

1 **Archaeogenomic analysis of the first steps of Neolithisation in Anatolia and the Aegean**

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25 **Abstract**

26 The Neolithic transition in west Eurasia occurred in two main steps: the gradual development of sedentism  
27 and plant cultivation in the Near East, and the subsequent spread of Neolithic cultures into the Aegean and  
28 across Europe after 7,000 cal BCE. Here we use published ancient genomes to investigate gene flow events  
29 in west Eurasia during the Neolithic transition. We confirm that the early Neolithic central Anatolians in  
30 the ninth millennium BCE were likely descendants of local hunter-gatherers, rather than immigrants from  
31 the Levant or Iran. We further study the emergence of post-7,000 cal BCE north Aegean Neolithic  
32 communities. Although Aegean farmers have frequently been assumed to be colonists originating from  
33 either central Anatolia or from the Levant, our findings raise alternative possibilities: north Aegean  
34 Neolithic populations may have been the product of multiple westward migrations, including south  
35 Anatolian emigrants, or they may have been descendants of local Aegean Mesolithic groups who adopted  
36 farming. These scenarios are consistent with the diversity of material cultures among Aegean Neolithic  
37 communities and the inheritance of local forager know-how. The demographic and cultural dynamics  
38 behind the earliest spread of Neolithic culture in the Aegean could therefore be distinct from the subsequent  
39 Neolithization of mainland Europe.

40

41 **Keywords**

42 Ancient DNA, archaeogenomics, Neolithic, migration, acculturation, population genetics

43

## 44 **1. Introduction**

45 The primary zone of Neolithisation in western Eurasia encompassed the Levant, Taurus-Zagros ranges of  
46 Mesopotamia, central Anatolia and Cyprus [1–4]. The earliest evidence for sedentary life and food storage  
47 in this region goes back to the Natufians (c.12,500–10,800 cal BCE) [5,6]. Sedentary communities were  
48 established across this zone during the first phase of the Pre-Pottery Neolithic (PPN, or Aceramic Neolithic,  
49 c.10,000-8,500 cal BCE), and the first indications of plant cultivation appeared [7–9]. Between c.8,500–  
50 7,000 cal BCE, community sizes increased, architectural elaboration intensified, and a subsistence economy  
51 based on agriculture gradually became the norm [10–14]. Meanwhile, portable artifacts such as figurines  
52 and stamps evolved into staples of sedentary life, and pottery production became widespread around c.7,000  
53 cal BCE [10,11]. The elements of the subsequent Pottery Neolithic culture (PN, c.7,000-5,500 cal BCE),  
54 including integrated cultivation practices of domestic plants and animals, the architectural practices of  
55 sedentary life, together with portable artifacts, have been collectively described as the Near Eastern  
56 “Neolithic Package” [15–18].

57

58 During the same period, there were no signs of a Neolithisation in west Anatolia and the Aegean. Only after  
59 c.7,000 cal BCE did elements of the “Neolithic Package” appear in these regions, eventually spreading  
60 toward Europe [19–21]. Some archaeologists suggest that the emergence of the Neolithic elements in the  
61 Aegean and in Europe without a preceding PPN development period indicates the role of demic processes,  
62 i.e. migrations from the Neolithic primary zones through land and sea routes, frequently described as a leap-  
63 frog model where migrants form enclaves in new territory [15, 16, 22-27]. Others, in contrast, favour a role  
64 for interaction between local foragers and primary zone Neolithic populations, including the adoption of  
65 Neolithic elements by locals and acculturation [16, 28-30].

66

67 Recent archaeogenomic data has shown that the Neolithization of central, western and northern Europe  
68 involved migration from a single eastern source, frequently termed “Anatolian farmer” [31-34], while in  
69 other regions, such as in the Baltic [34,35] and in South Greece [34], acculturation may have played role.

70 In most of Europe, there is limited genetic evidence for early admixture between farmers and local European  
71 Mesolithic (WHG) communities in the 6th millennium BCE, such that early European farmers studied to  
72 date (with few exceptions [36]) carry ancestry similar to farmers from northwest Anatolian Barcin [32-34]  
73 (**electronic supplementary material, figure S1**). In subsequent millenia, however, WHG-like ancestry  
74 appears in middle and late Neolithic European populations [37-39]. These observations support a leap-frog  
75 model of Neolithic spread in Europe [28]: farmers only occupied enclaves in the new territories while  
76 Mesolithic groups persisted in the same regions [40-43].

77

78 The processes behind the earliest steps of Neolithization and the Neolithic spread in the Aegean are less  
79 understood. For instance, whether Aegean Neolithic populations were recent colonists originating from  
80 areas of the primary zone of Neolithisation (e.g. [27]), descendants of indigenous foragers (e.g. [20]), or  
81 admixed groups (e.g. [15,16]) is still contentious. Additionally, whether Aegeans' demographic or cultural  
82 relationships were stronger with central Anatolians [16] or with Levantine seafaring populations [27]  
83 remains unclear. We re-analyse published ancient human genomes to answer these questions and to dissect  
84 the demographic dynamics behind the Neolithic transition in Anatolia and the Aegean.

85

## 86 **2. Methods**

87

### 88 **(a) Compiling and mapping genomic data**

89

90 We obtained DNA sequencing data of 99 published ancient individuals (**electronic supplementary**  
91 **material, table S1**), generated using either whole genome shotgun sequencing and/or sequencing of  
92 libraries enriched by hybridization capture [31-33, 36, 38, 39, 43-47]. We mapped sequencing reads to the  
93 human reference genome (hs37d5) using the Burrows-Wheeler Aligner (BWA, v.0.7.12) [48], with the  
94 parameters “-l 16500, -n 0.01, -o 2”. We filtered PCR duplicates using FilterUniqSAMCons.py [49]. We

95 filtered reads shorter than 35 base pairs, with >10% mismatches to the reference, and <30 mapping quality  
96 per read.

97

## 98 **(b) Preparation of population genetics analysis data sets**

99

100 We restricted our analysis to known present-day DNA variants to minimize false positives. We used two  
101 different modern reference panels, calling genotypes of ancient individuals for SNPs overlapping with (i)  
102 the Human Origins genotype dataset [43,50] and (ii) the 1000 Genomes whole genome sequence data [51]  
103 using SAMtools *mpileup* (v.1.3) [52]. For (i) we obtained a curated version of the Human Origins panel of  
104 594,924 autosomal SNP genotype calls for 2,730 present-day individuals from [43]. We determined the  
105 SNPs of the ancient samples overlapping with this dataset. We encoded transitions as missing to avoid  
106 confounding with cytosine deamination in ancient DNA. To prepare (ii) we obtained the BAM and VCF  
107 files for the African Yoruba individuals from 1000 Genomes Project phase 3 from [51]. Using vcftools  
108 [53], we extracted a total of 1,938,919 transversion SNPs with minor allele frequencies of  $\geq 10\%$  in the  
109 Yoruba population to avoid false positive calls [37, 41]. We determined the positions in the ancient samples  
110 overlapping with this dataset. We merged ancient genotypes with these two datasets using PLINK [54]  
111 requiring base quality  $\geq 30$  per overlapping position. We haploidised each full dataset by randomly selecting  
112 one allele per position. The Human Origins-merged dataset, which has higher number of present-day  
113 populations, was used for principal component analysis and for calculating  $f_3$ -statistics. The 1000 Genomes-  
114 merged dataset, with a higher number of SNPs, was used for  $D$ -statistics, where we require high statistical  
115 power.

