). These graphs have  $Z=1.7$ ,  $0.4$  and  $2.7$  (left to right).

## Limitations of the admixture graph framework

Although the *qpGraph* framework allows us to test whether a specific model is consistent with the observed data, there are a number of limitations to the inferences we can draw. In particular:

- 1) We assume that each population is homogeneous. If there is undetected ancestry or heterogeneity in one of the populations (as seems possible for Peloponnese\_Neolithic), the admixture graph will require extra admixture events to model it.
- 2) The topology of the model is specified in advance. Thus, while we can reject that specific topology, or conclude that it is consistent with the data, we cannot say anything confident about other topologies that might also be consistent. In general, finding the correct topology is a trial-and-error process, and usually has to be informed by external information.
- 3) The fitted admixture graphs are consistent with respect to the populations used in the graph, but a topology that fits in a given case might not fit at all as a subgraph of a larger graph that includes more populations. Thus the results of admixture graph fitting should always be interpreted only in the context of the populations in the graph. In particular, two populations in the graph can be made to share ancestry either by having shared drift, or by admixture from a shared populations. In the cases discussed here, we have not attempted to model structure within the admixing hunter-gather population, which might confound the estimation of shared drift.