

A genomic history of the North Pontic Region from the Neolithic to the Bronze Age

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The North Pontic Region was the meeting point of the farmers of Old Europe and the foragers and pastoralists of the Eurasian steppe^{1,2}, and the source of migrations deep into Europe^{3–5}. Here we report genome-wide data from 81 prehistoric North Pontic individuals to understand the genetic makeup of its people. North Pontic foragers had ancestry from Balkan and Eastern hunter-gatherers⁶ as well as European farmers and, occasionally, Caucasus hunter-gatherers. During the Eneolithic period, a wave of migrants from the Caucasus–Lower Volga area⁷ bypassed local foragers to mix in equal parts with Trypillian farmers, forming the people of the Usatove culture around 4500 BCE. A temporally overlapping wave of migrants from the Caucasus–Lower Volga blended with foragers instead of farmers to form Serednii Stih people⁷. The third wave was the Yamna—descendants of the Serednii Stih who formed by mixture around 4000 BCE and expanded during the Early Bronze Age (3300 BCE). The temporal gap between Serednii Stih and the Yamna is bridged by a genetically Yamna individual from Mykhailivka, Ukraine (3635–3383 BCE), a site of archaeological continuity across the Eneolithic–Bronze Age transition and a likely epicentre of Yamna formation. Each of these three waves of migration propagated distinctive ancestries while also incorporating outsiders, a flexible strategy that may explain the success of the peoples of the North Pontic in spreading their genes and culture across Eurasia^{3–5,8–10}.

The area north of the Black Sea called the North Pontic Region (NPR; Fig. 1 and Supplementary Information section 1.1) has been proposed as the homeland for communities that spoke core-Indo-European languages¹¹, which began to spread across Eurasia by the late fourth millennium BCE following an expansion of the Yamna archaeological complex (hereafter referred to as Yamna). The Yamna expansion largely superseded the rich tapestry of genetic ancestry of preceding populations.

Genome-wide studies of ancient DNA have revealed that the genetic ancestry of post-glacial hunter-gatherer groups in the NPR was derived from a mixture of ancestries related to western hunter-gatherers (WHGs) in the west, and Danubian Iron Gates Balkan hunter-gatherers⁶ (BHG) and eastern hunter-gatherers³ (EHGs) in the east. In Ukraine, the Mesolithic–Neolithic transition (after 5800 BCE) was marked by WHG admixture with the EHG ancestry of previously established local populations⁶.

During the Neolithic period, the western NPR was home to Balkan and central European farming cultures, such as Criș, Starčevo and Linearbandkeramik (LBK), carrying early European farmer (EEF) ancestry, stemming from Anatolian Neolithic farmers (ANF) with different proportions of WHG admixture¹². The Neolithic hunter-gatherer populations of the Dniro Valley (hereafter UNHG (labelled Ukraine_N)) continued to retain the EHG/WHG-based genetic ancestry⁶.

In the early Eneolithic (around 4800 BCE), farming groups of the Cucuteni–Trypillia archaeological complex (hereafter Trypillia) spread eastwards across the Carpathians to the Dniro Valley^{13,14}. The ancestry of Trypillia was primarily EEF-derived with admixtures from BHGs/WHGs and Caucasus hunter-gatherers (CHGs)^{6,15–18}.

During their eastward expansion, Trypillia encountered mobile communities of the Serednii Stih archaeological complex¹³ (hereafter referred to as Stih), which probably formed in the Azov–Dniro–Donets

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Fig. 1 | Map of sampling locations including newly generated data and key context populations. The map was drawn using public domain Natural Earth data with the `rnaturalearth` package in R²⁵. New data from the present study are listed in bold. EBA, Early Bronze Age; MBA, Middle Bronze Age.

area in the first half of the fifth millennium BCE^{19–21}. The presence of early Stih in the Azov steppe around 4700–4500 BCE is supported by strontium isotope analysis of an early Stih individual from the Mariupol necropolis (Supplementary Information section 1). However, knowledge about the genetic ancestry of steppe populations such as Stih (referred to as steppe ancestry^{3–6,10,13}) has been limited until now owing to small sample sizes that revealed highly variable ancestry^{6,13,18}.

In the fourth millennium BCE, a distinctive archaeological complex known as Usatove was established in the northwestern NPR. Sampled Usatove individuals had EEF and steppe ancestries, as well as a Caucasus Eneolithic/Maykop-related genetic component⁵, but the proximate sources of the composing ancestries remain unclear. In the second half of the fourth millennium BCE, the NPR was occupied by diverse groups, characterized by distinct burial rites and pottery types and techniques, and increased mobility, possibly including wheeled wagon transportation². This diversity was eclipsed in the last third of the fourth millennium BCE by the expansion of the Yamna, persisting into the first half of the following millennium.

Genetic ancestry data on the Epipaleolithic to Early Bronze Age populations of the NPR come from a limited number of sites, hampering the understanding of population dynamics, particularly in the time preceding the genetic turnover precipitated by Yamna-related people^{3,4,6,10,18,22}. Here we analyse prehistoric NPR individuals from a much wider selection of archaeological sites than has previously been available, including substantially larger sample sizes from Trypillia, Usatove and Stih. Co-analysing these data with the data reported in the accompanying Article⁷, we examine the contribution of these groups to the genetic ancestry of Yamna with a particular focus on integrating the results of the present study with the archaeological evidence to produce a holistic picture of genetic and archaeological transformations preceding and following the formation of the Yamna.

We generated whole-genome ancient DNA data for 81 ancient individuals from the NPR from the Neolithic to the Bronze Age (data for 76 of these individuals are reported for the first time) (Supplementary Table 1). To generate these data, we sampled 206 skeletal elements and built 462 next-generation sequencing libraries; after screening we took 245 forward into analysis (Supplementary Table 2). We enriched our

analyses by generating 51 direct radiocarbon dates (Supplementary Table 3) and used comparative data to analyse isotopic ratios (Supplementary Information section 1 and Supplementary Table 4). We co-analysed these data with data from the accompanying study⁷ of steppe populations, which includes 291 newly reported individuals and 63 individuals with improved data.

We carried out principal component analysis²³ (PCA), forming the axes using a set of populations⁷ (Fig. 2a and Methods) that are designed to capture Siberian–European hunter-gatherer (top) to West Asian (bottom) differentiation and Eastern–Western European (horizontally top) and Inland–Highland vs Mediterranean²⁴ (horizontally bottom) differentiation. This analysis reveals five major clines. Four—the Caucasus–Lower Volga (CLV) cline, the Volga cline, the Dnipro cline and the European Hunter-Gatherer (EuHG) cline—are described formally in the accompanying study⁷. The fifth, the European Farmer and Hunter-Gatherer (EFHG) cline, is formed by European farmers (central European LBK and populations related to Gumelnița or Karanovo from the Yunatsite site in Bulgaria (Yunatsite Chalcolithic (YUN_CA))) on one side, and BHGs (Serbia_IronGates_Mesolithic), on the other²⁵ (Fig. 2a).

UNHG individuals are located on the ‘eastern’ end of the EuHG cline towards BHG, and the ‘northern’ edge of the Dnipro cline. This suggests that UNHG contributed to later Eneolithic and Bronze Age people on the Dnipro cline, with Core Yamna⁷ at the ‘southern’ end.

The Eneolithic (apart from the Stih) and Bronze Age individuals in Fig. 2a are mostly located towards the ‘farmer’ end of the EFHG cline. Four NPR individuals form a cline between the Core Yamna and steppe Maykop, and although seemingly proximate in PCA to the BP group population consisting of Eneolithic individuals from Lower Volga Berezhnovka and Caucasus Progress-2, qpAdm shows them to be ancestrally different, tracing about half of their ancestry to Siberian/Central Asian Neolithic sources⁷. Two of these (Usatove_I20078 and Zhivotilovka_I17974) are late Eneolithic (3300–3000 BCE) individuals from Moldova. The other two, Csongrád_I5124 from Hungary⁷ and I20072 (Giurgiulești) from Moldova (4300–4000 BCE) are archaeologically associated with the people that left Ochre Graves across the NPR and adjacent Balkan–Carpathian area^{26,27}.

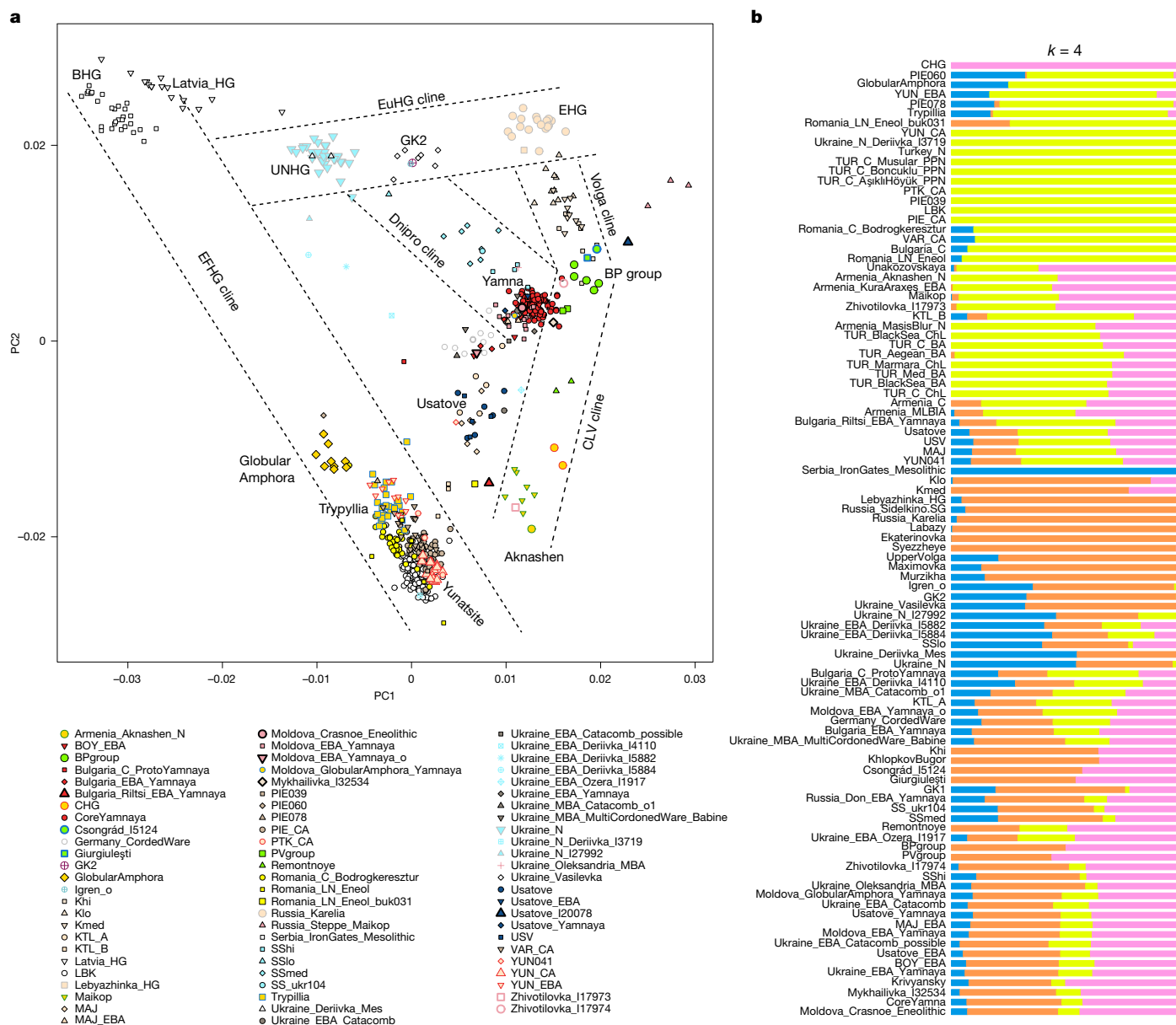


Fig. 2 | Genetic variation in the North Pontic Region. a, PCA of the NPR samples in relation to the three steppe clines (Volga, Dnipro and CLV) and respective samples from ref. 7. Raw coordinates of the plotted points can be found in Supplementary Table 5. **b**, Unsupervised ADMIXTURE summary graph of

populations from this report and ref. 7 (Supplementary Information section 3). Components broadly correspond to CHG (pink), Anatolian–European Neolithic (yellow), BHG (blue), and EHG (orange).