116

## 117 **(c) Principal component analysis**

118

119 We performed principal component analysis (PCA) by calculating principal components using west  
120 Eurasian populations from the Human Origins dataset using the *smartpca* program of EIGENSOFT [55]

121 with the “numoutlieriter:0” parameter. We projected ancient genomes onto the reference space using the  
122 “lsqproject:YES” option and plotted the results using R (v.3.3.0).

123

#### 124 **(d) $D$ - and $f_3$ -statistics**

125

126 We computed  $D$ -statistics using the *qpDstat* program of ADMIXTOOLS package [50]. We assessed  
127 statistical significance by calculating standard errors using a block jackknife of 0.5 Mbp. We used the Yoruba  
128 population as outgroup for the  $D$ -statistics [33]. We computed  $f_3$ -statistics, *i.e.* genetic affinity between pairs  
129 of populations based on an estimate of shared drift between them since their divergence from an outgroup  
130 population, using the *qp3Pop* program of the ADMIXTOOLS package [50]. The Human Origins data set’s  
131 African Mbuti population was used as outgroup for calculating  $f_3$ -statistics [43]. We performed multiple  
132 testing correction using Benjamini-Yekutieli method for all 207  $D$ -statistics results and reported adjusted  
133  $p$ -values together with  $Z$  scores per each test [56]. For the pairwise  $f_3$ -statistics, as genetic distance measure  
134 between a pair of populations,  $X$  and  $Y$ , we used:  $1 - f_3(\text{Mbuti}; X, Y)$  [31]. These pairwise distances were  
135 summarized with the multidimensional scaling (MDS) method using the *cmdscale* function of R. We  
136 evaluated the goodness of fit for MDS using “GOF” component obtained from *cmdscale* function.

137

#### 138 **(e) Heterozygosity estimates**

139

140 We calculated heterozygosity as a measure of genetic diversity in a population, using genome sequence  
141 data of (i) Bon002 (from Boncuklu, central Anatolia, pre-7,000 cal BCE) [31], Tep003 (Tepecik-Çiftlik,  
142 central Anatolia, post-7,000 cal BCE) [31], Bar8 (Barcın, north Aegean, post-7,000 cal BCE) [32], and  
143 Rev5 (Revenia, north Aegean, post-7,000 cal BCE) [32]. We calculated genome coverage per sample using  
144 GenomeCoverageBed [57]. We downsampled the genome sequences of Bon002 and Bar8 to similar levels  
145 as the other two samples using SAMtools (v.1.3) [52]. We calculated heterozygosity per sample using  
146 ANGSD [58] as “angsd -GL 1 -doGlf 2 -doMajorMinor 1 -sites ReferenceSNP.pos -bam bamlist -doSaf 1

147 -anc referencegenome.fasta". To minimise false positives, we only considered transversions overlapping  
148 with of Yoruba individuals from 1000 Genomes Project phase 3 from [51].

149

#### 150 **(f) Modelling of admixture**

151

152 We used the *qpWave/qpAdm* framework [39, 59] in the AdmixTools package [50] to model populations as  
153 mixtures of two or more sources. The following worldwide set of ancient and present-day outgroups, which  
154 most probably did not experience any post-split gene flow from Anatolian/Aegean populations, was used:  
155 Mbuti, Yoruba, Ust Ishim, El Miron, Goyet Q116, Villabruna, Kostenki14, Vestonice16, Papuan, Onge,  
156 Karitiana, Mixe, Chipewyan, Oroqen, Koryak, Dai, Japanese. Adding East European hunter-gatherers  
157 (EHG) as a close outgroup to increase the resolution did not change the results.

158

#### 159 **(g) Serial coalescent simulations**

160

161 We performed serial coalescent simulations using fastsimcoal [60] under 4 various demographic models  
162 involving Neolithic central Anatolians, Aegeans, Iranians, and WHG (not including Levantine populations,  
163 for whom we lack whole genome data). The simulations were designed to mimick the data with respect to  
164 tree topology, divergence times, and sample sizes. We then performed *D*-statistics on the simulated DNA  
165 and compared these with the observed data to gain understanding into the plausibility of different models.  
166 Specifically, we generated data to represent Iranian Neolithics (10,000 BP), WHGs (Loschbour: 7,200 BP),  
167 central Anatolian Neolithics (Tepecik-Çiftlik: 8,500 BP; Boncuklu: 10,000 BP), the Aegean Neolithics  
168 (Revenia: 8,300 BP) and present-day sub-Saharan Africans (Yoruba-YRI). We launched 100 runs for each  
169 model defined in the parameter file (input.par) for testing different population histories. For all models, we  
170 sampled 30 Mb DNA sequences for: 5 present-day Yoruba, 2 Iranian Neolithics, 2 WHGs, 4 central  
171 Anatolian Neolithics (2 Tepecik-Çiftlik, 2 Boncuklu) and 2 Aegean Neolithics (Revenia). We assumed a  
172 mutation rate of 1.00E-09 bp/year, and a recombination rate of 1.00E-08 bp/year, and assumed 25 years per

173 generation, again following [46]. We set the effective population size ( $N_e$ ) of these populations and times  
174 of divergence between Anatolian Neolithic, WHGs and Iranian Neolithic populations based on [46]. We  
175 converted all outputs (arp file) to plink format and computed  $D$ -statistics with topology of  $D(YRI, Test,$   
176  $central\ Anatolian\ N, Aegean\ N)$  to test the relationships among populations via AdmixTools [50]. Note that  
177 the tree topology involving the Anatolian/Aegean populations, Iran, WHG, and the Africans, were based  
178 on the phylogenetic analysis from [46]. The Anatolian/Aegean populations were assumed to diverge  
179 simultaneously from the same source (star-shaped).

180

181

### 182 **3. Results**

183

#### 184 **(a) Early Holocene gene pools of west Eurasia and the Anatolian/Aegean gene pool**

185

186 We compiled published genome sequence data of 99 ancient individuals (sample ages: c.11,840-4,360 cal  
187 BCE) (**electronic supplementary material, figure S2, table S1**). Both a PCA using present-day and  
188 ancient populations (**electronic supplementary material, figure S1**) and an MDS analysis using only  
189 ancient genomes (**figure 1,2, electronic supplementary material, table S2**) revealed the presence of four  
190 distinct gene pools in early Holocene west Eurasia: (a) a “Caucasia/Iran gene pool”, (b) a “Levant gene  
191 pool”, (c) a “European pre-Neolithic gene pool”, (d) an “Anatolian/Aegean gene pool”. To objectively  
192 measure clustering in gene pools a-c, we used  $D$ -statistics of the form  $D(Yoruba, p1; p2, p3)$  where “ $p$ ”  
193 refers to the Caucasia/Iran, the Levant, or European pre-Neolithic gene pools, correcting for multiple  
194 testing. In 80% comparisons ( $p < 0.05$ ;  $Z \geq 3$ ), populations belonging to the same gene pool shared more  
195 alleles with each other compared to external populations (**figure 1a, electronic supplementary material,**  
196 **figure S3a, table S3**). The only exceptions were comparisons involving a single pre-Neolithic individual  
197 from Iran for which we had relatively few SNPs and low statistical power.

