

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

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**The north Black Sea (Pontic) Region was the nexus of the farmers of Old Europe and the foragers and pastoralists of the Eurasian steppe<sup>1,2</sup>, and the source of waves of migrants that expanded deep into Europe<sup>3-5</sup>. We report genome-wide data from 78 prehistoric North Pontic individuals to understand the genetic makeup of the people involved in these migrations and discover the reasons for their success. First, we show that native North Pontic foragers had ancestry not only from Balkan and Eastern hunter-gatherers<sup>6</sup> but also from European farmers and, occasionally, Caucasus hunter-gatherers. More dramatic inflows ensued during the Eneolithic, when migrants from the Caucasus-Lower Volga area<sup>7</sup> moved westward, bypassing the local foragers to mix with Trypillian farmers advancing eastward. People of the Usatove archaeological group in the Northwest Pontic were formed ca. 4500 BCE with an equal measure of ancestry from the two expanding groups. A different Caucasus-Lower Volga group, moving westward in a distinct but temporally overlapping wave, avoided the farmers altogether, and blended with the foragers instead to form the people of the Serednii Stih archaeological complex<sup>7</sup>. A third wave of expansion occurred when Yamna descendants of the Serednii Stih forming ca. 4000 BCE expanded during the Early Bronze Age (3300 BCE). The temporal gap between Serednii Stih and the Yamna expansion is bridged by a genetically Yamna individual from Mykhailivka in Ukraine (3635-3383 BCE), a site of uninterrupted archaeological continuity across the Eneolithic-Bronze Age transition, and the likely epicenter of Yamna formation. Each of these three waves propagated distinctive ancestries while also incorporating outsiders during its advance, a flexible strategy forged in the North Pontic region that may explain its peoples' outsized success in spreading their genes and culture across Eurasia<sup>3-5,8-10</sup>.**

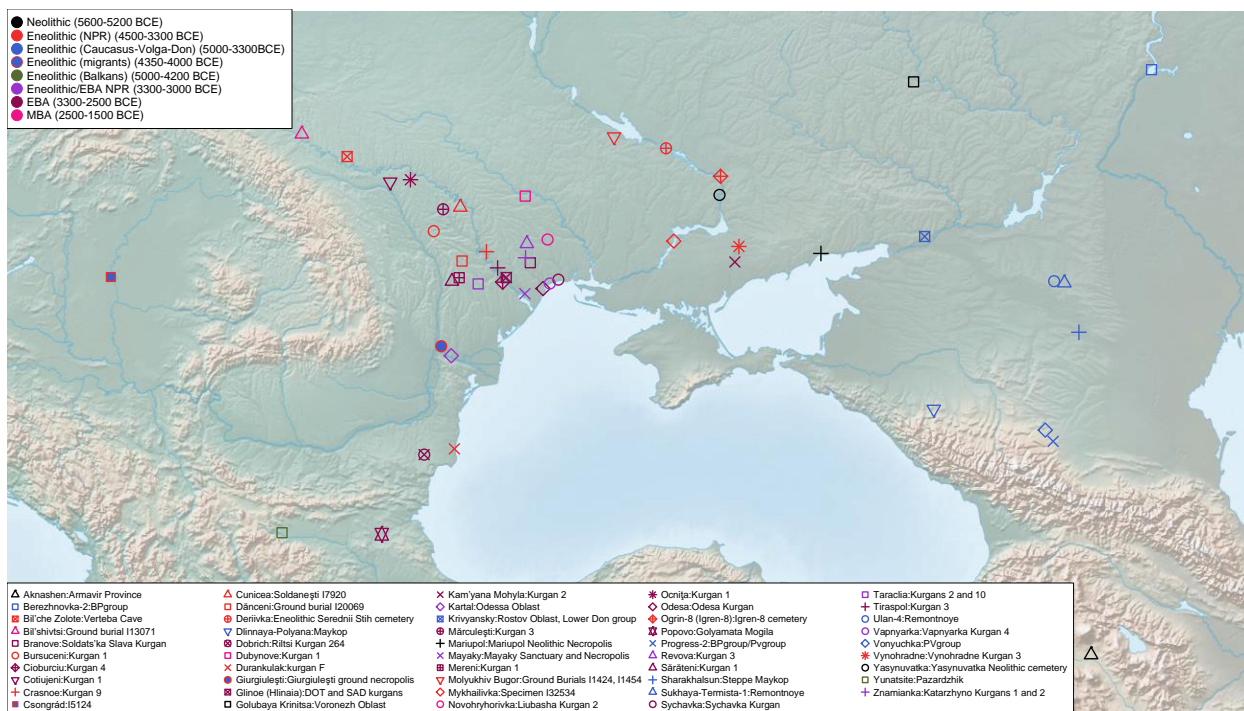
## 1 Introduction

2  
3 The steppe and forest-steppe regions north of the Black Sea, known as the North Pontic Region  
4 (NPR, Fig. 1, Supplementary Information, section 1), have been proposed as the homeland for  
5 communities that developed core Indo-European language terminology<sup>11</sup>, which began spreading  
6 across Eurasia facilitated by the turn-of-the-third-millennium expansion of the Yamna  
7 archaeological complex (YAC)<sup>10</sup>. During the Early Metal Ages (Eneolithic and the Early Bronze  
8 Age, EBA), a diverse array of archaeological groups inhabited the NPR. In principle, important  
9 information about how these populations interacted with each other can be learned from their  
10 genetic relationships—complementing the archaeological evidence—but key aspects remain  
11 poorly understood.

12  
13 Genome-wide ancient DNA studies have revealed that from the beginning of the Holocene to the  
14 end of the Neolithic (approximately 9200-5000 BCE), the genetic ancestry of hunter-gatherer  
15 groups in the NPR and adjacent areas was derived from a mixture of ancestral populations whose  
16 ancestry was on a genetic gradient ranging in the west from “Western Hunter-Gatherers”  
17 (WHGs) and “Balkan Hunter Gatherers” (BHG) who lived in the Danubian Iron Gates region<sup>6</sup>,  
18 to “Eastern Hunter-Gatherers”<sup>3</sup> (EHGs) in the east. In Ukraine, the transition from the Mesolithic  
19 to the Neolithic was marked by WHG admixture with the EHG ancestry of previously  
20 established local populations<sup>6</sup>.

21  
22 During the Neolithic, after ca. 5800 BCE, the western NPR saw an expansion of Balkan and  
23 central European farming groups, such as Criş, Starčevo, and LBK, carrying Early European

24 Farmer (EEF) ancestry, who, in turn, were descended from Anatolian Neolithic Farmers (ANF)  
 25 with different proportions of WHG admixture<sup>12</sup>. In the northeastern NPR, the Neolithic  
 26 populations of the Dnipro Valley continued to retain the EHG/WHG-based genetic ancestry<sup>6</sup>.  
 27



28  
 29  
 30 **Fig. 1: Map of sampling locations including newly generated data and key context**  
 31 **populations.**  
 32

33 In the early Eneolithic (ca. 4800 BCE), farming groups of the Cucuteni-Trypillia archaeological  
 34 complex (CTAC) began spreading over the forest-steppe part of the western NPR, reaching the  
 35 middle Dnipro Valley by the first half of the 4<sup>th</sup> millennium BCE<sup>13</sup>. Archaeologists trace the  
 36 origin of CTAC to western Transylvania<sup>13,14</sup>. The genetic ancestry of CTAC was primarily EEF-  
 37 derived with admixture from WHG, EHG and Caucasus Hunter-Gatherers (CHG)<sup>6,15–18</sup>.

38  
 39 During their eastward expansion, CTAC encountered mobile steppe communities of the Serednii  
 40 Stih archaeological complex (SSAC)<sup>13</sup>, which likely emerged from the Azov-Dnipro-Donets area  
 41 in the first half of the 5<sup>th</sup> millennium BCE<sup>19–21</sup>. The presence of early SSAC in the Azov steppe  
 42 ca. 4700-4500 BCE is supported by Sr isotope analysis of an early SSAC individual from the  
 43 Mariupol necropolis (Supplementary Information, section 1). However, knowledge about the  
 44 genetic ancestry of steppe populations like SSAC (referred to as “steppe ancestry”<sup>3–6,10,13</sup>) has  
 45 been limited until now due to small sample sizes which revealed highly variable ancestry<sup>6,13,18</sup>.

46  
 47 In the 4<sup>th</sup> millennium BCE, a distinctive archaeological complex known as Usatove was  
 48 established in the northwestern NPR. Sampled individuals from Usatove harbored EEF and  
 49 steppe ancestries, as well as a Caucasus Eneolithic/Maykop-related genetic component<sup>5</sup>, but the  
 50 knowledge of the proximate sources of the composing ancestries has been unclear.

52 In the second half of the 4<sup>th</sup> millennium BCE, the NPR witnessed an expanding diversity of  
53 archaeological groups, characterized by distinct burial rites and pottery types/techniques, and  
54 increased mobility, possibly including wheeled wagon transportation<sup>2</sup>. This diversity came to an  
55 end in the last third of the 4<sup>th</sup> millennium with the expansion of the YAC, persisting into the  
56 Early Bronze Age (EBA) during the first half of the following millennium.

57  
58 Genetic ancestry data on the Epipaleolithic-Early Bronze Age populations of the NPR come  
59 from a limited number of sites, hampering the understanding of population dynamics,  
60 particularly during the time that preceded a genetic turnover in Europe precipitated by YAC-  
61 related people<sup>3,4,10,22</sup>. This report analyses prehistoric NPR individuals from a much wider  
62 selection of archaeological sites than has previously been available, including substantially larger  
63 sample sizes from key groups, in particular CTAC, Usatove, and SSAC. Co-analyzing with the  
64 data reported in the linked paper<sup>7</sup>, we examine the contribution of these groups to the genetic  
65 ancestry of YAC and have a particular focus on integrating the results of the present study with  
66 the archaeological evidence to produce a holistic picture of genetic and archaeological  
67 transformations preceding and following the formation of the Yamna.

## 68 **Results**

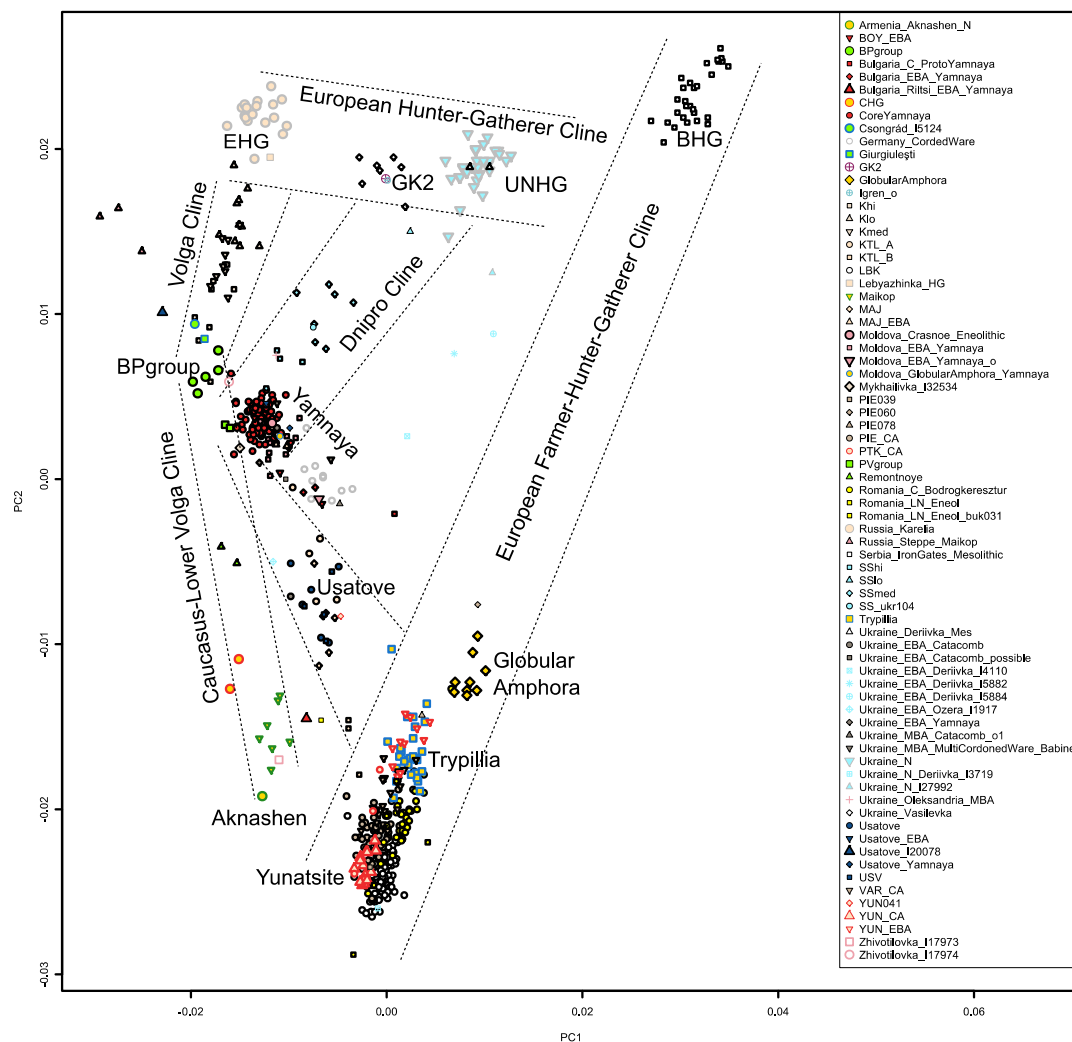
69  
70 We sequenced ancient DNA for 78 ancient individuals from the NPR from the Neolithic to the  
71 Bronze Age. For 73 we report whole genome data for the first time including an individual from  
72 the Neolithic Mariupol necropolis, 11 SSAC individuals from Ukraine, 10 CTAC individuals  
73 from Moldova and Ukraine, and 23 YAC individuals from Moldova and Ukraine; for five  
74 individuals with previously reported results, we increased data quality (Online Table 1). To  
75 generate these data, we sampled 203 skeletal elements and built 452 next-generation sequencing  
76 libraries; after screening we took 235 forward into analysis (Online Table 2). We enriched our  
77 analyses by generating 51 new radiocarbon dates (Online Table 3). We co-analysed these data  
78 with that from a parallel study of steppe populations including 299 newly reported individuals  
79 and 55 individuals with improved data<sup>7</sup>; both studies co-analyze the full dataset.

