

The genetic prehistory of the Greater Caucasus

Chuan-Chao Wang^{1,2,*}, Sabine Reinhold³, Alexey Kalmykov⁴, Antje Wissgott¹, Guido Brandt¹, Choongwon Jeong¹, Olivia Cheronet^{5,6,7}, Matthew Ferry^{8,9}, Eadaoin Harney^{8,9,10}, Denise Keating^{5,7}, Swapan Mallick^{8,9,11}, Nadin Rohland^{8,11}, Kristin Stewardson^{8,9}, Anatoly R. Kantorovich¹², Vladimir E. Maslov¹³, Vladimira G. Petrenko¹³, Vladimir R. Erlikh¹⁴, Biaslan Ch. Atabiev¹⁵, Rabadan G. Magomedov¹⁶, Philipp L. Kohl¹⁷, Kurt W. Alt^{18,19,20}, Sandra L. Pichler¹⁹, Claudia Gerling¹⁹, Harald Meller²¹, Benik Vardanyan^{22,23}, Larisa Yeganyan²³, Alexey D. Rezepkin²⁴, Dirk Mariaschk³, Natalia Berezina²⁵, Julia Gresky²⁶, Katharina Fuchs²⁷, Corina Knipper²⁸, Stephan Schiffels¹, Elena Balanovska²⁹, Oleg Balanovsky^{29,30}, Iain Mathieson³¹, Thomas Higham³², Yakov B. Berezin²⁵, Alexandra Buzhilova²⁵, Viktor Trifonov³³, Ron Pinhasi³⁴, Andrej B. Belinskij⁴, David Reich^{8,9,11,35}, Svend Hansen^{3,*}, Johannes Krause^{1,35,*} & Wolfgang Haak^{1,36,*}

¹*Department of Archaeogenetics, Max-Planck Institute for the Science of Human History, Kahlaische Strasse 10, D-07745 Jena, Germany*

²*Department of Anthropology and Ethnology, Xiamen University 361005 Xiamen, China*

³*German Archaeological Institute, Eurasia Department, Im Dol 2-6, D-14195 Berlin, Germany*

⁴*'Nasledie' Cultural Heritage Unit, 355006 Stavropol, Russia*

⁵*Earth Institute, University College Dublin, Dublin 4, Ireland*

⁶*Department of Anthropology, University of Vienna, 1090 Vienna, Austria*

⁷*School of Archaeology, University College Dublin, Dublin 4, Ireland*

⁸*Department of Genetics, Harvard Medical School, Boston 02115 MA, USA*

⁹*Howard Hughes Medical Institute, Harvard Medical School, Boston 02115 MA, USA*

¹⁰*Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA02138, USA*

¹¹*Broad Institute of Harvard and MIT, Cambridge 02142 MA, USA*

¹²*Department of Archaeology, Faculty of History, Lomonosov Moscow State University, Lomonosovsky pr. 27/4, 119192, Moscow, Russia*

¹³*Institute of Archaeology RAS, Ul. Dm. Ulyanova 19, 117036 Moscow, Russian Federation*

¹⁴*State Museum of Oriental Art, 12a Nikitskiy Boulevard, 119019 Moscow, Russian Federation*

¹⁵*Ltd. Institute for Caucasus Archaeology, Ul. Katkhanova 30, 361401 Nalchik, Republic Kabardino-Balkaria, Russian Federation*

¹⁶*Institute of History, Archaeology and Ethnography DNC RAS, Ul. M. Jaragского 75, 367030 Makhachkala, Republic Dagestan, Russian Federation*

¹⁷*Department of Anthropology, Wellesley College, Pendleton East 331, 106 Central Street, Wellesley, MA 02481, USA*

¹⁸*Danube Private University, A-3500 Krems-Stein, Austria*

¹⁹*IPAS – Institute of Prehistory and Archaeological Science, University of Basel, CH-4055 Basel, Switzerland*

²⁰*Department of Biomedical Engineering, University of Basel, CH-4123 Allschwil, Switzerland*

- 50 ²¹*State Heritage Museum, Saxony-Anhalt, D-06114 Halle/Saale, Germany*
51 ²²*Martin-Luther-Universität Halle-Wittenberg, Germany*
52 ²³*Shirak Center for Armenological Studies of National Academy of Science RA,*
53 *Armenia*
54 ²⁴*Institute for the History of Material Culture, Russian Academy of Sciences,*
55 *Dvortsovaya nab.,18, 191186 Saint-Petersburg, Russia*
56 ²⁵*Research Institute and Museum of Anthropology of Lomonosov Moscow State*
57 *University, Mokhovaya 11, Moscow, Russia*
58 ²⁶*German Archaeological Institute, Department of Natural Sciences, Im Dol 2-6, D-*
59 *14195 Berlin, Germany*
60 ²⁷*CRC 1266 "Scales of Transformation", Institut für Ur- und Frühgeschichte,*
61 *Christian-Albrechts-Universität, Johanna-Mestorf-Straße 2-6, 24118 Kiel, Germany*
62 ²⁸*Curt Engelhorn Center for Archaeometry gGmbH, 68159 Mannheim, Germany*
63 ²⁹*Research Centre for Medical Genetics, Moscow 115478, Russia*
64 ³⁰*Vavilov Institute for General Genetics, Moscow 119991, Russia*
65 ³¹*Department of Genetics, Perelman School of Medicine, University of Pennsylvania,*
66 *Philadelphia PA 19104, USA*
67 ³²*Oxford Radiocarbon Accelerator Unit, RLAHA, University of Oxford, OX13QY, UK*
68 ³³*Institute for the History of Material Culture, Russian Academy of Sciences,*
69 *Dvortsovaya nab.,18, 191186 Saint-Petersburg, Russia*
70 ³⁴*Department of Evolutionary Anthropology, University of Vienna, 1010 Vienna,*
71 *Austria*
72 ³⁵*Max Planck-Harvard Research Center for the Archaeoscience of the Ancient*
73 *Mediterranean, Cambridge, MA 02138, USA*
74 ³⁶*School of Biological Sciences, The University of Adelaide, Adelaide 5005, Australia*
75
76
77
78 *corresponding authors: haak@shh.mpg.de, krause@shh.mpg.de,
79 wang@xmu.edu.cn, svend.hansen@dainst.de
80

81 **Abstract**

82 Archaeogenetic studies have described the formation of Eurasian ‘steppe ancestry’ as
83 a mixture of Eastern and Caucasus hunter-gatherers. However, it remains unclear
84 when and where this ancestry arose and whether it was related to a horizon of cultural
85 innovations in the 4th millennium BCE that subsequently facilitated the advance of
86 pastoral societies likely linked to the dispersal of Indo-European languages. To
87 address this, we generated genome-wide SNP data from 45 prehistoric individuals
88 along a 3000-year temporal transect in the North Caucasus. We observe a genetic
89 separation between the groups of the Caucasus and those of the adjacent steppe. The
90 Caucasus groups are genetically similar to contemporaneous populations south of it,
91 suggesting that – unlike today – the Caucasus acted as a bridge rather than an
92 insurmountable barrier to human movement. The steppe groups from Yamnaya and
93 subsequent pastoralist cultures show evidence for previously undetected farmer-
94 related ancestry from different contact zones, while Steppe Maykop individuals
95 harbour additional Upper Palaeolithic Siberian and Native American related ancestry.
96

97 The 1100-kilometre long Caucasus mountain ranges extend between the Black Sea
98 and the Caspian Sea and are bound by the rivers Kuban and Terek in the north and by
99 the Kura and Araxes rivers in the south. With Mount Elbrus in Russian Kabardino-
100 Balkaria rising to a height of 5642 metres and Mount Shkhara in Georgia to 5201
101 metres, the Caucasus mountain ranges form a natural barrier between the Eurasian
102 steppes and the Near East (Fig. 1).

103

104 The rich archaeological record suggests extensive periods of human occupation since
105 the Upper Palaeolithic^{1, 2, 3}. The density of languages and cultures in the region is
106 mirrored by faunal and floral diversity, and the Caucasus has often been described as
107 a contact zone and natural refuge with copious ecological niches. However, it also
108 serves as a bio-geographic border between the steppe and regions to the south such as
109 Anatolia and Mesopotamia rather than a corridor for human^{4, 5} and animal movement^{6,}
110 ^{7, 8}. The extent to which the Caucasus has played an important role for human
111 population movements between south and north over the course of human history is
112 thus a critical question, and one that until now has been unanswered by
113 archaeogenetic studies.

114

115 A Neolithic lifestyle based on food production began in the Caucasus after 6000
116 calBCE⁹. In the following millennia the Caucasus region began to play an
117 increasingly important role in the economies of the growing urban centres in northern
118 Mesopotamia¹⁰ as a region rich in natural resources such as ores, pastures and
119 timber¹¹. In the 4th millennium BCE the archaeological record attests to the presence
120 of the Maykop and Kura-Araxes cultural complexes, with the latter being found on
121 both flanks of the Caucasus mountain range, thus clearly demonstrating the
122 connection between north and south¹¹. The Maykop culture was an important player
123 in the innovative horizon of the 4th millennium BCE in Western Eurasia. It is well
124 known for its rich burial mounds, especially at the eponymous Maykop site in today's
125 Adygea, which reflect the rise of a new system of social organization¹². The 4th
126 millennium BCE witnesses a concomitant rise in commodities and technologies such
127 as the wheel and wagon including associated technology, copper alloys, new
128 weaponry, and new breeds of domestic sheep^{13, 14}.