198



199 We then investigated the relationships among ancient Anatolians and other west Eurasian gene pools, using  
200 the oldest Anatolian population yet sequenced: Boncuklu from central Anatolia (sample ages: c.8,300-7,952  
201 cal BCE), an Aceramic Neolithic population previously predicted to be the descendants of local Epi-  
202 paleolithic groups [31, 61]. We computed  $D$ -statistics of the form  $D(\text{Yoruba}, p1; p2, \text{Boncuklu})$ , where  
203 “ $p1$ ” and “ $p2$ ” refer to populations belonging to different gene pools: Caucasia/Iran, the Levant, or the  
204 European pre-Neolithic. In 56% of the comparisons ( $p < 0.05$ ;  $Z \geq 2.8$ ), all three regional gene pools showed  
205 higher affinity to Boncuklu than to each other (figure 1a-1b, electronic supplementary material, table  
206 S4). Using the qpWave/qpAdm algorithm [39, 59] we further modelled the Boncuklu population as a  
207 mixture of CHG (59.1%), Levant (31.4%) and WHG (9.5%) (electronic supplementary material, table  
208 S5).

209

210 We next included three post-7,000 cal BCE Neolithic populations from Anatolia and Aegean in the  
211 analyses: Tepecik-Çiftlik in central Anatolia [31,62], Barcın in northwest Anatolia [32, 38, 63], and  
212 “Revenia” in Pieria of northeast Greece [32]. We computed  $D$ -statistics of the form  $D(\text{Yoruba}, p1; p2,$   
213  $p3)$  where “ $p1$ ” and “ $p2$ ” are Anatolian/Aegean populations and “ $p3$ ” is an external population  
214 (Caucasia/Iran, Levant, or European pre-Neolithic). In 94% of the comparisons ( $p < 0.05$ ;  $Z \geq 2.8$ ) all  
215 Anatolian/Aegean populations were genetically closer to each other than to any other gene pool (electronic  
216 supplementary material, figure S3b, table S5).

217

218 Given archaeological indication that Aegean Neolithic was influenced by east Mediterranean sources [27],  
219 we further studied the genetic affinities of Aegean Neolithic people to central Anatolian Neolithics and to  
220 the Levantines. Calculating  $D$ -statistics of the form  $D(\text{Yoruba}, \text{northAegean}; \text{Levant}, \text{centralAnatolia})$   
221 revealed that the post-7,000 cal BCE Neolithic north Aegean individuals (Barcın and Revenia) consistently  
222 share more alleles with central Anatolians compared to south Levantines, where 50% of the comparisons  
223 were significant ( $p < 0.05$ ;  $Z \geq 2.8$ ) (electronic supplementary material, figure S3b, table S5).

224

225 **(b) Notable genetic diversity in the Aegean**

226

227 To assess demographic events in the Near East during the Neolithic transition we studied signatures of  
228 regional admixture using diachronic populations from the same region (**figure 1c**). In 83% of the  
229 comparisons, pre-7,000 cal BCE Neolithic populations of the Levant and of Iran were genetically closer to  
230 all post-7,000 cal BCE Anatolian/Aegean populations (Tepecik, Barcin, Revenia) compared to the pre-  
231 7,000 cal BCE Anatolian Boncuklu ( $p < 0.05$ ;  $Z \geq 3$ ) (**electronic supplementary material, figure S3c,**  
232 **table S6**). Considering the radiocarbon dates of the investigated individuals, this is consistent with gene  
233 flow from both the Levant and from Iran into Anatolia, within a period ranging from the PPN to the PN  
234 (**figure 1c, arrows “e” and “f”**). These results are also compatible with a regional increase in the levels of  
235 admixture during the Neolithic [**31, 33**], although alternative explanations to gene flow remain plausible,  
236 such as population structure confounding the analysis results [**64**].

237

238 Next, to gain understanding into Aegean Neolithization we studied the population genetic characteristics  
239 of the PN Aegean groups relative to central Anatolian groups. We first compared heterozygosity estimates  
240 among these populations. If the Aegeans were recent colonists from a single origin, due to a founder effect,  
241 one might expect lower heterozygosity in the Aegean than in central Anatolia. In contrast, Barcin and  
242 Revenia individuals had higher heterozygosity levels (mean 0.25 and 0.26, respectively) than those of  
243 Boncuklu and Tepecik (0.22 and 0.19, respectively) (**electronic supplementary material, table S7**).

244

245 Second, we calculated *D*-statistics focused on the Aegeans, which suggested higher admixture in this region  
246 than in central Anatolia:

247

248 (i)  $D(\text{Yoruba}, \text{Natufian}; \text{northAegean}_-, \text{centralAnatolia}, \text{northAegean})$  revealed that pre-Neolithic  
249 population of Levant had stronger genetic affinity to the two north Aegean Neolithic populations (Barcin  
250 and Revenia, post-7,000 cal BCE), than to the two central Anatolian Neolithic groups (Boncuklu and

251 Tepecik, pre- and post-7,000 cal BCE) ( $p < 0.05$ ;  $Z \geq 3$ ) (**figure 2a, arrow “a”, electronic supplementary**  
252 **material, figure S4a, table S8**). Given the above-proposed gene flow event from Levant into Anatolia  
253 during the Neolithic this result might imply additional genetic interactions between Natufian-related  
254 populations and the ancestors of north Aegean populations that bypassed central Anatolia.

255

256 (ii)  $D(\text{Yoruba}, \text{Caucasia/Iran}; \text{~~northAegean}~~, \text{centralAnatolia}, \text{northAegean})$  revealed that in 50% of the  
257 comparisons CHGs and Neolithic Iran individuals shared more alleles with the two north Aegean PN  
258 populations than with the two central Anatolians ( $p < 0.05$ ;  $Z \geq 2.8$ ) (**figure 2a, arrows “b” and “c”,**  
259 **electronic supplementary material, figure S4a, table S9**).

260

261 (iii) Likewise, WHG individuals showed higher affinity to the two north Aegean PN populations than PN  
262 central Anatolian group groups ( $p < 0.05$ ;  $Z \geq 3$ ) (**figure 2a, arrow “d”, electronic supplementary**  
263 **material, figure S4a, table S10**).

264

265 (iv) Natufians, WHGs and Iranian PPN individuals were consistently more similar to the Revenia individual  
266 than to those in Barcın (**electronic supplementary material, figure S4b, table S11**).

267

268 (v) Both the Boncuklu (PPN) and the Tepecik (PN) groups of central Anatolia had stronger affinity to the  
269 north Aegean PN populations, Barcın and Revenia, than to each other (**figure 2b, electronic**  
270 **supplementary material, table S12**). Likewise, all Anatolian groups (Boncuklu, Tepecik-Çiftlik and  
271 Barcın) were genetically closer to Revenia, than they were to each other (**figure 2b, electronic**  
272 **supplementary material, table S13**).