80  
81 To obtain a qualitative picture of population structure in the NPR, we began by using smartpca<sup>23</sup>  
82 to perform principal component analysis (PCA) using the same set of populations to form the  
83 axes as in<sup>7</sup> (Fig. 2a).

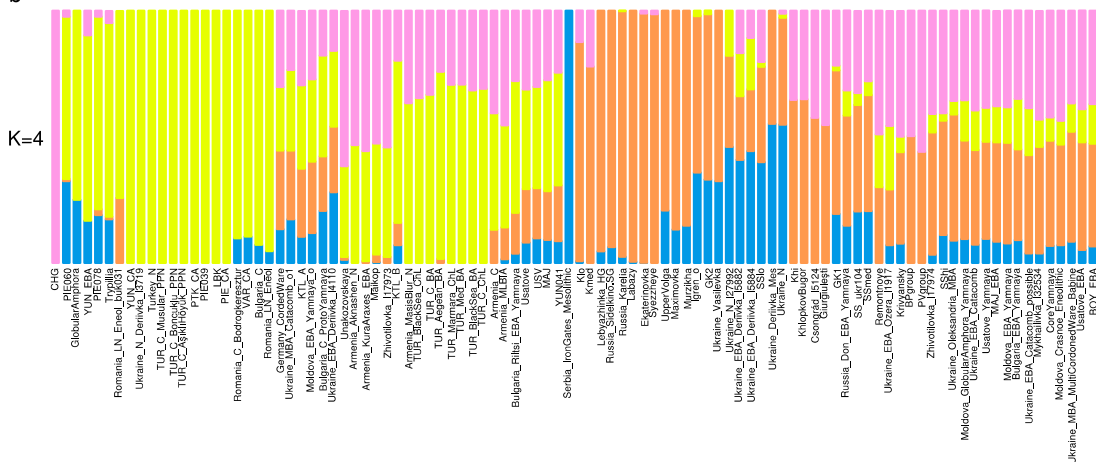
84  
85 The PCA reveals five major clines. Four of them—the Caucasus-Lower Volga (CLV) Cline, the  
86 Volga Cline, the Dnipro Cline, and the European Hunter-Gatherer (EuHG) Cline—are described  
87 formally in the accompanying paper<sup>7</sup>. The fourth, the European Farmer and Hunter Gatherer  
88 cline (EFHG), is formed by European farmers (central European LBK and populations related to  
89 Gumelnița/Karanovo from the Yunatsite site in Bulgaria (Yunatsite Chalcolithic, YUN\_CA), on  
90 one side, and BHG, on the other (Fig. 2a).

91  
92 The UNHG individuals presented in this report are located on the “eastern” end of the EuHG  
93 cline towards the BHG and at the “northern” edge of the Dnipro cline. This PCA placement  
94 suggests that UNHG contributed to the later (Eneolithic and Bronze Age (BA)) people on the  
95 Dnipro cline that are positioned along the length of that cline, with Core Yamna<sup>7</sup> at the  
96 “southern” end.

a



b



**Fig. 2: a, PCA of the NPR samples in relation to the three steppe clines and respective samples from<sup>7</sup>. b, Unsupervised ADMIXTURE summary graph of populations and individuals from this report and<sup>7</sup>.**



97 The Eneolithic (apart from the SSAC) and BA individuals in Fig. 2a are mostly located towards  
98 the “farmer” end of the EFHG cline. Four NPR individuals form a cline stretching from the Core  
99 Yamna cluster towards steppe Maykop and traversing the CLV-Volga cline proximate to a key  
100 Eneolithic population represented by the Berezhnovka-2-Progress 2 individuals (BPgroup), a  
101 genetically homogeneous people between the northeast Caucasus and lower Volga that can be  
102 approximately modeled as a mixture of EHG, CHG, and Siberian/Central Asian Neolithic  
103 ancestries<sup>7</sup>. Two of these (I20078 and I17974) are late Eneolithic (3300-3000 BCE) individuals  
104 from Moldova. The other two, I18740 from Hungary<sup>7</sup> and I20072 from Moldova, dated to ca.  
105 4300-4000 BCE, are archaeologically associated with the Volga-Caucasus – lower Dnipro pulse  
106 of the steppe people that left “ochre graves” across the NPR and adjacent Balkan-Carpathian  
107 area<sup>24,25</sup>.

108

### 109 **Formal modeling of sources of Neolithic NPR ancestry**

110

111 We computed  $f_3$  statistics with Ukraine Neolithic as a target and a wide variety of possible  
112 sources (Supplementary Information, section 2; Extended Data Table 1). Consistent with their  
113 position in the PCA on Fig. 2a, the Ukraine Neolithic population is admixed with the most  
114 significantly negative ( $Z=-17.2$ ) statistic when a sample from Karelia in northwestern Russia  
115 (EHG) and the BHG are used as sources, suggesting that the Ukraine Neolithic population is, to a  
116 first approximation, composed of sources related to the EHG and BHG populations.

117

118 However, it is evident from the PCA in Fig. 2a that the UNHG end of the EuHG cline is shifted  
119 towards populations with EEF ancestry. In unsupervised ADMIXTURE analysis (Supplementary  
120 Information, section 3; Fig. 2b), we find that the UNHG are assigned small components of  
121 Anatolian Farmer/CHG ancestry, not present in other Mesolithic Deriivka (Dnipro Valley), EHG  
122 (Karelia) or BHG (Iron Gates) groups. When samples from individuals labeled Ukraine\_N  
123 (UNHGs) are modeled with other EuHG populations from<sup>7</sup> only a single 2-source model  
124 ( $p=0.576$ ) with  $72.5\pm 2.9\%$  GK2 from the Golyubaya Krinitisa site on the Lower Don<sup>7</sup> and  
125  $27.5\pm 2.9\%$  BHG ancestry, remains viable (here and in what follows, we indicate statistical  
126 uncertainty through standard errors; a 95% confidence interval corresponds to 1.96 standard  
127 errors in either direction of the point estimate). A fitting to a broader cline between EHG and  
128 BHG as a mixture of these two sources with either Lebyazhinka or Karelia as the EHG source,  
129 fails ( $p<1e-9$ ) and qpAdm output suggests that these models underestimate shared genetic drift  
130 with Turkey\_N ( $Z<-3.5$ ).

131

132 To obtain insights into population admixture histories that could explain these patterns, we  
133 explored 3-source models detailed in Supplementary Information, section 2. The feasible models  
134 all include EHG-BHG sources (Lebyazhinka and BHG), but they all also include ~7-9% of EEF  
135 ancestry, with the source of this ancestry (in a specific EEF-derived group) being unclear. The  
136 presence of EEF ancestry (who were largely of Anatolian Neolithic-related origin) accounts for  
137 the underestimated drift with Turkey\_N in the model without such ancestry.

138

139 To test whether the inferred EEF ancestry is a population-wide feature of UNHGs, we fit a  
140 model that included central European LBK farmer ancestry representing EEF populations to 35  
141 individuals with the Ukraine\_N label (Supplementary Information, section 2; Extended Data

142 Table 2). The results show that EEF ancestry is a general feature of UNHG populations, and thus  
143 this pattern is not driven by a few outliers.

144  
145 The UNHG was modeled with significant BHG and EHG ancestry and represents an *increase* of  
146 BHG ancestry relative to the Mesolithic specimens from Vasylivka III<sup>6</sup> and Vasylivka I<sup>26</sup> (Fig.  
147 2). A migration of people from the Iron Gates area in the Dnipro Valley in the 7<sup>th</sup> millennium  
148 BCE<sup>27</sup> may be responsible for this shift. As the BHG population from the Iron Gates has been  
149 shown to carry sporadic EEF ancestry<sup>6</sup>, the admixture of Iron Gates migrants could be a way to  
150 account for both BHG and EEF admixture compared to the Mesolithic Vasylivka.

151  
152 Diverse hunter-gatherers of WHG-EHG mixed background in Sweden<sup>3,28,29</sup> and Latvia provide  
153 no evidence for the EEF ancestry we detect in the UNHG (Supplementary Information, section  
154 2), highlighting the uniqueness of the UNHG in that respect. As an additional control, we used  
155 the Pitted Ware/Battle Axe Culture populations from Ajvide in Sweden<sup>30,31</sup> and Västerbjers<sup>32</sup>,  
156 finding that these populations in which EEF ancestry was incorporated into groups of  
157 predominantly hunter-gatherer background are correctly inferred by our model to have ~1/5  
158 EEF-related ancestry. Our finding of EEF-related ancestry in Ukraine Neolithic hunter-gatherers  
159 provides a separate and much earlier instance of the incorporation of farmer ancestry into the  
160 hunter-gatherer communities at the periphery of the Neolithic expansion in Europe.

161  
162 UNHG individuals I31730 and I1738 that failed the LBK-EHG-BHG model can be modeled  
163 with CHG instead of LBK as a source (Extended Data Table 2), suggestive of CHG-related  
164 ancestry extending past the middle Don<sup>7,33</sup> to the Azov Sea coast and the Dnipro Valley during  
165 the second half of the 6<sup>th</sup> millennium BCE. This sporadic and isolated CHG admixture in UNHG  
166 reflects a qualitatively different phenomenon from the generalized shift of ancestry towards the  
167 CLV cline of all Serebnii Stih and Yamna individuals of the Dnipro Cline (Fig. 2a). Nonetheless,  
168 this find extends the zone of early contacts with the Caucasus that were also transforming  
169 populations of the Don and Volga rivers in the east<sup>7</sup> and generating ancestry profiles radiating  
170 out of the lower Volga-Caucasus area in the Eneolithic.

171  
172 **CLV cline admixture and long-range migration in the early Eneolithic Pontic steppe**

173  
174 Serebnii Stih culture individuals had highly variable genetic ancestry—we subdivided them into  
175 “SShi”, “SSmed”, and “SSlo” subsets based on their degree of relatedness to UNHG—and their  
176 relationship with individuals on the three steppe clines are examined in detail in<sup>7</sup>. In that study,  
177 Serebnii Stih could be modeled without European farmer populations as sources. A summary of  
178 our findings is that the Serebnii Stih can be modeled with one source being the Core Yamna as  
179 the endpoint of the Dnipro cline (a proxy for earlier populations in the Eneolithic for which the  
180 Yamna descend with little or no mixture<sup>7</sup>), and Dnipro-Don HGs (UNHGs or GK2). Because  
181 Core Yamna themselves are formed as mixture of about 2/3 ancestry of populations of the CLV  
182 cline (proxied by PVgroup, BPgroup, Remontnoye, or Maykop) mixed with Dnipro-Don HGs<sup>7</sup>,  
183 the SSAC ancestry formation can be seen as the result of the fusion of CLV cline migrants with  
184 Dnipro-Don HGs.