Sources of Neolithic NPR ancestry

We computed f_3 -statistics with UNHG as a target and a wide variety of possible sources (Extended Data Table 1, Supplementary Information section 2 and Supplementary Table 2.3). The results suggest that the UNHG population is, to a first approximation, composed of sources related to EHG and BHG.

However, it is evident from the PCA in Fig. 2a that the UNHG end of the EuHG cline is shifted towards populations with EEF ancestry. In unsupervised analysis with the ADMIXTURE algorithm (Fig. 2b, Supplementary Information section 3 and Supplementary Fig. 3.1), the UNHG are assigned small components of Anatolian Farmer/CHG ancestry, which are not present in Mesolithic Ukraine (Deriivka), EHG (Karelia) or BHG (Iron Gates) groups. When samples from individuals labelled Ukraine_N (UNHG) are modelled with other EuHG populations from ref. 7, only a single 2-source model ($P = 0.576$) with $72.5 \pm 2.9\%$ Golubaya Krinitsa individual GK2 on the Lower Don⁷ and

$27.5 \pm 2.9\%$ BHG ancestry, remains viable (point estimate $\pm$ standard error (a 95% confidence interval corresponds to $1.96 \times$ s.e.m. in either direction of the point estimate)). Fitting to a broader cline between EHG and BHG as a mixture of these two sources with either Lebyazhinka or Karelia as the EHG source fails ($P < 10^{-9}$) and qpAdm output suggests that these models underestimate shared genetic drift with Turkey_N ($Z < -3.5$).

Three-source models (Supplementary Information section 2 and Supplementary Table 2.42) all include EHG and BHG sources along with 7–9% of EEF ancestry, the latter accounting for the underestimated drift with Turkey_N in a model without such ancestry.

To test whether EEF ancestry is a general feature of UNHG populations, we fit a model that included central European LBK, representing EEF ancestry, to 35 individuals with the Ukraine_N label (Extended Data Table 2, Supplementary Information section 2 and Supplementary Information appendix V). The results show that this pattern is not driven by a few outliers.

The UNHG are inferred to have significant BHG and EHG ancestry, and have an increase of BHG ancestry relative to Mesolithic individuals from Vasylivka III⁶ and Vasylivka I²⁸ (Fig. 2). Thus, the genetic evidence is consistent with a migration of people from the Iron Gates area to the Dnipro Valley in the seventh millennium BCE²⁹ being the cause of this shift. As BHG individuals from the Iron Gates have been shown to carry sporadic EEF ancestry⁶, the existence of some Iron Gates-like migrants with such ancestry could account for both BHG and EEF admixture compared to Mesolithic Ukraine.

Hunter-gatherers of mixed WHG–EHG background in the Baltic^{3,24,30} do not carry the EEF ancestry that we detect in the UNHG (Supplementary Information section 2 and Supplementary Information appendix V). The Pitted Ware/Battle Axe Culture populations from Ajvide in Sweden^{31,32} and Västbergs³³, in which EEF ancestry was incorporated into groups of predominantly hunter-gatherer background, are correctly inferred by our model to have around one-fifth EEF-related ancestry. Our finding of EEF-related ancestry in UNHGs provides a separate and much earlier instance of the incorporation of farmer ancestry into the hunter-gatherer communities at the periphery of the Neolithic expansion in Europe.

UNHG individuals I31730 (Mariupol Necropolis, this report) and I1738 (Vovnigi 2⁶), which are inconsistent with the LBK–EHG–BHG model, can be modelled with CHG instead of LBK as a source (Extended Data Table 2), consistent with CHG-related ancestry occasionally extending past the middle Don^{7,34} to the Dnipro Valley¹⁸ during the second half of the sixth millennium BCE⁷.

CLV admixture and long-range mobility

The ancestry of Serednii Stih individuals is examined in detail in ref. 7. Stih could be modelled with one source being the Core Yamna as the endpoint of the Dnipro cline (a proxy for earlier populations in the Eneolithic from which the Yamna descend⁷) and Dnipro–Don hunter-gatherers (UNHG or GK2). Because Core Yamna themselves are consistent with being an approximately 4:1 mixture of CLV cline and Dnipro–Don hunter-gatherer populations⁷, the Stih ancestry formation can be seen as the result of the fusion of CLV cline migrants with Dnipro–Don hunter-gatherers.

The ancestry of a Stih outlier from Igren-8 (I27930; Igren_o; 4400–4000 BCE)⁷ appears to be similar to the Neolithic GK2 individual (5610–5390 BCE) from the Middle Don³⁴ and to Mesolithic hunter-gatherers from Vasylivka I and Vasylivka 2^{6,28} (Fig. 2a) and could be modelled as having approximately two-thirds EHG and one-third BHG ancestry⁷. Individual I27930 thus represents a Neolithic ancestry carry-over in a burial context of Stih³⁵, plausibly appearing in the Dnipro Valley as a result of a long-range migration from the Middle Don or continuing the Mesolithic ancestry of the nearby Vasylivka.

Individual I20072 (4330–4058 cal BCE) from Giurgiulești on the Lower Danube is cladal with the Lower Volga–North Caucasus Eneolithic groups (Supplementary Information section 2 and Supplementary Table 2.1). Along with the contemporaneous Csongrád individual from Hungary, they represent an example of long-distance migration across an even larger range than individual I27930 from Igren (Igren_o), spanning from the Volga to the heart of Central Europe.

Trypillia and Usatove

Admixture f_3 statistics involving Trypillian individuals from this report and refs. 6, 15–17 show that they are admixed⁶ (Extended Data Table 1), with more hunter-gatherer ancestry than EEF groups such as Yunatsite or LBK, but without a more refined understanding of ancestry sources³⁶. A qpAdm model with BP group, YUN_CA and BHG is feasible for 23 out of the 24 Trypillians, all of which include some CLV (Extended Data Table 3 and Supplementary Information section 2, page 107). For these 23 Trypillia individuals, genetic ancestry is, on average, 81%

Balkan Eneolithic (such as in YUN_CA), 14% BHG and 5% CLV-derived BP group (Table 1 and Extended Data Table 4). According to DATES³⁷ (distribution of ancestry tracts of evolutionary signals), the formative admixture of Trypillia took place in 4595 ± 121 BCE (95% credible interval 4832–4358 BCE) (Fig. 3, Table 1 and Extended Data Table 4).

Usatove individuals from our study and ref. 5 are genetically varied and occupy the space in the PCA between the Trypillians and the area where the CLV, Volga and Dnipro clines intersect. Formal modelling with qpAdm reveals that the Usatove population can only be fit ($P = 0.128$) as a mixture of around 45% PV group (intermediate group on the CLV cline) and ~55% Trypillians (Table 1). A generalized three-way model (Supplementary Information section 2) confirmed that the CLV ancestry in Usatove was not from the Lower Volga-centred BP group, but had a significant proportion of southern Caucasus Neolithic (Aknashen)-related ancestry⁵. In contrast to Usatove, the CLV admixture in the Cernavodă I population from Kartal (KTL_A⁵) in the Danube delta is best estimated as BP group-derived, with relatively less or no Aknashen-related ancestry (Table 1). We estimate using DATES³⁷ that the formative admixture of Usatove took place in 4471 ± 51 BCE (95% credible interval 4571–4371 BCE) (Fig. 3 and Table 1).

Yamna ancestry and Caucasus admixture

Following ref. 7, we define a group that we call Core Yamna, which is represented by a genetically homogeneous set of 104 individuals with high data quality that are archaeologically assigned to the Yamna and Afanasievo cultures. In ref. 7, these individuals are shown to be from mixed origins around 4000 BCE and to have formed an ancestral population that expanded from a small founding size around 3750–3350 BCE. Core Yamna is also the largest ancestral source in all individuals with Yamna ancestry, who differ only in having additional admixture from local populations who the Core Yamna must have encountered during their expansion⁷. In ref. 7, multiple lines of evidence indicate that the Core Yamna and probably the Yamna itself formed in the Dnipro–Don area of the northeastern NPR region, but do not narrow their geographic origin on the basis of genetic evidence alone.

Reference 7 further showed that the Core Yamna can be modelled as a mixture of CLV and NPR hunter-gatherer groups. When EEF ancestry is forced as an additional source into the Core Yamna beyond CLV and NPR hunter-gatherer sources (Extended Data Fig. 1, Supplementary Information section 2 and Supplementary Information appendix III), its proportion is not significantly greater than zero ($3.2 \pm 3.1\%$), whereas that of the Caucasus Neolithic is ($15.6 \pm 4.3\%$), suggesting that Anatolian-related ancestry¹⁰ in the Core Yamna mediated mainly from Caucasus Neolithic populations (like Aknashen in Armenia¹⁰) and not from European farmers of Anatolian origin³⁸. Further support for this hypothesis comes from the fact that qpAdm models of exclusively CLV and NPR hunter-gatherer ancestry conform with unsupervised ADMIXTURE estimates of ancestry (Fig. 2b and Supplementary Information section 3, page 141). Although EEF ancestry in the Core Yamna is conjectural, it was clearly present in the western Yamna from Bulgaria, Hungary, Moldova, Romania and Serbia⁷. Yamna admixture became a general ancestry feature in southeast Europe, postdating this culture's expansion, except in the southernmost corner of the Balkan Peninsula in the Aegean^{10,39–41}.

Seeking to narrow down the location from which the Yamna originated, we focused on the chronologically earliest Core Yamna individual, Mykhailivka_I32534 (3635–3383 cal BCE), from the second (proto-Yamna) layer of the Mykhailivka site in the lower Dnipro Valley in Ukraine, pre-dating the onset of Yamna expansion and forming a clade with it ($P = 0.684$). Mykhailivka_I32534 continues to fit as a clade with Core Yamna when CLV groups are placed on the right set of qpAdm analysis (Supplementary Information section 2 and Supplementary Table 2.2). Moreover, when either UNHG or EEF are added as a second source, both are not significant ($|Z| < 1$) and nominally negative,

Table 1 | Genetic profiles of individuals in the North Pontic Region from 4500–2500 BCE

Individual or group identifier and date	Model fit to data
Wave 1: early pioneers from the genetically northern end of the CLV cline and descendants	
I20072: Giurgiulești burial 6, 4330–4058 calBCE	BP group ^a
I5124: Csongrád burial 1, 4331–4073 calBCE	87% BP group/13% Lebyazhinka_HG
Trypillia formation by mixture 4832–4358 BCE	5% BP group/14% BHG/81% YUN_CA ^b
Usatove, formation by mixture 4571–4371 BCE	44–48% PV group ^c /52–56% Trypillians
Cernavodă I, KTL_A, formation by mixture 4340–4058 BCE	54% BP group/46% Trypillians
Wave 2: migration from a genetically intermediate part of the CLV cline and establishment of Core Yamna ancestry	
Serednii Stih, formation by mixture around 4400 BCE ³⁵	13–17% Aknashen Neolithic/8–56% BP group/31–56% Dnipro–Don
Core Yamna, formation by mixture ⁷ 4132–3944 BCE	26% Remontnoye ^d /74% SShi of Serednii Stih
Cernavodă I, KTL_B, formation by mixture 4438–3898 BCE	27% Remontnoye/73% European farmers
I1428: Riltsi kurgan 264, burial 5, 3360–2890 calBCE	50% Remontnoye/50% YUN_CA
I17973: Bursuceni kurgan 1, burial 21, skeleton 1, 3354–3103 calBCE	Consistent with being Maykop direct descendant
Wave 3: Yamna expansion	
Core Yamna genetic ancestry in the Eneolithic and Bronze Age NPR	
I32534: Mykhailivka 1, square VI, 3635–3383 calBCE	Core Yamna
I20196: Crasnoe kurgan 9, burial 9, skeleton 2, 3352–3101 calBCE	Core Yamna
I12229: Mayaky, kurgan 1, burial 9, 3088–2911 calBCE	Core Yamna
I20079: Taraclia II, kurgan 10, burial 2, 2571–2355 calBCE	Core Yamna
I12840: Dubynove, kurgan 1, burial 10, 2453–2148 calBCE	Core Yamna
I16668: Revova, kurgan 3, burial 10, 2800–2000 BCE	Core Yamna
Mixtures of Core Yamna and European farmers	
I1456: Durankulak, kurgan F, burial 15, 3500–3000 BCE	45% Core Yamna/55% Globular Amphora
Bulgaria Yamna, 3300–2500 BCE	78–100% Core Yamna/0–22% YUN_CA
Moldova Yamna, 3300–2500 BCE	84–100% Core Yamna/0–16% YUN_CA
Ukraine Yamna, 3300–2500 BCE	92–100% Core Yamna/0–8% YUN_CA
I17747: Tiraspol kurgan 3, burial 15, 2865–2576 calBCE	61% Core Yamna/39% Trypillia
I20076: Ocnîța kurgan 1, burial 3, 2906–2702 calBCE	88% Core Yamna/12% Globular Amphora
I4110, I5882, I5884: Deriivka I cemetery, 3500–2700 BCE ⁶	36–46% Core Yamna/23–44% BHG/15–32% Trypillia
I13071: Bil'shivtsi individual 1, 2201–2032 calBCE	72% Core Yamna/28% YUN_CA
I12234: Liubasha and Sychavka kurgans, 2434–1127 BCE	77% Core Yamna/15% Globular Amphora/8% UNHG
Mixture of Core Yamna and Dnipro–Don hunter-gatherer descendants	
Don Yamna, 3200–2600 BCE	40% Core Yamna/60% SSmed
Continued	

