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1 **Editor Summary:** Gelabert et al examine genomic and archaeological data from Europe's earliest farming communities in Central  
 2 Europe (5500-5000BCE). They find differentiated genetic networks but no evidence of unequal access to resources linked to sex or  
 3 kin.  
 4 **Peer Review Information:** *Nature Human Behaviour* thanks Eszter Bánffy, and the other, anonymous, reviewer(s) for their contribution  
 5 to the peer review of this work.

## 6 **Inventory of Supporting Information**

7  
 8 **Manuscript #:NATHUMBEHAV-23072145C**

9  
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### 11 **1. Extended Data**

12  
 13

Figure or Table #	Figure/Table title	Filename	Figure/Table Legend
Please group Extended Data items by type, in sequential order. Total number of items (Figs. + Tables) must not exceed 10.	One sentence only	Whole original file name including extension. i.e.: Smith_ED_Fig1.jpg	If you are citing a reference for the first time in these legends, please include all new references in the main text Methods References section, and carry on the numbering from the main References section of the paper. If your paper does not have a Methods section, include all new references at the end of the main Reference list.
Extended Data Fig. 1	Extended Figure 1	Extended1.pdf	
Extended Data Fig. 2	Extended Figure 2	Extended3.pdf	
Extended Data Fig. 3	Extended Figure 3	Extended3.pdf	

Extended Data Fig. 4	Extended Figure 4	Extended4.pdf	
Extended Data Fig. 5	Extended Figure 5	Extended5.pdf	
Extended Data Fig. 6	Extended Figure 6	Extended6.pdf	
Extended Data Fig. 7	Extended Figure 7	Extended7.pdf	

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15 **1. Supplementary Information:**

16 **A. PDF Files**

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Item	Present?	Filename	A brief, numerical description of file contents.
		Whole original file name including extension. i.e.: Smith_SI.pdf. The extension must be .pdf	i.e.: <i>Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.</i>
Supplementary Information	Yes	SI.pdf	
Reporting Summary	Yes	Reporting.pdf	
Peer Review Information	No	<i>OFFICE USE ONLY</i>	

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22 **B. Additional Supplementary Files**

23

Type	<b>Number</b> Each type of file (Table, Video, etc.) should be numbered from 1 onwards. Multiple files of the same type should be listed in sequence, i.e.: Supplementary Video 1, Supplementary Video 2, etc.	<b>Filename</b> Whole original file name including extension. i.e.: <i>Smith_Supplementary_Video_1.mov</i>	<b>Legend or Descriptive Caption</b> Describe the contents of the file
Supplementary Table	Supplementary Tables 1 to 12	Tables-SI.xlsx	

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26

## 27 **Social and genetic diversity in first farmers of central Europe**

28

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117 **Abstract**

118 The Linearbandkeramik (LBK) Neolithic communities were the first to spread farming  
119 across large parts of Europe. We report genome-wide data for 250 individuals: 178  
120 individuals from whole-cemetery surveys of the Alföld Linearbankeramik Culture (ALPC)  
121 eastern LBK site of Polgár-Ferenci-hát, the western LBK site of Nitra Horné Krškany, and the  
122 western LBK settlement and massacre site of Schletz, as well as 48 LBK from 16 other sites  
123 and 24 earlier Körös and Starčevo from 17 more sites. Here we show a systematically higher  
124 percentage of western hunter-gatherer (WHG) ancestry in eastern than western LBK sites,  
125 showing these two distinct LBK groups had different genetic trajectories. We find evidence  
126 for patrilocality, with more structure across sites on the male than female lines and a higher  
127 rate of within-site relatives for males. At Schletz we find almost no relatives, showing that  
128 the massacred individuals were from a large population, not a small community.