185

186 A SSAC individual ukr104<sup>18</sup> (the same as I28319 of our study) clusters with the subset of SSAC  
187 individuals with medium contribution from the UNHG group (SSmed)<sup>7</sup> (Fig. 2a) and forms a  
188 clade with it using qpWave ( $p=0.281$ ).

189  
190 The SSAC outlier from Igren-8 (I27930), a detailed analysis of which is presented in<sup>7</sup>, appears to  
191 be of hunter-gatherer ancestry similar to the Neolithic GK2 individual (5610-5390 BCE) from  
192 the Middle Don. These individuals were similar in their ancestry sources to much earlier  
193 Mesolithic hunter-gatherers from Vasylivka<sup>6,26</sup> (Fig. 2a) and could be modeled as having  $\sim 2/3$   
194 EHG and  $\sim 1/3$  BHG ancestry<sup>7</sup>. Individual I27930 thus represents a Neolithic ancestry carry-over  
195 in a burial context of SSAC (Supplementary Information, section 1), likely appearing in the  
196 Dnipro Valley as a result of a long-range migration from the Middle Don.

197  
198 In the northwest NPR, individual I20072 (4330-4058 calBCE) from Giurgiulești on the Lower  
199 Danube is cladal with the Lower Volga-North Caucasus Eneolithic groups (BPgroup and, with  
200 lower confidence, PVgroup, Supplementary Information, section 2) and, along with  
201 contemporaneous Csongrád individual from Hungary, represents an example of long-distance  
202 migration, across an even larger range than individual I27930 from Igren, spanning from the  
203 Volga to the heart of Central Europe.

204

## 205 **Trypillia and Usatove**

206

207 Trypillian individuals<sup>6,15-17</sup> are on the farmer end of the EFHG cline in the PCA (Fig. 2a).  
208 Admixture  $f_3$ -statistics show that they are admixed (Extended Data Table 1) with a highly  
209 significant negative statistic with Yunatsite Chalcolithic<sup>5</sup> and BHG<sup>6</sup> as sources ( $Z=-23.8$ ) which  
210 parallels the PCA in suggesting that Trypillians have more hunter-gatherer ancestry than the EEF  
211 populations such as Yunatsite or LBK, but without identifying EEF ancestry sources<sup>34</sup>.

212

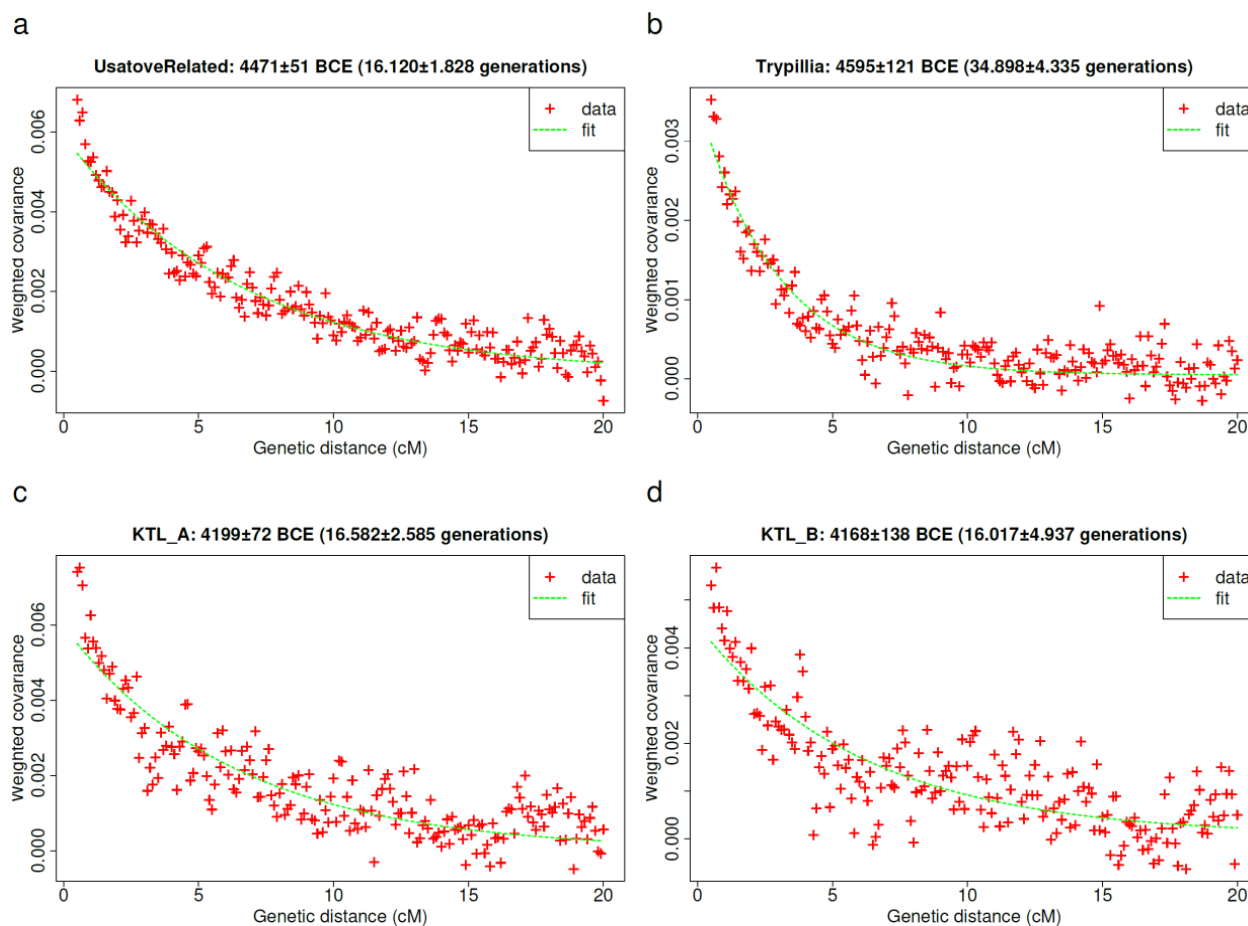
213 When we attempt to model Trypillians as a mixture of two or three sources using qpAdm, we  
214 find no fitting model for them as a whole. We explored removing the Trypillian individual that is  
215 the strongest genetic outlier (I20069 from Dănceni, 3323-2935 calBCE). However, even after  
216 excluding I20069, we still were not able to model Trypillians successfully ( $p < 1e-5$  even for  $N=3$   
217 models).

218

219 In an alternate approach to model individuals under the “Trypillia” label, 24 of 28 Trypillian  
220 individuals can be modeled in our framework. The four exceptions are discussed in the  
221 Supplementary Information, section 2. A single model (with BPgroup, YUN\_CA, and BHG) is  
222 feasible for 23 of the 24 individuals. Three other models are qualitatively similar to the identified  
223 model and are feasible for 22 out of 24 individuals, all models including some CLV (Extended  
224 Data Table 3). Our calculations show that for many individuals there is no significant BPgroup-  
225 related ancestry, but this kind of ancestry is highest in the PCA outlier (I20069;  $25.8 \pm 2.4\%$ ). The  
226 BP ancestry is positive for all but three, and positive by more than two standard errors for 10 of  
227 the 28 individuals. For the 23 Trypillia individuals modeled in our framework, we estimate that  
228 their genetic ancestry is, on average, 81% Balkan Eneolithic (such as in YUN\_CA), 14% BHG,  
229 and the remaining 5% comes from the CLV cline (BPgroup) (Table 1). We estimate using  
230 DATES<sup>35</sup> that the formative admixture of Trypillia took place  $4595 \pm 121$  BCE (95% C.I. 4832-  
231 4358 BCE) (Table 1, Fig. 3).



232



233

234

235 **Fig. 3: DATES estimates of admixture timing of CLV and European farmer ancestry**  
236 **admixture. (a) Usatove-related individuals from this study and<sup>5</sup>. (b) Trypillians from this**  
237 **study and<sup>17</sup>. Kartal cluster A (c) and B (d) from<sup>5</sup>.**

238

239 Usatove individuals from our study, combined with previous reports to provide a substantial  
240 sample size, are genetically varied and occupy the space in the PCA between the Trypillians and  
241 the point where the three great clines of the steppe (CLV, Volga, and Dnipro) diverge from each  
242 other. Evidence of their admixed origin comes from the significantly negative admixture  $f_3$ -  
243 statistic with Karelia (EHG) and Yunatsite Chalcolithic as sources ( $Z=-10.1$ ; Extended Data  
244 Table 1) suggesting that the Usatove had ancestry from east of the NPR as well as ancestry  
245 related to European farmers. Formal modeling with qpAdm reveals that the Usatove population  
246 can be modeled uniquely ( $p=0.128$ ) as a mixture of ~45% PVgroup (and intermediate group on  
247 the CLV cline) and ~55% Trypillians (Table 1). The model with BPgroup+Trypillians fails quite  
248 clearly ( $p<1e-4$ ). A generalized 3-way model with BPgroup+Armenia\_Aknashen\_N (South  
249 Caucasus) ancestry, substituting PVgroup for Aknashen+BPgroup, and allowing the eastern  
250 source to vary to any position along the CLV Cline, fits ( $p=0.393$ ) with an estimated  $14.4\pm 3.1\%$   
251 Aknashen-related ancestry (Supplementary Information, section 2), confirming that the CLV  
252 ancestry in Usatove was not from the lower Volga-centered BPgroup, but had a significant  
253 proportion of southern Caucasus Neolithic-related ancestry.

254 Our conclusions about the Usatove being a PVgroup+Trypillia model were confirmed on two  
255 other populations from<sup>5</sup>: MAJ, a different Eneolithic sample set from Mayaky ( $p=0.231$ ) as well  
256 as the USV population from Usatove-Velykyj Kuyalnik ( $p=0.083$ ). Neither YUN\_CA nor  
257 Globular Amphora works as a source for the Usatove, which is well explained by local Trypillian  
258 origins for their farmer-related ancestry ( $p<1e-4$ ). In contrast to Usatove, the CLV admixture in  
259 the Cernavodă I population from Kartal (KTL\_A) in the Danube delta is best estimated as  
260 BPgroup-derived, with relatively less or no Aknashen-related ancestry (Table 1). We estimate  
261 using DATES<sup>35</sup> that the formative admixture of Usatove took place  $4471\pm 51$  BCE (95% C.I.  
262 4571-4371 BCE) (Table 1, Fig. 3).

## 264 Yamna ancestry and Maykop/steppe Maykop migrations in the Late Eneolithic NPR

265  
266 Following<sup>7</sup>, we define a group we call “Core Yamna,” who we represent by a set of 104  
267 individuals that are archaeologically assigned to the Yamna and Afanasievo cultures, all of  
268 whom have excellent data quality (at least 400,000 of the targeted autosomal SNPs), and that are  
269 genetically homogeneous according to qpWave ( $p\geq 0.2$ ). In<sup>7</sup> we show that these individuals  
270 descend with little or no mixture from an ancestral population that began expanding from a small  
271 founding group around 3750-3350 BCE. Core Yamna is also the largest ancestral source in all  
272 individuals carrying Yamna ancestry, who differ only in having additional admixture from local  
273 populations the Core Yamna must have encountered during their expansion<sup>7</sup>. In<sup>7</sup> we provide  
274 multiple lines of evidence that the Core Yamna and likely the YAC itself formed in the Dnipro-  
275 Don area of the northeastern NPR region, while not being able to narrow their geographic origin  
276 further based on genetic evidence alone.

277  
278 In<sup>7</sup> we show that the Core Yamna can be modeled without any EEF ancestry but as a mixture of  
279 CLV and NPR hunter-gatherer groups. When we force EEF ancestry as an additional source into  
280 the Core Yamna (Supplementary Information, section 2) its proportion is not significantly  
281 greater than zero ( $3.2\pm 3.1\%$ ) while that of the Caucasus Neolithic is ( $15.6\pm 4.3\%$ ), suggesting  
282 that the Anatolian-related ancestry<sup>10</sup> in the Core Yamna was mediated mainly from the Caucasus  
283 Neolithic populations (like Aknashen in Armenia<sup>10</sup>) and not from European farmers of Anatolian  
284 origin<sup>36</sup>. Further confirmation of this hypothesis comes from the fact that qpAdm models of  
285 exclusively CLV+NPR hunter-gatherer ancestry (Supplementary Information, section 3)  
286 conform closely with independently derived unsupervised ADMIXTURE estimates of ancestry  
287 (Fig. 2b). While EEF ancestry in the Core Yamna itself is conjectural and not necessary from the  
288 point of view of modeling this population, it was clearly present in the western Yamna from  
289 Bulgaria, Hungary, Moldova, Romania, and Serbia (<sup>7</sup>, Supplementary Information, section 2). In  
290 this paper, we seek to narrow down the location from which the Yamna originated, synthesizing  
291 archaeological and genetic evidence from the very beginning instead of relying on the genetic  
292 data alone and only combining with archaeological information at the end.