Mixtures of Core Yamna and Steppe Maykop descendants

I20078: Taraclia II kurgan 2, burial 14, 3340–3034 calBCE	39% Core Yamna/61% Steppe Maykop
I17974: Bursuceni kurgan 1, burial 21, skeleton 2, 3334–3030 calBCE	82% Core Yamna/18% Steppe Maykop
Yamna and Maykop descendants	
I1917: Oзера kurgan 18, burial 14, 3096–2913 calBCE	50% Core Yamna/50% Maykop
Mayaky Yamna, 2900–2500 BCE	81% Don Yamna/19% Maykop

Genetic profiles of individuals in the North Pontic Region from 4500–2500 BCE are well described as a result of 3 expansion waves: 2 waves of CLV cline expansion and 1 wave of Yamna expansion (a more detailed version is presented in Extended Data Table 4). For admixture dates we give one standard error, and a 95% confidence interval. For direct dates on bones analysed for DNA, we indicate the 95% calibrated confidence with suffix 'calBCE'; all other dates are archaeologically estimated ranges.

^aBP group is a homogeneous group from the CLV at the bend between CLV and Volga (EHG-rich) clines (Fig. 2a) from Berezhnovka and Progress 2 that carries CHG, EHG and Siberian/Central Asian Neolithic-related ancestries⁷. ^bBalkan farmers of Gumelnița/Karanovo ancestry from Yunatsite in Bulgaria. ^cPV group is a BP-related group from the CLV cline with more Aknashen (south Caucasus) ancestry than the BP group, from Berezhnovka and Vonyukha⁷. ^dRemontnoye represents a population composed of a southern ancestry represented by either the Aknashen Neolithic of Armenia or the Bronze Age Maykop, and a northern ancestry from the low-EHG end of the Volga Cline, such as the BP group⁷.

providing no evidence for ancestry other than Core Yamna. Mykhailivka_I32534 thus bridges the temporal gap between the Late Serednii Stih populations and the main Yamna expansion that spanned south Siberia to eastern Europe and from which it is impossible to determine the origin of Yamna formation as any information about geography has been obscured by expansion over thousands of kilometres of distance.

Of the three other early (around 3350–3100 BCE) individuals with predominantly Core Yamna ancestry, all from Moldova, individual I20196 from Crasnoe (Moldova_Crasnoe_Eneolithic) was cladal with Core Yamna ($P = 0.683$). Of the other two, I17743 from Mereni (part of Moldova_EBA_Yamnaya) exhibited 6.9% EEF admixture ($P = 0.593$) and Zhivotilovka_I17974 from Bursuceni exhibited 18.2% Steppe Maykop admixture ($P = 0.324$; Supplementary Information section 2 and Supplementary Table 2.9).

Besides Mykhailivka_I32534, four Yamna individuals from Ukraine, I12168, I20975, I3141_enhanced and I21056 are cladal with the Core Yamna group in showing no evidence of EEF admixture. Three Yamna Ukraine individuals from the northwest NPR exhibit significant admixture of this type from proximate sources such as Bulgaria Eneolithic or Trypillia (Supplementary Information section 2 and Supplementary Tables 2.4 and 2.13). Thus, the northwest NPR is consistent with being the place where the Yamna first received substantial EEF admixture during their western expansion.

A substantial proportion of EEF ancestry in two Yamna outlier individuals from Moldova is best fitted by Core Yamna plus Trypillia or Globular Amphora models (Supplementary Information section 2 and Supplementary Table 2.10). One of the Yamna individuals from Bulgaria exhibited 22.3% YUN_CA-related admixture, whereas another individual from the same site was cladal with the Core Yamna (Supplementary Information section 2 and Supplementary Tables 2.6 and 2.7). Thus, the Yamna expansion, beginning in Ukraine and reaching the South Balkans, included individuals who maintained the Core Yamna genetic profile as well as others admixing with local farmers and initiating the transmission of Yamna ancestry and, probably, Indo-European languages beyond the steppe.

Two of the Steppe Maykop-shifted individuals in PCA (Fig. 2a), Zhivotilovka_I17974 and Usatove_I20078 from Moldova were formed of the same Yamna plus Steppe Maykop-associated admixture process, with I17974 carrying about one-third of the Steppe Maykop-associated ancestry found in I20078 ($18.2 \pm 6.0\%$ versus $60.6 \pm 6.2\%$) (Table 1, Extended Data Fig 2, Supplementary Information section 2 and

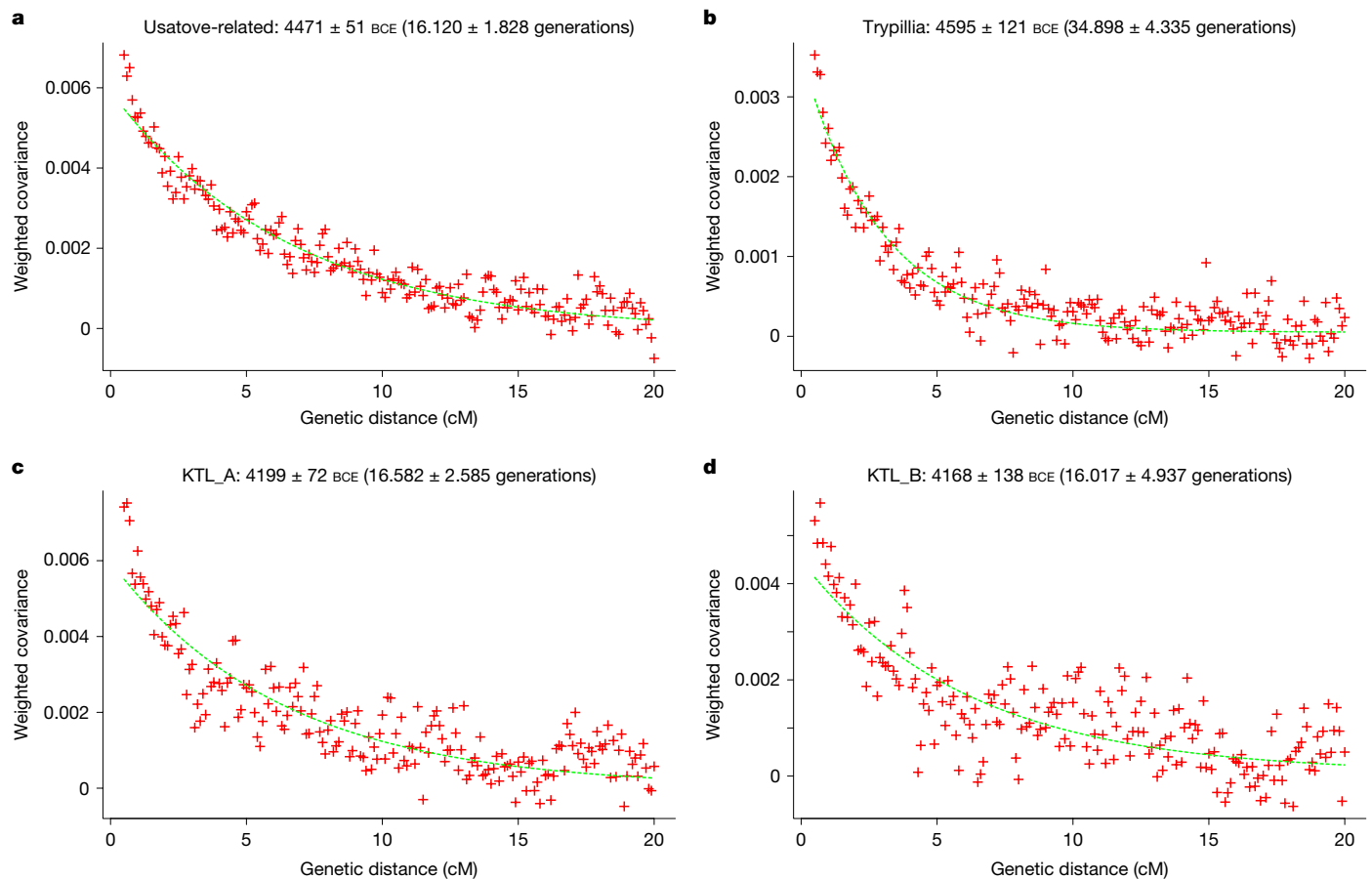


Fig. 3 | DATES estimates of timing of CLV and European farmer ancestry admixture. **a**, Usatove-related individuals from this study and ref. 5. **b**, Trypillians from this study and ref. 17. **c**, **d**, Kartal cluster A (**c**) and B (**d**) from ref. 5. We show ± 1 standard error, both for the raw admixture date, and the

translation to years BCE (assuming fixed generation length of 28 years and not including uncertainty about the age of the admixed individuals; Methods). Red crosses represent the data and the green dashed line is the fit.

Supplementary Tables 2.15 and 2.16). Zhivotilovka_I17973, co-buried with I17974, cannot be well-modelled with any of the sources available to us, but is nearest to the ‘southern’ end of the CLV cline (Maykop of the North Caucasus ($P = 0.0025$) or the Aknashen Neolithic of the South Caucasus ($P = 0.0047$)), which is corroborated by the position of I17973 in the PCA (Fig. 2a). In the northeastern NPR, an early Yamna individual Ukraine_EBA_Ozera_I1917⁶ is best modelled as an even mix of Core Yamna and Maykop, providing, similar to individual I17973, a clear link to the Caucasus. More evidence for this link comes from the Early Bronze Age population from Mayaky⁵, which is discontinuous with the Usatove from the same region but represented a unique combination of one-fifth Maykop ancestry with the remainder best represented by the Yamna of the Lower Don, a population which was itself a mix of Core Yamna and NPR hunter-gatherers⁷.

Yamna ancestry in the Bronze Age

We find that individuals of the Catacomb archaeological complex, which chronologically partially overlaps and succeeds Yamna in the NPR, continued to exhibit Yamna genetic ancestry. The population, labelled ‘Ukraine_EBA_Catacomb’, including individuals I12840 and I16668 from our dataset, is cladal with the Core Yamna ($P = 0.075$, Supplementary Information section 2 and Supplementary Table 2.1). Yamna ancestry persisted in the NPR into the second half of the third millennium BCE.

The Catacomb group was succeeded in the NPR by the Babyne (Multi-Cordoned Ware) complex (Supplementary Information

section 1.4). Feasible models for Babyne ancestry involve Core Yamna, a European farmer source, and considerable hunter-gatherer ancestry (Table 1, Supplementary Information section 2 and Supplementary Table 2.14). Similarly admixed populations have been described from the Bronze Age of present-day Romania at the sites of Arman (Cârlomănești) and Târșoru Vechi in Muntenia¹⁰, indicating that populations of high hunter-gatherer ancestry contributed to some post-Yamna people in the NPR and Southern Carpathians.