129

130 **Main text**

131 **Introduction**

132 The archaeological roots of the Linear Pottery culture (Linearbandkeramik, LBK) ca. 5500-  
133 5000 BCE are conventionally traced to the Starčevo culture of central Transdanubia <sup>1-3</sup>, as  
134 well as the Körös culture of the Great Hungarian Plain (Alföld) <sup>4</sup>. The LBK is often divided  
135 into two subgroups: the 'eastern LBK' Alföld Linearbankeramik Culture (ALPC) on the Great  
136 Hungarian Plain and the much more geographically expansive 'western LBK'. The western  
137 LBK has been reconstructed to have spread in two waves, first from Transdanubia, at ca.  
138 5500 BCE, to Slovakia, Austria, Moravia, Bohemia, and central and eastern Germany. Several  
139 centuries later, a second wave reached the Paris basin and adjacent areas of France as far  
140 west as Normandy and as far east as Poland, Ukraine, Moldova, and Romania <sup>1,2</sup>.

141

142 LBK material culture appears strikingly uniform, given its geographic extent, with the typical  
143 LBK settlement pattern consisting of clusters of sites along the alluvial plains of rivers.  
144 Nevertheless, archaeological studies<sup>5</sup> have documented subtle but significant differences in  
145 subsistence, settlement patterns, health, and lifeways among LBK communities<sup>6</sup>. The LBK

146 culture is no longer recognized after around 5000-4900 BCE. Studies of the temporal  
147 distributions of radiocarbon dates suggest a demographic collapse in that century<sup>7</sup>,  
148 potentially linked with violence exemplified at the Late LBK massacres sites of Vrable in  
149 Slovakia<sup>8</sup>, Talheim in Southern Germany, and Asparn-Schletz<sup>9,10</sup> in Lower Austria.

150  
151 Studies of variation in strontium (Sr) isotope ratios across individuals have provided insight  
152 into mobility patterns in the LBK, notably at Nitra, Schwetzingen, and Vedrovice. These  
153 analyses revealed higher variability in Sr ratios in females than males<sup>5</sup>, showing that women  
154 originated from outside the communities where they were buried more often than men,  
155 implying different patterns of mobility between the two sexes and providing evidence of  
156 patrilocal practices. Further evidence for patrilocality came from a study showing that males  
157 buried with polished stone adzes, likely indicative of high social status, had less strontium  
158 variation than males without them, suggesting that the former tended to be born and live in  
159 their natal communities<sup>11</sup>. In the archaeological context of settlement patterns, these results  
160 suggest that LBK society may have been organised into patrilocal kin-like groups<sup>12</sup>, with  
161 land inherited through the male line. Most LBK sites are located on loess soils, and  
162 subsequently, movements of individuals within loess regions are not easily detectable based  
163 on strontium isotope ratios. In contrast, paleogenomic methods have the potential to reveal  
164 differences between male and female behaviours regardless of local geology. A caveat is that  
165 cross-cultural studies of where people live after marriage have shown that women tending  
166 to be buried away from their natal homes does not necessarily mean patrilocality; the  
167 observed patterns could also reflect more complex kinship systems including ones where  
168 couples tended to reside in either their paternal or maternal line family homes<sup>13</sup>.

169  
170 Analyses of whole genome data from 157 LBK individuals published before this study  
171 showed that they inherited their predominant ancestry from Early European farmers (EEF)  
172 who then mixed with local European Mesolithic populations, resulting in admixed groups  
173 with typically 5% Western Hunter-Gatherer (WHG) ancestry<sup>14-19</sup>, with a possible  
174 differential contribution of Starčevo to LBK and Körös to the ALPC<sup>20</sup>. However, some LBK  
175 individuals have a much higher percentage of WHG ancestry (e.g. an individual at the LBK

176 site of Brunn, Austria) <sup>18</sup>, suggesting a more complex admixture process <sup>16</sup>. The only  
177 published cemetery-scale studies of LBK substructure focused on the western LBK sites of  
178 Derenburg-Meerenstieg II and Stuttgart-Mühlhausen in Germany, both with homogenous  
179 ancestry <sup>16,21</sup>. As the LBK also practised settlement burials, this leaves open the question as  
180 to whether cemeteries only represent a selected portion of the population.