293  
294 The chronologically earliest individuals in our sample set who are cladal with Core Yamna are  
295 I32534 (3635-3383 calBCE) from Ukraine and I20196 from Moldova ( $p=0.684$  and  $p=0.683$ ,  
296 respectively). Individual I17743 from Moldova had predominantly Core Yamna ancestry but also  
297 harbored 6.9% Balkan EEF admixture ( $p=0.593$ ). Individuals I20196 and I17743 both date to ca.  
298 3350-3100 BCE (Supplementary Information, section 2) and their contemporary I17974 from

299 Moldova is 81.8% Core Yamna and 18.2% Steppe Maykop ( $p=0.324$ ; Supplementary  
300 Information, section 2).

301  
302 For individual I32534, from the second (proto-Yamna) layer of the Mykhailivka site in the lower  
303 Dnipro Valley, only the Core Yamna model remains feasible when either BPgroup or PVgroup is  
304 placed on the right set in qpAdm analysis (Supplementary Information, section 2). As a further  
305 test, when we force either EEF or UNHG as a second ancestry source (on top of the Core Yamna  
306 ancestry), neither one is significantly different from zero ( $|Z|<1$ ) and both are, in fact, nominally  
307 slightly negative. Thus, there is no evidence for the presence of either the EEF or UNHG-related  
308 ancestries of the NPR region on top of the Core Yamna ancestry and I32534 is consistent with  
309 being simply a member of the Core Yamna group. Beyond qpAdm modeling, the Mykhailivka  
310 individual clusters with Core Yamna in PCA (Fig. 2a) and in unsupervised ADMIXTURE  
311 analysis (Fig. 2b). All these lines of evidence converge in showing that I32534 is indeed an early  
312 Core Yamna individual who bridges the temporal gap between the geographically proximate  
313 Late Serebnii Stih populations and those of the main Yamna expansion that are sampled from  
314 south Siberia to eastern Europe and in which any associations with the locale of Yamna  
315 formation have been wiped out by thousands of kilometers of distance.

316  
317 Four Yamna individuals from Ukraine are cladal with the Core Yamna group in showing no  
318 evidence of EEF admixture (Supplementary Information, section 2). Three Yamna Ukraine  
319 individuals, as well as one Catacomb Individual I12617, all from the northwest NPR, harbor  
320 significant European farmer-associated admixture from proximate sources like Bulgaria  
321 Eneolithic or Trypillia (Supplementary Information, section 2). Thus, the northwest NPR can be  
322 identified as the place in where the Yamna first received substantial EEF admixture during their  
323 western expansion.

324  
325 The substantial proportion of farmer ancestry in Yamna outlier individuals I20076 and I17747  
326 (2865-2576 calBCE) from Moldova is best fitted by Core Yamna + Trypillia or Globular  
327 Amphora models (Supplementary Information, section 2). One of the Yamna individuals from  
328 Bulgaria contained 22.3% YUN\_CA admixture, while another individual from the same site was  
329 cladal with the Core Yamna (Supplementary Information, section 2). The Yamna expansion,  
330 beginning in Ukraine and reaching the South Balkans, included both individuals who maintained  
331 the Core Yamna genetic profile, as well as those who had begun to admix with local farmers,  
332 initiating the transmission of Yamna ancestry and probably Indo-European languages beyond the  
333 steppe.

334  
335 We also present genetic evidence of the westward expansion of the North Caucasus/lower  
336 Volga-Don ancestry at the early stages or pre-dating the Yamna expansion. This is reflected in  
337 individuals I17974 and I20078 from Moldova, who were formed of the same Yamna+Steppe  
338 Maykop-associated admixture process, with I17974 carrying about  $\sim 1/3$  of the Steppe Maykop-  
339 associated ancestry found in I20078 (Table 1, Supplementary Information, section 2). The  
340 Caucasus affinity was also observed for individual, I17973, co-buried with I17974, who cannot  
341 be well-modeled with any of the sources available to us, but is nearest to the “southern” end of  
342 the CLV cline (Maykop of the North Caucasus ( $p=0.0025$ ) or the Aknashen Neolithic of the  
343 South Caucasus ( $p=0.0047$ , Supplementary Information, section 2), which is corroborated by the  
344 position of I17973 on the PCA (Fig. 2a). In the northeastern NPR, an early Yamna individual

345 I1917 from Ožera<sup>6</sup> is best modeled as an even mix of Core Yamna and Maykop, providing, like  
346 individual I17973, a clear link to the Caucasus. More evidence for this link comes from the Early  
347 Bronze Age population from Mayaky<sup>5</sup>, which is discontinuous with the Usatove from the same  
348 region but represented a unique combination of 1/5 Maykop ancestry with the remainder best  
349 represented by the Yamna of the Lower Don, a population which was itself a mix of Core Yamna  
350 and NPR hunter-gatherers<sup>7</sup>.

351

### 352 **Yamna ancestry in the Bronze Age NPR**

353

354 We find that individuals of the Catacomb archaeological complex (CAC), which chronologically  
355 partially overlaps and succeeds Yamna in the NPR, continued to harbor Yamna genetic ancestry.  
356 The population labeled “Ukraine\_EBA\_Catacomb”, including individuals I12840 and I16668  
357 from our dataset, is cladal with the Core Yamna ( $p=0.075$ , Supplementary Information, section  
358 1). Yamna ancestry persisted in the NPR into the second half of the 3<sup>rd</sup> millennium BCE.

359

360 The Catacomb group was succeeded in the NPR by the Multi-Cordoned Ware/Babyne (MCW/B)  
361 complex (Supplementary Information, section 1). The only feasible models for the ancestry of  
362 two MCW/B individuals in our sample selection involve Core Yamna, a European farmer  
363 source, and additional hunter-gatherer ancestry above and beyond what was present in even the  
364 most hunter-gatherer admixed farmer populations of the farmer-hunter-gatherer cline  
365 (Supplementary Information, section 2). Three-way modeling with varying hunter-gatherer  
366 ancestry for BA Ukraine (Supplementary Information, section 2) confirms that MCW/B in  
367 Ukraine experienced gene flow from a population that had considerable hunter-gatherer ancestry.  
368 Such populations have been described from the BA of what is today Romania at the sites of  
369 Arman (Cârlomănești) and Târgșoru Vechi in Muntenia<sup>10</sup>, indicating that populations of high  
370 hunter-gatherer ancestry contributed to some post-Yamna people in the NPR and Southern  
371 Carpathians.

**Table 1.** A compendium of the ancestral landscape of the North Pontic Region in the Eneolithic and Early Bronze Age (ca. 4500-2500 BCE) showing two waves of Caucasus-Lower Volga (CLV) cline ancestry migration in the NPR.

Genetic ID, Archaeological ID, date	Population Source(s)	P-value	Comment
<b>Wave 1: Early pioneers from northern part of Caucasus-Lower Volga (CLV) cline and their descendants</b>			
I20072 Giurgiulești Burial 6 (3), 4330-4058 calBCE	BPgroup <sup>a</sup>	0.896	Eneolithic Individual from Moldova who was a descendant of Lower-Volga North Caucasus Eneolithic 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	Eneolithic 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 4595±121 BCE (4832-4358 BCE)	Median: 4% BPgroup, 14% BHG, 82% YUN_CA <sup>b</sup>	7e-6	Heterogeneous Eneolithic Trypillia population from Ukraine and Moldova was formed on the basis of the European farmer-hunter-gatherer cline and included some CLV ancestry with admixture from Usatove-related groups in the second half of the 4 <sup>th</sup> millennium BCE. The given model fits 23 of 28 Trypillian individuals but does not fit the Trypillian population as a whole.
Usatove (Mayaky), genetic ancestry forming 4471±51 BCE (4571-4371 BCE)	45% PVgroup <sup>c</sup> and 55% Trypillians	0.128	Eneolithic 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 <sup>5</sup>
Usatove (Usatove-Velykyj Kuyalnik), USV	48% PVgroup and 52% Trypillians	0.083	Usatove individuals from Usatove-Velykyj Kuyalnik in Ukraine <sup>5</sup>
Cernavodă I, KTL_A, genetic ancestry forming 4199±72 BCE (4340-4058 BCE)	54% BPgroup and 46% Trypillians	0.618	Eneolithic Cernavodă I population from Kartal in Ukraine (cluster A <sup>5</sup> ) was 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.
<b>Wave 2: Migration from intermediate part of the CLV cline and establishment of the Core Yamna ancestry</b>			
Serednii Stih, genetic ancestry forming ca. 4400 BCE <sup>35</sup> (SShi, SSmed, SSlo subsets)	CLV ancestry: 13-17% Aknashen Neolithic and 8-56% BPgroup; Dnipro-Don ancestry: 31-56% GK2 ancestry	0.102-0.851	Eneolithic SSAC Individuals from Ukraine were genetically heterogeneous but formed a cline of ancestry on the basis of CLV people (themselves a mixture of Caucasus Neolithic / Aknashen-related) and North Caucasus-Lower Volga Eneolithic (BPgroup-related) with Dnipro-Don people (who had Ukraine Neolithic hunter-gatherer-related ancestry) <sup>7</sup>
Core Yamna, genetic ancestry forming 4038±48 BCE (4132-3944 BCE)	26% Remontnoye <sup>d</sup> 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 Dnipro-Don people. Their emergence occurred likely in the North Pontic Region as a descendant of a late SSAC population who are unique in possessing this combination of ancestries <sup>7</sup>
	CLV ancestry: 21% Aknashen Neolithic and 57% BPgroup; Dnipro-Don ancestry: 23% GK2 ancestry	0.934	
Cernavodă I, KTL_B, genetic ancestry forming 4168±138 BCE (4438-4898 BCE)	27% Remontnoye and 73% European farmers (YUN_CA+Globular Amphora)	0.294	Eneolithic Cernavodă I population from Kartal in Ukraine (cluster B <sup>5</sup> ) had much less CLV ancestry and this ancestry was not from the Lower Volga (BPgroup) end of the CLV cline, but rather from a population like Maykop or Remontnoye
<b>Wave 3: Yamna expansion</b>			
<b>Core Yamna</b>			
I32534 Mykhailivka 1, Square VI, 3635-3383 calBCE	Core Yamna	0.684	Eneolithic Individual from Ukraine is the earliest <sup>14</sup> C-dated Yamna in NPR



I20196 Crasnoe Kurgan 9, Burial 9, Skeleton 2, 3352-3101 calBCE	Core Yamna	0.683	Eneolithic Individual from Moldova was 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 a Yamna descendant
I20079 Taraclia II, Kurgan 10, Burial 2, 2571-2355 calBCE	Core Yamna	0.864	Early-Middle Bronze Age (EMBA) Individual from Moldova was a Yamna descendant
Catacomb Archaeological Complex (CAC) I12840 Dubynove, Kurgan 1, Burial 10, 2453-2148 calBCE I16668 Revova, Kurgan 3, Burial 10, 2800-2000 BCE	Core Yamna	0.075	EMBA CAC individuals from Ukraine (MJ-09 from Mamaj Gora <sup>37</sup> , I12840 and I16668, this study) were Yamna descendants
<b>Core Yamna + European Farmer descendants</b>			
I1456 Durankulak, Kurgan F, burial 15 (main burial), 3500-3000 BCE	45% Core Yamna and 55% Globular Amphora	0.099	Eneolithic 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	-	EBA Yamna individuals from Bulgaria, Moldova, and Ukraine ( <sup>7</sup> , this report) included unadmixed Core Yamna as well as others with European farmer ancestry. The source of the farmer ancestry could be Trypillia or Globular Amphora and is unclear.
Bulgaria Yamna, Boyanovo subset, 3300-2500 BCE <sup>5</sup>	94% Core Yamna and 6% YUN_CA	0.211	
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	36-46% Core Yamna and 23-44% Balkan Hunter Gatherer and 15-32% Trypillia	0.179- 0.889	Three Eneolithic-EBA individuals from Ukraine had some Yamna ancestry but substantial Balkan hunter-gatherer (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	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 2, Burial 3, 1499-1127 calBCE I16674 Liubasha kurgan 2 burial 15, 2434-1943 calBCE	92% Core Yamna and 3% Globular Amphora and 5% BHG	0.148	These two Middle Bronze Age individuals of Multi-Cordoned Ware/Babyne archaeological circle from Ukraine were mostly of Yamna descent but had admixed with a population with even more hunter-gatherer ancestry than in the Globular Amphora
<b>Core Yamna + Dnipro-Don Hunter Gatherer descendants</b>			
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 Ukraine Neolithic hunter-gatherer ancestry <sup>7</sup>
<b>Core Yamna + Steppe Maykop descendants</b>			
I20078 Taraclia II, Kurgan 2, Burial 14, 3340-3034 calBCE	39% Core Yamna and 61% Steppe Maykop	0.432	Late Eneolithic Individual from a ZV/III-C type burial from Moldova was mix of Yamna with Steppe Maykop
I17974 Bursuceni, Kurgan 1 Burial 21, Skeleton 2, 3334-3030 calBCE	82% Core Yamna and 18% Steppe Maykop	0.324	Late Eneolithic Individual from a ZV/III-C type burial from Moldova represents another mixture of Yamna with Steppe Maykop
<b>Yamna + Maykop descendants</b>			
I1917 Ozera Kurgan 18 Burial 14, 3096-2913 calBCE	50% Core Yamna and 50% Maykop	0.345	This individual from Ukraine <sup>6</sup> 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 <sup>5</sup> were a mixture of Don