Discussion

This study presents a comprehensive reconstruction of the population dynamics in the North Pontic steppe and forest steppe, leading up to and following the emergence of the Yamna.

We demonstrate that the Neolithic populations of the Dnipro Valley were admixed, roughly with BHG and EHG sources, along with approximately 7–9% EEF ancestry in the UNHG population except for some outliers, such as individual I27992 who was buried in a boat-shaped grave from Yasynivka ($27 \pm 6.0\%$ EEF, this report) and an unadmixed EEF individual I3719 from Deriivka I⁶ ($103.5 \pm 1.6\%$ EEF). CHG ancestry was also sporadically present at around 7–10%, notably in the Neolithic necropolis at Mariupol. The proximal sources of EEF ancestry in UNHG remain unclear, but may have been mediated by BHG migrants in the Dnipro Valley or individuals of EEF genetic background such as individual I3719 who were included in UNHG communities⁶.

The Eneolithic Trypillia population was mainly formed from the sources along the EFHG cline that received limited (approximately 5%)

admixture from people with BP group CLV ancestry. Usatove was formed from PV group CLV people evenly intermixing with Trypillian ancestry.

The evidence from Usatove and Trypillia clarifies the process of the CLV admixture in the NPR in the Eneolithic. Some carriers of Volga–CLV ancestry, as in Giurgiulești and Csongrád, advanced across the NPR steppe to the Balkans and Carpathian region largely without admixing with the people they encountered along the way. By contrast, the eastward-bound Trypillian farmers incorporated the ancestry of the Volga–CLV incomers. An intriguing possibility raised by our findings is that Usatove was formed around an outpost in the Danube–Dniester delta area where migrants of Trypillia and early CLV–PV group and their economic interests converged. A similar scenario is feasible for the Cernavodă I population of Kartal_A, but with BP group-derived carriers of CLV ancestry such as in Giurgiulești and Csongrád individuals. Alternatively, Usatove and Kartal A could have formed as a ‘commonwealth’ of co-existing and interdependent cultures in which Trypillia and populations from the Caucasus–Volga both participated. A third scenario places egalitarian Trypillians under the dominance of hierarchically organized patriarchal societies carrying CLV ancestry, extending into the northwestern NPR.

In contrast to Usatove, Serednii Stih carriers of CLV and UNHG-related ancestries in the NPR⁷ lacked appreciable EEF ancestry. The results in ref. 7 and herein establish the Core Yamna as a late Serednii Stih-derived population that had more CLV ancestry than sampled Serednii Stih individuals but was made of the same CLV and UNHG/GK2 derived components. CLV ancestry comprised approximately 5% in Trypillia and approximately 50% of Usatove ancestry, whereas in Yamna⁷ it was approximately 77%. In Usatove, around 14% of CLV ancestry was southern Caucasus Aknashen-related (Supplementary Information section 2, page 118), whereas in the Core Yamna the Aknashen-related ancestry was approximately 21%, thus suggesting that the westward CLV migration may not originate at a single point⁷.

The existence of unadmixed Core Yamna in a wide area from the Altai to Bulgaria is most parsimoniously explained as a consequence of rapid Yamna expansion. The question of whether the remarkable homogeneity of the Core Yamna cluster was a consequence of relative isolation during their formative period or a purposeful avoidance of heterogamy remains to be answered. In contrast with the formative period, the Yamna taking part in the western expansion carried hunter-gatherer-enriched ancestries related to that seen in Don Yamna¹⁰, and ancestries from Maykop and Steppe Maykop, while absorbing local EEF ancestry. This shift in interpopulation interaction strategy could potentially be a result of a shifting balance of power that enabled or encouraged broader mating opportunities. The integrative nature of these communities, coupled with their remarkable mobility, plausibly contributed to the success of the Yamna in disseminating their Indo-European language and culture across geographic and population boundaries.

The chronologically earliest (3635–3383 cal BCE) individual with the Core Yamna ancestry comes from the Mykhailivka settlement, which displays a succession of uninterrupted cultural layers from the late Eneolithic to the EBA^{42,43}. In the context of the archaeological evidence, these results increase the plausibility of arguments that the lower Dnipro, specifically the area around the Mykhailivka site at a crossroads of ancient steppe ‘highway’ network across the Pontic–Caspian steppe (Supplementary Information section 1.5), is where Yamna first emerged. The Catacomb and Babyne groups that succeeded Yamna in the NPR continued to carry Yamna genetic ancestry and displayed a resurgence of hunter-gatherer ancestry towards the Middle Bronze Age. The geographic dispersal of individuals with Babyne genetic ancestry may reflect the high mobility of this group, similar to that of the Yamna but smaller in scale.

Waves of CLV expansion

Our analysis suggests a history of three CLV-related partially overlapping waves of migrations into the NPR in the Eneolithic (Table 1).

Potentially the earliest BP group/PV group-related Lower Volga end of CLV wave started around 4500 BCE. It was associated with Giurgiulești–Csongrád ‘Ochre Graves’ (Supplementary Fig. 1.1) and left admixture in Trypillia, Usatove and Kartal_A⁵. A second and more protracted wave carried an intermediate (West Manych–Remontnoye type) part of the CLV cline, and became associated, in its initial pulse, with the formation of Serednii Stih around 4500 BCE, and contributing to the formation of Kartal_B⁵. Otherwise, however, this second wave remained largely contained in the Lower Dnipro Valley region, notably during the steppe ‘hiatus’ in the late fifth to early fourth millennium BCE, characterized by a sharp climatic shift towards aridity and cooler temperatures and relative lack of archaeological material^{2,44,45}.

The Core Yamna genetic mixture is estimated⁷ to have taken place at 4038 ± 48 BCE (95% credible interval 3944–4132 BCE), at the height of the steppe hiatus. It is unclear whether this date corresponds to an admixture of populations that happened rapidly, or if it corresponds to a process that unfolded over generations, in which case the date we estimate is an average. Thus, the steppe hiatus may be a reason for the emergence of the Core Yamna ancestry from a nascent Stih-derived proto-Yamna population that was isolated owing to the climatic upheaval. In this scenario, the individual from Mykhailivka represents such a proto-Yamna population near the geographical origin of the Core Yamna and sampled from the time where its genetic distinctiveness had already developed.

The third wave of CLV ancestry expansion is that of the Yamna proper, beginning around 3300 BCE and lasting into the middle of the following millennium. All three expansion waves spread ancestry from different points on the geographically and genetically diverse CLV cline.

It is remarkable that the three genetic waves of CLV ancestry expansion align, spatially and temporally, with the three waves of Kurgan People proposed by Marija Gimbutas in the 1950s to explain the spread of Indo-European influences and the fall of ‘Old Europe’ (summarized in refs. 1,46). Although Gimbutas envisioned the spread of Kurgan ancestry as a result of a conquest and emphasized cultural transformation, our results present evidence of massive genetic transformations effected by the spread of CLV ancestry during waves 1 and 2, and especially, with the spread of the Yamna during wave 3. Such genetic changes must have involved complex cultural dynamics, in which both conflict and peaceful synthesis may have had a role. Future studies that explore the cultural impact of these three expansion waves must be informed by the new understanding of the immense genetic impacts that accompanied them.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-024-08372-2>.

1. Gimbutas, M. A. Three waves of the Kurgan people into Old Europe, 4500–2500 B.C. *J. Indo-Eur. Stud.* **18**, 240–268 (1997).
2. Anthony, D. W. *The Horse, the Wheel, and Language: How Bronze-Age Riders from the Eurasian Steppes Shaped the Modern World* (Princeton Univ. Press, 2007).
3. Haak, W. et al. Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* **522**, 207–211 (2015).
4. Allentoft, M. E. et al. Population genomics of Bronze Age Eurasia. *Nature* **522**, 167–172 (2015).
5. Penske, S. et al. Early contact between late farming and pastoralist societies in southeastern Europe. *Nature* **620**, 358–365 (2023).
6. Mathieson, I. et al. The genomic history of southeastern Europe. *Nature* **555**, 197–203 (2018).
7. Lazaridis, I. et al. The genetic origin of the Indo-Europeans. *Nature* <https://doi.org/10.1038/s41586-024-08531-5> (2024).
8. de Barros Damgaard, P. et al. The first horse herders and the impact of early Bronze Age steppe expansions into Asia. *Science* **360**, eaar7711 (2018).
9. Narasimhan, V. M. et al. The formation of human populations in South and Central Asia. *Science* **365**, eaat7487 (2019).

10. Lazaridis, I. et al. The genetic history of the Southern Arc: a bridge between West Asia and Europe. *Science* **377**, eabm4247 (2022).
11. Kroonen, G., Jakob, A., Palmér, A. I., van Sluis, P. & Wigman, A. Indo-European cereal terminology suggests a Northwest Pontic homeland for the core Indo-European languages. *PLoS ONE* **17**, e0275744 (2022).
12. Lipson, M. et al. Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature* **551**, 368–372 (2017).
13. Nikitin, A. G., Videiko, M., Patterson, N., Renson, V. & Reich, D. Interactions between Trypillian farmers and North Pontic forager-pastoralists in Eneolithic central Ukraine. *PLoS ONE* **18**, e0285449 (2023).
14. Burdo, N. B. Kul'turno-istoricheskiye kontakty ranne-tripol'skikh plemen. In *Drevneyshiye Obshchnosti Zemledel'tsev i Skotovodov Severnogo Prichernomor'ya* (ed. Yarovoy, E. V.) 49–51 (Nauchno-issledovatel'skaya laboratoriya «Arkheologiya» PGU im.T. G. Shevchenko, 2002).
15. Nikitin, A. G. et al. Mitochondrial DNA analysis of Eneolithic Trypillians from Ukraine reveals Neolithic farming genetic roots. *PLoS ONE* **12**, e0172952 (2017).
16. Nikitin, A. G., Sokhatsky, M. P., Kovalyukh, M. M. & Videiko, M. Y. Comprehensive site chronology and ancient mitochondrial DNA analysis from Verteba Cave—a Trypillian Culture site of Eneolithic Ukraine. *Interdiscip. Archaeol.* **1**, 9–18 (2010).
17. Gelabert, P. et al. Genomes from Verteba cave suggest diversity within the Trypillians in Ukraine. *Sci Rep.* **12**, 7242 (2022).
18. Mattila, T. M. et al. Genetic continuity, isolation, and gene flow in Stone Age Central and Eastern Europe. *Commun. Biol.* **6**, 793 (2023).
19. Kotova, N. S. *Early Eneolithic in the Pontic Steppes* (British Archaeological Reports, 2008).
20. Telegin, D. Ya. & Potekhina I. D. *Neolithic Cemeteries and Populations in the Dnieper Basin* (BAR International Series 383, 1987).
21. Telegin, D. Y. Keramika rann'oho eneolitu typu Zasukha v lisostepovomu Livoberezhzhi Ukrainy. *Arkheolohiya* **64**, 73–84 (1988).
22. Nielsen, R. et al. Tracing the peopling of the world through genomics. *Nature* **541**, 302–310 (2017).
23. Patterson, N., Price, A. L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* <https://doi.org/10.1371/journal.pgen.0020190> (2006).
24. Lazaridis, I. et al. Ancient DNA from Mesopotamia suggests distinct Pre-Pottery and Pottery Neolithic migrations into Anatolia. *Science* **377**, 982–987 (2022).
25. South, A., Michael, S. & Massicotte, P. rnatuarearthdata: World vector map data from Natural Earth used in 'rnatuarearth'. R package version 1.0.0.9000 <https://github.com/ropensci/rnatuarearthdata>, <https://docs.ropensci.org/rnatuarearthdata/> (2024).
26. Ecsedy, I. *The People of the Pit-Grave Kurgans in Eastern Hungary* (Akadémiai Kiadó, 1979).
27. Govedarica, B. *Zepterträger, Herrscher Der Steppen: Die Frühen Ockergräber Des Älteren Äneolithikums Im Karpatenbalkanischen Gebiet Und Im Steppenraum Südost-Und Osteuropas* (Philipp von Zabern, 2004).
28. Posth, C. et al. Palaeogenomics of Upper Palaeolithic to Neolithic European hunter-gatherers. *Nature* **615**, 117–126 (2023).
29. Haskevych, D. Late Mesolithic individuals of the Danube Iron Gates origin on the Dniro River Rapids (Ukraine)? Archaeological and Bioarchaeological Records. *Open Archaeol.* **8**, 1138–1169 (2022).
30. Mathieson, I. et al. Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499–503 (2015).
31. Skoglund, P. et al. Genomic diversity and admixture differs for Stone-Age Scandinavian foragers and farmers. *Science* **344**, 747–750 (2014).
32. Malmström, H. et al. The genomic ancestry of the Scandinavian Battle Axe culture people and their relation to the broader Corded Ware horizon. *Proc. R. Soc. B* **286**, 20191528 (2019).
33. Coutinho, A. et al. The Neolithic Pitted Ware culture foragers were culturally but not genetically influenced by the Battle Axe culture herders. *Am. J. Phys. Anthropol.* **172**, 638–649 (2020).
34. Allentoft, M. E. et al. Population genomics of post-glacial western Eurasia. *Nature* **625**, 301–311 (2024).
35. Rassamakin, Y. Y. Mohyl'nyky Ihren' (Ohrin') 8 ta Oleksandriya doby eneolitu: problemy datuvannya ta kul'turnoyi prynezhnosti. *Arheologia* **4**, 26–48 (2017).
36. Patterson, N. et al. Ancient admixture in human history. *Genetics* **192**, 1065–1093 (2012).
37. Chintalapati, M., Patterson, N. & Moorjani, P. The spatiotemporal patterns of major human admixture events during the European Holocene. *eLife* **11**, e77625 (2022).
38. Wang, C.-C. et al. Ancient human genome-wide data from a 3000-year interval in the Caucasus corresponds with eco-geographic regions. *Nat. Commun.* **10**, 590 (2019).
39. Lazaridis, I. et al. Genetic origins of the Minoans and Mycenaeans. *Nature* **548**, 214–218 (2017).
40. Skourtanioti, E. et al. Ancient DNA reveals admixture history and endogamy in the prehistoric Aegean. *Nat. Ecol. Evol.* **7**, 290–303 (2023).
41. Clemente, F. et al. The genomic history of the Aegean palatial civilizations. *Cell* **184**, 2565–2586.e21 (2021).
42. Korobkova, G. F. & Shaposhnikova, O. G. *Poselenie Mikhailovka: Etalonnij Pamyatnik Drevneyamnoj Dkury* (Evropejskij Dom, 2005).
43. Kotova, N. S. *Dereivskaya Kul'tura i Pamyatniki Nizhnemikhaylovskogo Tipa* (Maidan: Kiev, Kharkov, 2013).
44. Rassamakin, Y. Y. in *Late Prehistoric Exploitation of the Eurasian Steppe* (eds Levine, M. et al.) 59–182 (McDonald Institute Monographs, 1999).
45. Nikitin, A. G. & Ivanova, S. in *Steppe Transmissions* (eds. Preda-Bălănică, B. & Ahola, M.) 9–27 (Archaeolingua, 2023); <https://doi.org/10.33774/coe-2022-7m315>.
46. Gimbutas, M. The Indo-Europeanization of Europe: the intrusion of steppe pastoralists from south Russia and the transformation of Old Europe. *Word* **44**, 205–222 (1993).