129

130 The adjacent Pontic-Caspian and Eurasian steppe also played an important role in this
131 linked economic system, being the most likely region for the domestication of the
132 horse that revolutionised transport¹³. In addition, many steppe kurgans (large burial
133 mounds that are first observed in the context of the Maykop culture) have yielded the
134 remains of wheels and ox-drawn carts, highlighting a mobile economy focused on
135 cattle and sheep/goat herding¹⁵. The adoption of the horse almost certainly
136 contributed to the intensification of pastoralist practices in the Eurasian steppes,
137 allowing more efficient keeping of larger herds^{16, 17, 18} and facilitating the massive
138 range expansions of pastoralists associated with the Yamnaya cultural community and
139 related groups from the East European steppe^{19, 20}. This transformation changed the
140 European gene pool during the early 3rd millennium BCE and descendants of the
141 Yamnaya eventually also transformed the ancestry of South Asia as well²¹. However,
142 flow of goods and ideas between the eastern European steppe zone, the Caucasus, the
143 Carpathians, and Central Europe has been documented by archaeological and ancient
144 DNA research as early as the 5th millennium BCE, long before the massive migration
145 took place^{22, 23, 24}. Taken together, the Caucasus region played a crucial role in the
146 prehistory of Western Eurasia and this study aims to shed new light on events in the

147 key period between the 4th and 3rd millennium BCE.

148

149 Recent ancient DNA studies have enabled the resolution of several long-standing
150 questions regarding cultural and population transformations in prehistory. One of
151 these is the Mesolithic-Neolithic transition in Europe, which saw a change from a
152 hunter-gatherer lifestyle to a sedentary, food-producing subsistence strategy.

153 Genome-wide data from pre-farming and farming communities have identified
154 distinct ancestral populations that largely reflect subsistence patterns in addition to
155 geography²⁵. One important feature is a cline of European hunter-gatherer (HG)
156 ancestry that runs roughly from West to East (hence WHG and EHG; blue component
157 in Fig. 2A, 2C), which differs greatly from the ancestry of Early European farmers
158 that in turn is closely related to that of northwest Anatolian farmers^{26,27} and more
159 remotely also to pre-farming individuals from the Levant²³. The Near East and
160 Anatolia have long been seen as the regions from which European farming and animal
161 husbandry emerged. Surprisingly, these regions harboured three divergent
162 populations, with Anatolian and Levantine ancestry in the western part and a group
163 with a distinct ancestry in the eastern part first described in Upper Pleistocene
164 individuals from Georgia (Caucasus hunter-gatherers; CHG)²⁸ and then in Mesolithic
165 and Neolithic individuals from Iran^{23,29}. The following two millennia, spanning from
166 the Neolithic to Chalcolithic and Early Bronze Age periods in each region, witnessed
167 migration and admixture between these ancestral groups, leading to a pattern of
168 genetic homogenization and reduced genetic distances between these Neolithic source
169 populations²³. In parallel, Eneolithic individuals from the Samara region (5200-4000
170 BCE) also exhibit population mixture, specifically EHG- and CHG/Iranian ancestry, a
171 combination that forms the so-called 'steppe-ancestry'²⁸. This ancestry eventually
172 spread further west^{19,20}, where it contributed substantially to the ancestry of present-
173 day Europeans, and east to the Altai region as well as to South Asia²³.

174

175 To understand and characterize the genetic variation of Caucasian populations,
176 present-day groups from various geographic, cultural/ethnic and linguistic
177 backgrounds have been analyzed previously at the autosomal, Y-chromosomal and
178 mitochondrial level^{4,5,30}. Yunusbayev and colleagues described the Greater Caucasus
179 region as an asymmetric semipermeable barrier based on a higher genetic affinity of
180 southern Caucasus groups to Anatolian and Near Eastern populations and a genetic
181 discontinuity between these and populations of the North Caucasus and of adjacent
182 Eurasian steppes. While autosomal and mitochondrial DNA data appear relatively
183 homogeneous across diverse ethnic and linguistic groups and the entire mountainous
184 region, the Y-chromosome diversity reveals a deeper genetic structure attesting to
185 several male founder effects, with striking correspondence to geography, language
186 groups and historical events^{4,5}.

187

188 In our study we aimed to investigate when and how the genetic patterns observed
189 today were formed and test whether they have been present since prehistoric times by
190 generating time-stamped human genome-wide data. We were also interested in
191 characterizing the role of the Caucasus as a conduit for gene-flow in the past and in
192 shaping the cultural and genetic makeup of the wider region (Supplementary
193 Information 1). This has important implications for understanding the means by
194 which Europe, the Eurasian steppe zone, and the earliest urban centres in the Near
195 East were connected³¹. We aimed to genetically characterise individuals from cultural
196 complexes such as the Maykop and Kura-Araxes and assessing the amount of gene

197 flow in the Caucasus during times when the exploitation of resources of the steppe
198 environment intensified, since this was potentially triggered by the cultural and
199 technological innovations of the Late Chalcolithic and Early Bronze Age 6000-5000
200 years ago¹¹. Lastly, since the spread of steppe ancestry into central Europe and the
201 eastern steppes during the early 3rd millennium BCE (5000-4500 BP) was a striking
202 migratory event in human prehistory^{19, 20}, we also wanted to retrace the formation of
203 the steppe ancestry profile and whether this might have been influenced by
204 neighbouring farming groups to the west or from regions of early urbanization further
205 south.

206

207 **Results**

208

209 **Genetic clustering and uniparentally-inherited markers**

210 We report genome-wide data at a targeted set of 1.2 million single nucleotide
211 polymorphisms (SNPs)^{19, 32} for 59 Eneolithic/Chalcolithic and Bronze Age
212 individuals from the Caucasus region. After filtering out 14 individuals that were
213 first-degree relatives or showed evidence of contamination or reference bias
214 (Supplementary Information 3 and Data 1) we retained 45 individuals for downstream
215 analyses using a cut-off of 30,000 SNPs. We merged our newly generated samples
216 with previously published ancient and modern data^{19, 20, 23, 24, 26, 27, 29, 33, 34, 35, 36, 37, 38, 39,}
217 ^{40, 41, 42, 43} (Supplementary Data 2). We first performed principal component analysis
218 (PCA)⁴⁴ and ADMIXTURE⁴⁵ analysis to assess the genetic affinities of the ancient
219 individuals qualitatively (Fig. 2) and followed up quantitatively with formal *f*- and *D*-
220 statistics, *qpWave*, *qpAdm*, and *qpGraph*⁴⁴. Based on PCA and ADMIXTURE plots
221 we observe two distinct genetic clusters: one cluster falls with previously published
222 ancient individuals from the West Eurasian steppe (hence termed ‘*Steppe*’), and the
223 second clusters with present-day southern Caucasian populations and ancient Bronze
224 Age individuals from today’s Armenia (henceforth called ‘*Caucasus*’), while a few
225 individuals take on intermediate positions between the two. The stark distinction seen
226 in our temporal transect is also visible in the Y-chromosome haplogroup distribution,
227 with R1/R1b1 and Q1a2 types in the *Steppe* and L, J, and G2 types in the *Caucasus*
228 cluster (Fig. 3A, Supplementary Data 1). In contrast, the mitochondrial haplogroup
229 distribution is more diverse and almost identical in both groups (Fig. 3B,
230 Supplementary Data 1).

231

232 The two distinct clusters are already visible in the oldest individuals of our temporal
233 transect, dated to the Eneolithic period (~6300-6100 yBP/4300-4100 calBCE). Three
234 individuals from the sites of Progress 2 and Vonjuchka 1 in the North Caucasus
235 piedmont steppe (‘Eneolithic steppe’), which harbor Eastern and Caucasian hunter-
236 gatherer related ancestry (EHG and CHG, respectively), are genetically very similar
237 to Eneolithic individuals from Khalynsk II and the Samara region^{19, 27}. This extends
238 the cline of dilution of EHG ancestry via CHG/Iranian-like ancestry to sites
239 immediately north of the Caucasus foothills (Fig. 2D).