			Yamna (itself a mixture of Core Yamna and Dnipro-Don hunter-gatherers) and Maykop
<b>CLV cline admixing with European Farmers</b>			
I1428 Riltsi Kurgan 264, Burial 5, 3360-2890 calBCE	50% Remontnoye and 50% YUN_CA	0.558	Eneolithic individual from Bulgaria who was a mixture of CLV people (PVgroup or Remontnoye) and European farmers such as YUN_CA
<b>CLV cline outlier</b>			
I17973 Bursuceni, Kurgan 1, Burial 21, Skeleton 1, 3354-3103 calBCE	Maykop (?)	0.0025	Late Eneolithic Individual from the same burial as I17974 is related to populations from the Caucasus (Fig. 2) but with some unspecified ancestry

Notes: For admixture dates we give one standard error of uncertainty, as well as a 95% confidence interval computed as  $\pm 1.96$  standard errors. For direct dates on the bones we analyzed for DNA, we indicate the 95% calibrated confidence for the date with the suffix “calBCE”; all other dates are archaeologically estimated ranges. <sup>a</sup>BPgroup is a genetically homogeneous group of people from the Lower Volga-North Caucasus Eneolithic (CLV) at the bend between CLV and Volga (EHG-rich) clines (Fig. 2a) from the sites of Berezhnovka and Progress 2 that carries CHG, EHG, and Siberian/Central Asian Neolithic-related ancestries<sup>7</sup>. <sup>b</sup>Balkan farmers of Gumelnița/Karanovo from the Yunatsite site in Bulgaria. <sup>c</sup>PVgroup BP-related group from the CLV cline with more Aknashen (south Caucasus) ancestry than BPgroup represented by individuals from the sites of Berezhnovka and Vonjucka<sup>7</sup>. <sup>d</sup>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 a population from the low-EHG end of the Volga Cline such as the BPgroup<sup>7</sup>.

## 372 Discussion

373  
374 This study presents the first comprehensive reconstruction of the population dynamics in the  
375 North Pontic steppe and forest steppe, clarifying genetic transformations in this region leading up  
376 to and following the emergence of the YAC.

377  
378 We demonstrate that the Neolithic populations of the Dnipro Valley were admixed, roughly with  
379 BHG and EHG sources, along with approximately ~7-9% EEF ancestry throughout the UNHG  
380 population except for some outliers such as individual I27992 from Yasynyvatka ( $27 \pm 6.0\%$  EEF,  
381 this report) and an unadmixed EEF individual I3719 from Deriivka I<sup>6</sup> ( $103.5 \pm 1.6\%$  EEF). CHG  
382 ancestry is also sporadically present at comparably low levels relative to the EEF ancestry (~7-  
383 10%), including in the region most proximate to the North Caucasus in the NPR Neolithic  
384 necropolis at Mariupol. The proximal sources of EEF ancestry in UNHGs remain unclear but  
385 may have been mediated by BHG migrants in the Dnipro Valley or individuals of EEF genetic  
386 background such as individual I3719<sup>6</sup> that were included in UNHG communities.

387  
388 We infer that the Eneolithic Trypillia population was mainly formed from the sources along the  
389 EFHG cline that received limited (~5%) admixture from people that had BPgroup CLV ancestry.  
390 Usatove was formed on the basis of PVgroup CLV people evenly intermixing with Trypillian  
391 ancestry. The Trypillia and Usatove populations thus both harbored ancestry from near the bend  
392 of the Volga-CLV clines (Fig. 2a) and differed from each other in that this ancestry was minor in  
393 Trypillia and more BPgroup-related, while comprising approximately half of the Usatove  
394 ancestry and being more PVgroup-related (shifted towards the Maykop-Aknashen end of the  
395 CLV cline).

396  
397 The evidence from Usatove and Trypillia clarifies the process of the CLV admixture in the NPR  
398 in the Eneolithic. As people bearing the Volga-CLV ancestry moved across the North Pontic

399 steppe and into the Balkan-Carpathian region, they encountered local farmer populations. Some  
400 carriers of Volga-CLV ancestry, as in Giurgiulești and Csongrád, reached the Balkans and  
401 Carpathian region with no genetic admixture with the people they encountered along the way; if  
402 some migrants did admix, they left little demographic impact, perhaps because they were small  
403 in number relative to the local populations. In contrast, their farmer counterparts in the NPR such  
404 as Trypillia were more demographically affected, incorporating the Volga-CLV incomers'  
405 genetic ancestry as well as elements of their material culture. An intriguing possibility raised by  
406 our findings is that Usatove was formed around an outpost in the Danube-Dniester interfluvium to  
407 oversee the economic interests of Trypillia, on the one hand, and early carriers of the southern  
408 Caucasus-enriched PVgroup of the CLV cline ancestry, on the other. A similar scenario is  
409 feasible for the Cernavodă I population of Kartal\_A, but with BPgroup-derived carriers of CLV  
410 ancestry such as in Giurgiulești and Csongrád individuals. Alternatively, Usatove and Kartal A  
411 could have formed as a “commonwealth” of co-existing and interdependent cultures in which  
412 Trypillia and populations from the Caucasus-Volga both participated. A third potential scenario  
413 places egalitarian Trypillians under the dominance of hierarchically organized patriarchal  
414 societies carrying CLV ancestry, extending into the northwestern NPR.

415  
416 The other great Eneolithic culture of the NPR, the Serechnii Stih, also consisted of people who  
417 received varying degrees of CLV and UNHG-related ancestries<sup>7</sup>. The Serechnii Stih cline lacks  
418 appreciable EEF ancestry in contrast to Usatove and, especially, Trypillia. The results in<sup>7</sup> and the  
419 current analysis establish the Core Yamna as a late Serechnii Stih-derived population that had  
420 more CLV ancestry than the sampled Serechnii Stih individuals but was made of the same CLV  
421 and UNHG-GK2 derived components. CLV ancestry comprised only 5% in Trypillia and  
422 roughly 50% in Usatove, while in Yamna it was 77%<sup>7</sup>. In Usatove, ca. 14% of CLV ancestry was  
423 southern Caucasus Aknashen-related (Supplementary Information, section 2), while in the Core  
424 Yamna the Aknashen-related ancestry was ca. 21%, so this was not a single-source CLV  
425 migration into the steppe and Pontic region<sup>7</sup>.

426  
427 Evidence presented in<sup>7</sup> argues for a YAC origin in the Dnipro-Don area of the northeastern NPR.  
428 Yamna ancestry became a feature of almost all individuals in southeast Europe postdating the  
429 Yamna expansion, except for the southernmost corner of the Balkan Peninsula in the  
430 Aegean<sup>10,38–40</sup>. The expansion of the YAC eastward brought its bearers to near the foot of the  
431 Urals (where the Samara Yamna were sampled) and to west Siberia, where they formed the  
432 Afanasievo culture of the Altai.

433  
434 The existence of unadmixed Core Yamna in a wide area from the Altai to Bulgaria can be seen  
435 as evidence of the rapidity of the Yamna expansion, providing little opportunity for admixture  
436 during its initial pulse. The question of whether the remarkable homogeneity of the Core Yamna  
437 cluster was a consequence of relative isolation during their formative period or a cultural  
438 avoidance of heterogamy that was later abandoned, remains to be answered.

439  
440 During their western expansion, the Yamna absorbed EEF ancestry from the populations of the  
441 west-northwest edge of the NPR and southeastern Europe, while at the same time integrating  
442 individuals with ancestries from the Don Yamna or the Maykop and Steppe Maykop. Thus, the  
443 Yamna variably incorporated ancestries from nearly every encountered group during their  
444 expansion pulse. This integrative nature of their communities, coupled with their remarkable

445 mobility, likely contributed to the Yamna's success in disseminating their Indo-European  
446 language and culture across geographic and population boundaries.

447  
448 The chronologically earliest (3635-3383 calBCE) individual with the Core Yamna ancestry  
449 comes from the Mykhailivka settlement displaying a succession of uninterrupted cultural layers  
450 from the late Eneolithic to the EBA<sup>41,42</sup>, without the evidence of site depopulation moving into  
451 the Yamna period seen at almost all other Eneolithic sites. In the context of the archaeological  
452 evidence, the presented results increase the plausibility of arguments that the lower Dnipro,  
453 specifically the area around the Mykhailivka site at a crossroads of ancient steppe "highway"  
454 network across the Pontic-Caspian steppe (Supplementary Information, section 1), is a place  
455 where Yamna first emerged.

456  
457 The groups that succeeded Yamna in the NPR in the second half of the 3<sup>rd</sup> millennium BCE  
458 continued to harbor Yamna genetic ancestry, as well as displaying a resurgence of hunter-  
459 gatherer ancestry towards the Middle Bronze Age, the latter evidenced by the MCW/B  
460 individuals from Ukraine and Romania. The geographic dispersal of individuals with MCW/B  
461 genetic ancestry may reflect high mobility of this group, like that of the Yamna but smaller in  
462 scale.

463

#### 464 **The three waves of CLV ancestry expansion in the NPR**

465

466 Our analysis suggests a history of three partially overlapping waves of CLV migrations into the  
467 NPR in the Eneolithic (Table 1). A first and, potentially earliest wave spread before ca. 4500  
468 BCE (the DATES-estimated Trypillia and Usatove genetic ancestry formation), bringing a  
469 mostly BPgroup/PVgroup-related pulse from the genetically "northern"/Lower Volga part of the  
470 CLV cline. It was associated with Giurgiulești-Csongrád Suvorove-type burials, and left an  
471 admixture in Trypillia, Usatove (with participation of the Neolithic Caucasus ancestry), and  
472 Kartal\_A. A second and more protracted wave carried an intermediate part of the CLV cline,  
473 involved a more genetically "intermediate" ancestry (an example of which is Remontnoye) and  
474 became associated, in its initial pulse, with the formation of Serebnii Stih ca. 4500 BCE. In its  
475 westernmost reach, the second wave extended to the northwest NPR, contributing to the  
476 formation of Kartal\_B, but otherwise remaining largely contained in the Lower Dnipro Valley  
477 region, notably during the steppe "hiatus" in the late 5<sup>th</sup>-early 4<sup>th</sup> millennium BCE, characterized  
478 by a relative lack of archaeological material.

479

480 The Core Yamna genetic mixture is estimated to have taken place at 4038±48 BCE (95% C.I.:  
481 3944-4132 BCE)<sup>7</sup>, which is the height of the steppe hiatus inferred from archaeological  
482 information. It is unclear whether this date corresponds to an admixture of populations that  
483 happened very rapidly, or whether it corresponds to a process that unfolded over generations, in  
484 which case the date we estimate is an average. Nonetheless, it does coincide with a sharp  
485 climatic shift towards aridity and cooler temperatures. Thus, the steppe hiatus may be a reason  
486 for the emergence of the core Yamna ancestry from a nascent SSAC-derived Yamna population  
487 that was relatively isolated due to the climatic upheaval.