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Methods

Wet laboratory work

In clean rooms where the goal was to protect bones and teeth from contamination by the individuals handling them, we processed human skeletal remains into powder⁴⁷, extracted DNA using a method designed to retain short molecules^{47–49}, in some cases using automated liquid handlers⁵⁰, and converted the extracts into double-stranded⁵¹ and single-stranded⁵² libraries, which were molecularly barcoded with appended dual barcodes (for double-stranded libraries) and dual indices (for both double-stranded and single-stranded libraries) to allow them to be pooled together and then bioinformatically deconvoluted at the analysis stage. We enriched the libraries for sequences overlapping more than 1.2 million single nucleotide polymorphisms (SNPs) as well as the mitochondrial genome⁵³, and then sequenced on NextSeq500, HiSeqX or NovaSeq instruments, targeting on the order of 100,000 sequences for unenriched libraries and on the order of 30 million molecules for enriched ones. Supplementary Table 2 provides information on each library that we analysed.

Bioinformatic analysis

Following sequencing, we used identifying sequences (barcodes and indices) to demultiplex reads into the appropriate library, before trimming these and sequence adapters. We then used paired-end sequences requiring an overlap of at least 15 base pairs (allowing for 1 mismatch), using a modified version of SeqPrep 1.1 (<https://github.com/jstjohn/SeqPrep>); at overlapping bases, we selected the highest quality nucleotide to represent the sequence at that position. We aligned sequences to both the human reference genome sequence (hg19) (<https://www.internationalgenome.org/category/grch37/>) and to the inferred ancestral reconstructed sapiens sequence (RSRS) mitochondrial sequence⁵⁴, using the BWA samse command⁵⁵. We removed duplicated molecules based on having the same start and stop positions and orientation in their alignment and the same barcodes. The computational pipelines we used are publicly available on GitHub at <https://github.com/dReichLab/ADNA-Tools> and <https://github.com/dReichLab/adna-workflow>. We called variants using a pseudohaploid genotyping approach, where a single base is randomly selected from a pool of possible bases at each SNP, filtering by a minimum mapping quality of least 10, and base quality of at least 20, trimming each sequence by two base pairs to remove damage artifacts. To assess ancient DNA authenticity, we used contamMix-1.0.1051⁵⁶ to search for heterogeneity in mitochondrial DNA sequences that are expected to be non-variable in uncontaminated individuals, and ANGSD⁵⁷ to search for heterogeneity in X chromosome sequences which should be non-variable in contaminated male individuals⁵⁷. We also evaluated authenticity by searching for an increase in cytosine-to-thymine errors in the final nucleotide (in untrimmed reads) which is expected for genuine ancient DNA⁵⁸ and by computing the ratio of Y chromosome to the sum of X and Y chromosome sequences which is expected to be very low for females and to have a very much higher value for males. We determined a consensus sequence for mitochondrial DNA using bcftools (<https://github.com/samtools/bcftools>) and SAMtools⁵⁹ requiring a minimum of twofold coverage to call the nucleotide and a majority rule to determine its value. We used HaploGrep2 to determine the mitochondrial haplogroups based on this consensus sequence, leveraging the phylo tree database (mtDNA tree build 17)⁶⁰.

Population genetic analysis

We performed principal components in smartpca²³ using lsqproject: YES and newshrink: YES parameters and the populations OberkasselCluster (set of trans-Alpine WHG individuals identified in²⁸), Russia_Firsovo_N, Iran_HajjiFiruz_C⁹, Iran_C_SehGabi⁶¹, Iran_C_TepeHissar⁶², Israel_C⁶³, and Germany_EN_LBK^{3,12,30,64} to form the axes (Fig. 2).

We used qpWave and qpAdm^{3,65} to test whether $n + 1$ 'left' populations (one test and n sources) are consistent with descending from n ancestral sources with respect to a set of 'right' populations as in ref. 7 (OldAfrica^{66–68}, Russia_AfontovaGora⁶⁹, CHG⁷⁰, Iran_GanjDareh_N⁶¹, Italy_Villabruna⁶⁹, Russia_Sidelkino.SG⁸ and Turkey_N³⁰).

We performed a subset of unsupervised ADMIXTURE analysis⁷¹ using a new data processing pipeline focusing on 'summary individuals' that prevents the formation of population-specific ancestry components. This provides a complementary approach to qpAdm, allowing us to obtain insights into the ancestry of diverse population from the NPR and neighbouring regions (Fig. 2b).

We dated the admixture time of Usatove-related populations (individuals from Mayaky presented in this report and from Mayaky (MAJ) and Usatove–Velykyj Kuyalnik (USV) (from ref. 5)) and Trypillians, using DATES³⁷ to infer the number of generations prior to the ¹⁴C date of the studied individuals, and converted to a calendar date assuming 28 years per generation⁷². Uncertainty ranges reflect the standard error computed by DATES and not the uncertainty of the average ¹⁴C date of admixed individuals.

Ethics statement

All applicable regulations were followed when sampling human remains and exporting them for analysis. All samples originating from Ukraine were excavated or sampled from museum or archival collections prior to 2022. Authors obtained consent, when available, from the individuals who conducted the excavations, who are either co-authors of the study or are acknowledged for their contribution. Human remains were processed using a minimal amount of skeletal material with the goal of minimizing damage. Geographic names as well as names of archaeological groups were transliterated following their spelling in the countries from which samples originate. Geographic boundaries of political entities were respected following international law. Open science principles require making all data used to support the conclusions of a study maximally available, and we support these principles here by making fully publicly available not only the digital copies of molecules (the uploaded sequences) but also the molecular copies (the ancient DNA libraries themselves, which constitute molecular data storage). Those researchers who wish to carry out deeper sequencing of libraries published in this study should make a request to D.R. We commit to granting reasonable requests as long as the libraries remain preserved in our laboratories, with no requirement that we be included as collaborators or co-authors on any resulting publications.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Genotype data for individuals included in this study can be obtained from the Harvard Dataverse repository at <https://doi.org/10.7910/DVN/CJTV3Q>. The DNA sequences reported in this paper have been deposited in the European Nucleotide Archive under accession number PRJEB81468. Other newly reported data such as radiocarbon dates and archaeological context information are included in the manuscript and supplementary files.

47. Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl Acad. Sci. USA* **110**, 15758–15763 (2013).
48. Korlević, P. et al. Reducing microbial and human contamination in DNA extractions from ancient bones and teeth. *Biotechniques* **59**, 87–93 (2015).
49. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S. & Reich, D. Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. B* **370**, 20130624 (2014).

50. Rohland, N., Glocke, I., Aximu-Petri, A. & Meyer, M. Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nat. Protoc.* **13**, 2447–2461 (2018).
51. Prendergast, M. E. et al. Ancient DNA reveals a multistep spread of the first herders into sub-Saharan Africa. *Science* **365**, eaaw6275 (2019).
52. Gansauge, M.-T., Aximu-Petri, A., Nagel, S. & Meyer, M. Manual and automated preparation of single-stranded DNA libraries for the sequencing of DNA from ancient biological remains and other sources of highly degraded DNA. *Nat. Protoc.* **15**, 2279–2300 (2020).
53. Fu, Q. et al. An early modern human from Romania with a recent Neanderthal ancestor. *Nature* **524**, 216–219 (2015).
54. Behar, D. M. et al. A “Copernican” reassessment of the human mitochondrial DNA tree from its root. *Am. J. Hum. Genet.* **90**, 675–684 (2012).
55. Li, H. & Durbin, R. Fast and accurate long-read alignment with Burrows–Wheeler transform. *Bioinformatics* **26**, 589–595 (2010).
56. Fu, Q. et al. A revised timescale for human evolution based on ancient mitochondrial genomes. *Curr. Biol.* **23**, 553–559 (2013).
57. Korneliussen, T. S., Albrechtsen, A. & Nielsen, R. ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics* **15**, 356 (2014).
58. Briggs, A. W. et al. Removal of deaminated cytosines and detection of in vivo methylation in ancient DNA. *Nucleic Acids Res.* **38**, e87–e87 (2010).
59. Li, H. et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
60. Weissensteiner, H. et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Res.* **44**, W58–W63 (2016).
61. Lazaridis, I. et al. Genomic insights into the origin of farming in the ancient Near East. *Nature* **536**, 419–424 (2016).
62. Shinde, V. et al. An ancient Harappan genome lacks ancestry from Steppe pastoralists or Iranian farmers. *Cell* **179**, 729–735.e10 (2019).
63. Harney, É. et al. Ancient DNA from Chalcolithic Israel reveals the role of population mixture in cultural transformation. *Nat. Commun.* **9**, 3336 (2018).
64. Rivollat, M. et al. Ancient genome-wide DNA from France highlights the complexity of interactions between Mesolithic hunter-gatherers and Neolithic farmers. *Sci. Adv.* **6**, eaaz5344 (2020).
65. Reich, D. et al. Reconstructing Native American population history. *Nature* **488**, 370–374 (2012).
66. Skoglund, P. et al. Reconstructing prehistoric African population structure. *Cell* **171**, 59–71.e21 (2017).
67. Wang, K. et al. Ancient genomes reveal complex patterns of population movement, interaction, and replacement in sub-Saharan Africa. *Sci. Adv.* **6**, eaaz0183 (2020).
68. Lipson, M. et al. Ancient DNA and deep population structure in sub-Saharan African foragers. *Nature* **603**, 290–296 (2022).
69. Fu, Q. et al. The genetic history of Ice Age Europe. *Nature* **534**, 200–205 (2016).
70. Jones, E. R. et al. Upper Palaeolithic genomes reveal deep roots of modern Eurasians. *Nat. Commun.* **6**, 8912 (2015).
71. Alexander, D. H., Novembre, J. & Lange, K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* **19**, 1655–1664 (2009).
72. Fenner, J. N. Cross-cultural estimation of the human generation interval for use in genetics-based population divergence studies. *Am. J. Phys. Anthropol.* **128**, 415–423 (2005).