181  
182 A centerpiece of this study is a large sample-size analysis of intra- and inter-site variation in  
183 the LBK at three locations with different archaeological characteristics: 1) the ALPC  
184 settlement site of Polgár-Ferenci-hát (5500-5100 BCE) in eastern Hungary in which  
185 individuals were buried between houses rather than in a cemetery, 2) the cemetery of Nitra,  
186 western Slovakia, dated to the LBK expansion phase, 5200–5000 BCE, and 3) the enclosed  
187 settlement and massacre LBK site of Asparn-Schletz in Lower Austria dated to the final phase  
188 of the LBK at around 5000 BCE. We co-analyzed the newly generated genomic data for  
189 individuals from these sites together with new genomic data from 31 other archaeological  
190 sites and previously published data to address the following: 1) the extent of genetic  
191 differentiation between the LBK and ALPC; 2) kinship patterns of LBK communities and the  
192 extent of their correlation to variations in burial location, strontium isotopic values and  
193 grave goods; 3) correlations between kinship and differences in diet and mobility (which  
194 have previously been hypothesised to be related to LBK social structure); and 4) the genetic  
195 structure of the individuals of the settlement and massacred at Asparn-Schletz.

196  
197 We generated genome-wide data passing standard metrics for authentication for 250 newly-  
198 reported individuals of the Starčevo, Körös, and LBK/ALPC cultures from Austria, Slovakia,  
199 Croatia, Romania, Serbia, and Hungary, using target enrichment for 1.24 million single  
200 nucleotide polymorphisms (SNPs), and reported improved quality data from an additional 7  
201 individuals, generating a total of 282 new sequencing libraries (Figure 1, 2B, Supplementary  
202 Tables 1-2). The new data include 18 Starčevo, 6 Körös (pre-LBK), 80 Hungarian ALPC  
203 (henceforth "Hungary\_ALPC"), 2 Transdanubian LBK ("Transdanubia\_LBK"), 87 Austrian  
204 LBK ("Austria\_LBK") and 57 Slovakian LBK ("Slovakia\_LBK") from a total of 31  
205 archaeological sites (Figure 1, 2B). Individuals with fewer than 30,000 SNPs covering the

206 autosomal targets were not included in ancestry analyses, but their data are reported. In  
207 addition, we did not use data from 1st-degree relatives of higher coverage individuals in the  
208 data set for ancestry analyses. We co-analyzed these individuals with published data for 171  
209 Starčevo, Körös, ALPC, and LBK individuals<sup>15,16,18-23</sup>. We also generated 19 new radiocarbon  
210 dates and built Bayesian date models for four sites of the ALPC and LBK (Supplementary  
211 Section 2, Supplementary Figures 18-21).

212

### 213 **Elevated WHG ancestry in the eastern LBK**

214 We used *smartpca*<sup>24</sup> to perform a Principal Components Analysis (PCA) (Extended Figure 1,  
215 Supplementary Figure 22) on genome-wide data from present-day European populations  
216 genotyped on the Affymetrix Human Origins SNP array and then projected the ancient  
217 individuals. The PCA shows that the individuals from the ALPC sites are located closer to the  
218 WHG-like individuals in the PCA. The Körös and Starčevo individuals cluster with the  
219 western LBK, suggesting that the analysed ALPC individuals might be the result of a mixture  
220 between an early Neolithic population and additional WHG.

221

222 We grouped individuals based on archaeological culture and geography (proxied by present-  
223 day country): Austria\_LBK, Slovakia\_LBK, Transdanubia\_LBK, Hungary\_ALPC, and  
224 Germany\_LBK. We estimated ancestry proportions with *qpAdm*, using as proxies for the  
225 sources a pool of Balkan early farmers with little or no WHG admixture (Balkan\_N) and a  
226 pool of western European hunter-gatherers (WHGA)<sup>25</sup>. As Right reference outgroups, we  
227 used pools of Turkey\_N, ancient Africans, and Mesolithic European hunter-gatherers more  
228 divergent in time or space (WHGB) (Supplementary Section 5). We used *qpWave* to identify  
229 significant outliers from the main cultural and geographical groups at  $p\text{-value}<0.05$ , adding  
230 the tags HGEXC (“hunter-gatherer excess”) and EEFEXC (“Early European Farmer excess”)  
231 (Supplementary Table 1, Extended Figure 2). Eastern LBK Hungary\_ALPC individuals have,  
232 on average,  $11\pm 0.3\%$  WHG ancestry ( $p=0.77$  for fit) (Figure 2A). In contrast, Slovakia\_LBK  
233 and Austria\_LBK individuals have an average of  $4.5\pm 0.4\%$  WHG ( $p=0.01$  and  $0.09$  for fit). Ten  
234 western LBK individuals from Transdanubia (Transdanubia\_LBK), have an estimated 3%  
235 WHGA ancestry, although the *qpAdm* model is not a statistical fit ( $p<0.001$ ) so this