273

274 (vi) All European early farmer populations examined were genetically closer to Revenia than to each other  
275 (**electronic supplementary material, figure S4c, table S14**)

276

277 Observations (ii), (iii), and in particular (v) are intriguing. We asked whether these could be consistent with  
278 a number of demographic scenarios, assuming a phylogenetic topology that included Iran, WHG, and  
279 Aegean/Anatolian populations, estimated by [46]. We considered the following scenarios: (a) separate  
280 extreme bottlenecks in the ancestors of the two central Anatolian populations (possibly causing  
281 differentiation between the central Anatolian populations from each other, and from all other groups, (b)  
282 independent gene flow events from external sources (WHG and Iran) into the two central Anatolian groups  
283 (possibly causing differentiation between the two), (c) independent gene flow from WHG and Iran into the  
284 Aegean, (d) independent gene flow from WHG, Iran, and the two central Anatolian lineages into the  
285 Aegean. We performed serial coalescent simulations using realistic settings and compared the results with  
286 the observed *D*-statistics. We could only replicate the observed results under scenario (d) that describes  
287 rampant admixture in the Aegean (**electronic supplementary material, figure S5**).

288

#### 289 **4. Discussion**

290

291 The analyses presented here highlight two points regarding the process of Neolithization. First, the  
292 observation that the two central Anatolian populations cluster together to the exclusion Neolithic  
293 populations of south Levant or of Iran restates the conclusion that farming in central Anatolia in the PPN  
294 was established by local groups instead of immigrants, which is consistent with the described cultural  
295 continuity between central Anatolian Epipaleolithic and Aceramic communities [9, 65]. This reiterates the  
296 earlier conclusion [33] that the early Neolithization in the primary zone was largely a process of cultural  
297 interaction instead of gene flow.

298

299 The second point relates to whether Aegean Neolithization (post-7,000 cal BCE) involved similar  
300 acculturation processes, or was driven by migration similar to Neolithization in mainland Europe - a long-  
301 standing debate in archaeology [16, 20, 22, 27, 28]. Here we discuss the two scenarios based on the genetic  
302 analysis.

303

304 **Model 1: Migration from Anatolia to the Aegean.** A recent study reported that by the 7<sup>th</sup> millennium BCE  
305 the eastward border of the WHG gene pool extended to the Iron Gates (on the border between Romania and  
306 Serbia) [34]. Plausibly, during the early Holocene, the WHG population could also have been present along  
307 the Aegean coastline, such that the border between central Anatolian and WHG gene pools ran along west  
308 Anatolia. If so, the Aegean Neolithization must have involved replacement of a local, WHG-related  
309 Mesolithic population by incoming easterners.

310

311 If migration occurred, where did it originate? Because Revenia and Barcın cluster with PPN and PN central  
312 Anatolian Neolithic groups to the exclusion of the south Levant (**figure 1c, electronic supplementary**  
313 **material, figure S3c**), the latter is unlikely to be the source, leaving central Anatolia or south Anatolia  
314 (north Levant) as potential origins.

315

316 Notably, the north Aegeans (Revenia and Barcın) show higher diversity than the central Anatolians. We  
317 had earlier shown that the highest quality Barcın genome carries a smaller proportion of short runs of  
318 homozygosity than the highest quality Boncuklu genome [31], which also supports the notion that the  
319 ancestral effective population size of the Aegeans was larger than those of central Anatolians. Moreover,  
320 we find that the north Aegeans share more alleles with eastern, western and southern gene pools, as  
321 estimated using the *D*-statistic (**figure 2**). Although the *D*-statistic can be sensitive to technical biases, our  
322 result is unlikely to be a technical artifact because, (a) the north Aegean data were derived from two  
323 independent studies [32, 38], (b) the Barcın data was produced using two different techniques, whole  
324 genome shotgun sequencing and SNP capture, and (c) both Barcın and Revenia display the same population  
325 genetic patterns, suggesting that the admixture signals in the Aegean individuals are reproducible. In  
326 addition, although unknown population structure can complicate interpretation of the *D*-statistic [64], we  
327 note that the admixture estimates are consistent with the estimated higher genetic diversity in the Aegean.

328

329 If the Revenia and Barcin individuals studied here were descendants of Anatolian Neolithic immigrants,  
330 they must have been recent settlers, as all samples analyzed here date to early stages of the Aegean Neolithic  
331 (Revenia: 6,438-6,264 and Barcin: 6,500-6,200 cal BCE). Furthermore, if the migration was *directly* of  
332 central Anatolian origin (represented by Boncuklu and Tepecik-Çiftlik), the putative migrants must have  
333 admixed with populations carrying alleles of distinct gene pools (Levant, Caucasus/Iran, and WHG) within  
334 a few centuries, in order to explain our observations above (**figure 2a**).

335

336 Alternatively, the migration event could have originated from the Anatolian south coast or north Levant  
337 [27] (currently no genome data is available from these groups). This region could have hosted a hypothetical  
338 central Anatolian-related population exposed to admixture from CHG-, Iran-, and Levant-related gene pools  
339 in earlier millenia. A south Anatolian population could have been in contact with different central Anatolian  
340 populations from the Konya Plain (Boncuklu) and Cappadocia (Tepecik-Çiftlik), explaining the affinity of  
341 both Boncuklu and Tepecik-Çiftlik to Barcin. A seafaring population could also be in genetic contact with  
342 putative WHG-related populations of the Aegean. This hypothetical population could have initiated the  
343 Cyprus Neolithic in the 11<sup>th</sup> millenium BCE and later Aegean Neolithic communities in the 7<sup>th</sup> millenium  
344 BCE [27].

345 One surprising observation here is the apparent absence of WHG-like ancestry in Late  
346 Neolithic/Chalcolithic Aegean genomes: ADMIXTURE analysis results from two individuals from  
347 northwest Anatolia (Kumtepe, ~5000 BCE) [74] and four individuals from south Greece (Franchti Cave  
348 and Diros, ~4000 BCE) [34], all lack noticeable WHG-like ancestry components [31,32,34]. This contrasts  
349 with WHG admixture emerging in European farmer populations in the Middle and Late Neolithic [37, 39],  
350 and perhaps earlier in the Balkans [34], indicating the persistence of Mesolithic populations in Europe after  
351 Neolithic migrations. Therefore, if the Mesolithic populations of the Aegean coast had indeed been WHG-  
352 related, they must have been fully replaced by the eastern migrant farmers.

353

354 **Model 2: Adoption of Neolithic elements by local foragers.** Alternatively, the Aegean coast Mesolithic  
355 populations may have been part of the Anatolian-related gene pool that occupied the Aegean seaboard  
356 during the early Holocene. Under this scenario, the north Aegean PN populations would be at least partial  
357 descendants of local hunter-gatherers who adopted Neolithic lifestyle post-7,000 cal BCE, triggered by  
358 contacts with central Anatolian and Levantine populations. The following events would be conceivable: (a)  
359 During the Last Glacial Maximum (LGM), the Aegean evolved into a refuge hosting a significant human  
360 population, which is in line with climatic modeling [66-69]; estimates of human population density during  
361 the Marine Isotope Stage 2 in west (but not central) Anatolia reach one of their highest levels in Europe  
362 [70, 71]. The existence of an Aegean human population going back to the LGM is also consistent with  
363 mitochondrial haplogroup-based analyses [72], and that Anatolian-like mitochondrial haplogroups are  
364 found also in Mesolithic Balkan and Aegean populations [32, 34]. (b) Following the LGM, Aegean  
365 emigrants dispersed into central Anatolia and established populations that eventually gave rise to the local  
366 Epi-Paleolithic and later Neolithic communities, in line with the earliest direct evidence for human presence  
367 in central Anatolia c.14,000 cal BCE [61]. This hypothetical out-of-the-Aegean event coincides with the  
368 post-LGM Near East-related migration signatures in European Mesolithic genomes [73]. (c) Between the  
369 LGM and post-7,000 cal BCE Neolithization, WHG, Natufian, and Caucasus/Iran-related groups admixed  
370 with north Aegeans, differentiating the latter from their central Anatolian relatives and leading to our  
371 observations in **figure 2a**. (d) Post-7,000 cal BCE, there occurred additional, albeit limited central  
372 Anatolian gene flow back into the Aegean, giving rise to our observation in **figure 2b**.