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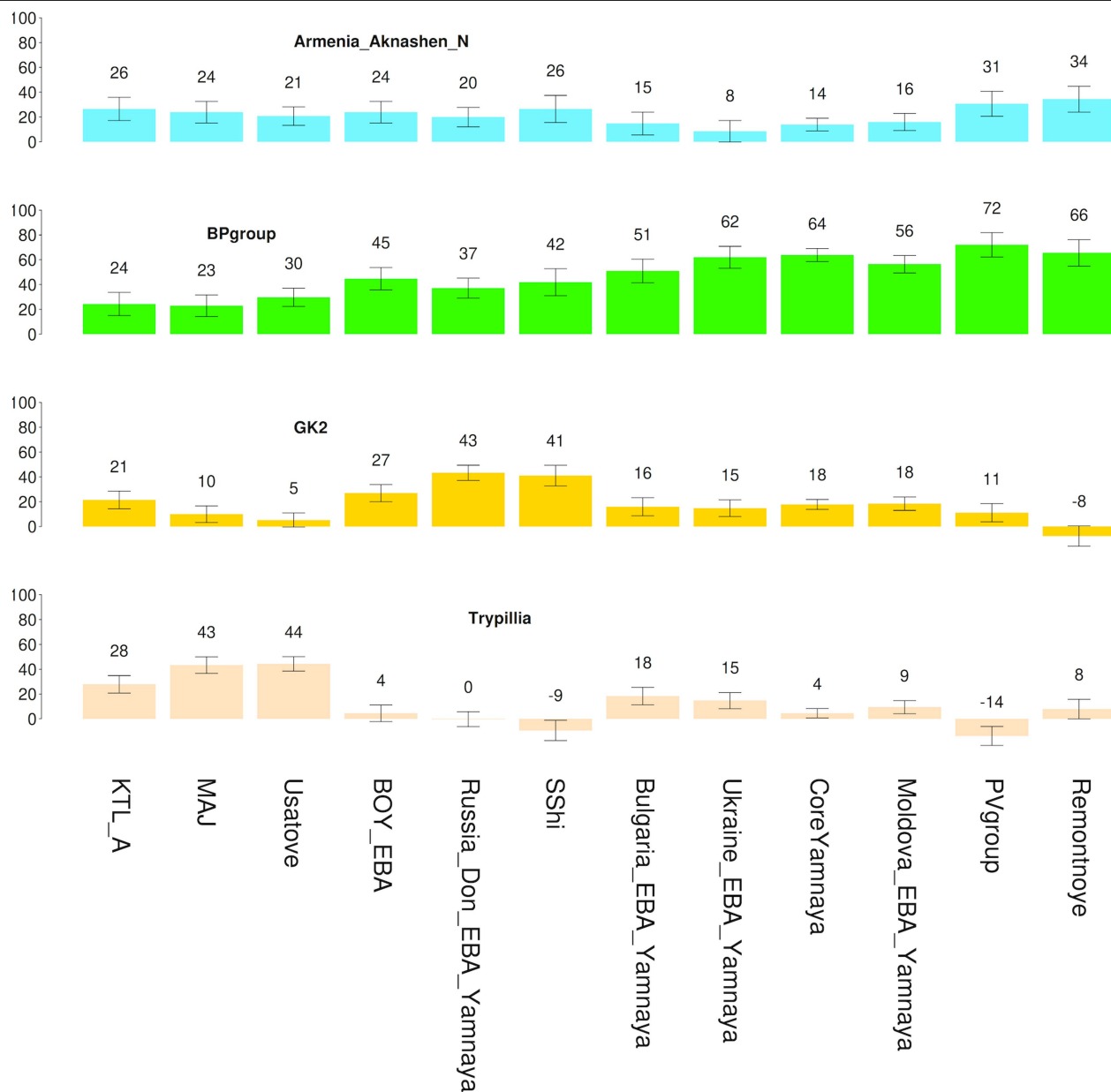
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-024-08372-2>.

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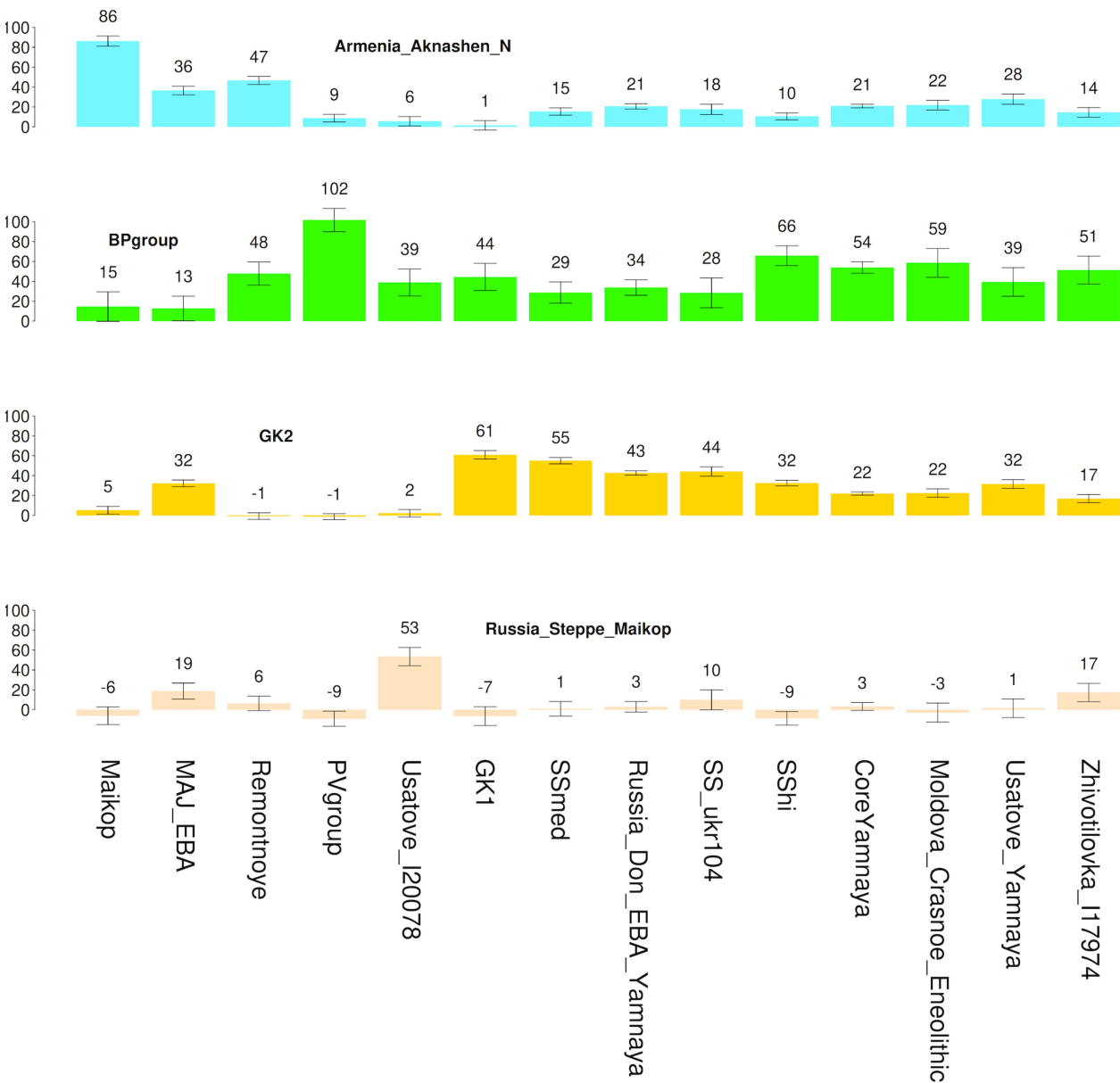
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Extended Data Fig. 1 | Admixture proportions of 4-source model with Trypillians as the 4th source. Plotted populations fit the model ($p > 0.05$) and we only show populations where the RMSE of standard errors (S.E.) is less than

10% of the point estimate (shown above each bar). For full list of tested populations and alternative choices of modelling, see Supplementary Information Section 2, Appendix I. Sample sizes are in Online Table 4 of ref. 7.



Extended Data Fig. 2 | Admixture proportions of 4-source model with Steppe Maykop as the 4th source. Plotted populations fit the model ($p > 0.05$) and we only show populations where the RMSE of standard errors (S.E.) is less than 10% of the point estimate (shown above each bar). For full list of tested

populations including sample sizes and alternative choices of modelling, see Supplementary Information Section 2, Appendix II. Sample sizes are in Online Table 4 of ref. 7.

Extended Data Table 1 | Statistics of the form $f_3(\text{Source}_1, \text{Source}_2; \text{Test})$

Test	Source1	Source2	$f_3(\text{Source}_1, \text{Source}_2; \text{Test})$	Z-score
BOY_EBA	TTK	Trypillia	-0.016097	-7.0
Bulgaria_EBA_Yamna	Russia_Karelia	YUN_CA	-0.011836	-9.2
CoreYamna	Maykop	Russia_Karelia	-0.006310	-13.6
GlobularAmphora	Serbia_IronGates_Mesolithic	YUN_CA	-0.005914	-8.2
KTL_A	Russia_Karelia	YUN_CA	-0.014186	-17.5
KTL_B	Russia_Karelia	YUN_CA	-0.009922	-9.1
MAJ	Russia_Karelia	YUN_CA	-0.009438	-12.7
MAJ_EBA	GlobularAmphora	TTK	0.004403	1.6
Moldova_EBA_Yamna	Maykop	Russia_Karelia	-0.007198	-10.0
PIE_CA	Serbia_IronGates_Mesolithic	YUN_CA	-0.002351	-6.9
PTK_CA	TTK	YUN_CA	0.001444	0.3
Romania_LN_Eneol	Armenia_Aknashen_N	Serbia_IronGates_Mesolithic	0.002525	0.5
SShi	Armenia_Aknashen_N	Russia_Karelia	-0.010140	-6.3
SSmed	BPgroup	Serbia_IronGates_Mesolithic	-0.012501	-10.6
Trypillia	Serbia_IronGates_Mesolithic	YUN_CA	-0.008350	-23.8
Ukraine_Deriivka_Mes	Russia_Karelia	Serbia_IronGates_Mesolithic	-0.003244	-1.3
Ukraine_EBA_Catacomb	Armenia_Aknashen_N	Russia_Karelia	-0.022783	-1.7
Ukraine_EBA_Yamna	Maykop	Russia_Karelia	-0.009610	-8.1
Ukraine_MBA_MultiCordonedWare_Babyne	GK2	YUN_CA	-0.017018	-2.5
Ukraine_N	Russia_Karelia	Serbia_IronGates_Mesolithic	-0.007871	-17.2
Ukraine_Vasilevka	Serbia_IronGates_Mesolithic	TTK	-0.005716	-3.0
Usatove	Russia_Karelia	YUN_CA	-0.008941	-10.1
USV	Russia_Karelia	YUN_CA	-0.011918	-12.0
VAR_CA	Serbia_IronGates_Mesolithic	YUN_CA	-0.003861	-9.3
YUN_EBA	Serbia_IronGates_Mesolithic	YUN_CA	-0.001677	-2.6

The statistic with the lowest Z-score of all the considered pairs is shown. P-values from *qpAdm* are based on a Hotelling T2 test.