240 In contrast, the oldest individuals from the northern mountain flank itself, which are
241 three first degree-related individuals from the Unakozovskaya cave associated with
242 the Darkveti-Meshoko Eneolithic culture (analysis label ‘Eneolithic Caucasus’) show
243 mixed ancestry mostly derived from sources related to the Anatolian Neolithic
244 (orange) and CHG/Iran Neolithic (green) in the ADMIXTURE plot (Fig. 2C). While
245 similar ancestry profiles have been reported for Anatolian and Armenian Chalcolithic
246 and Bronze Age individuals^{20, 23}, this result suggests the presence of the mixed

247 Anatolian/Iranian/CHG related ancestry north of the Great Caucasus Range as early
248 as ~6500 years ago.

249

250 **Ancient North Eurasian ancestry in ‘Steppe Maykop’ individuals**

251 Four individuals from mounds in the grass steppe zone, which are archaeologically
252 associated with the ‘Steppe Maykop’ cultural complex (Supplementary Information
253 1), lack the Anatolian farmer-related component when compared to contemporaneous
254 Maykop individuals from the foothills. Instead they carry a third and fourth ancestry
255 component that is linked deeply to Upper Paleolithic Siberians (maximized in the
256 individual Afontova Gora 3 (AG3)^{36, 37} and Native Americans, respectively, and in
257 modern-day North Asians such as North Siberian Nganasan (Supplementary Fig. 1).
258 To illustrate this affinity with ‘ancient North Eurasians’ (ANE)²⁶, we also ran PCA
259 with 147 Eurasian (Supplementary Fig. 2A) and 29 Native American populations
260 (Supplementary Fig. 2B). The latter represent a cline from ANE-rich steppe
261 populations such as EHG, Eneolithic individuals, AG3 and Mal’ta 1 (MA1) to
262 modern-day Native Americans at the opposite end. To formally test the excess of
263 alleles shared with ANE/Native Americans we performed f_4 -statistics of the form
264 $f_4(\text{Mbuti}, X; \text{Steppe Maykop}, \text{Eneolithic steppe})$, which resulted in significantly
265 positive Z scores $|Z| > 3$ for AG3, MA1, EHG, Clovis and Kennewick for the ancient
266 populations and many present-day Native American populations (Supplementary
267 Table 1). Based on these observations we used *qpWave* and *qpAdm* methods to model
268 the number of ancestral sources contributing to the Steppe Maykop individuals and
269 their relative ancestry coefficients. Simple two-way models of Steppe Maykop as an
270 admixture of Eneolithic steppe, AG3 or Kennewick do not fit (Supplementary Table
271 2). However, we could successfully model Steppe Maykop ancestry as being derived
272 from populations related to all three sources (p-value 0.371 for rank 2): Eneolithic
273 steppe (63.5±2.9 %), AG3 (29.6±3.4%) and Kennewick (6.9±1.0%) (Fig. 4;
274 Supplementary Table 3). We note that the Kennewick related signal is most likely
275 driven by the East Eurasian part of Native American ancestry as the f_4 -statistics
276 (Steppe_Maykop, Fitted Steppe_Maykop; Outgroup1, Outgroup2) show that the
277 Steppe Maykop individuals share more alleles not only with Karitiana but also with
278 Han Chinese when compared with the fitted ones using Eneolithic steppe and AG3 as
279 two sources and Mbuti, Karitiana and Han as outgroups (Supplementary Table 2).

280

281 **Characterising the *Caucasus* ancestry profile**

282 The Maykop period, represented by twelve individuals from eight Maykop sites
283 (Maykop, n=2; a cultural variant ‘Novosvobodnaya’ from the site Klady, n=4; and
284 Late Maykop, n=6) in the northern foothills appear homogeneous. These individuals
285 closely resemble the preceding Caucasus Eneolithic individuals and present a
286 continuation of the local genetic profile. This ancestry persists in the following
287 centuries at least until ~3100 yBP (1100 calBCE) in the mountains, as revealed by
288 individuals from Kura-Araxes from both the northeast (Velikent, Dagestan) and the
289 South Caucasus (Kaps, Armenia), as well as Middle and Late Bronze Age individuals
290 (e.g. Kudachurt, Marchenkova Gora) from the north. Overall, this *Caucasus* ancestry
291 profile falls among the ‘Armenian and Iranian Chalcolithic’ individuals and is
292 indistinguishable from other Kura-Araxes individuals (‘Armenian Early Bronze Age’)
293 on the PCA plot (Fig. 2), suggesting a dual origin involving Anatolian/Levantine and
294 Iran Neolithic/CHG ancestry, with only minimal EHG/WHG contribution possibly as
295 part of the Anatolian farmer-related ancestry²³.

296 Admixture f_3 -statistics of the form $f_3(X, Y; \text{target})$ with the *Caucasus* cluster as target
297 resulted in significantly negative Z scores $|Z| < -3$ when CHG (or AG3 in Late
298 Maykop) were used as one and Anatolian farmers as the second potential source
299 (Supplementary Table 4). We also used *qpWave* to determine the number of streams
300 of ancestry and found that a minimum of two is sufficient (except for Eneolithic
301 Caucasus or Dolmen LBA, for which one source is sufficient (Supplementary Table
302 5).

303 We then tested whether each temporal/cultural group of the *Caucasus* cluster could be
304 modelled as a simple two-way admixture by exploring all possible pairs of sources in
305 *qpWave*. We found support for CHG as one source and Anatolian farmer-related
306 ancestry or a derived form such as is found in southeastern Europe as the other
307 (Supplementary Table 6). We focused on model of mixture of proximal sources (Fig.
308 4B) such as CHG and Anatolian Chalcolithic for all six groups of the Caucasus
309 cluster (Eneolithic Caucasus, Maykop and Late Makyop, Maykop-Novosvobodnaya,
310 Kura-Araxes, and Dolmen LBA), with admixture proportions on a genetic cline of 40-
311 72% Anatolian Chalcolithic related and 28-60% CHG related (Supplementary Table
312 7). When we explored Romania_EN and Greece_Neolithic individuals as alternative
313 southeast European sources (30-46% and 36-49%), the CHG proportions increased to
314 54-70% and 51-64%, respectively. We hypothesize that alternative models, replacing
315 the Anatolian Chalcolithic individual with yet unsampled populations from eastern
316 Anatolia, South Caucasus or northern Mesopotamia, would probably also provide a fit
317 to the data from some of the tested Caucasus groups. The models replacing CHG with
318 Iran Neolithic-related individuals could explain the data in a two-way admixture with
319 the combination of Armenia Chalcolithic or Anatolia Chalcolithic as the other source.
320 However, models replacing CHG with EHG individuals received no support
321 (Supplementary Table 8), indicating no strong influence for admixture from the
322 adjacent steppe to the north. In an attempt to account for potentially un-modelled
323 ancestry in the Caucasus groups, we added EHG, WHG and Iran Chalcolithic as
324 additional sources in the previous two-way modelling. The resulting ancestry
325 coefficients do not deviate substantially from 0 (high standard errors) when adding
326 EHG or WHG, suggesting very limited direct ancestry from both hunter-gatherer
327 groups (Supplementary Table 9). Alternatively, when we added Iran Chalcolithic
328 individuals as a third source to the model, we observed that Kura-Araxes and
329 Maykop-Novosvobodnaya individuals had likely received additional Iran
330 Chalcolithic-related ancestry (24.9% and 37.4%, respectively; Fig. 4; Supplementary
331 Table 10).

332

333 **Characterising the *Steppe* ancestry profile in the North Caucasus**

334 Individuals from the North Caucasian steppe associated with the Yamnaya cultural
335 formation (5300-4400 BP, 3300-2400 calBCE) appear genetically almost identical to
336 previously reported Yamnaya individuals from Kalmykia²⁰ immediately to the north,
337 the middle Volga region^{19,27}, Ukraine and Hungary, and to other Bronze Age
338 individuals from the Eurasian steppes who share the characteristic ‘steppe ancestry’
339 profile as a mixture of EHG and CHG/Iranian ancestry^{23,28}. These individuals form a
340 tight cluster in PCA space (Figure 2) and can be shown formally to be a mixture by
341 significantly negative admixture f_3 -statistics of the form $f_3(\text{EHG}, \text{CHG}; \text{target})$
342 (Supplementary Fig. 3). This also involves individuals assigned to the North Caucasus
343 culture (4800-4500 BP, 2800-2500 calBCE) in the piedmont steppe of the central
344 North Caucasus, who share the steppe ancestry profile. Individuals from the
345 Catacomb culture in the Kuban, Caspian and piedmont steppes (4600-4200 BP, 2600-

346 2200 calBCE), which succeeded the Yamnaya horizon, also show a continuation of
347 the ‘steppe ancestry’ profile.

348

349 The individuals of the Middle Bronze Age (MBA) post-Catacomb horizon (4200-
350 3700 BP, 2200-1700 calBCE) such as Late North Caucasus and Lola culture represent
351 both ancestry profiles common in the North Caucasus region: individuals from the
352 mountain site Kabardinka show a typical steppe ancestry profile, whereas individuals
353 from the Late North Caucasus site Kudachurt 90 km to the west retain the ‘southern’
354 Caucasus profile. The latter is also observed in our most recent individual from the
355 western Late Bronze Age Dolmen culture (3400-3200 BP, 1400-1200 calBCE). In
356 contrast, one individual assigned to the Lola culture resembles the ancestry profile of
357 the Steppe Maykop individuals.

358

359 **Admixture into the steppe zone from the south**

360 Evidence for interaction between the *Caucasus* and the *Steppe* clusters is visible in
361 our genetic data from individuals associated with the later Steppe Maykop phase
362 around 5300-5100 years ago. These ‘outlier’ individuals were buried in the same
363 mounds as those with steppe and in particular Steppe Maykop ancestry profiles but
364 share a higher proportion of Anatolian farmer-related ancestry visible in the
365 ADMIXTURE plot and are also shifted towards the *Caucasus* cluster in PC space
366 (Fig. 2D). This observation is confirmed by formal *D*-statistics (Steppe Maykop
367 outlier, Steppe Maykop; X; Mbuti), which are significantly positive when X is a
368 Neolithic or Bronze Age group from the Near East or Anatolia (Supplementary Fig.
369 4). By modelling Steppe Maykop outliers successfully as a two-way mixture of
370 Steppe Maykop and representatives of the *Caucasus* cluster (Supplementary Table 3),
371 we can show that these individuals received additional ‘Anatolian and Iranian
372 Neolithic ancestry’, most likely from contemporaneous sources in the south. We
373 estimated admixture time for the observed farmer-related ancestry individuals using
374 the linkage disequilibrium (LD)-based admixture inference implemented in
375 ALDER⁴⁶, using Steppe Maykop outliers as the test population and Steppe Maykop as
376 well as Kura-Araxes as references. The average admixture time for Steppe Maykop
377 outliers is about 20 generations or 560 years ago, assuming a generation time of 28
378 years⁴⁷ (Supplementary Information 6).