373

374 **The archaeological evidence.** Both the migration and acculturation models for Aegean Neolithisation enjoy  
375 support from material culture investigations, but the overall evidence points to a complex process where  
376 Aegean societies were culturally influenced by diverse sources, including the central Anatolian Neolithic,  
377 the Levant Neolithic, and possibly local Mesolithic traditions. In contrast to the relative homogeneity of  
378 European Neolithic cultures, such as the LBK and Cardial, the Aegean Neolithic is noted for its diversity  
379 [65]. Variation in Neolithic Package elements and primary zone traditions is notable across Aegean sites,

380 among regions (e.g. east and west of Marmara), even between closely neighbouring villages  
381 [16,17,20,65,75-80]. This diversity includes, for example, obsidian, with Greek Aegean (Melos) [81,82] or  
382 mainland Anatolian (Cappadocian) [83] sources being preferred in some settlements, and yet other  
383 settlements showing no evidence of obsidian use [65]. Cultural trait diversity involves architecture, tool  
384 types, ceramics, and symbolic elements (such as figurines and intramural burial), which may show partial  
385 similarities to either central Anatolia or to the Levant, or may be unique [16,65,76]. For instance, intramural  
386 burial, a common feature among primary zone sites, is also widespread in east Marmara early Neolithic  
387 villages (including Barcın), but totally absent in settlements only two hundred kilometers west [16].  
388 Mesolithic-like lithic industries and the prominence of seafood in some settlements further imply the  
389 continuing presence of Aegean Mesolithic traditions into the Neolithic [16,20,27,84,85]. Indeed, lively  
390 seafaring activity was prevalent in the Mediterranean and the Aegean already by the 11<sup>th</sup> millennium BCE  
391 [86,87], as evidence from Cyprus, Crete, Franchti, Cyclops Cave, Ouirakos and other Aegean island and  
392 coastal mainland Mesolithic sites demonstrate [20,27,84,87-95].

393

394 Instead of a single-sourced colonisation process, the Aegean Neolithization may thus have flourished upon  
395 already existing coastal and interior interaction networks connecting Aegean foragers with Levantine and  
396 central Anatolian PPN populations, and involved multiple cultural interaction events from its early steps  
397 onward [16,20,65,75]. This wide diversity of cultural sources and the potential role of local populations in  
398 Neolithic development may set apart Aegean Neolithization from that in mainland Europe. While  
399 Mesolithic Aegean genetic data is awaited to fully resolve this issue, researchers should be aware of the  
400 possibility that the initial emergence of the Neolithic elements in the Aegean, at least in the north Aegean,  
401 involved cultural and demographic dynamics different than those in European Neolithization.

402

403

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642 **Figure 1.** Genetic differentiation among ancient west Eurasians and predicted admixture events. Panels  
643 (a) and (c) show results of the same multidimensional scaling (MDS) analysis, summarising  $f_3$ -statistics  
644 (shared genetic drift) between ancient population pairs (electronic supplementary material, table S2). The  
645 goodness of fit was estimated as 0.17 and 0.17 for both dimensions. Admixture events among gene pools  
646 inferred using  $D$ -statistics are represented as arrows on each MDS plot. The circles where the arrow tips  
647 touch indicate which population is involved in the inferred admixture. Tepecik-Çiftlik is labeled as  
648 Tepecik. **(a)** Admixture in Boncuklu (central Anatolian PPN). For clarity, the other Anatolian/Aegean  
649 populations are not plotted. Arrow “a”: gene flow between Boncuklu and pre-Neolithic populations of  
650 mainland Europe (relative to other gene pools). Arrow “b”: gene flow between Boncuklu and Levant  
651 populations (relative to other gene pools). Arrow “c”: gene flow between Boncuklu and Caucasia/Iran  
652 populations (relative to other gene pools). **(b)** Results of  $D$ -statistics in the form of  $D(\text{Yoruba}, p1; p2,$   
653  $\text{Boncuklu})$ . Multiple testing correction was performed using the Benjamini-Yekutieli method [56]. **(c)**  
654 Arrow “d”: estimated gene flow between Boncuklu and Levantine populations. This is based on testing  
655 the topology  $D(\text{Outgroup}, \text{Boncuklu}; \text{Levant}_{\text{preNeolithic}}, \text{Levant}_{\text{Neolithic}})$ , showing that the Boncuklu  
656 population showed higher genetic affinity to Levantine Neolithics (sample ages: c.8,300-6,750 cal BCE)  
657 than to Levantine pre-Neolithics (sample ages: c.11,840-9,760 cal BCE), although the result was only

658 marginally significant ( $p > 0.05$ ,  $Z > 2.5$ ) (figure S2c, table S5). Arrow “e”: gene flow from the Iran PPN  
659 population into Anatolian/Aegean PN populations. Arrow “f”: gene flow from the Levant PPN population  
660 into Anatolian/Aegean PN populations (electronic supplementary material, table S5 and table S6).