488

489 It is conceivable that the steppe groups of the post-hiatus chronological period (3900-3300 BCE)  
490 such as Lower Mykhailivka, Mykhailivka 2 (proto-Yamna), and Konstantinovka in the lower



491 Don, forming under the increasing influence of the North Caucasus<sup>42</sup>, stem from a SSAC-  
492 derived steppe population that became isolated during the climate-influenced hiatus, but re-  
493 emerged (now as proto-Yamna) following it. Such a scenario would explain two features in the  
494 population history of the Core Yamna: its population bottleneck prior to ca. 3750-3350 BCE<sup>7</sup>,  
495 potentially occurring in the context of climatic-induced isolation, and its genetic position at the  
496 low-UNHG end of the Dnipro Cline as the result of the proximity and influence of the North  
497 Caucasus. Genetically, the Core Yamna can be modeled as a mixture of ~3/4 of the high CLV  
498 ancestry subset of Serechnii Stih (SShi) and ~1/4 of the genetically intermediate (along the CLV  
499 cline) population represented by two individuals from the Manych Depression at Remontnoye  
500 (Table 1; <sup>7</sup>) who date to this key period (4152-3637BCE). In this scenario, the individual from  
501 Mykhailivka represents a proto-Yamna population near the geographical origin of the Core  
502 Yamna and sampled from the time where its genetic distinctiveness had already appeared. Other  
503 early individuals from the NPR, such as Bursuceni and Taraclia II.2.14, also carried the Core  
504 Yamna ancestry, while connecting the NPR with other populations of the North Caucasus<sup>43,44</sup>.

505  
506 The third wave of CLV ancestry expansion is that of the YAC proper, beginning ca. 3300 BCE  
507 and lasting into the middle of the following millennium. All three expansion waves stem from  
508 different points on the geographically and genetically diverse CLV cline.

509  
510 It is remarkable that the three genetic waves of CLV ancestry expansion align, spatially and  
511 temporally, with the three waves of Kurgan People proposed by Marija Gimbutas in the 1950s to  
512 explain the spread of Indo-European influences and the fall of “Old Europe” (summarized in<sup>1,45</sup>).  
513 In Gimbutas’ theory and in our genetic analysis, the three waves originated in the Lower Volga-  
514 North Caucasus area and acted as constituent elements of a single process that unfolded in time  
515 and space throughout the Eneolithic and into the Bronze Age, transforming the cultural  
516 landscape of Western Eurasia. We must note, however, that Gimbutas envisioned the spread of  
517 Kurgan ancestry as a military conquest and emphasized *cultural* transformation of the conquered  
518 people encountered by the Kurgan culture bearers. Our results present evidence of massive  
519 *genetic* transformations effected by the spread of CLV ancestry during Waves 1 and 2, and  
520 especially, with the spread of the Yamna during Wave 3. Such genetic changes must have  
521 involved complex cultural dynamics, in which both conflict and peaceful synthesis may have  
522 played a role. Future studies can further explore the cultural impact these three expansion waves  
523 brought about, informed by the new understanding of the immense genetic impacts that  
524 accompanied them.

## 525 526 **Conclusion**

527  
528 Our detailed survey of individuals from the Neolithic to Bronze Age in the NPR shows a shifting  
529 landscape of ancestry. The earliest inhabitants of the NPR that carried BHG/WHG-EHG-EEF  
530 ancestry components had lived there from Paleolithic to Neolithic times. Caucasus ancestry,  
531 having made its tentative and sporadic appearance already among the UNHG of the NPR,  
532 appears in large proportion among all the Eneolithic NPR populations that followed, derived  
533 from the diverse people of the Caucasus-Lower Volga cline who appear in the Lower Danube  
534 and its tributaries in central Europe unadmixed, and leaving traces of their ancestry in larger  
535 NPR-area populations (as in Trypillia), or as equipotent ancestry contributors (as in Usatove and  
536 Kartal\_A), and as highly variable clinal populations (as in the Serechnii Stih). Many diverse



537 blends of autochthonous NPR inhabitants and CLV newcomers were formed in which both  
538 farmers and hunter-gatherers of the NPR contributed and in which people from different sections  
539 of the CLV cline participated (Table 1). The distinctive Serebnii Stih-descended population  
540 ancestral to the Core Yamna dominates, after its 4<sup>th</sup> millennium BCE appearance and subsequent  
541 expansion, absorbing and incorporating, in diverse blends of its own (Table 1), ancestries of the  
542 people they encountered along the way. The later history of the NPR, in which Cimmerians,  
543 Scythians, Greeks, Sarmatians, Turks, Bulgars, and Slavs, and numerous others who made their  
544 mark on the cultural and genetic landscape, mirrors the region's more distant past that we study  
545 here: a continuous process of transformation and change that not only shaped its modern  
546 inheritors, but also played a central role in shaping events across the wider Eurasian continent.

## 547 **Materials and Methods**

548

### 549 **Wet laboratory work**

550 In clean rooms where the goal was to protect bones and teeth from contamination by the  
551 individuals handling them, we processed human skeletal remains into powder<sup>46</sup>, extracted DNA  
552 using a method designed to retain short molecules<sup>46-48</sup> in some cases using automated liquid  
553 handlers<sup>49</sup>, and converted the extracts into double-stranded<sup>50</sup> and single-stranded<sup>51</sup> libraries,  
554 which were molecularly barcoded with appended dual barcodes (for double-stranded libraries)  
555 and dual indices (for both double-stranded and single-stranded libraries) to allow them to be  
556 pooled together and then bioinformatically deconvoluted at the analysis stage. We enriched the  
557 libraries for sequences overlapping more than 1.2 million SNPs as well as the mitochondrial  
558 genome<sup>52</sup>, and then sequenced on NextSeq500, HiSeqX, or NovaSeq instruments, targeting on  
559 the order of a hundred thousand molecules for unenriched libraries and on the order of 30 million  
560 molecules for enriched one. Online Table 2 provides information on each library we analyzed.

561

### 562 **Bioinformatic analysis**

563 Following sequencing, we used the identification markers (barcodes and indices) to demultiplex  
564 reads into the to the appropriate library, before trimming these and sequence adapters. Paired-end  
565 reads were then merged requiring an overlap of at least 15 base pairs (allowing for 1 mismatch),  
566 using a modified version of SeqPrep 1.1 (<https://github.com/jstjohn/SeqPrep>); at overlapping bases,  
567 we selected the highest quality nucleotide to represent the sequence at that position. We aligned  
568 sequences to both the human reference genome sequence (hg19)  
569 (<https://www.internationalgenome.org/category/grch37/>) and to the inferred ancestral  
570 Reconstructed Sapiens Reference Sequence (RSRS) mitochondrial sequence<sup>53</sup>, using BWA's  
571 `samse` command<sup>54</sup>. We removed duplicated molecules based on having the same start/stop  
572 positions and orientation in their alignment and the same barcodes. The computational pipelines  
573 we used are publicly available on GitHub at <https://github.com/dReichLab/ADNA-Tools> and  
574 <https://github.com/dReichLab/adna-workflow>. We call variants by using a 'pseudohaploid  
575 genotyping' approach, where a single base is randomly selected from a pool of possible bases at  
576 each SNP, filtering by a minimum mapping quality of least 10, and base quality of at least 20,  
577 trimming each read by two base pairs to remove damage artifacts. To assess ancient DNA  
578 authenticity, we used both *contamMix-1.0.1051*<sup>55</sup> to search for heterogeneity in mitochondrial  
579 DNA sequences which are expected to be non-variable in uncontaminated individuals, and  
580 ANGSD (ref) to search for heterogeneity in X chromosome sequences which should be non-  
581 variable in contaminated male individuals<sup>56</sup>. We also evaluated authenticity by searching for an

582 increase in cytosine-to-thymine errors in the final nucleotide (in untrimmed reads) which is  
583 expected for genuine ancient DNA<sup>57</sup> and by computing the ratio of Y chromosome to sum of X  
584 and Y chromosome sequences which is expected to be very low for females and to have a very  
585 much higher value for males. We determined a consensus sequence for mitochondrial DNA  
586 using *bcftools* (<https://github.com/samtools/bcftools>) and *SAMTools*<sup>58</sup> requiring a minimum of 2-  
587 fold coverage to call the nucleotide and a majority rule to determine its value. We used  
588 *HaploGrep2* to determine the mitochondrial haplogroups based on this consensus sequence,  
589 leveraging the phylotree database (mtDNA tree build 17)<sup>59</sup>.

590

### 591 **Population genetic analysis**

592 We performed principal components in smartpca<sup>23</sup> using lsqproject: YES and newshrink: YES  
593 parameters and the populations OberkasselCluster (set of trans-Alpine WHG individuals  
594 identified in<sup>26</sup>), Russia\_Firsovo\_N, Iran\_HajjiFiruz\_C<sup>9</sup>, Iran\_C\_SehGabi<sup>60</sup>,  
595 Iran\_C\_TepeHissar<sup>61</sup>, Israel\_C<sup>62</sup>, Germany\_EN\_LBK<sup>3,12,28,63</sup> to form the axes (Fig. 2).  
596 We used qpWave and qpAdm<sup>3,64</sup> to test whether  $n+1$  “left” populations (one Test and  $n$  sources)  
597 are consistent with descending from  $n$  ancestral sources with respect to a set of Right populations  
598 as in<sup>7</sup> (OldAfrica<sup>65–67</sup>, Russia\_AfontovaGora<sup>368</sup>, CHG<sup>69</sup>, Iran\_GanjDareh\_N<sup>60</sup>,  
599 Italy\_Villabruna<sup>68</sup>, Russia\_Sidelkino.SG<sup>8</sup>, Turkey\_N<sup>28</sup>).

600

601 We performed unsupervised ADMIXTURE analysis<sup>70</sup> using a new methodology of “summary  
602 individuals” (SI) that prevents the formation of population-specific ancestry components, as a  
603 complementary approach (other than qpAdm) to assess the ancestry of diverse population from  
604 the NPR and neighboring regions (Fig. 2b).

605

606 We dated the admixture time of Usatove-related populations (individuals from Mayaky  
607 presented in this report and from Mayaky (MAJ) and Usatove-Velykyj Kuyalnik (USV) from<sup>5</sup>)  
608 and Trypillians, using DATES<sup>35</sup> to infer the number of generations prior to the <sup>14</sup>C date of the  
609 studied individuals, and converted to a calendar date assuming 28 years per generation<sup>71</sup>.  
610 Uncertainty ranges reflect the standard error computed by DATES and not the uncertainty of the  
611 average <sup>14</sup>C date of admixed individuals.

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620 the excavations that produced many of the samples featured in this report and for providing the  
621 theoretical groundwork that inspired many of the hypotheses tested here.

### 622 **Data Availability**

623 Genotype data for individuals included in this study can be obtained from the Harvard Dataverse  
624 repository through the following link (XXX). The DNA sequences reported in this paper have  
625 been deposited in the European Nucleotide Archive under the accession number XXX. Other

626 newly reported data such as radiocarbon dates and archaeological context information are  
627 included in the manuscript and supplementary files.

### 628 **Author Contributions**

629 AGN, IL, SI, VD, ML, IP, and DR conceived the study. AGN, IL, NP, and DR supervised data  
630 analysis. AGN, SS, VR, and DR secured funding for the study. AGN, SI, MV, VD, NK, ML, IP,  
631 MK-N, SL, SM, HS, GS, and TT provided samples for the study. IL, NP, and DR supervised or  
632 performed statistical analyses. AGN, VR, SS, KC, EC, EH, LI, AML, MeM, MaM, AM, JO, LQ,  
633 JNW, FZ, SwM, and NR performed laboratory and bioinformatic analyses. AGN and AK  
634 curated the samples. NP, ML, NK, SM, SL, HS, SS, PW, and DR critically reviewed and edited  
635 manuscript files. AGN and IL wrote the manuscript with input from all co-authors.

636

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646 Institute (HHMI) Open Access to Publications policy, as HHMI lab heads have previously  
647 granted a nonexclusive CC BY 4.0 license to the public and a sublicensable license to HHMI in  
648 their research articles. Pursuant to those licenses, the author-accepted manuscript can be made  
649 freely available under a CC BY 4.0 license immediately upon publication.

### 650 **Conflict of Interest Statement**

651 The authors declare no competing interests.

652

### 653 **Ethics Statement**

654 All applicable regulations were followed when handling human remains both in the lab and in  
655 the field. All samples originating from Ukraine were excavated or sampled from museum or  
656 archival collections in Ukraine prior to 2022. Authors obtained consent, when available, from the  
657 individuals who conducted the excavations, who are either co-authors of the study or are  
658 acknowledged for their contribution. Human remains were processed using a minimal amount of  
659 skeletal material with the goal of minimizing damage. The open-access publication of the results  
660 of this study ensures unrestricted access to the results by specialists as well as the general public.  
661 Geographic names as well as names of archaeological groups were transliterated following their  
662 spelling in the countries from which samples originate. Geographic boundaries of political  
663 entities were respected following international law.

**Extended Data Table 1. Statistics of the form  $f_3(\text{Source}_1, \text{Source}_2; \text{Test})$ .** The statistic with the lowest Z-score of all the considered pairs is shown. This is the same as Table S3 in the supplement.