379

380 **Contribution of Anatolian farmer-related ancestry to Bronze Age steppe groups**

381 In principal component space Eneolithic individuals (Samara Eneolithic) form a cline
382 running from EHG to CHG (Fig. 2D), which is continued by the newly reported
383 Eneolithic steppe individuals. However, the trajectory of this cline changes in the
384 subsequent centuries. Here we observe a cline from Eneolithic_steppe towards the
385 *Caucasus* cluster. We can qualitatively explain this ‘tilting cline’ by developments
386 south of the Caucasus, where Iranian and Anatolian/Levantine Neolithic ancestries
387 continue to mix, resulting in a blend that is also observed in the *Caucasus* cluster,
388 from where it could have spread onto the steppe. The first appearance of ‘Near
389 Eastern farmer related ancestry’ in the steppe zone is evident in Steppe Maykop
390 outliers. However, PCA results also suggest that Yamnaya and later groups of the
391 West Eurasian steppe carry some farmer related ancestry as they are slightly shifted
392 towards ‘European Neolithic groups’ in PC2 (Fig. 2D) compared to Eneolithic steppe.
393 This is not the case for the preceding Eneolithic steppe individuals. The tilting cline is
394 also confirmed by admixture f_3 -statistics, which provide statistically negative values
395 for AG3 as one source and any Anatolian Neolithic related group as a second source

396 (Supplementary Table 11). Detailed exploration via D -statistics in the form of
397 $D(\text{EHG, steppe group; X, Mbuti})$ and $D(\text{Samara_Eneolithic, steppe group; X, Mbuti})$
398 show significantly negative D values for most of the steppe groups when X is a
399 member of the *Caucasus* cluster or one of the Levant/Anatolia farmer-related groups
400 (Supplementary Figs. 5 and 6). In addition, we used f - and D -statistics to explore the
401 shared ancestry with Anatolian Neolithic as well as the reciprocal relationship
402 between Anatolian- and Iranian farmer-related ancestry for all groups of our two main
403 clusters and relevant adjacent regions (Supplementary Fig. 4). Here, we observe an
404 increase in farmer-related ancestry (both Anatolian and Iranian) in our *Steppe* cluster,
405 ranging from Eneolithic steppe to later groups. In Middle/Late Bronze Age groups
406 especially to the north and east we observe a further increase of Anatolian farmer-
407 related ancestry consistent with previous studies of the Poltavka, Andronovo,
408 Srubnaya and Sintashta groups^{23, 27} and reflecting a different process not especially
409 related to events in the Caucasus.

410

411 The exact geographic and temporal origin of this Anatolian farmer-related ancestry in
412 the North Caucasus and later in the steppe is difficult to discern from our data. Not
413 only do the *Steppe* groups vary in their respective affinity to each of the two, but also
414 the *Caucasus* groups, which represent potential sources from a geographic and
415 cultural point of view, are mixtures of them both²³. We therefore used $qpWave$ and
416 $qpAdm$ to explore the number of ancestry sources for the Anatolian farmer-related
417 component to evaluate whether geographically proximate groups plausibly
418 contributed to the subtle shift of Eneolithic ancestry in the steppe towards those of the
419 Neolithic groups. Specifically, we tested whether any of the Eurasian steppe ancestry
420 groups can be successfully modelled as a two-way admixture between Eneolithic
421 steppe and a population X derived from Anatolian- or Iranian farmer-related ancestry,
422 respectively. Surprisingly, we found that a minimum of four streams of ancestry is
423 needed to explain all eleven steppe ancestry groups tested, including previously
424 published ones (Fig. 2; Supplementary Table 12). Importantly, our results show a
425 subtle contribution of both Anatolian farmer-related ancestry and WHG-related
426 ancestry (Fig.4; Supplementary Tables 13 and 14), which was likely contributed
427 through Middle and Late Neolithic farming groups from adjacent regions in the West.
428 A direct source of Anatolian farmer-related ancestry can be ruled out (Supplementary
429 Table 15). At present, due to the limits of our resolution, we cannot identify a single
430 best source population. However, geographically proximal and contemporaneous
431 groups such as Globular Amphora and Eneolithic groups from the Black Sea area
432 (Ukraine and Bulgaria), which represent all four distal sources (CHG, EHG, WHG,
433 and Anatolian_Neolithic) are among the best supported candidates (Fig. 4;
434 Supplementary Tables 13,14 and 15). Applying the same method to the subsequent
435 North Caucasian *Steppe* groups such as Catacomb, North Caucasus, and Late North
436 Caucasus confirms this pattern (Supplementary Table 17).

437

438 Using $qpAdm$ with Globular Amphora as a proximate surrogate population (assuming
439 that a related group was the source of the Anatolian farmer-related ancestry), we
440 estimated the contribution of Anatolian farmer-related ancestry into Yamnaya and
441 other steppe groups. We find that Yamnaya individuals from the Volga region
442 (Yamnaya Samara) have $13.2 \pm 2.7\%$ and Yamnaya individuals in Hungary $17.1 \pm 4.1\%$
443 Anatolian farmer-related ancestry (Fig.4; Supplementary Table 18)– statistically
444 indistinguishable proportions. Replacing Globular Amphora by Iberia Chalcolithic,
445 for instance, does not alter the results profoundly (Supplementary Table 19). This

446 suggests that the source population was a mixture of Anatolian farmer-related
447 ancestry and a minimum of 20% WHG ancestry, a profile that is shared by many
448 Middle/Late Neolithic and Chalcolithic individuals from Europe of the 3rd millennium
449 BCE analysed thus far.

450 To account for potentially un-modelled ancestry from the *Caucasus* groups, we added
451 ‘Eneolithic Caucasus’ as an additional source to build a three-way model. We found
452 that Yamnaya Caucasus, Yamnaya Ukraine Ozera, North Caucasus and Late North
453 Caucasus had likely received additional ancestry (6% to 40%) from nearby *Caucasus*
454 groups (Supplementary Table 20). This suggests a more complex and dynamic picture
455 of steppe ancestry groups through time, including the formation of a local variant of
456 steppe ancestry in the North Caucasian steppe from the local Eneolithic, a
457 contribution of Steppe Maykop groups, and population continuity between the early
458 Yamnaya period and the Middle Bronze Age (5300-3200 BP, 3300-2200 calBCE).
459 This was interspersed by additional, albeit subtle gene-flow from the West and
460 occasional equally subtle gene flow from neighbouring groups in the Caucasus and
461 piedmont zones.

462

463 **Insights from micro-transects through time**

464 The availability of multiple individuals from one site (here burial mounds or kurgans)
465 allowed us to test genetic continuity on a micro-transect level. By focusing on two
466 kurgans (Marinskaya 5 and Sharakhalsun 6), for which we could successfully
467 generate genome-wide data from four and five individuals, respectively, we observe
468 that the genetic ancestry varied through time, alternating between the *Steppe* and
469 *Caucasus* ancestries (Supplementary Fig. 8). This shows that the apparent genetic
470 border between the two distinct genetic clusters was shifting over time. We also
471 detected various degrees of kinship between individuals buried in the same mound,
472 which supports the view that particular mounds reflected genealogical lineages.
473 Overall, we observe a balanced sex ratio within our sites across the individuals tested
474 (Supplementary Information 4).

475

476 **A joint model of ancient populations of the Caucasus region**

477 We used *qpGraph* to explore models that jointly explain the population splits and
478 gene flow in the Greater Caucasus region by computing f_2 -, f_3 - and f_4 - statistics
479 measuring allele sharing among pairs, triples, and quadruples of populations and
480 evaluating fits based on the maximum $|Z|$ -score comparing predicted and observed
481 values of these statistics. Our fitted model recapitulates the genetic separation
482 between the *Caucasus* and *Steppe* groups with the Eneolithic steppe individuals
483 deriving more than 60% of ancestry from EHG and the remainder from a CHG-
484 related basal lineage, whereas the Maykop group received about 86.4% from CHG,
485 9.6% Anatolian farming related ancestry, and 4% from EHG. The Yamnaya
486 individuals from the Caucasus derived the majority of their ancestry from Eneolithic
487 steppe individuals but also received about 16% from Globular Amphora-related
488 farmers (Fig. 5).

489

490

491 **Discussion**

492

493 Our data from the Greater Caucasus region cover over 3000 years of prehistory as a
494 transect through time, ranging from the Eneolithic (starting 6500 yBP, 4500 calBCE)
495 to the Late Bronze Age (ending 3200 yBP, 1200 calBCE). We observe a genetic

496 separation between the groups in the piedmont steppe, i.e. the northern foothills of the
497 Greater Caucasus, and those groups of the bordering herb, grass and desert steppe
498 regions in the north (i.e. the ‘real’ steppe). We have summarised these broadly as
499 *Caucasus* and *Steppe* groups in correspondence with the eco-geographic vegetation
500 zones that characterise the socio-economic basis of the associated archaeological
501 cultures.

502
503 When compared to present-day human populations from the Caucasus, which show a
504 clear separation into North and South Caucasus groups along the Great Caucasus
505 mountain range (Fig. 2D), our new data highlights that the situation during the Bronze
506 Age was quite different. The fact that individuals buried in kurgans in the North
507 Caucasian piedmont and foothill zone are more closely related to ancient individuals
508 from regions further south in today’s Armenia, Georgia and Iran allows us to draw
509 two major conclusions.