236 measurement should be viewed with caution (Supplementary Table 3 and Supplementary  
237 Section 5).

238

### 239 **No evidence for sex biased population mixture**

240 We used DATES <sup>26</sup> to estimate the age of admixture in WHG and Early European Farmers.  
241 Consistent with previous findings, but now with higher resolution<sup>22</sup>, we infer that the  
242 mixture occurred on average ~400 years before the sampled Austrian\_LBK, Slovakia\_LBK,  
243 and Germany\_LBK lived (range of 95% CI: 6,010-5,460 BCE) and 530 years before the ALPC  
244 individuals (range of 95% CI: 5,875-5,796 BCE), assuming an average date of 5,300 years of  
245 ALPC and 5,100 for the LBK (Supplementary Table 4). This suggests a scenario in which the  
246 dawn of the archaeologically defined LBK culture was marked by the completion of a period  
247 of mixture, reflecting a social incorporation of WHG communities, which plausibly could  
248 have been part of the process by which the LBK distinguished itself from preceding cultures.

249

250 Some degree of mixture with WHG continued into the LBK period itself, as documented by  
251 individuals at the early LBK site of Brunn (Austria) with evidence of admixture in the last  
252 couple of generations before they lived <sup>18</sup> (Supplementary Table 1). We found further  
253 evidence for this using the RFMix <sup>27</sup> method, where we inferred the locations and size of  
254 segments of WHG ancestry in each LBK individual after filling in missing genotypes and  
255 phasing the data using the imputation engine GLIMPSE <sup>28</sup> (Supplementary Figure 23,  
256 Supplementary Table 5, Supplementary Section 5). We correlated the summed length of  
257 inferred WHG segments from RFMix greater than 0.2 cM to *qpAdm* estimates of WHG  
258 ancestry and observed a high Pearson correlation coefficient of 0.85, suggesting that these  
259 inferred segments often reflect true WHG admixture (Supplementary Figure 24), although  
260 there are inevitably errors in this inference and we do not have a well-calibrated  
261 understanding of their rate or genomic distribution. We identified long putative WHG  
262 segments in some ALPC individuals (up to 55 cM, individual I21902 from Polgár-Ferenci-hát,  
263 5371-5216 cal BCE), which if true suggest mixture in the last few generations in their history,  
264 similar to the pattern at Brunn. At the ALPC site of Polgár-Ferenci-hát, with its elevated rate  
265 of WHG ancestry, we also detected significant within-community variation in WHG ancestry.

266 In one genetic group, henceforward referred to as “Family B” (Supplementary Figure 28),  
267 three individuals from this cluster (I21898, I21902, I18660), father, son and daughter,  
268 respectively, had significantly elevated WHG: (36%, 26% and 29%, respectively). The  
269 daughter, who we estimate to have been 27-28 years old at the time of her death, was buried  
270 with many grave goods which were otherwise uncommon at this settlement (Supplementary  
271 Figure 29). These individuals are related to a 3-4th-degree to two others (I21827 and  
272 I18695), father and daughter. The daughter of this second group had significantly elevated  
273 WHG ancestry (20%)(Supplementary Figure 28, Supplementary Table 1), while the father's  
274 ancestry was typical for the majority of individuals from this site (13% WHG). In the first  
275 group, the mother is unsampled, but we assessed her WHG ancestry to be ~9% lower than  
276 the father's (which explains the offspring's intermediate WHG proportions). In contrast and  
277 by a similar calculation, in the second group, we estimate that the unsampled mother had  
278 ~7% higher WHG ancestry than the father. Thus, WHG admixture patterns appear to vary by  
279 family.