Article

Extended Data Table 2 | Ancestry of UNHG individuals

Modeling Ukraine Neolithic individuals with LBK as a source									
Individual	P-value	Proportions			Std. errors			Z-score of LBK	Population Label
		LBK	EHG	BHG	LBK	EHG	BHG		
I5878_enhanced	1.22E-01	9.7%	58.3%	32.0%	2.4%	3.7%	4.1%	4.0	Ukraine_N_father.or.son.I5883
I5886_enhanced	5.56E-04	7.4%	58.1%	34.4%	1.9%	3.3%	3.5%	3.9	Ukraine_N
I5886_published	2.23E-02	9.6%	57.6%	32.7%	3.0%	4.6%	5.0%	3.2	Ukraine_N
I5892	3.59E-01	3.2%	57.2%	39.6%	2.7%	4.1%	4.6%	1.2	Ukraine_N
I5870	6.63E-01	7.8%	56.2%	36.0%	2.3%	3.7%	3.9%	3.4	Ukraine_N
I3716_published	2.61E-01	8.7%	56.1%	35.2%	2.7%	4.8%	5.0%	3.2	Ukraine_N
I31730	3.72E-03	6.5%	54.9%	38.5%	2.3%	3.9%	4.1%	2.8	Ukraine_N
I1736	8.22E-01	6.5%	54.8%	38.7%	1.9%	3.2%	3.4%	3.4	Ukraine_N
I27992	3.95E-01	27.0%	54.5%	18.5%	6.0%	9.5%	10.6%	4.5	Ukraine_N_I27992
I3720	1.00E-01	5.8%	53.8%	40.4%	3.6%	5.3%	5.7%	1.6	Ukraine_N
I5872_published	6.42E-01	10.1%	53.2%	36.7%	3.0%	4.3%	4.8%	3.4	Ukraine_N
I3717	6.08E-01	9.4%	53.1%	37.5%	2.0%	3.3%	3.5%	4.7	Ukraine_N
I6133_published	2.55E-01	1.9%	52.5%	45.7%	3.8%	6.0%	6.7%	0.5	Ukraine_N
I5957_published	8.41E-01	3.7%	52.5%	43.8%	3.0%	5.0%	5.5%	1.2	Ukraine_N
I5869	5.99E-01	10.4%	51.9%	37.7%	2.7%	4.5%	5.0%	3.9	Ukraine_N_1d.rel.I5870
I3713_published	9.28E-02	5.8%	51.4%	42.8%	3.4%	5.5%	6.0%	1.7	Ukraine_N
I1732	3.43E-01	3.5%	51.4%	45.1%	1.8%	3.1%	3.3%	1.9	Ukraine_N
I1378_enhanced	5.91E-02	4.0%	51.4%	44.6%	2.2%	3.8%	4.0%	1.8	Ukraine_N_son.I1732
I3715	3.50E-01	5.1%	51.1%	43.8%	1.8%	3.5%	3.7%	2.8	Ukraine_N
I5888_enhanced	2.30E-02	6.3%	50.9%	42.9%	1.8%	3.0%	3.3%	3.5	Ukraine_N_father.or.son.I5875
I27982	1.80E-03	11.9%	50.9%	37.2%	4.8%	7.5%	8.0%	2.5	Ukraine_N
I27994	2.39E-01	7.6%	50.8%	41.6%	2.0%	3.1%	3.3%	3.8	Ukraine_N
I5883	6.50E-01	7.2%	50.4%	42.4%	2.5%	3.9%	4.3%	2.9	Ukraine_N
I4112_enhanced	3.14E-02	6.4%	50.2%	43.4%	2.1%	3.7%	3.7%	3.0	Ukraine_N_dup.I4112
I5889_published	3.31E-01	10.2%	50.0%	39.8%	3.6%	5.4%	5.6%	2.8	Ukraine_N
I3721	5.35E-01	15.2%	49.6%	35.3%	3.1%	5.0%	5.2%	4.9	Ukraine_N
I5893_enhanced	5.52E-01	4.0%	48.9%	47.1%	2.3%	3.5%	3.8%	1.7	Ukraine_N_1d.rel.I5881
I3714	4.43E-01	8.1%	48.8%	43.1%	2.6%	4.0%	4.5%	3.1	Ukraine_N
I5879	9.33E-01	5.0%	48.7%	46.3%	2.5%	4.2%	4.4%	2.0	Ukraine_N_father.or.son.I3718
I5891_enhanced	2.98E-01	2.6%	48.3%	49.0%	2.9%	4.4%	5.0%	0.9	Ukraine_N_1d.rel.I4114
I3712_published	9.16E-01	14.3%	47.7%	38.1%	3.4%	5.2%	5.7%	4.2	Ukraine_N
I5875	2.34E-01	7.0%	46.8%	46.2%	1.9%	3.3%	3.5%	3.7	Ukraine_N
I1734	8.96E-01	7.2%	46.8%	46.0%	1.9%	3.0%	3.2%	3.8	Ukraine_N
I4114	7.20E-01	7.3%	46.0%	46.7%	1.9%	2.9%	3.1%	3.8	Ukraine_N
I5873_published	7.85E-01	12.3%	45.9%	41.8%	4.9%	7.9%	8.2%	2.5	Ukraine_N
I5881_published	8.66E-01	5.6%	45.8%	48.6%	3.0%	5.1%	5.4%	1.9	Ukraine_N
I4112_published	3.76E-01	7.6%	45.6%	46.8%	3.5%	5.6%	5.7%	2.2	Ukraine_N
I4111	2.08E-02	8.6%	45.1%	46.3%	1.8%	3.0%	3.3%	4.8	Ukraine_N
I1738	2.69E-03	5.8%	44.1%	50.2%	1.8%	3.2%	3.4%	3.2	Ukraine_N
I5890	2.39E-01	7.9%	43.7%	48.4%	2.0%	3.4%	3.8%	4.0	Ukraine_N
I5881_enhanced	4.98E-02	8.2%	43.2%	48.6%	1.8%	3.1%	3.3%	4.6	Ukraine_N
I3718	6.34E-01	8.0%	42.9%	49.1%	1.9%	3.1%	3.4%	4.2	Ukraine_N
I27990	2.40E-01	10.3%	39.5%	50.2%	2.7%	4.7%	4.9%	3.8	Ukraine_N
I5868_published	7.67E-01	12.5%	38.1%	49.4%	4.8%	7.8%	8.5%	2.6	Ukraine_N
I3719_enhanced	9.27E-01	103.5%	4.2%	-7.6%	1.6%	2.2%	2.4%	64.7	Ukraine_N_Deriivka_I3719
Modeling Ukraine Neolithic individuals with CHG as a source									
Individual	P-value with LBK	P-value with CHG	CHG	EHG	BHG	CHG	EHG	BHG	
I5886_enhanced	5.60E-04	2.20E-03	8.50%	50.80%	40.70%	2.50%	3.70%	2.90%	
I5886_published	2.20E-02	4.70E-02	13.10%	47.60%	39.40%	3.90%	6.00%	4.70%	
I31730	3.70E-03	9.30E-02	7.40%	48.30%	44.40%	2.80%	4.50%	3.70%	
I5888_enhanced	2.30E-02	1.10E-02	6.50%	45.60%	47.90%	2.20%	3.70%	3.10%	
I27982	1.80E-03	1.00E-03	16.70%	38.20%	45.10%	6.50%	9.90%	7.40%	
I4112_enhanced	3.10E-02	2.00E-02	7.10%	44.40%	48.50%	2.80%	4.30%	3.30%	
I4111	2.10E-02	1.80E-03	7.90%	39.80%	52.40%	2.30%	3.60%	3.10%	
I1738	2.70E-03	2.30E-01	10.20%	37.10%	52.70%	2.20%	3.40%	3.10%	
I5881_enhanced	5.00E-02	3.90E-04	6.90%	39.60%	53.50%	2.30%	3.50%	3.20%	

EHG = Lebyazhinka_HG; BHG = Serbia_IronGates_Mesolithic; CHG = Caucasus_Hunter_Gatherer. We include close relatives and outliers. P-values from qpAdm are based on a Hotelling T2 test.

Extended Data Table 3 | By-individual modelling of Trypillians

Trypillian individual	P-value	Proportions			Std. errors			Z-score of BPgroup
		BPgroup	Iron Gates	YUN_CA	BPgroup	Iron Gates	YUN_CA	
I2111_enhanced	0.6637	-5.1%	20.4%	84.7%	3.7%	3.4%	3.3%	-1.4
VERT117_wNonUDG.SG	0.0863	-3.9%	14.9%	89.0%	2.6%	2.5%	2.3%	-1.5
I7586	0.3971	-1.4%	14.3%	87.1%	2.3%	2.2%	2.1%	-0.6
VERT029_wNonUDG.SG	0.3637	0.6%	13.5%	86.0%	2.3%	2.2%	2.0%	0.3
VERT035_wNonUDG.SG	0.0279	0.9%	17.8%	81.4%	2.4%	2.1%	2.1%	0.4
VERT028_wNonUDG.SG	0.1660	1.0%	15.8%	83.1%	2.5%	2.2%	2.1%	0.4
VERT100B_wNonUDG.SG	0.2974	1.7%	15.2%	83.0%	2.3%	2.1%	2.1%	0.7
I1929	0.5967	1.8%	14.7%	83.5%	6.6%	5.7%	5.2%	0.3
I13064	0.1473	3.0%	14.9%	82.1%	2.2%	2.1%	1.9%	1.4
VERT030_wNonUDG.SG	0.1079	3.2%	12.7%	84.1%	2.4%	2.2%	2.0%	1.3
VERT115_wNonUDG.SG	0.3177	3.4%	14.2%	82.3%	3.0%	2.7%	2.6%	1.1
VERT106C_wNonUDG.SG	0.9459	3.5%	15.5%	81.1%	3.1%	2.7%	2.7%	1.1
VERT015_wNonUDG.SG	0.0019	3.8%	13.5%	82.7%	2.3%	2.1%	2.0%	1.7
VERT033_wNonUDG.SG	0.0606	3.9%	12.2%	83.9%	2.6%	2.3%	2.2%	1.5
VERT107_wNonUDG.SG	0.0914	3.9%	17.4%	78.7%	2.3%	2.2%	2.0%	1.7
I7584	0.3849	5.1%	12.6%	82.2%	5.0%	4.4%	4.1%	1.0
I2110	0.4913	5.3%	13.5%	81.1%	2.4%	2.3%	2.2%	2.2
VERT105B_wNonUDG.SG	0.0105	5.4%	12.3%	82.3%	2.5%	2.3%	2.1%	2.2
VERT111_wNonUDG.SG	0.0004	5.5%	10.2%	84.3%	2.7%	2.5%	2.3%	2.0
I1926_enhanced	0.3223	5.9%	16.0%	78.1%	2.3%	2.3%	2.1%	2.6
VERT104B_wNonUDG.SG	0.2516	5.9%	12.0%	82.2%	2.4%	2.0%	2.0%	2.5
I3151_enhanced	0.4581	6.1%	14.8%	79.1%	3.9%	3.6%	3.3%	1.6
VERT118_wNonUDG.SG	0.3989	7.1%	12.2%	80.7%	2.6%	2.3%	2.2%	2.7
I7920	0.1891	7.5%	13.5%	79.0%	2.4%	2.0%	2.1%	3.1
VERT103B_wNonUDG.SG	0.0252	8.2%	10.6%	81.2%	2.6%	2.2%	2.2%	3.2
I7923	0.7187	9.2%	15.3%	75.5%	5.6%	5.1%	4.2%	1.6
VERT031_wNonUDG.SG	0.5192	13.5%	11.5%	75.0%	2.5%	2.2%	2.2%	5.4
I20069	0.0926	25.8%	9.9%	64.3%	2.4%	2.2%	2.1%	10.8

P-values from qpAdm are based on Hotelling T2 test.