510
511 First, sometime after the Bronze Age present-day North Caucasian populations must
512 have received additional gene-flow from populations north of the mountain range that
513 separates them from southern Caucasians, who largely retained the Bronze Age
514 ancestry profile. The archaeological and historic records suggest numerous incursions
515 during the subsequent Iron Age and Medieval times⁴⁸, but ancient DNA from these
516 time periods is needed to test this directly.

517
518 Second, our results reveal that the Greater Caucasus Mountains were not an
519 insurmountable barrier to human movement in prehistory. Instead the foothills to the
520 north at the interface of the steppe and mountain ecozones could be seen as a transfer
521 zone of cultural innovations from the south and the adjacent Eurasian steppes to the
522 north, as attested by the archaeological record. The latter is best exemplified by the
523 two Steppe Maykop outlier individuals dating to 5100-5000 yBP/3100-3000 calBCE,
524 which carry additional Anatolian farmer-related ancestry likely derived from a
525 proximate source related to the *Caucasus* cluster. We could show that individuals
526 from the contemporaneous Maykop period in the piedmont region are likely
527 candidates for the source of this ancestry and might explain the regular presence of
528 ‘Maykop artefacts’ in burials that share Steppe Eneolithic traditions and are
529 genetically assigned to the *Steppe* group. Hence the diverse ‘Steppe Maykop’ group
530 indeed represents the mutual entanglement of *Steppe* and *Caucasus* groups and their
531 cultural affiliations in this interaction sphere.

532
533 Concerning the influences from the south, our oldest dates from the immediate
534 Maykop predecessors Darkveti-Meshoko (Eneolithic Caucasus) indicate that the
535 *Caucasus* genetic profile was present north of the range ~6500 BP, 4500 calBCE.
536 This is in accordance with the Neolithization of the Caucasus, which had started in the
537 flood plains of the great rivers in the South Caucasus in the 6th millennium BCE from
538 where it spread to the West and Northwest Caucasus during the 5th millennium BCE⁹,
539 ⁴⁹. It remains unclear whether the local CHG ancestry profile (represented by Late
540 Upper Palaeolithic/Mesolithic individuals from Kotias Klde and Satsurblia in today’s
541 Georgia) was also present in the North Caucasus region before the Neolithic.
542 However, if we take the Caucasus hunter-gatherer individuals from Georgia as a local
543 baseline and the oldest Eneolithic Caucasus individuals from our transect as a proxy
544 for the local Late Neolithic ancestry, we notice a substantial increase in Anatolian
545 farmer-related ancestry. This in all likelihood is linked to the process of

546 Neolithization, which also brought this type of ancestry to Europe. As a consequence,
547 it is possible that Neolithic groups could have reached the northern flanks of the
548 Caucasus earlier⁵⁰ (Supplementary Information 1) and in contact with local hunter-
549 gatherers facilitated the exploration of the steppe environment for pastoralist
550 economies. Hence, additional sampling from older individuals is needed to fill this
551 temporal and spatial gap.

552
553 Our results show that at the time of the eponymous grave mound of Maykop, the
554 North Caucasus piedmont region was genetically connected to the south. Even
555 without direct ancient DNA data from northern Mesopotamia, the new genetic
556 evidence suggests an increased assimilation of Chalcolithic individuals from Iran,
557 Anatolia and Armenia and those of the Eneolithic Caucasus during 6000-4000
558 calBCE²³, and thus likely also intensified cultural connections. Within this sphere of
559 interaction, it is possible that cultural influences and continuous subtle gene flow from
560 the south formed the basis of Maykop (Fig. 4; Supplementary Table 10). In fact, the
561 Maykop phenomenon was long understood as the terminus of the expansion of South
562 Mesopotamian civilisations in the 4th millennium BCE^{11, 12, 51}. It has been further
563 suggested that along with the cultural and demographic influence the key
564 technological innovations that had revolutionised the late 4th millennium BCE in
565 western Asia had ultimately also spread to Europe⁵². An earlier connection in the late
566 5th millennium BCE, however, allows speculations about an alternative archaeological
567 scenario: was the cultural exchange mutual and did e.g. metal rich areas such as the
568 Caucasus contribute substantially to the development and transfer of these
569 innovations^{53, 54}?

570
571 We also observe a degree of genetic continuity within each cluster. While this
572 continuity in each cluster spans the 3000 years covered in this study, we also detect
573 occasional gene-flow between the two clusters as well as from outside sources.
574 Moreover, our data shows that the northern flanks were consistently linked to the
575 Near East and had received multiple streams of gene flow from the south, as seen e.g.
576 during the Maykop, Kura-Araxes and late phase of the North Caucasus culture.
577 Interestingly, this renewed appearance of the southern genetic make-up in the
578 foothills corresponds to a period of climatic deterioration (known as 4.2 ky event) in
579 the steppe zone, that put a halt to the exploitation of the steppe zone for several
580 hundred years⁵⁵. Further insight arises from individuals that were buried in the same
581 kurgan but in different time periods, as highlighted in the two kurgans Marinskaya 5
582 and Sharakhalsun 6. Here, we recognize that the distinction between *Steppe* and
583 *Caucasus* with reference to vegetation zones (Fig. 1) is not strict but rather reflects a
584 shifting border of genetic ancestry through time, possibly due to climatic shifts and/or
585 cultural factors linked to subsistence strategies or social exchange. It seems plausible
586 that the occurrence of *Steppe* ancestry in the piedmont region of the northern foothills
587 coincides with the range expansion of the Yamnaya pastoralists. However, more time-
588 stamped data from this region will be needed to provide further details on the
589 dynamics of this contact zone.

590
591 An interesting observation is that steppe zone individuals directly north of the
592 Caucasus (Eneolithic Samara and Eneolithic steppe) had initially not received any
593 gene flow from Anatolian farmers. Instead, the ancestry profile in Eneolithic steppe
594 individuals shows an even mixture of EHG and CHG ancestry, which argues for an
595 effective cultural and genetic border between the contemporaneous Eneolithic

596 populations in the North Caucasus, notably *Steppe* and *Caucasus*. Due to the temporal
597 limitations of our dataset, we currently cannot determine whether this ancestry is
598 stemming from an existing natural genetic gradient running from EHG far to the north
599 to CHG/Iran in the south or whether this is the result of farmers with Iranian farmer/
600 CHG-related ancestry reaching the steppe zone independent of and prior to a stream
601 of Anatolian farmer-like ancestry, where they mixed with local hunter-gatherers that
602 carried only EHG ancestry.

603
604 Another important observation is that all later individuals in the steppe region, starting
605 with Yamnaya, deviate from the EHG-CHG admixture cline towards European
606 populations in the West. This documents that these individuals had received
607 Anatolian farmer-related ancestry, as documented by quantitative tests and recently
608 also shown for two Yamnaya individuals from Ukraine (Ozera) and one from
609 Bulgaria²⁴. For the North Caucasus region, this genetic contribution could have
610 occurred through immediate contact with groups in the *Caucasus* or further south. An
611 alternative source, explaining the increase in WHG-related ancestry, would be contact
612 with contemporaneous Chalcolithic/EBA farming groups at the western periphery of
613 the Yamnaya culture distribution area, such as Globular Amphora and Tripolye
614 (Cucuteni–Trypillia) individuals from Ukraine, which also have been shown to carry
615 Anatolian Neolithic farmer-derived ancestry²⁴.

616
617 Archaeological arguments would be consonant with both scenarios. Contact between
618 early Yamnaya and late Maykop groups at the end of the 4th millennium BCE is
619 suggested by impulses seen in early Yamnaya complexes. A western sphere of
620 interaction is evident from striking resemblances of imagery inside burial chambers of
621 Central Europe and the Caucasus⁵⁶ (Supplementary Fig. 9), and particular similarities
622 also exist in geometric decoration patterns in stone cist graves in the Northern Pontic
623 steppe⁵⁷, on stone *stelae* in the Caucasus⁵⁸, and on pottery of the Eastern Globular
624 Amphora Culture, which links the eastern fringe of the Carpathians and the Baltic
625 Sea⁵⁶. This implies an overlap of symbols with a communication and interaction
626 network that formed during the late 4th millennium BCE and operated across the
627 Black Sea area involving the Caucasus^{59, 60}, and later also involved early Globular
628 Amphora groups in the Carpathians and east/central Europe⁶¹. The role of early
629 Yamnaya groups within this network is still unclear⁵⁷. However, this interaction zone
630 pre-dates any direct influence of Yamnaya groups in Europe or the succeeding
631 formation of the Corded Ware^{62, 63} and its persistence opens the possibility of subtle
632 bidirectional gene-flow, several centuries before the massive range expansions of
633 pastoralist groups that reached Central Europe in the mid-3rd millennium BCE^{19, 35}.

634
635 We were surprised to discover that Steppe Maykop individuals from the eastern desert
636 steppes harboured a distinctive ancestry component that relates them to Upper
637 Palaeolithic Siberian individuals (AG3, MA1) and Native Americans. This is
638 exemplified by the more commonly East Asian features such as the derived EDAR
639 allele, which has also been observed in EHG from Karelia and Scandinavian hunter-
640 gatherers (SHG). The additional affinity to East Asians suggests that this ancestry
641 does not derive directly from Ancestral North Eurasians but from a yet-to-be-
642 identified ancestral population in north-central Eurasia with a wide distribution
643 between the Caucasus, the Ural Mountains and the Pacific coast²¹, of which we have
644 discovered the so far southwestern-most and also youngest (e.g. the Lola culture
645 individual) genetic representative.