280

281 We tested directly for sex bias in WHG admixture patterns by comparing qpAdm estimates  
282 of ancestry on the X-chromosome, with 2/3 female ancestry, and the autosomes, without sex  
283 bias. The estimates are statistically indistinguishable in all tests (Supplementary Table 3)  
284 (Figure 2A), providing no evidence for either primarily male WHG contribution to early  
285 farmers<sup>29</sup>, or hunter-gatherer Mesolithic women preferring farmers due to perceived higher  
286 status<sup>30</sup>. A caveat is that these null results may reflect limited precision in X chromosome  
287 qpAdm estimates.

288

### 289 **Differential mating and social strategies in the LBK/ALPC**

290 The large sample size of LBK individuals analysed in this study allows us to perform a  
291 continental-scale comparison of patterns of variation on the Y chromosome reflecting the  
292 history of the entire male line, and mitochondrial DNA, reflecting the history of the entire  
293 female line. In the Y-chromosome analysis, we detect previously unappreciated geographic  
294 variation across the LBK (Extended Figure 3) (a  $\chi^2(209,42)=242$  test for heterogeneity is  
295 highly significant at  $p<10^{-12}$ ), with haplogroup G dominant in the Slovakian, German, and

296 Hungarian LBK; haplogroup C in the Austrian\_LBK; and the majority of the Hungary\_ALPC  
297 individuals with haplogroup I (36%), associated with Mesolithic populations such as those  
298 of the Iron Gates regions of Serbia and Romania <sup>20,31</sup>. We present the list of mutations  
299 supporting each assignment in Supplementary Table 6. In contrast, we do not detect  
300 significant structure in mitochondrial DNA haplogroup frequencies, with no haplotype with  
301 a frequency greater than 30%, and no evidence for haplotype frequency differences across  
302 the regional groups (Extended Figure 3, Supplementary Table 1), ( $\chi^2$  (420)=58.8.3,  $p=0.30$ ).  
303 These results provide evidence of limited gene flow among LBK communities on the male  
304 line, and one possible reason for this is a much higher rate of movement of females between  
305 communities. Previous studies already suggested homogeneity in Y-chromosomes in the 6th  
306 Millennium BCE<sup>32</sup>. Here, we provide evidence that these differences are regionally variable,  
307 which could be explained by the limited movements of males. However, we do not have  
308 sufficient sampling to make any general claim about patrilocality practices in the ALPC <sup>13</sup>.

309  
310 By studying the distributions of close relatives in the two burial locations where we detect  
311 many relationships (Figure 3A-B), we find genetic evidence for patrilocality in Polgár-Ferenci-  
312 hát but not Nitra Horné Krškany. At Nitra Horné Krškany, we detect ten families, including a  
313 pedigree spanning four generations, and at Polgár-Ferenci-hát, we detect four families,  
314 including one with 12 individuals. Combining the two cemeteries, we find that relatives up  
315 to the 3<sup>rd</sup> degree (Supplementary Table 7, Supplementary Section 6) tend to be buried  
316 together more often than random pairs of individuals. At Nitra Horné Krškany, we did not  
317 detect significant differences in the number of relatives between males and females  $\chi^2$   
318 (47,1)=0.14,  $p=0.70$ . In contrast, we detect strong evidence of patrilocality at Polgár-Ferenci-  
319 hát, with more relatives for males (21 of 22) than for females (14 of 23):  $\chi^2(45,1)=7.78$ ,  
320  $p=0.005$  (Table 1). We also identified that all the individuals from Rákóczifalva-Bagi-földek  
321 Site-8/A are from a single family group (Supplementary Section 6, Supplementary Table 7).

322

### 323 **No kinship-associated differences in mobility and diet**

324 We analysed the findings of genetic relatedness together with dietary (carbon,  $\delta^{13}\text{C}$ , and  
325 nitrogen,  $\delta^{15}\text{N}$ ) and strontium isotope data ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) <sup>33</sup> (Supplementary Section 3, Figure

326 3C, Extended Figure 4, Supplementary Table 8). We did not perform similar analyses for  
327 Asparn-Schletz as we had dietary isotopic data for too few individuals and too few detected  
328 genetic relatives.