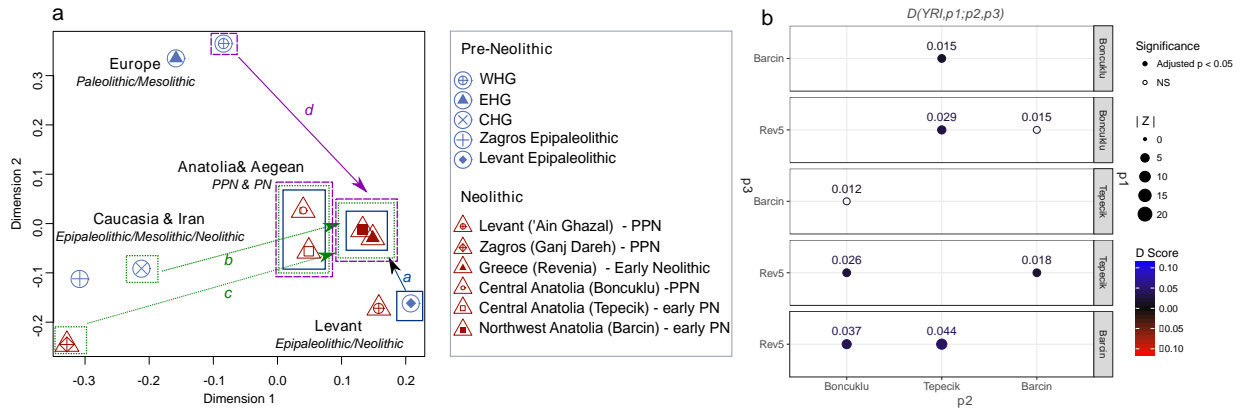
661  
662 **Figure 2.** Summary of  $D$ -statistics describing population relationships within the Anatolian/Aegean gene  
663 pool and between Anatolians/Aegeans and neighboring groups. The Yoruba genome was used as  
664 outgroup in  $D$ -statistics. The Tepecik-Çiftlik is labeled as Tepecik. All  $D$ -statistics results are reported in  
665 electronic supplementary material, table S8-S13. (a)  $D$ -statistics results summarised as arrows on the  
666 MDS plot (same as figure 1). Each triple population compared in  $D$ -tests are framed in the same colour. If  
667 a test population has greater genetic affinity to the second population compared to a third one, an arrow  
668 with same color as the frames is drawn from the test population to the second population (the arrows’  
669 direction or lengths are not representative of gene flow magnitudes). Arrow “a” and navy frames  
670 summarise  $D(\text{Yoruba}, \text{Natufian}; \text{northAegean}, \text{centralAnatolian}, \text{northAegean})$ , where Natufians had  
671 stronger genetic affinity to north Aegean PN than to central Anatolian PPN or PN groups (electronic  
672 supplementary material, table S8). Arrows “b” and “c” and green frames summarise  $D(\text{Yoruba},$   
673  $\text{CHG\&Iran\_Neolithic}; \text{northAegean}, \text{centralAnatolian})$ . In 6/8 comparisons CHGs and Iran PPN  
674 populations had stronger genetic affinity to the north Aegean PN than to central Anatolian PPN and PN  
675 (electronic supplementary material, table S9). Arrow “d” and purple frames summarise  $D(\text{Yoruba}, \text{WHG};$   
676  $\text{northAegean}, \text{centralAnatolian})$ . In all comparisons WHGs had stronger genetic affinity to the north  
677 Aegean PN than to central Anatolian PPN and PN, with the exception of  $D(\text{Yoruba}, \text{WHG}; \text{Boncuklu},$   
678  $\text{Barcin})$  being non-significant (electronic supplementary material, table S10). (b) Results of  $D$ -tests  
679 calculated as  $D(\text{Outgroup}, \text{RightPopulation}; \text{BottomPopulation}, \text{LeftPopulation})$ , where right, bottom and  
680 left refer to the positions of the populations on the matrix. For instance the top row shows that Boncuklu  
681 has significantly higher affinity to Barcin than to Tepecik-Çiftlik. The  $D$ -statistic magnitude is  
682 represented by color,  $Z$  score by size, and significance by being filled or not. Multiple testing correction



688 **Figure 1.** Genetic differentiation among ancient west Eurasians and predicted admixture events.  
689 Panels a and c show the results of the same multidimensional scaling (MDS) analysis,  
690 summarising  $f_3$ -statistics (shared genetic drift) between ancient population pairs (electronic  
691 supplementary material, table S2). The goodness of fit was estimated as 0.17 and 0.17 for two  
692 dimensions. Admixture events among gene pools inferred using  $D$ -statistics are represented as  
693 arrows on each MDS plot. The circles where the arrow tips touch indicate which population is  
694 involved in the inferred admixture. Tepecik-Çiftlik is labeled as Tepecik. **(a)** Admixture in  
695 Boncuklu (central Anatolian PPN). For clarity, the other Anatolian/Aegean populations are not  
696 plotted. Arrow “a”: gene flow between Boncuklu and pre-Neolithic populations of mainland  
697 Europe (relative to other gene pools). Arrow “b”: gene flow between Boncuklu and Levant  
698 populations (relative to other gene pools). Arrow “c”: gene flow between Boncuklu and  
699 Caucasia/Iran populations (relative to other gene pools). **(b)** Results of  $D$ -statistics in the form of  
700  $D(\text{Yoruba}, p1; p2, \text{Boncuklu})$ . Multiple testing correction was performed including all  $D$ -  
701 statistics results discussed in the paper using Benjamini-Yekutieli method [56]. **(c)** Arrow “d”:  
702 estimated gene flow between Boncuklu and Levantine populations. This is based on testing the  
703 topology  $D(\text{Outgroup}, \text{Boncuklu}; \text{Levant}_{preNeolithic}, \text{Levant}_{Neolithic})$ , showing that the  
704 Boncuklu population showed higher genetic affinity to Levantine Neolithics (sample ages:  
705 c.8,300-6,750 cal BCE) than to Levantine pre-Neolithics (sample ages: c.11,840-9,760 cal BCE),  
706 although the result was only marginally significant ( $p > 0.05, Z > 2.5$ ) (figure S2c, table S5).  
707 Arrow “e”: gene flow from the Iran PPN population into Anatolian/Aegean PN populations.  
708 Arrow “f”: gene flow from the Levant PPN population into Anatolian/Aegean PN populations  
709 (electronic supplementary material, table S5 and table S6).