646

647 The insight that the Caucasus mountains served not only as a corridor for the spread
648 of CHG/Neolithic Iranian ancestry but also for later gene-flow from the south also has
649 a bearing on the postulated homelands of Proto-Indo-European (PIE) languages and
650 documented gene-flows that could have carried a consecutive spread of both across
651 West Eurasia^{17, 64}. Perceiving the Caucasus as an occasional bridge rather than a strict
652 border during the Eneolithic and Bronze Age opens up the possibility of a homeland
653 of PIE south of the Caucasus, which itself provides a parsimonious explanation for an
654 early branching off of Anatolian languages. Geographically this would also work for
655 Armenian and Greek, for which genetic data also supports an eastern influence from
656 Anatolia or the southern Caucasus. A potential offshoot of the Indo-Iranian branch to
657 the east is possible, but the latest ancient DNA results from South Asia also lend
658 weight to an LMBA spread via the steppe belt²¹. The spread of some or all of the
659 proto-Indo-European branches would have been possible via the North Caucasus and
660 Pontic region and from there, along with pastoralist expansions, to the heart of
661 Europe. This scenario finds support from the well attested and now widely
662 documented ‘steppe ancestry’ in European populations, the postulate of increasingly
663 patrilinear societies in the wake of these expansions (exemplified by R1a/R1b), as
664 attested in the latest study on the Bell Beaker phenomenon³⁵.

665

666

667 **Materials and Methods**

668

669 **Sample collection**

670 Samples from archaeological human remains were collected and exported under a
671 collaborative research agreement between the Max-Planck Institute for the Science of
672 Human History, the German Archaeological Institute and the Lomonosov Moscow
673 State University and Anuchin Research Institute and Museum of Anthropology
674 (permission no. № 114-18/204-03).

675

676 **Ancient DNA analysis**

677 We extracted DNA and prepared next-generation sequencing libraries from 107
678 samples in two dedicated ancient DNA laboratories at Jena and Boston. Samples
679 passing initial QC were further processed at the Max Planck Institute for the Science
680 of Human History, Jena, Germany following the established protocols for DNA
681 extraction and library preparation^{65, 66}. Fourteen of these samples were processed at
682 Harvard Medical School, Boston, USA following a published protocol by replacing
683 the extender-MinElute-column assembly with the columns from the Roche High Pure
684 Viral Nucleic Acid Large Volume Kit to extract DNA from about 75mg of sample
685 powder from each sample. All libraries were subjected to partial (“half”) Uracil-
686 DNA-glycosylase (UDG) treatment before blunt end repair. We performed in-solution
687 enrichment (1240K capture)²⁷ for a targeted set of 1,237,207 SNPs that comprises two
688 previously reported sets of 394,577 SNPs (390k capture) and 842,630 SNPs, and then
689 sequenced on an in-house Illumina HiSeq 4000 or NextSeq 500 platform for 76bp
690 either single or paired-end.

691

692 The sequence data was demultiplexed, adaptor clipped with leehom⁶⁷ and then further
693 processed using *EAGER*⁶⁸, which included mapping with *BWA (v0.6.1)*⁶⁹ against
694 human genome reference GRCh37/hg19, and removing duplicate reads with the same
695 orientation and start and end positions. To avoid an excess of remaining C-to-T and

696 G-to-A transitions at the ends of the reads, three bases of the ends of each read were
697 clipped for each sample using trimBam
698 (https://genome.sph.umich.edu/wiki/BamUtil:_trimBam). We generated “pseudo-
699 haploid” calls by selecting a single read randomly for each individual at each of the
700 targeted SNP positions using the in-house genotype caller *pileupCaller*
701 (<https://github.com/stschiff/sequenceTools/tree/master/src-pileupCaller>).

702

703 **Quality control**

704 We report, but have not analyzed, data from individuals that had less than 30,000
705 SNPs hit on the 1240K set. We removed individuals with evidence of contamination
706 based on heterozygosity in the mtDNA genome data, a high rate of heterozygosity on
707 the X chromosome despite being male estimated with *ANGSD*⁷⁰, or an atypical ratio
708 of the reads mapped to X versus Y chromosomes.

709

710 **Merging new and published ancient and modern population data**

711 We merged our newly generated ancient samples with ancient populations from the
712 publicly available datasets^{13, 19, 20, 24, 27, 28, 33, 35, 37} (Supplementary Data 2), as well as
713 genotyping data from worldwide modern populations using Human Origins arrays
714 published in the same publications. We also included newly genotyped populations
715 from the Caucasus and Asia, described in detail in Jeong et al.⁷¹.

716

717 **Principal Component Analysis**

718 We carried out principal component analysis on Human Origins Dataset using the
719 *smartpca* program of *EIGENSOFT*⁴⁴, using default parameters and the lsqproject:
720 YES, numoutlieriter: 0, and shrinkmode:YES options to project ancient individuals
721 onto the first two components.

722

723 **ADMIXTURE analysis**

724 We carried out *ADMIXTURE* (v1.23)⁴⁵ analysis after pruning for linkage
725 disequilibrium in *PLINK*⁷² with parameters --indep-pairwise 200 25 0.4, which
726 retained 301,801 SNPs for the Human Origins Dataset. We ran *ADMIXTURE* with
727 default 5-fold cross-validation (--cv=5), varying the number of ancestral populations
728 between K=2 and K=22 in 100 bootstraps with different random seeds.

729

730 ***f*-statistics**

731 We computed *D*-statistics and *f*₄-statistics using *qpDstat* program of *ADMIXTOOLS*⁴⁴
732 with default parameters. We computed the admixture *f*₃-statistics using the *qp3Pop*
733 program of *ADMIXTOOLS* with the flag inbreed: YES. *ADMIXTOOLS* computes
734 standard errors using the default block jackknife.

735

736 **Testing for streams of ancestry and inference of mixture proportions**

737 We used *qpWave* and *qpAdm*¹⁹ as implemented in *ADMIXTOOLS* to test whether a set
738 of test populations is consistent with being related via *N* streams of ancestry from a
739 set of outgroup populations and estimate mixture proportions for a *Test* population as
740 a combination of *N* ‘reference’ populations by exploiting (but not explicitly modeling)
741 shared genetic drift with a set of outgroup populations. Mbuti.DG, Ust_Ishim.DG,
742 Kostenki14, MA1, Han.DG, Papuan.DG, Onge.DG, Villabruna, Vestonice16,
743 ElMiron, Ethiopia_4500BP.SG, Karitiana.DG, Natufian, Iran_Ganj_Dareh_Neolithic.
744 The “DG” samples are extracted from high coverage genomes sequenced as part of
745 the Simons Genome Diversity Project³³. For some analyses, we used an extended set

746 of outgroup populations, including some of the following additional ancient
747 populations to constrain standard errors: WHG, EHG, and Levant Neolithic.

748

749 **Dating of gene-flow events**

750 We estimated the time depth of selected admixture events using the linkage
751 disequilibrium (LD)-based admixture inference implemented in *ALDER*⁴⁶.

752

753 **Admixture graph modelling**

754 Admixture graph modelling was carried out with the *qpGraph* software as
755 implemented in *ADMIXTOOLS*⁴⁴ using Mbuti.DG as an outgroup.

756

757 **Sex determination and Y chromosomal and mtDNA haplogroup assignment**

758 We determined the sex of the newly reported samples in this study by counting the
759 number of reads overlapping with the targets of 1240k capture reagent³⁷. We
760 extracted the reads of high base and mapping quality (*samtools* depth -q30 -Q37)
761 using *samtools v1.3.1*⁷³. We calculated the ratios of the numbers of reads mapped on
762 X chromosome or Y chromosome compared with that mapped on autosomes (X-rate
763 and Y-rate, respectively). Samples with an X-rate < 0.42 and a Y-rate > 0.26 were
764 assigned as males and those with an X-rate > 0.68 and a Y-rate < 0.02 were assigned
765 as females.

766 We used *EAGER* and *samtools v1.3.1* to extract reads from the 1240k SNP and
767 mitocapture data mapped to the rCRS. We used *Geneious R8.1.9*⁷⁴ to locally realign,
768 visually inspect the pileups for contamination, and to call consensus sequences, which
769 were used for haplotyping in *HaploGrep 2*⁷⁵. In addition, we used the software
770 *contamMix 1.0.10*, which employs a Bayesian approach to estimate contamination in
771 the mitochondrial genome⁷⁶.

772 We called Y chromosomal haplogroups for males using the captured SNPs on Y
773 chromosome by restricting to sequences with mapping quality ≥ 30 and bases with
774 base quality ≥ 30 . We determined Y chromosomal haplogroups by identifying the
775 most derived allele upstream and the most ancestral allele downstream in the
776 phylogenetic tree in the ISOGG version 11.89 (accessed March 31, 2016)
777 (<http://www.isogg.org/tree>).

778

779 **Kinship analysis**

780 We used outgroup-*f*₃ statistics and the methods *lcMLkin*⁷⁷ and *READ*⁷⁸ to determine
781 genetic kinship between individuals.