329  
330 We detect significant within-family variation in the measurements of isotope sensitive to  
331 mobility both at Nitra Horné Krškany (Levene's test for variances  $n=12$ ,  $p=0.01$ )  
332 (Supplementary Table 14) and at Polgár-Ferenci-hát (Levene statistic for the difference in  
333 variance = 16.74,  $p=0.001$ ) (Supplementary Table 17). This shows that people at both sites  
334 and even the same families varied in the places where they resided over their lifetimes.

335  
336 We next tested for significant differences across families in their dietary patterns but found  
337 no strong signals. The only notable correlation we detect is at Nitra, where we found a  
338 marginally significant signal of variation across families for  $\delta^{13}\text{C}$  carbon isotopes (Kruskal-  
339 Wallis=17.20,  $N=26$ ,  $p=0.04$ ) (Supplementary Table 16), providing some evidence that  
340 families sourced food from different landscape contexts, either through variation in direct  
341 consumption or through variation in consumption of animals eating these plants<sup>27</sup>. However,  
342 because we carried out multiple hypothesis tests, the observation of one marginally  
343 significant signal of correlation like this should not be interpreted as strong evidence.

344  
345 We do not detect significant variation in strontium isotope ratios across families at Nitra  
346 Horné Krškany (Mann-Whitney U test,  $n=21$ ,  $p=0.16$ ) (Supplementary Table 15), nor do we  
347 detect a correlation between family structure and the presence of grave goods  
348 (Supplementary Section 2.1, Supplementary Table 8) ( $\delta^{13}\text{C}$ , Kruskal-Wallis=4.99,  $p=0.17$ ;  
349  $\delta^{15}\text{N}$ , Kruskal-Wallis = 1.45,  $p=0.69$ ) (Supplementary Table 16). At Polgár-Ferenci-hát, we  
350 also do not detect variation in isotopic ratios across families:  $\delta^{13}\text{C}$ , Kruskal-Wallis = 4.99,  
351  $p=0.17$ ;  $\delta^{15}\text{N}$ , Kruskal-Wallis = 1.45,  $p=0.69$  (Extended Figure 4, Supplementary Table 20).  
352 This suggests that diet, mobility and funerary rites were mostly independent of biological  
353 kinship ties.

354

355 **Variation across the LBK in community size and mate choice**

356 We carried out ancient DNA analysis of all excavated skeletons from Asparn-Schletz,  
357 corresponding to 70 individuals from the ditch system associated with a massacre, three  
358 from a water well with older dates than the massacred and 20 individuals from settlement  
359 burials. A total of 92 of the 93 individuals yielded enough genomic data for genetic analyses  
360 (Supplementary Tables 1 and 2). Of the 69 individuals with genome-wide data from the base  
361 of the ditch system, including 48 genetic males and 21 genetic females, we detected only a  
362 single pair of 1st/2nd-degree relatives and possibly a pair of individuals between ditch and  
363 settlement contexts. Only 4 of the 69 analysed individuals from the Asparn-Schletz ditch  
364 system are related up to the 3<sup>rd</sup> degree, contrasting with much higher rates at Nitra Horné  
365 Krškany and Polgár-Ferenci-hát (Table 1). We identified a single first-degree relationship  
366 between an older male adult (I24892) and a non-adult (I24280) from within the massacre  
367 context, providing further evidence that this was not an event that affected only a small  
368 community that might have been expected to include more families and hence more close  
369 relatives.

370  
371 We used HapNe-LD<sup>34</sup> to infer the effective population size trajectory of unrelated individuals  
372 from the Asparn-Schletz massacre in the hundreds of years before they lived (n=54). We find  
373 no evidence for a contraction in the gene pool in this period, which could be explained if the  
374 people massacred at Schletz were drawn from many and not a single community. In contrast,  
375 at Nitra Horné Krškany (n=18), we observe the signatures expected for a small community  
376 of people isolated from their neighbours (Figure 4, Supplementary Section 7).