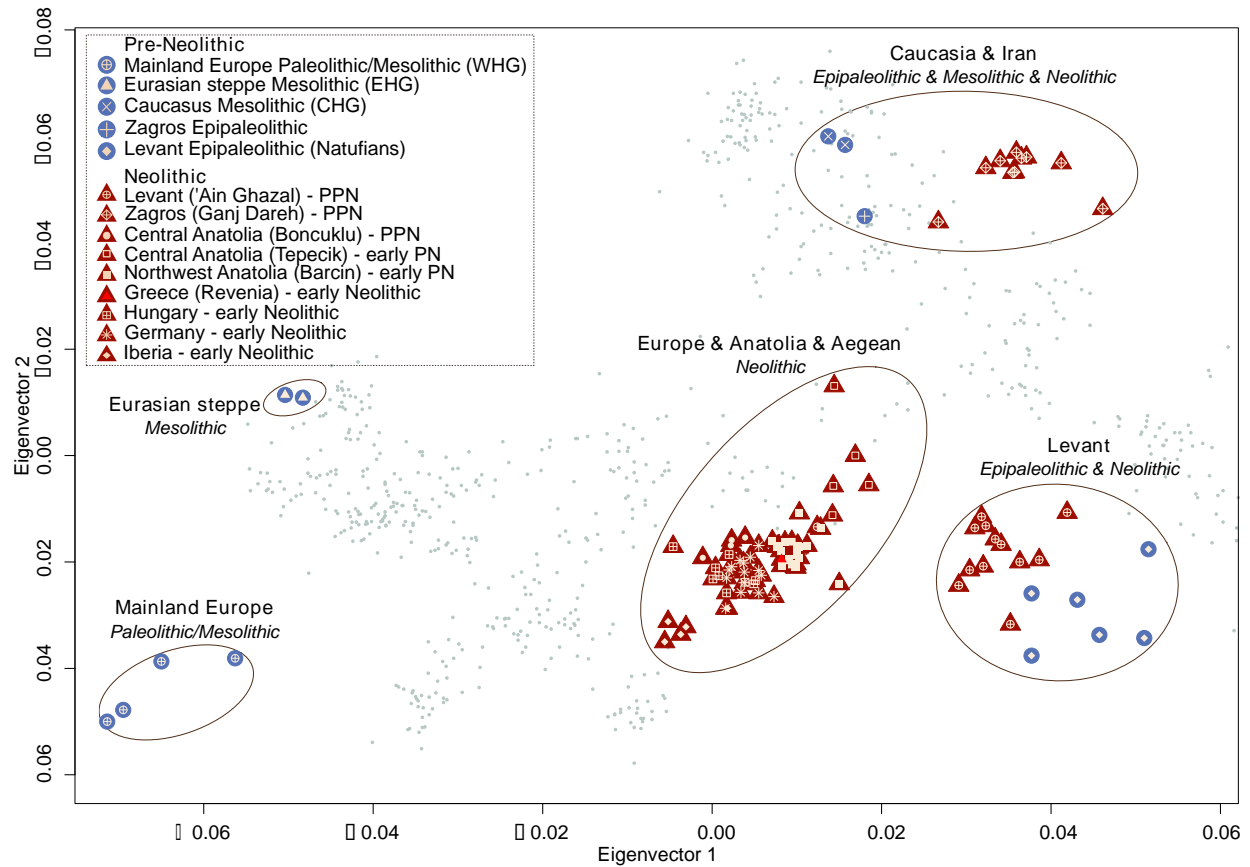
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734 **Figure 2**  
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 739 **Figure 2.** Summary of *D*-statistics describing population relationships within the  
 740 Anatolian/Aegean gene pool and between Anatolians/Aegeans and neighboring groups. The  
 741 Yoruba genome was used as outgroup in *D* statistics. The Tepecik-Çiftlik is labeled as Tepecik.  
 742 All *D* statistics results are reported in electronic supplementary material, table S8-S13. (a) *D*-  
 743 statistics results summarised as arrows on the MDS plot (same as figure 1). Each triple  
 744 population compared in *D* tests are framed in the same colour. If a test population has greater  
 745 genetic affinity to the second population compared to a third one, an arrow with same color as  
 746 the frames is drawn from the test population to the second population (the arrows' direction or  
 747 lengths are not representative of gene flow magnitudes). Arrow "a" and navy frames summarise  
 748  $D(Yoruba, Natufian; northAegean, centralAnatolian)$ , where Natufians had stronger genetic  
 749 affinity to north Aegean PN than to central Anatolian PPN or PN groups (electronic  
 750 supplementary material, table S8). Arrows "b" and "c" and green frames summarise  $D(Yoruba,$   
 751  $CHG\&Iran\_Neolithic; northAegean, centralAnatolian)$ . In 6/8 comparisons CHGs and Iran PPN  
 752 populations had stronger genetic affinity to the north Aegean PN than to central Anatolian PPN  
 753 and PN (electronic supplementary material, table S9). Arrow "d" and purple frames summarise  
 754  $D(Yoruba, WHG; northAegean, centralAnatolian)$ . In all comparisons WHGs had stronger  
 755 genetic affinity to the north Aegean PN than to central Anatolian PPN and PN, with the  
 756 exception of  $D(Yoruba, WHG; Boncuklu, Barcin)$  being non-significant (electronic  
 757 supplementary material, table S10). (b) Results of *D* tests calculated as  $D(Outgroup,$   
 758  $RightPopulation; BottomPopulation, LeftPopulation)$ . Multiple testing correction was performed  
 759 including all *D*-statistics results discussed in the paper using Benjamini-Yekutieli method  
 760 (electronic supplementary material, table S12 and S13).

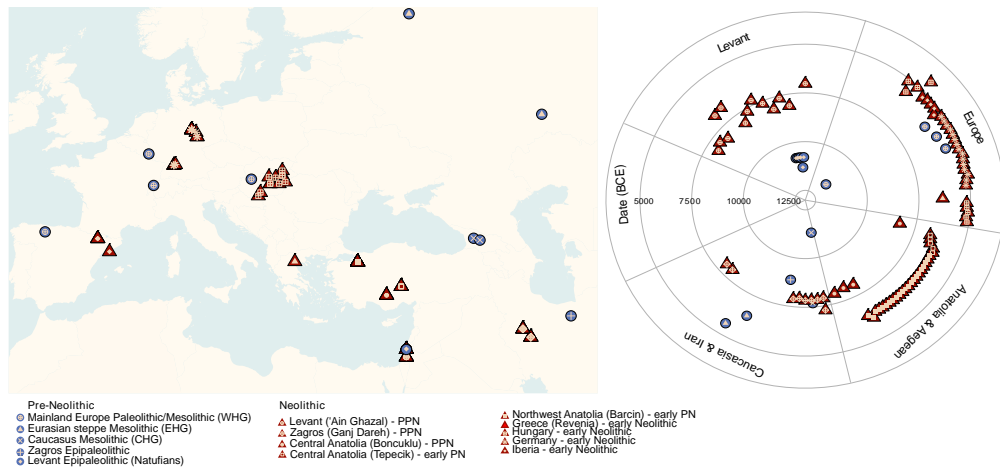
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765 **Figure S1.** Principal component analysis (PCA) with modern and ancient genomes. The  
 766 eigenvectors were calculated using 50 modern west Eurasian populations, onto which  
 767 genome data from ancient individuals were projected. The gray circles highlight the four  
 768 ancient gene pools of west Eurasia. Modern-day individuals are shown as gray points. In  
 769 the Near East, Pre-Neolithic (Epipaleolithic/Mesolithic) and Neolithic individuals  
 770 genetically cluster by geography rather than by cultural context. For instance, Neolithic  
 771 individuals of Anatolia cluster to the exclusion of individuals from the Levant or Iran). In  
 772 Europe, genetic clustering reflects cultural context but not geography: European early  
 773 Neolithic individuals are genetically distinct from European pre-Neolithic individuals but  
 774 tightly cluster with Anatolians. PPN: Pre-Pottery/Aceramic Neolithic, PN: Pottery  
 775 Neolithic, Tepecik: Tepecik-Çiftlik (electronic supplementary material, table S1 lists the  
 776 number of SNPs per ancient individual).