782

783 **Phenotypic SNP calls**

784 We determined the allele information of 5 SNPs (rs4988235, rs16891982, rs1426654,
785 rs3827760, rs12913832) thought to be affected by selection in our ancient samples
786 using the captured SNPs by restricting to sequences with mapping quality ≥ 30 and
787 bases with base quality ≥ 30 (Supplementary Information 7).

788

789 **Abbreviations**

790 We use the following abbreviated labels throughout the manuscript: E, Early; M,
791 Middle; L, Late; N, Neolithic; BA, Bronze Age; WHG, EHG, CHG, Western,
792 Eastern, Caucasus hunter-gatherers, respectively; Mal'ta 1, MA1; Afontova Gora 3,
793 AG3.

794

795 **Data availability**

796 Data is deposited in the European Nucleotide Archive under the accession numbers
797 XXX–XXX (will be made available during revision).

798

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808

809 **Author contributions**

810 SH, JK, CCW, SR and WH conceived the idea for the study design. AW, GB, OC,
811 MF, EH, DK, SM, NR, KS and WH performed and supervised wet and dry lab work.
812 SH, AK, ARK, VEM, VGP, VRE, BCA, RGM, PLK, KWA, SLP, CG, HM, BV, LY,
813 ADR, DM, NYB, JG, KF, CK, YBB, APB, VT, RP, SH and ABB assembled skeletal
814 material, contextual information and provided site descriptions. CCW, SR and WH
815 analysed data. CJ, IM, SS, EB, OB provided additional data and methods. WH, CCW,
816 SR, SH, VT, RP, TH, DR and JK wrote the manuscript with input from all authors.

817

818

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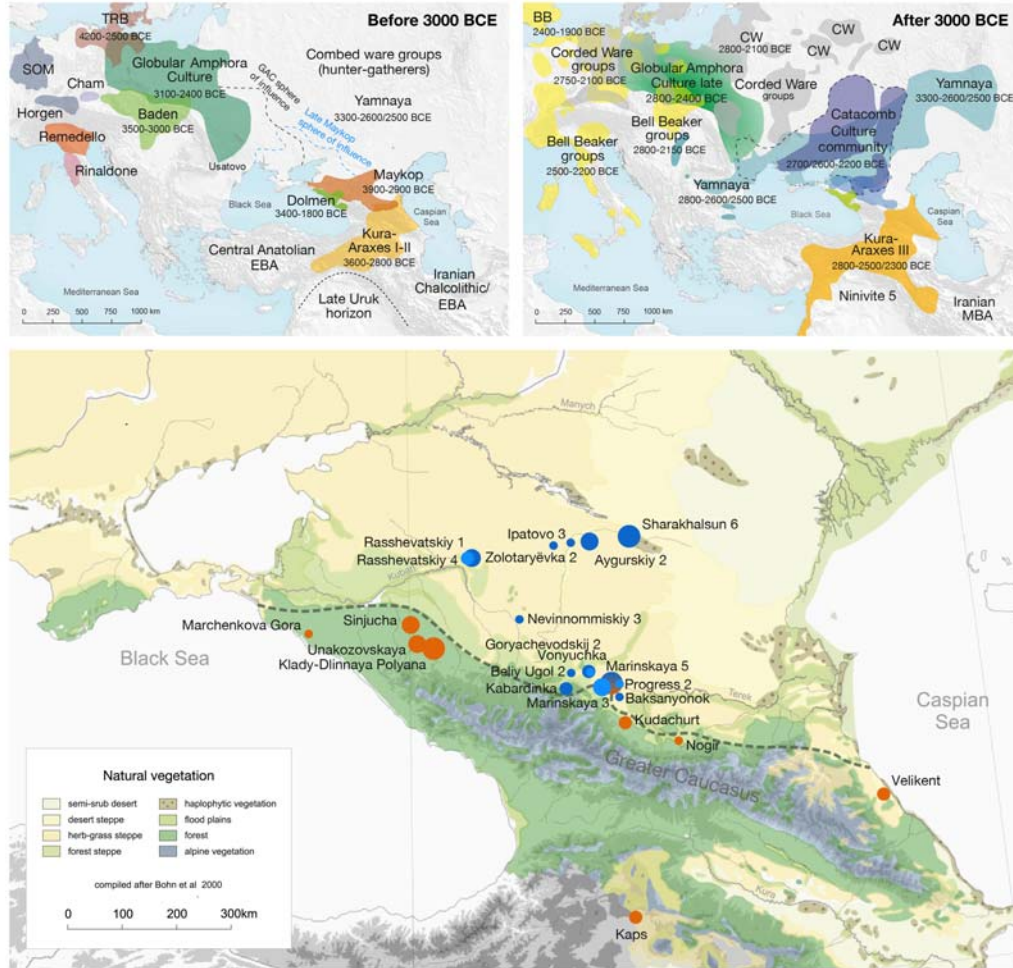
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1099 **Figures and Figure legends**

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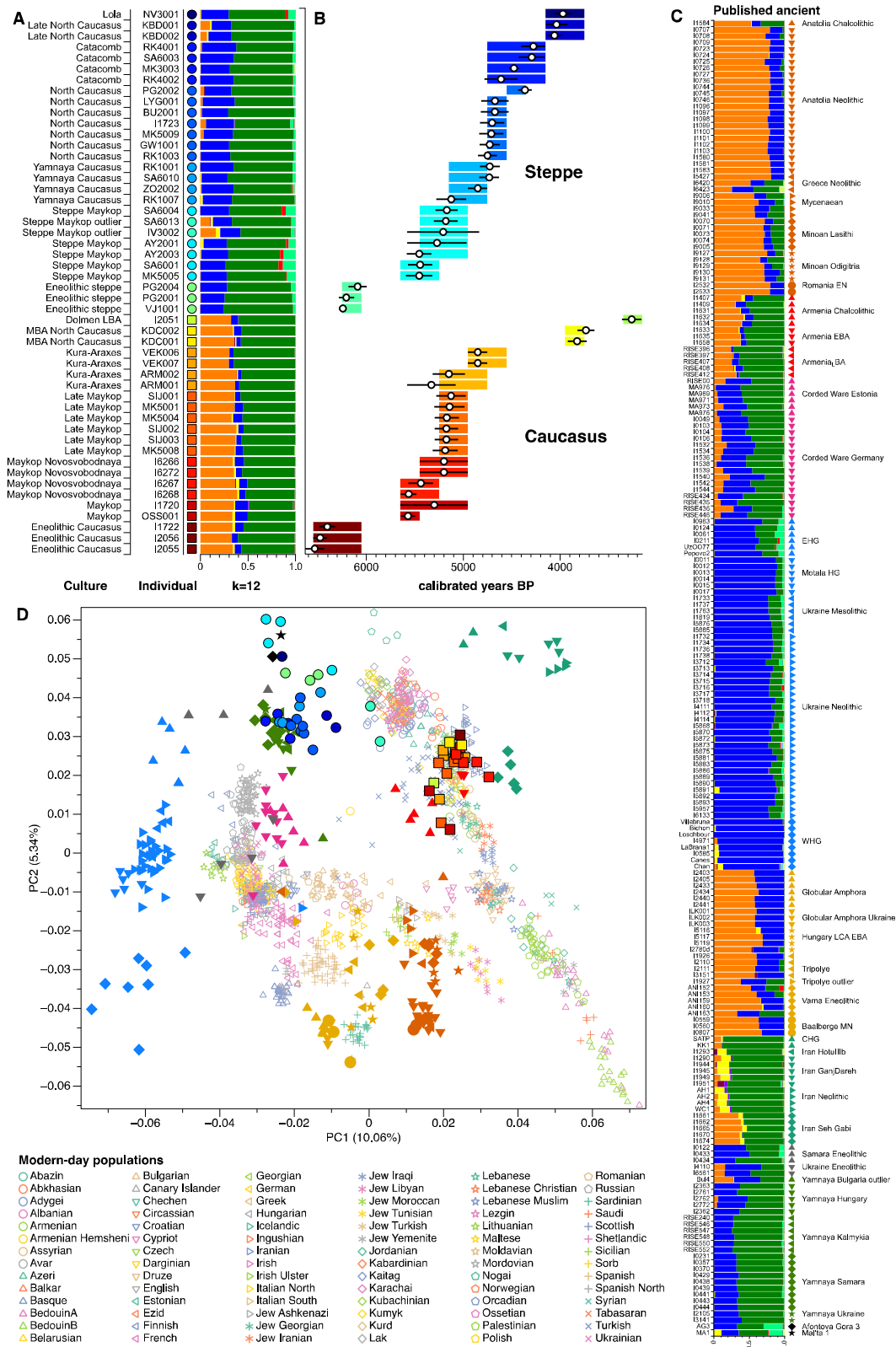
Fig. 1. Map of sites and archaeological cultures mentioned in this study.