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



**Extended Data Table 2. Ancestry of Ukraine Neolithic individuals.** EHG=Lebyazhinka\_HG; BHG=Serbia\_IronGates\_Mesolithic; CHG=Caucasus\_Hunter\_Gatherer. We include close relatives and outliers. These are the same as Tables S46 and S47 in the supplement.

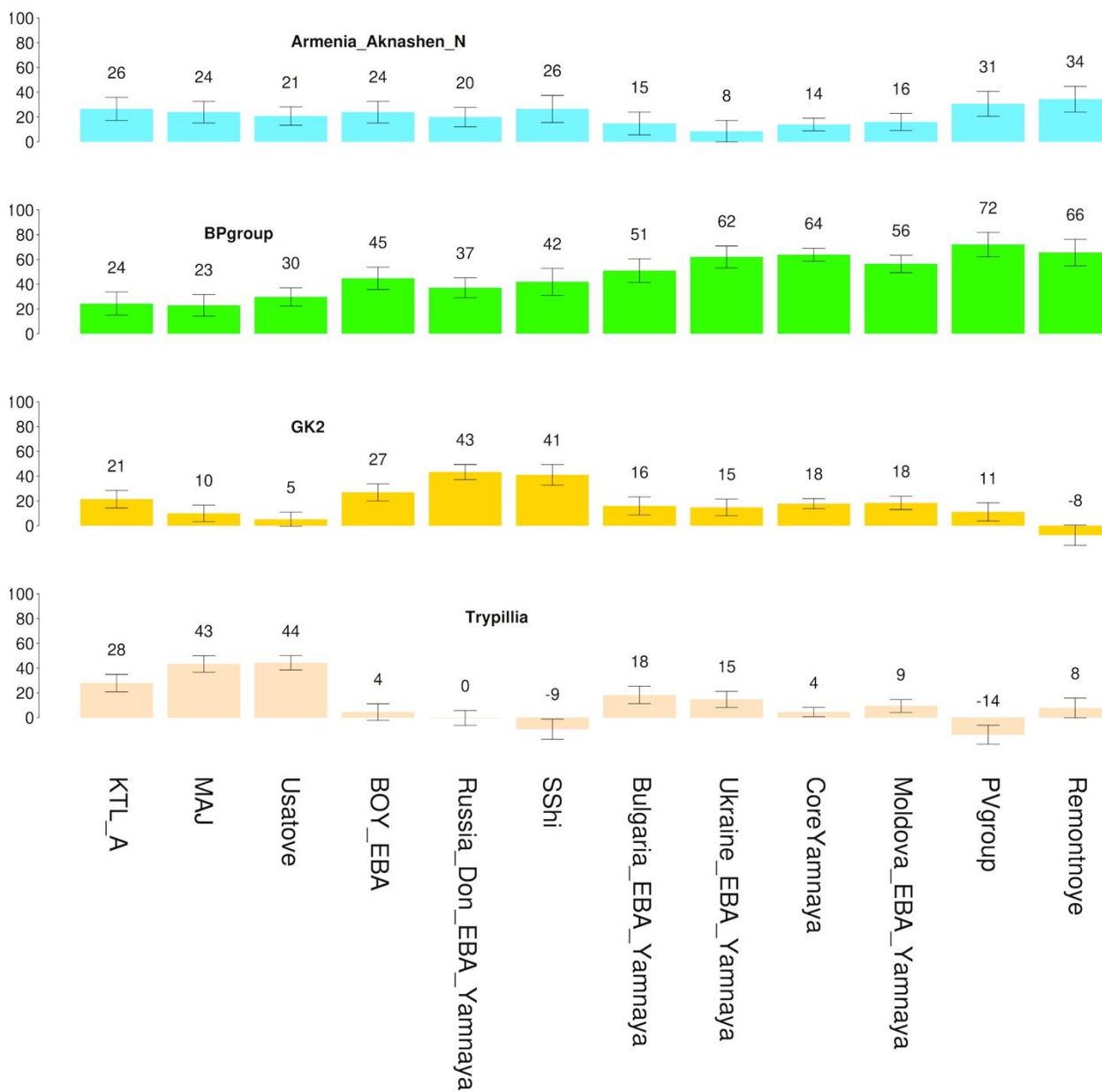
Modeling Ukraine Neolithic individuals with LBK as a source									
Individual	P-value	Proportions			Std. errors			Z-score of LBK	Population Label
		LBK	EHG	BHG	LBK	EHG	BHG		
I5878_enhanced	1.22E-01	9.7%	58.3%	32.0%	2.4%	3.7%	4.1%	4.0	Ukraine_N_father.or.son.I5883
I5886_enhanced	5.56E-04	7.4%	58.1%	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_Derivka_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%



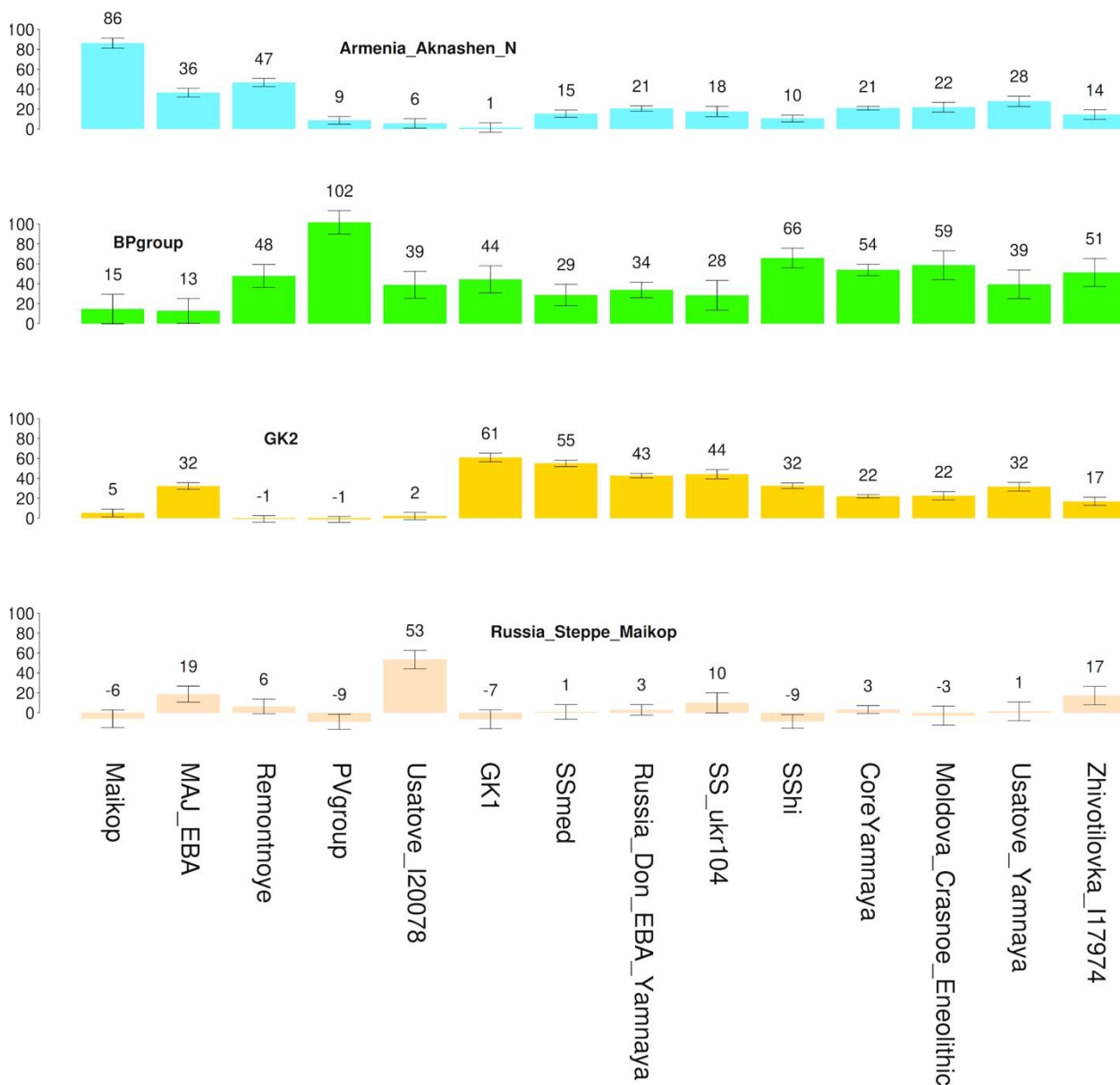
**Extended Data Table 3. By-individual modeling of Trypillians.** This is the same as Table S19 in the supplement.

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%	<b>2.2</b>
VERT105B_wNonUDG.SG	0.0105	5.4%	12.3%	82.3%	2.5%	2.3%	2.1%	<b>2.2</b>
VERT111_wNonUDG.SG	0.0004	5.5%	10.2%	84.3%	2.7%	2.5%	2.3%	<b>2.0</b>
I1926_enhanced	0.3223	5.9%	16.0%	78.1%	2.3%	2.3%	2.1%	<b>2.6</b>
VERT104B_wNonUDG.SG	0.2516	5.9%	12.0%	82.2%	2.4%	2.0%	2.0%	<b>2.5</b>
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%	<b>2.7</b>
I7920	0.1891	7.5%	13.5%	79.0%	2.4%	2.0%	2.1%	<b>3.1</b>
VERT103B_wNonUDG.SG	0.0252	8.2%	10.6%	81.2%	2.6%	2.2%	2.2%	<b>3.2</b>
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%	<b>5.4</b>
I20069	0.0926	25.8%	9.9%	64.3%	2.4%	2.2%	2.1%	<b>10.8</b>

**Extended Data Figure 1. Admixture proportions of 4-source model with Trypillians as the 4th source.** Plotted populations fit the model ( $p > 0.05$ ) and have RMSE of standard errors  $\leq 10\%$ .  $\pm 1$ S.E. are shown.



**Extended Data Figure 2. Admixture proportions of 4-source model with Steppe Maikop as the 4th source.** Plotted populations fit the model ( $p > 0.05$ ) and have RMSE of standard errors  $\leq 10\%$ .  $\pm 1$  S.E. are shown.



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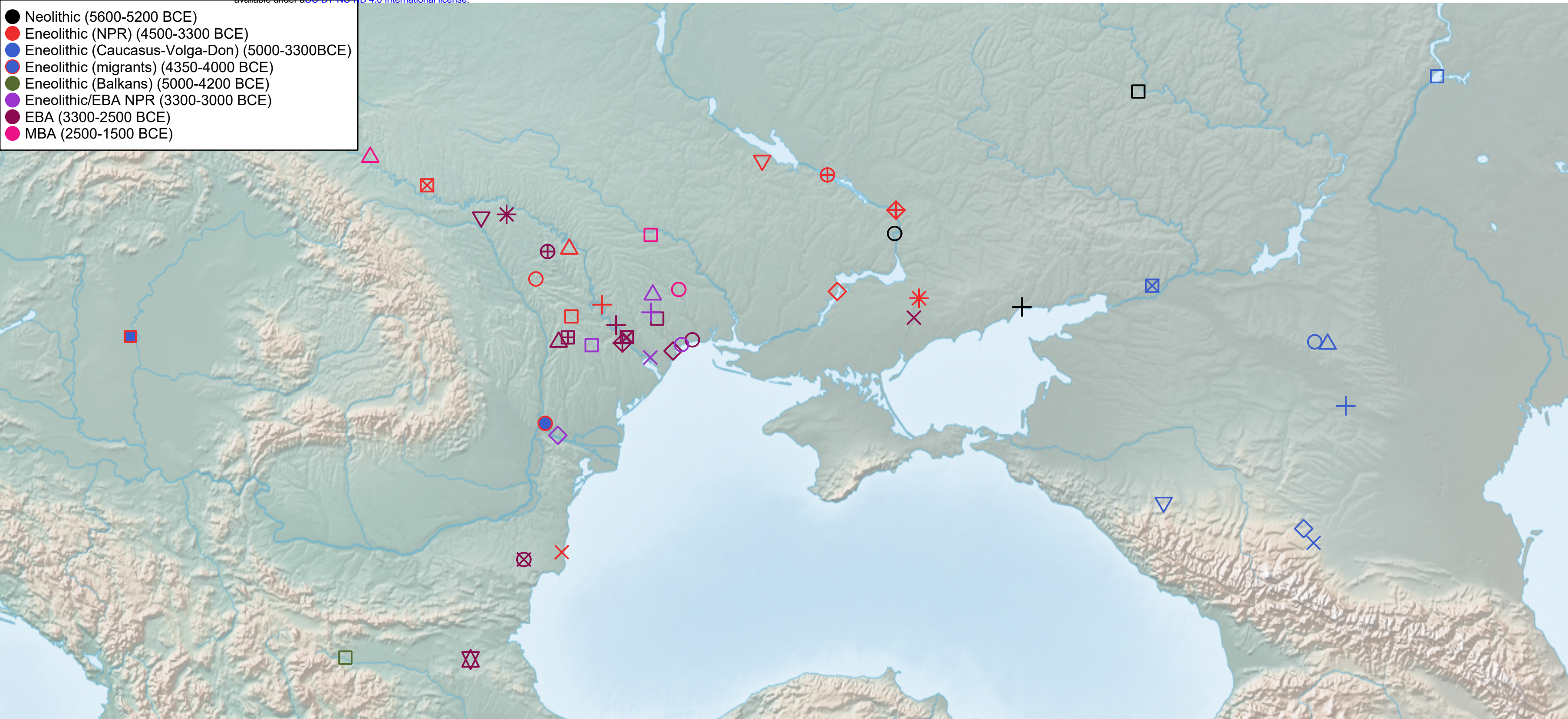