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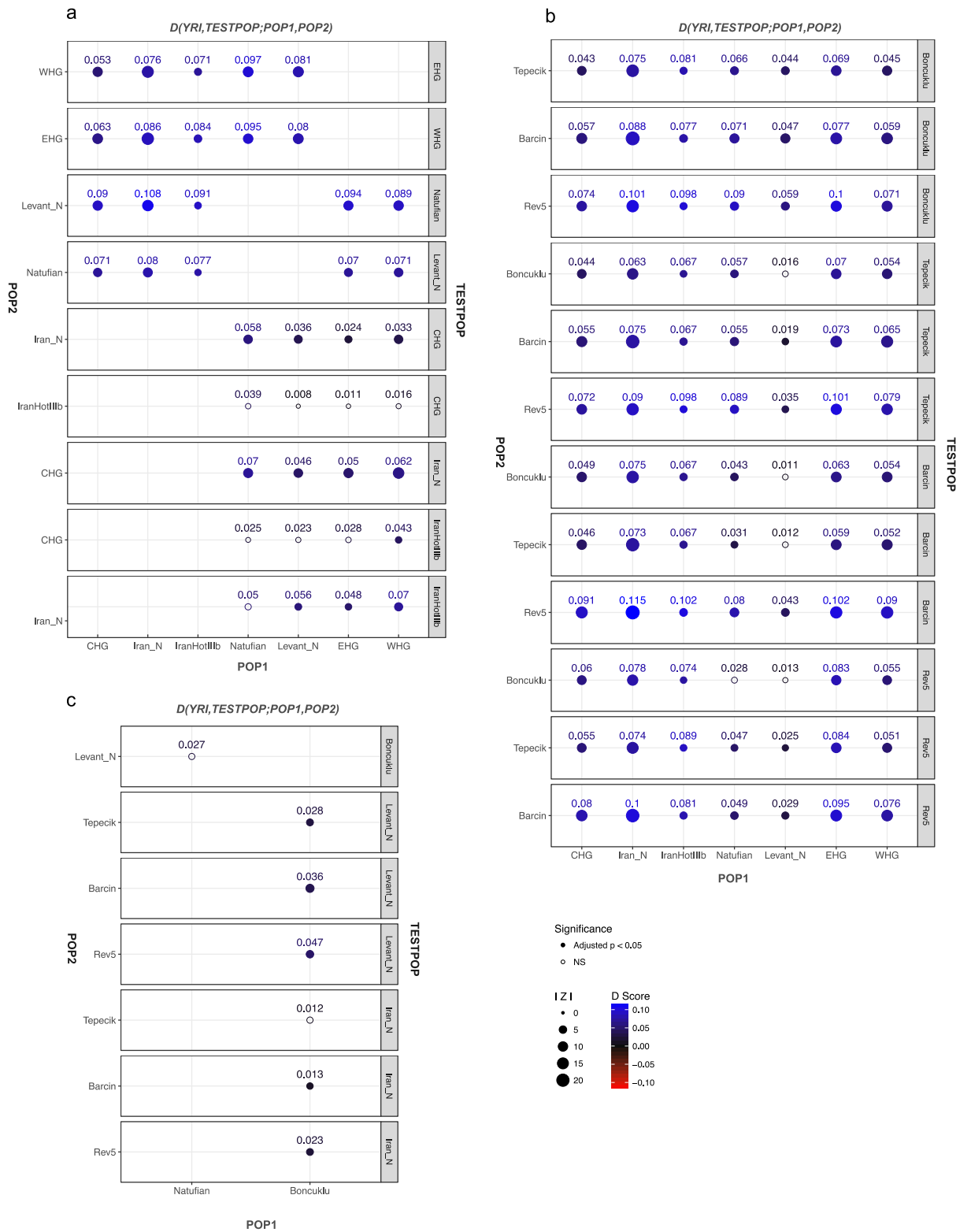
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**Figure S2.** Summary of the data analyzed in this study. (a) Map of west Eurasia showing the geographical locations and (b) timeline showing the time period (years BCE) of ancient individuals investigated in the study. Blue circles: individuals from pre-Neolithic context; red triangles: individuals from Neolithic contexts. For further information about the samples see electronic supplementary material, table S1.



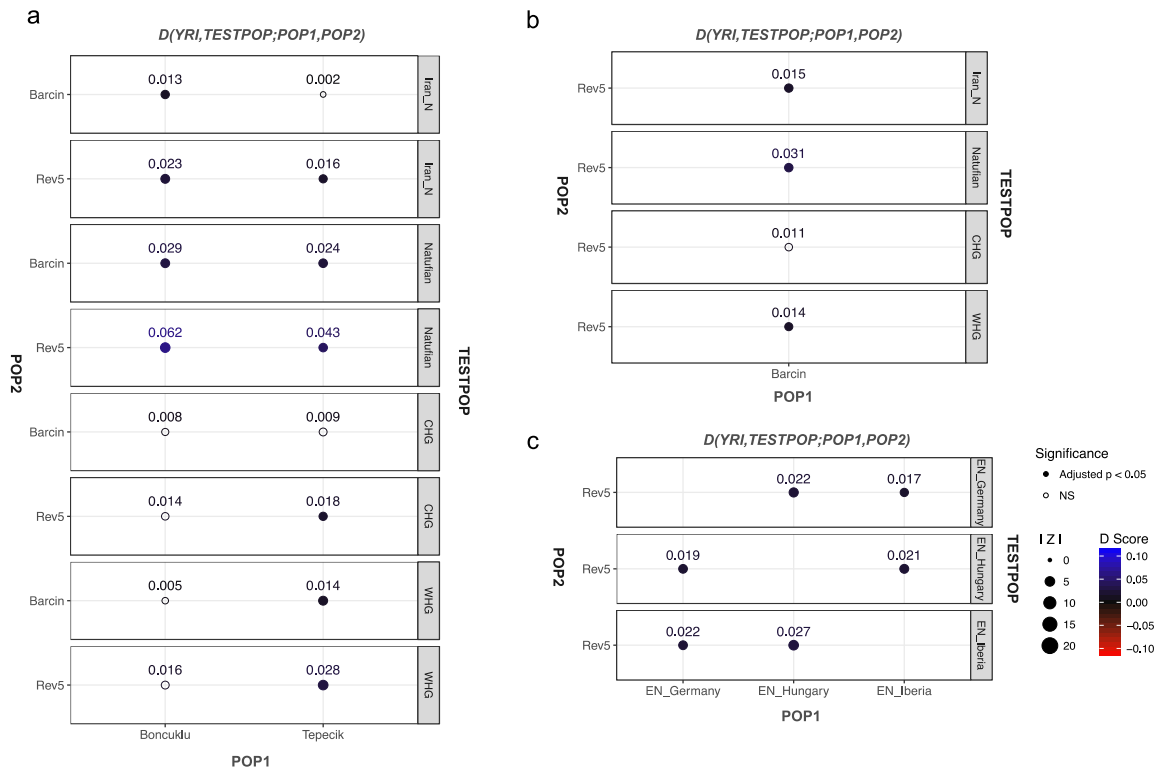
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**Figure S3.** Plots showing the results of  $D$ -statistics with topology  $D(\text{Yoruba}, \text{right population}, \text{bottom population}, \text{left population})$ . Multiple testing correction was performed

786 including all  $D$ -statistics results discussed in the paper using Benjamini-Yekutieli method  
 787 [56]. Results of  $D$ -statistics in the form of (a)  $D(\text{Yoruba}, p1; p2, p3)$ . “ $p$ ” refers to the  
 788 Caucasia/Iran, the Levant, or European pre-Neolithic gene pools (Table S3). (b)  $D(\text{Yoruba},$   
 789  $p1; p2, p3)$ . “ $p1$ ” and “ $p2$ ” are Anatolian/Aegean populations and “ $p3$ ” is either  
 790 Caucasia/Iran, Levant, or European pre-Neolithic (Table S5). (c)  $D(\text{Yoruba}, p1; p2, p3)$ .  
 791 “ $p1$ ” is Iran or Levant Neolithic, “ $p2$ ” is Boncuklu and  $p3$  is one of the PNs. (Table S6)  
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 794 **Figure S4.** Plots showing the results of  $D$ -statistics with topology  $D(\text{Yoruba}, \text{right}$   
 795  $\text{population}, \text{bottom population}, \text{left population})$ . Multiple testing correction was performed  
 796 including all  $D$ -statistics results discussed in the paper using Benjamini-Yekutieli method  
 797 [56]. Results of  $D$ -statistics in the form of (a)  $D(\text{Yoruba}, \text{Natufian}; \text{northAegean},$   
 798  $\text{centralAnatolia})$  (Table S8-S10). (b)  $D(\text{Yoruba}, \text{Iran Neolithic}\&\text{Natufian}\&\text{CHG}\&\text{WHG};$   
 799  $\text{Barcin}, \text{Revenia})$  (Table S11). (c)  $D(\text{Yoruba}, \text{European Neolithic}; \text{European Neolithic},$   
 800  $\text{Revenia})$  (Table S14)  
 801