377 Further evidence for the Asparn-Schletz individuals being drawn from a much larger  
378 population than those at the other sites comes from IBD sharing patterns between the  
379 studied individuals (Supplementary Table 9), inferred based on analysis of the imputed and  
380 phased dataset. We observe far less average sharing of IBD segments >12 cM among  
381 individuals at Asparn-Schletz (26 cM) than at Nitra Horné Krškany (174 cM) or Polgár-  
382 Ferenci-hát (158 cM). The reduction is significant ( $p=0.001$ ), even after excluding 1st, 2nd,  
383 and 3rd-degree relative pairs ( $p=0.005$ ), which suggests that the signal is driven by distant  
384 relatives in sites, not just close relatives (Figure 5).

385

386 Eight individuals from Polgár-Férenci-hát and four from Nitra Horné Krškany have elevated  
387 rates of Runs of Homozygosity (ROH), which reflects individuals reproducing within their  
388 own genetic lineages<sup>35</sup>. In contrast, the rest of the individuals at these sites, and all from  
389 Asparn-Schletz, have no segments with ROH >4 cM <sup>35</sup> (Extended Figure 5, Supplementary  
390 Table 10).

391  
392 The IBD analysis gives evidence of two qualitatively distinct regional networks of people  
393 linked by distant familial relationships: one for the Great Hungarian Plain, where the across-  
394 site rate of sharing averages 45.56 cM, and one for Central-Western Europe, where the  
395 across-site rate of sharing averages 9.19 cM, but with a far lower 0.19 cM of sharing across  
396 regions (Supplementary Table 10, Figure 5A). This is in accord with archaeological studies  
397 that imply that Nitra Horné Krškany and Polgár-Ferenci-hát are associated with different  
398 LBK expansions and periods<sup>22,36,37</sup>. We further observe that the rate of IBD sharing decreases  
399 significantly with distance from Polgár-Ferenci-hat ( $p=0.011$ ), which could be explained if  
400 there was a localised network of people within the ALPC. In contrast, there is weak or no  
401 detectable association of IBD sharing with geographic distance in the western LBK, as would  
402 be expected if the western LBK expansion was so rapid that nearby groups were hardly more  
403 closely related than groups far apart (Figure 5B). Finally, the observation that Hungary\_ALPC  
404 individuals have, on average, 16.64 cM in ROH (without 1st-degree relatives) and are the  
405 LBK group with the largest fractions of their genome in ROH, suggests that they may have  
406 had more restricted mating practices than the more widespread western LBK.

407

#### 408 **high-frequency long-range haplotype screens for selection**

409 We scanned the imputed diploid genotype data for the LBK and ALPC individuals for signals  
410 of selection by searching for haplotypes that had evidence of being very recent in origin  
411 based on their large scale and yet too common to have risen to such high frequency in the  
412 absence of selection. Because of the poor haplotype phasing expected for ancient genomes,  
413 we carried out these scans not only with the phased but also the unphased versions of the  
414 iHS and nSL scores, as implemented in Selscan 2.0<sup>38</sup>. We also used BetaScan<sup>39</sup> to test for loci  
415 affected by long-term balancing selection.

416

417 We detected evidence of long-term balancing selection in the HLA region on chromosome 6,  
418 with elevated B1 scores (Figure 6), consistent with previous evidence of balancing selection  
419 at this locus in Neolithic Europeans <sup>40</sup>. A second notable finding is 26 genes with evidence of  
420 balancing selection in the ALPC and LBK (Supplementary Table 11). Many were also  
421 reported as significant outliers based on analysis of patterns of variation in modern  
422 Europeans <sup>41</sup>.

423 We identified 3 and 37 genes with evidence of positive selection in the ALPC and LBK,  
424 respectively (Supplementary Tables 11-12, Supplementary Section 8, Extended Figure 6),  
425 including notable examples associated with pigmentation. The *PRKCH* gene encodes the  
426 PKC $\eta$  protein in melanocytes which is involved in the protein kinase C-dependent pathway  
427 regulating melanogenesis <sup>42</sup>. The *PTPRN2* gene had a higher level of expression in lightly  
428 pigmented melanocytes than in darkly pigmented melanocytes, similar to *SLC45A2* which  
429 contains one of the strongest known signals of pigmentation selection in Europe <sup>43</sup>. When we  
430 correlate the WHG local ancestry components with our selection signals, there is nominal  
431 evidence that non-WHG ancestry is more enriched at sites under selection (Supplementary  
432 Section 8, Extended Figure 7), although we have not ruled out the possibility that this could  
433 potentially be an artifact of greater sensitivity to selection signals at non-WHG regions.