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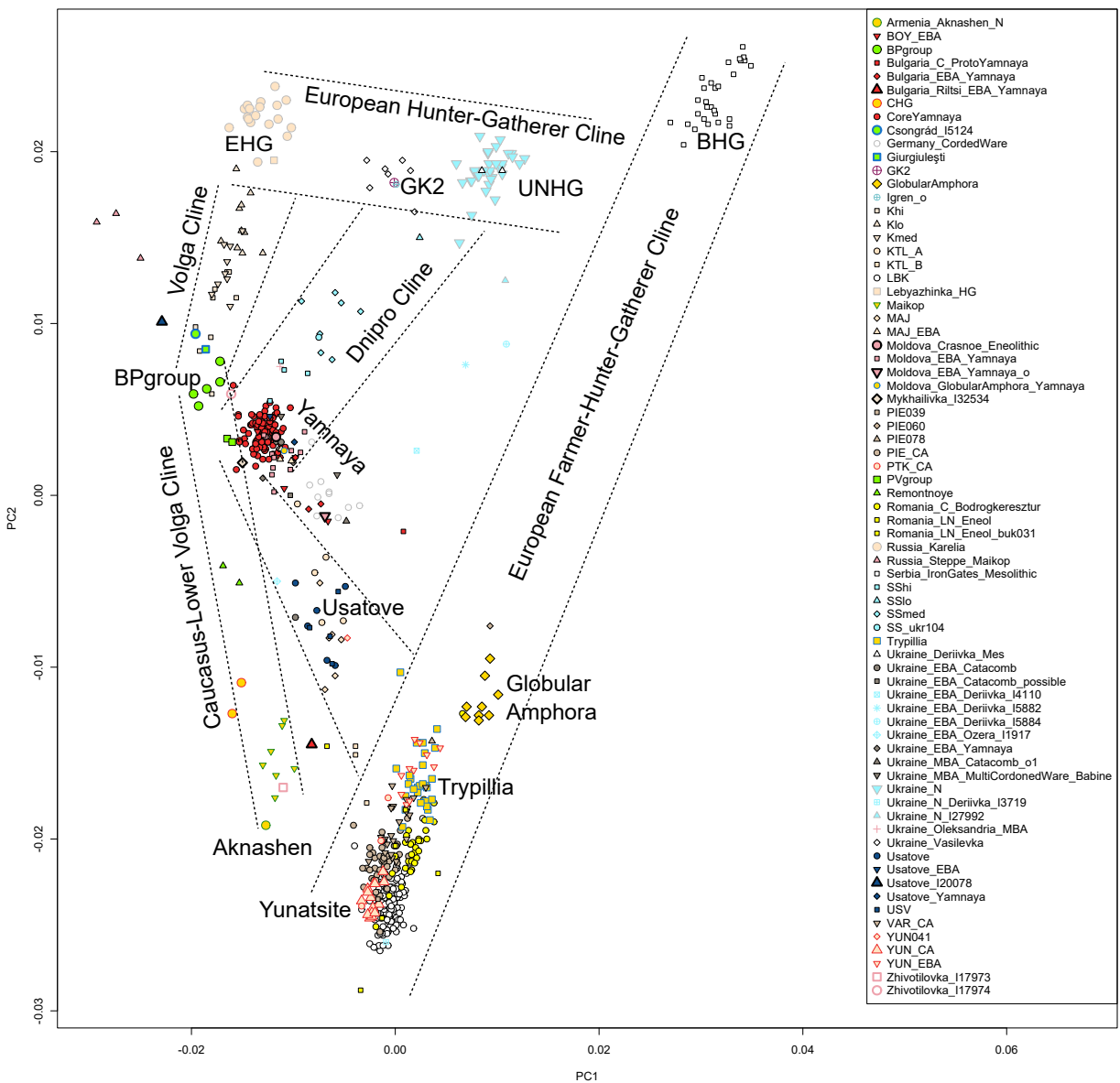


- Neolithic (5600-5200 BCE)
- Eneolithic (NPR) (4500-3300 BCE)
- Eneolithic (Caucasus-Volga-Don) (5000-3300BCE)
- Eneolithic (migrants) (4350-4000 BCE)
- Eneolithic (Balkans) (5000-4200 BCE)
- Eneolithic/EBA NPR (3300-3000 BCE)
- EBA (3300-2500 BCE)
- MBA (2500-1500 BCE)

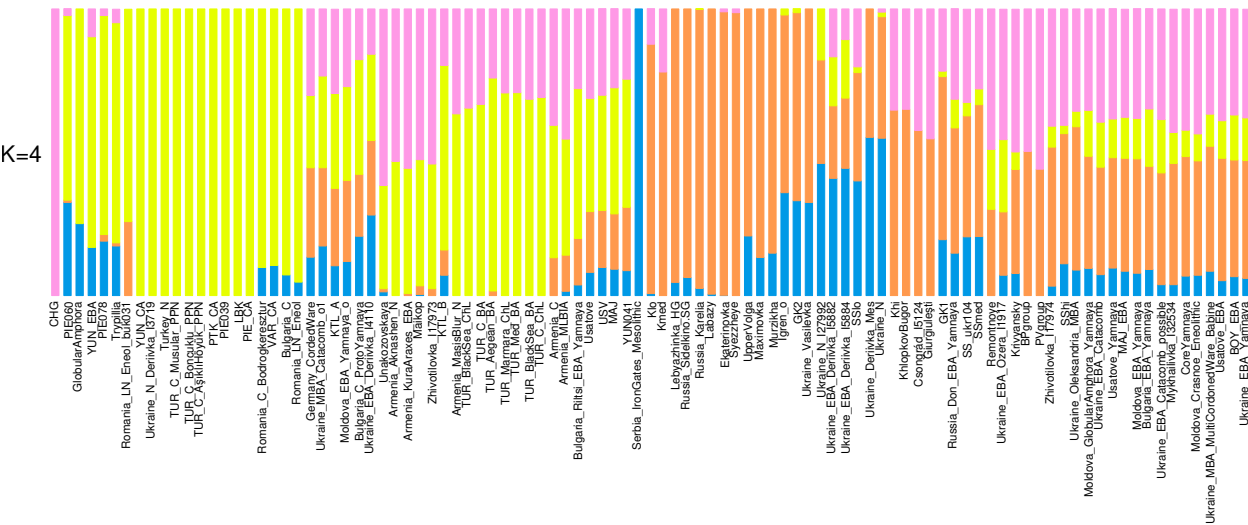


- |                                    |   |   |                                      |  |
|------------------------------------|---|---|--------------------------------------|--|
| ▲ Aknashen:Armavir Province        | ▲ Cuncea:Soldanești I7920                     | × Kam'yana Mohyla:Kurgan 2                    | ✱ Ocnîța:Kurgan 1                    | □ Taraclia:Kurgans 2 and 10                  |
| □ Berezhnovka-2:BPgroup            | □ Dănceni:Ground burial I20069                | ◇ Kartal:Odessa Oblast                        | ◇ Odesa:Odesa Kurgan                 | + Tiraspol:Kurgan 3                          |
| ⊠ Bil'che Zolote:Verteba Cave      | ⊕ Deriivka:Eneolithic Serednii Stih cemetery  | ⊠ Krivvansky:Rostov Oblast, Lower Don group   | ◇ Ogrin-8 (Igren-8):Igren-8 cemetery | ○ Ulan-4:Remontnoye                          |
| △ Bil'shivtsi:Ground burial I13071 | ▽ Dlinnaya-Polyana:Maykop                     | ⊕ Mărculești:Kurgan 3                         | ⊠ Popovo:Golyamata Mogila            | ○ Vapnyarka:Vapnyarka Kurgan 4               |
| □ Branove:Soldats'ka Slava Kurgan  | ⊠ Dobrich:Riltsi Kurgan 264                   | + Mariupol:Mariupol Neolithic Necropolis      | × Progress-2:BPgroup/Pvgroup         | ◇ Vonyuchka:PVgroup                          |
| ○ Bursuceni:Kurgan 1               | □ Dubynove:Kurgan 1                           | × Mayaky:Mayaky Sanctuary and Necropolis      | △ Revova:Kurgan 3                    | ✱ Vynohradne:Vynohradne Kurgan 3             |
| ⊠ Cioburciu:Kurgan 4               | × Durankulak:kurgan F                         | ⊠ Mereni:Kurgan 1                             | △ Sărăteni:Kurgan 1                  | ○ Yasynuvatka:Yasynuvatka Neolithic cemetery |
| ▽ Cotiujeni:Kurgan 1               | ● Giurgiulești:Giurgiulești ground necropolis | ▽ Molyukhiv Bugor:Ground Burials I1424, I1454 | + Sharakhalsun:Steppe Maykop         | □ Yunatsite:Pazardzhik                       |
| + Crasnoe:Kurgan 9                 | ⊠ Glinoe (Hlinaia):DOT and SAD kurgans        | ◇ Mykhailivka:Specimen I32534                 | △ Sukhaya-Termista-1:Remontnoye      | + Znamianka:Katarzhyno Kurgans 1 and 2       |
| ■ Csongrád:I5124                   | □ Golubaya Krinitza:Voronezh Oblast           | ○ Novohryhorivka:Liubasha Kurgan 2            | ○ Sychavka:Sychavka Kurgan           |  |

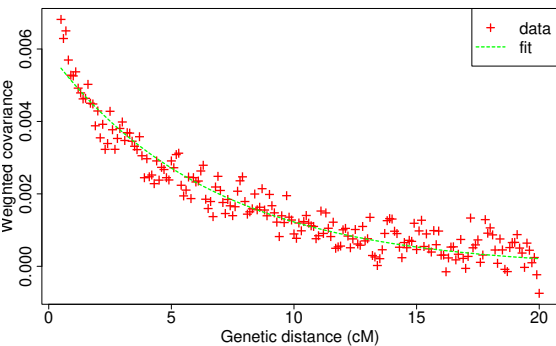
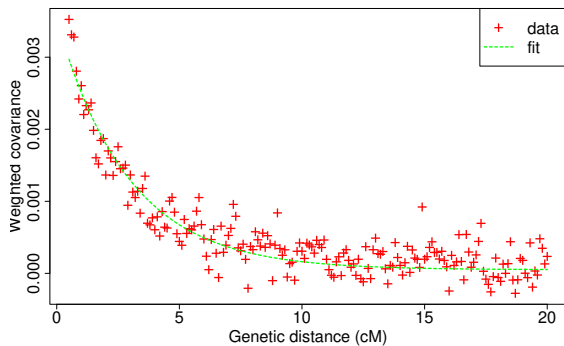
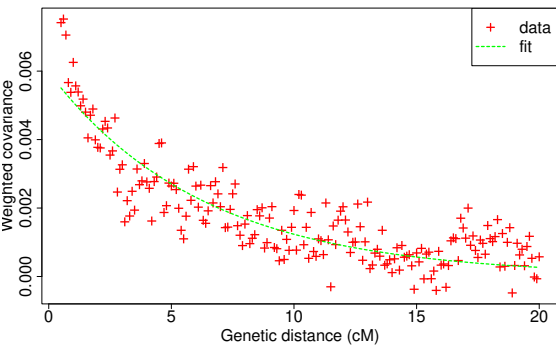




b





**a****UsatoveRelated: 4471±51 BCE (16.120±1.828 generations)****b****Trypillia: 4595±121 BCE (34.898±4.335 generations)****c****KTL\_A: 4199±72 BCE (16.582±2.585 generations)****d****KTL\_B: 4168±138 BCE (16.017±4.937 generations)**