Article

Extended Data Table 4 | Genetic profiles of individuals in the North Pontic Region 4500-2500 BCE are well described as a result of three expansion waves: two waves of Caucasus-Lower Volga (CLV) cline expansion and a wave of Yamna expansion (expanded version of Table 1)

Genetic ID, Arch. ID, Date	Pop. Source(s)	P-value	Comment
Wave 1: Early pioneers from the genetically northern end of the Caucasus-Lower Volga (CLV) cline & their descendants			
I20072: Giurgulești Burial 6 (3), 4330-4058 calBCE	BPgroup ^a	0.896	Neolithic Individual from Moldova who was a descendant of Lower-Volga North Caucasus Neolithic people (the low-EHG end (BPgroup endpoint, Fig. 2a) of the Volga Cline at a junction with the Caucasus-Lower Volga (CLV) cline), an example of long-range migration across the NPR
I5124: Csongrád Burial 1, 4331-4073 calBCE	87% BPgroup and 13% Lebyazhinka_HG	0.116	Neolithic Individual from Hungary with ancestry from the BPgroup end of the Volga cline, similar to a subset of Khvalynsk individuals, an example of long-range migration across the NPR
Trypillia genetic ancestry forming 4832-4358 BCE	Mean: 5% BPgroup, 14% BHG, 81% YUN_CA ^b	7e-6	Heterogeneous Neolithic Trypillia population from Ukraine and Moldova formed on the European farmer-hunter-gatherer cline and included CLV with admixture from Usatove-related groups in the second half of the 4 th millennium BCE. The given model fits 23 of 28 Trypillian individuals but not the Trypillians as a whole.
Usatove (Mayaky), genetic ancestry forming 4571-4371 BCE	45% PVgroup ^c and 55% Trypillians	0.128	Neolithic Usatove from Mayaky in Ukraine were an even mix of an intermediate PVgroup population on the CLV cline or, alternatively a mix of BPgroup and Caucasus Neolithic (Aknashen), and Trypillians
Usatove (Mayaky), MAJ	44% PVgroup and 56% Trypillians	0.231	Another group of Usatove individuals from Mayaky ^d
Usatove (Usatove-Velykyj Kuyalnik), USV	48% PVgroup and 52% Trypillians	0.083	Usatove individuals from Usatove-Velykyj Kuyalnik in Ukraine ^d
Cernavodă I, KTL_A, genetic ancestry forming 4340-4058 BCE	54% BPgroup and 46% Trypillians	0.618	Neolithic Cernavodă I population from Kartal in Ukraine (cluster A ⁴) an even mix of BPgroup and European farmers. This mix is similar to Usatove and related populations, but without the Caucasus Neolithic ancestry evident in Usatove; the mixture that formed KTL_A also occurred significantly later on average.
Wave 2: Migration from the genetically intermediate part of the CLV cline and establishment of Core Yamna ancestry			
Serednii Stih, genetic ancestry forming ca. 4400 BCE ³⁵ (SShi, SSmed, SSlo subsets)	CLV ancestry: 13-17% Aknashen Neolithic and 8-56% BPgroup; Dniro-Don ancestry: 31-56% GK2 ancestry	0.102-0.851	Neolithic Stih Individuals from Ukraine were genetically heterogeneous but formed a cline between CLV people (themselves a mix of Caucasus Neolithic (Aknashen-related) and North Caucasus-Lower Volga Neolithic (BPgroup-related) people) with Dniro-Don people (Ukraine Neolithic hunter-gatherer-related) ⁷
Core Yamna, genetic ancestry forming 4132-3944 BCE	26% Remontnoye ^a and 74% SShi subset of Serednii Stih	0.675	Early Bronze Age (EBA) Core Yamna cluster includes individuals across 5000 km from central Siberia to southeastern-central Europe and was formed on the basis of admixture of CLV people with Dniro-Don people. Their emergence likely occurred in the North Pontic Region as descendants of a late Stih population who are unique in possessing this combination of ancestries ⁷
Cernavodă I, KTL_B, genetic ancestry forming 4438-3898 BCE	CLV ancestry: 21% Aknashen Neolithic and 57% BPgroup; Dniro-Don ancestry: 23% GK2 ancestry	0.934	Neolithic Cernavodă I population from Kartal cluster B in Ukraine cluster B ⁵ had much less CLV ancestry than the cluster A individuals. This ancestry was also not from the Lower Volga (BPgroup) end of the CLV cline, but rather from a population like Maykop or Remontnoye
I1428: Rilti Kurgan 264, Burial 5, 3360-2890 calBCE	27% Remontnoye and 73% European farmers (YUN_CA+Globular Amphora)	0.294	Neolithic individual from Bulgaria who was a mixture of CLV people (PVgroup or Remontnoye) and European farmers such as YUN_CA
I17973: Bursuceni Kurgan 1, Burial 21, Skeleton 1, 3354-3103 calBCE	50% Remontnoye and 50% YUN_CA	0.558	Neolithic individual from Bulgaria who was a mixture of CLV people (PVgroup or Remontnoye) and European farmers such as YUN_CA
	Maykop (?)	0.0025	Late Neolithic Individual from the same burial as I17974 is related to populations from the Caucasus (Fig. 2) but with some unspecified ancestry
Wave 3: Yamna expansion			
Core Yamna			
I32534: Mykhailivka 1, Square VI, 3635-3383 calBCE	Core Yamna	0.684	Neolithic individual from Ukraine is the earliest ¹⁴ C-dated individual with Core Yamna ancestry in the NPR
I20196: Crasnoe Kurgan 9, Burial 9, Skeleton 2, 3352-3101 calBCE	Core Yamna	0.683	Neolithic Individual from Moldova was genetically a Yamna descendant
I12229: Mayaky, Kurgan 1, Burial 9, 3088-2911 calBCE	Core Yamna	0.178	EBA Individual from the Usatove site at Mayaky is discontinuous with the earlier Usatove people from Mayaky and was genetically a Yamna descendant
I20079: Taraclia II, Kurgan 10, Burial 2, 2571-2355 calBCE	Core Yamna	0.864	Early-Middle Bronze Age (EMBA) Individual from Zhyvotylivka-Volchansk/III-C (ZV/III-C) type burial from Moldova was genetically a Yamna descendant
Catacomb Archaeological Complex I12840: Dubynove, Kurgan 1, Burial 10, 2453-2148 calBCE I16668: Revova, Kurgan 3, Burial 10, 2800-2000 BCE	Core Yamna	0.075	EMBA Catacomb individuals from Ukraine (MJ-09 from Mamaj Gora ³⁷ , I12840 and I16668, this study) were Yamna descendants
Core Yamna + European Farmer-Hunter-Gatherer descendants			
I1456: Durankulak, Kurgan F, burial 15 (main burial), 3500-3000 BCE	45% Core Yamna and 55% Globular Amphora	0.099	Neolithic Individual from Bulgaria was a Yamna+Globular Amphora descendant representing a similar mix (but in different proportions) to the Corded Ware
Bulgaria Yamna, 3300-2500 BCE	Core Yamna and 0-22% YUN_CA	-	
Bulgaria Yamna, Boyanovo subset, 3300-2500 BCE ⁵	94% Core Yamna and 6% YUN_CA	0.211	EBA Yamna individuals from Bulgaria, Moldova, and Ukraine (7 and herein) included unadmixed Core Yamna as well as others with European farmer ancestry. This admixture started no later than the date of individual I17743 (Mereni II) from Moldova (3358-3100 BCE) which already had 6.9% such ancestry.
Moldova Yamna, 3300-2500 BCE	Core Yamna and 0-16% YUN_CA	-	
Ukraine Yamna, 3300-2500 BCE	Core Yamna and 0-8% YUN_CA	-	
I17747: Tiraspol Kurgan 3, Burial 15, 2865-2576 calBCE	61% Core Yamna and 39% Trypillia	0.523	Late EBA Yamna individual from Moldova had more farmer ancestry than other Yamna from the region
I20076: Ocnița Kurgan 1, Burial 3, 2906-2702 calBCE	88% Core Yamna and 12% Globular Amphora	0.180	Individual from an EBA Yamna burial in Moldova with Globular Amphora-style pot is analyzed separately but is of mostly Yamna descent
I4110, I5882, I5884: Deriivka I cemetery, 3500-2700 BCE ⁸	36-46% Core Yamna, 23-44% Balkan Hunter Gatherer (BHG), 15-32% Trypillia	0.179-0.889	Three Neolithic-EBA individuals from Ukraine had some Yamna ancestry but substantial (BHG) ancestry represented by Serbia Iron Gates hunter-gatherers
I13071: Bil'shivtsi Individual 1, 2201-2032 calBCE	72% Core Yamna and 28% YUN_CA	0.458	Middle Bronze Age (MBA) individual from a catacomb burial in western Ukraine with 2/3-1/3 Core Yamna-European Farmer ancestry, the source of the farmer ancestry being unclear.
I12234: Liubasha Kurgan Burial 3, 1499-1127 calBCE I7925: Liubasha Kurgan, Burial 9, 2119-1624 calBCE I12235: Liubasha Kurgan, Burial 11, 1686-1311 calBCE I16674: Liubasha kurgan Burial 15, 2434-1943 calBCE I12231: Sychavka Kurgan, Burial 18, 2118-1565 calBCE	77% Core Yamna and 15% Globular Amphora and 8% UNHG	0.148	These five MBA individuals of Multi-Cordoned Ware/Babyne archaeological circle from Ukraine were mostly of Yamna descent but mixed with a population of even more hunter-gatherer ancestry than the Globular Amphora
Core Yamna + Dniro-Don Hunter Gatherer descendants			
Don Yamna, 3200-2600 BCE	40% Core Yamna and 60% SSmed	0.237	Yamna from the lower Don were formed on the basis of the same elements as the Core Yamna and Serednii Stih but with more UNHG Ukraine Neolithic ancestry ⁷
Core Yamna + Steppe Maykop descendants			
I20078: Taraclia II Kurgan 2, Burial 14, 3340-3034 calBCE	39% Core Yamna, 61% Steppe Maykop	0.432	Late Neolithic Individual from a ZV/III-C type burial from Moldova was mix of Yamna with Steppe Maykop
I17974: Bursuceni Kurgan 1 Burial 21, Sk. 2, 3334-3030 calBCE	82% Core Yamna, 18% Steppe Maykop	0.324	Late Neolithic Individual from a ZV/III-C type burial from Moldova, another mix of Yamna & Steppe Maykop
Yamna + Maykop descendants			
I1917: Ozera Kurgan 18 Burial 14, 3096-2913 calBCE	50% Core Yamna and 50% Maykop	0.345	This individual from Ukraine ⁸ displaying mixed Maykop-Yamna burial traditions had half Maykop ancestry
Mayaky Yamna, 2900-2500 BCE	81% Don Yamna and 19% Maykop	0.424	Three EBA Yamna individuals from Kurgan 1 and a ground burial at the Usatove site of Mayaky ⁵ were a mixture of Don Yamna (itself a mixture of Core Yamna and Dniro-Don hunter-gatherers) and Maykop

Notes: For admixture dates we give one standard error, and a 95% confidence interval. For direct dates on bones analysed for DNA, we indicate the 95% calibrated confidence with suffix "calBCE"; all other dates are archaeologically estimated ranges. "BPgroup" is a homogeneous group from the Lower Volga-North Caucasus Neolithic (CLV) at the bend between CLV and Volga (EHG-rich) clines (Fig. 2a) from Berezhnovka and Progress 2 that carries CHG, EHG, and Siberian/Central Asian Neolithic-related ancestries⁷. "Balkan farmers of Gumelnița/Karanovo from Yunatsite in Bulgaria." "PVgroup BP-related group from the CLV cline with more Aknashen (south Caucasus) ancestry than BPgroup, from Berezhnovka & Vonyuchka⁷." "Remontnoye represents a population composed of a southern ancestry represented by either the Aknashen Neolithic of Armenia or the Bronze Age Maykop, and a northern ancestry from the low-EHG end of the Volga Cline such as the BPgroup⁷."