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Temporal and geographic distribution of archaeological cultures are shown for two windows in time that are critical for our data. The zoomed map shows the location of sites in the Caucasus. The size of the circle reflects number of individuals that produced genome-wide data. The dashed line illustrates a hypothetical geographic border between genetically distinct *Steppe* and *Caucasus* clusters. (*BB*=*Bell Beaker*; *CW*=*Corded Ware*; *TRB*=*Trichterbecher/Funnel Beaker*; *SOM*=*Seine-Oise-Marne complex*)

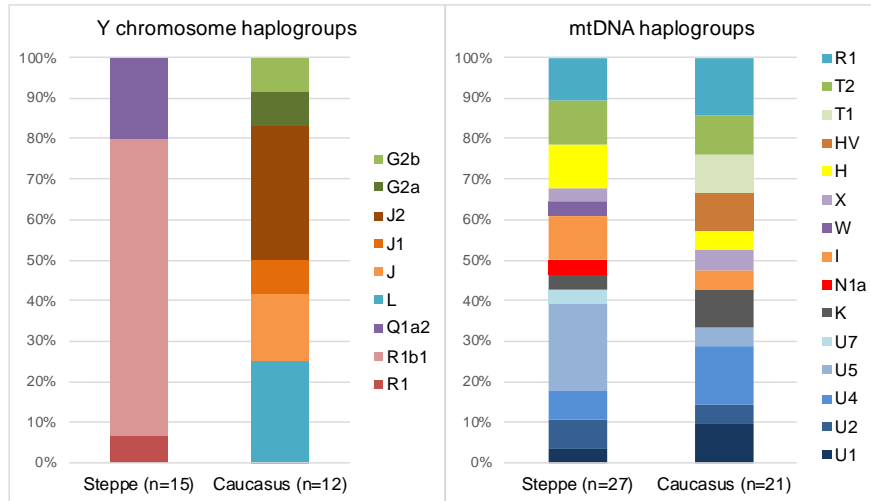
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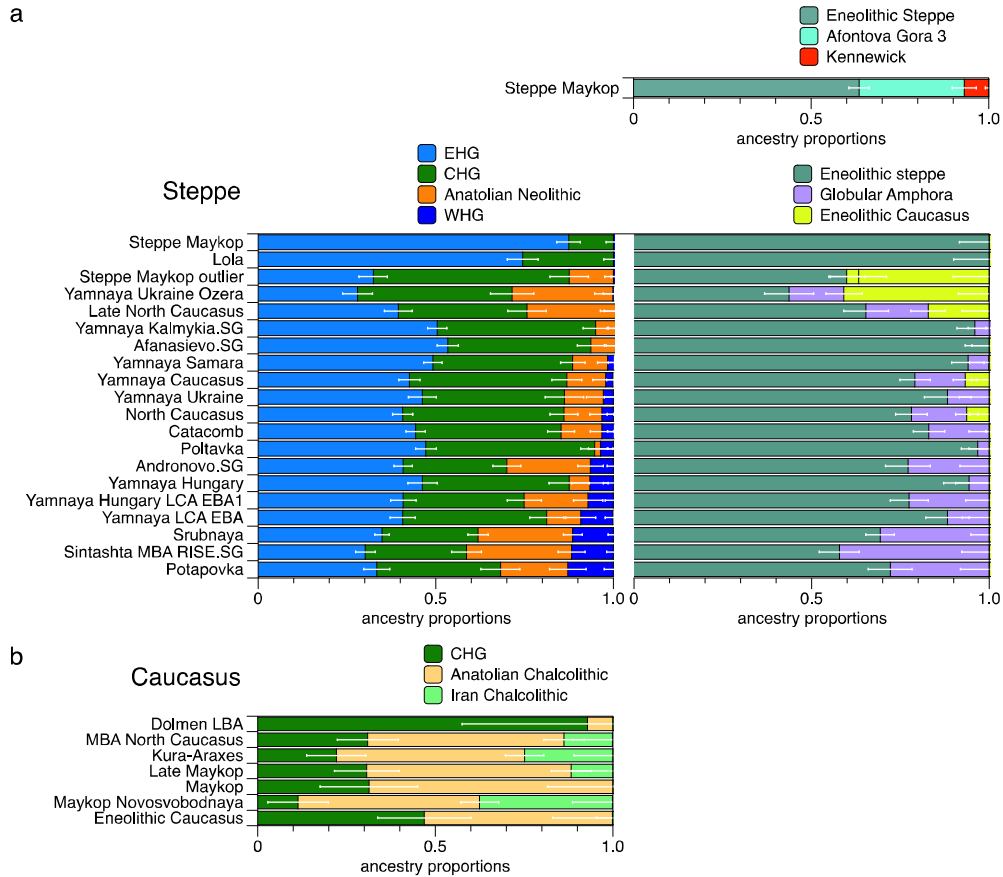
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1113 **Fig. 2. ADMIXTURE and PCA results, and chronological order of ancient**
1114 **Caucasus individuals.** (a) *ADMIXTURE* results (k=12) of the newly genotyped
1115 individuals (filled symbols with black outlines) sorted by genetic clusters (*Steppe* and
1116 *Caucasus*) and in chronological order (coloured bars indicate the relative
1117 archaeological dates, (b) white circles the mean calibrated radiocarbon date and the
1118 errors bars the 2-sigma range. (c) *ADMIXTURE* results of relevant prehistoric
1119 individuals mentioned in the text (filled symbols) and (d) shows these projected onto
1120 a PCA of 84 modern-day West Eurasian populations (open symbols).
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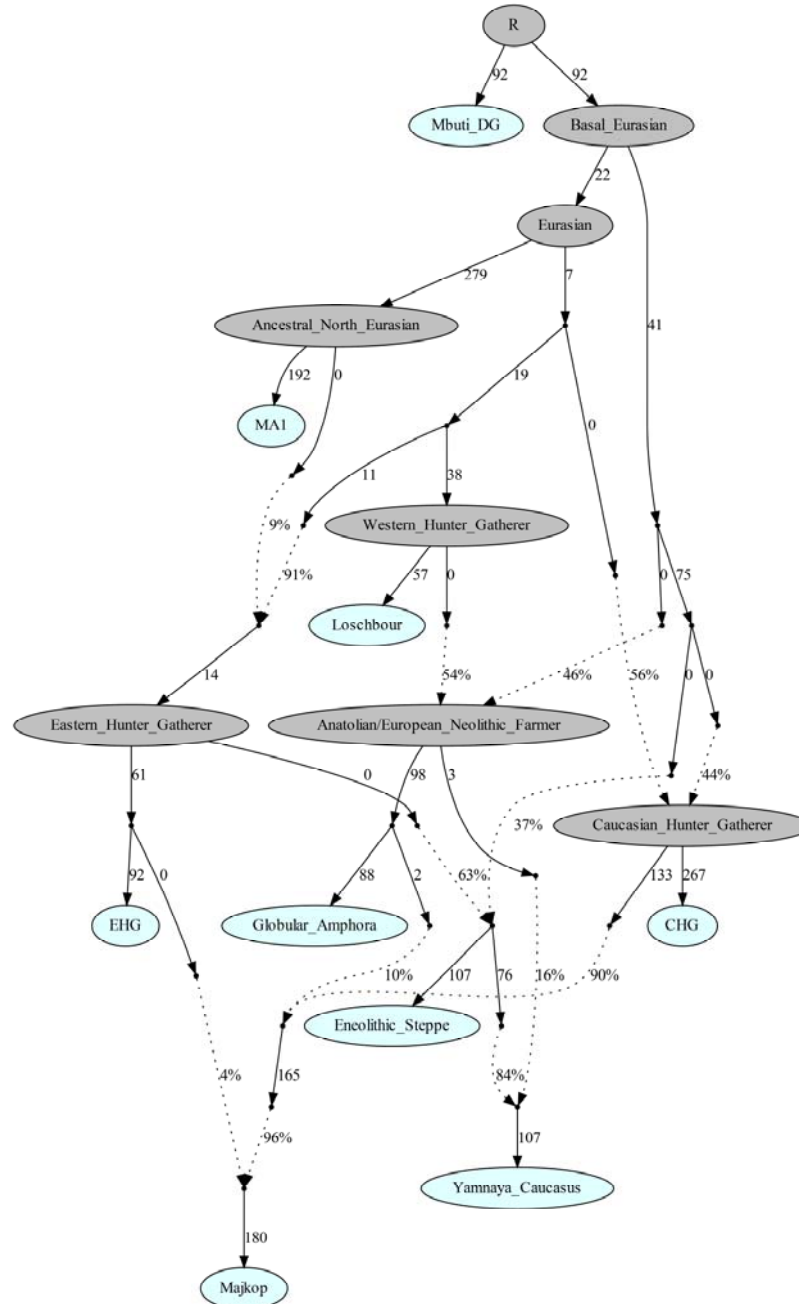
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Fig. 3. Comparison of Y-chromosome (A) and mitochondrial (B) haplogroup distribution in the *Steppe* and *Caucasus* cluster.



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Fig. 4. Modelling results for the Steppe and Caucasus cluster. Admixture proportions based on (temporally and geographically) distal and proximal models, showing additional Anatolian farmer-related ancestry in Steppe groups as well as additional gene flow from the south in some of the Steppe groups as well as the Caucasus groups (see also Supplementary Tables 10, 14 and 20).



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Fig. 5. Admixture Graph modelling of the population history of the Caucasus region. We started with a skeleton tree without admixture including Mbuti, Loschbour and MA1. We grafted onto this EHG, CHG, Globular_Amphora, Eneolithic_steppe, Maykop, and Yamnaya_Caucasus, adding them consecutively to all possible edges in the tree and retaining only graph solutions that provided no differences of $|Z| > 3$ between fitted and estimated statistics. The worst match is $|Z| = 2.824$ for this graph. We note that the maximum discrepancy is $f_4(\text{MA1}, \text{Maykop}; \text{EHG}, \text{Eneolithic_steppe}) = -3.369$ if we do not add the 4% EHG ancestry to Maykop. Drifts along edges are multiplied by 1000 and dashed lines represent admixture.