434

## 435 **Discussion**

436 Our study reveals differences in kinship structure, admixture, demography, and ancestry  
437 across the LBK. We report an average of around 11% WHG ancestry in the ALPC, a  
438 proportion that has reached as high as 35% in some individuals. This contrasts with the  
439 much lower average among the studied individuals from Austria (an average of 4,5% with a  
440 range of up to 14%) and Slovakia (an average of 4% with a range of up to 8%). This suggests  
441 that the admixture between farmers and hunters of the Great Hungarian Plain was more  
442 extensive than among the more westerly LBK communities. This admixture shows no sex-  
443 biased trend despite the high fraction of Y-chromosome haplogroups associated with WHG.

444

445 Correlation between isotopic and genetic shows no statistical relationships between diet and  
446 mobility patterns between families in Nitra Horné Krškany and Polgár-Ferenci-hát, but we  
447 find evidence for high variation in mobility within families, at least at Nitra. We observe no  
448 evidence of a correlation between genetic patterns and archaeological markers of social  
449 status. We can, therefore, make no claims regarding population substructure driven by social  
450 status in the LBK.

451  
452 We find that at both Nitra Horné Krškany and Polgár-Ferenci-hát, relatives were buried  
453 closer to each other than non-relatives. Polgár-Ferenci-hát males had significantly more  
454 relatives than females in the cemetery population we sampled. This pattern, combined with  
455 the evidence of limited regional diversity in the Y-chromosome and long IBD tracts, is  
456 consistent with limited mobility within the Great Hungarian Plain and patrilocal practices.  
457 We observed much less IBD across western LBK sites and approximately contemporary  
458 ALPC sites than within either community, suggesting they were part of different mating  
459 networks. We do observe IBD between sites of the Great Hungarian Plain.

460  
461 The proportion of relatives in Asparn-Schletz is lower than at any other LBK site analysed.  
462 We only identified relationships between males and children and only one with an adult  
463 male. This raises doubts regarding the idea that the individuals recovered at the ditch  
464 represent a local community and instead suggests that people massacred at this key were  
465 likely drawn from a widespread population <sup>44</sup>. When comparing Asparn-Schletz and Nitra  
466 Horné Krškany, we find evidence that Asparn-Schletz but not Nitra Horné Krškany  
467 represents a large genetic community. One possibility is that Asparn-Schletz was a central  
468 site that drew a population from a larger area in times of stress, such as outbreaks of violence  
469 <sup>9</sup>. Another explanation could be that communities in the broader LBK expansion area were  
470 formed with few biologically related individuals, as at Derenburg-Meerenstieg II and  
471 Stuttgart-Mühlhausen, Germany. In any case, our results suggest that frequent mobility  
472 between sites was a factor in many LBK communities <sup>45</sup>. A lack of related individuals has also  
473 been found in the Eneolithic massacre of Potocani, Croatia <sup>46</sup>.

474

475 Our results illuminate how combining whole-burial assemblage ancient DNA, sampled and  
476 processed with responsible protocols<sup>47</sup>, with isotopic and archaeological data, can reveal the  
477 structure of past societies as well as evidence for local variations in mobility and diet,  
478 shedding light on unappreciated aspects of past behaviour.

479

## 480 **Methods**

481

### 482 **Ancient DNA Data Generation**

483 The 319 individuals screened in this study were sampled with permission from the  
484 authorities responsible for each of them and in engagement with local archaeologist  
485 stakeholders, in a way consistent with recommendations for ethical analysis of ancient  
486 DNA<sup>47</sup>. Permits for ancient DNA analysis of the skeletal remains was issued to the authors of  
487 this work. The permission specified sampling of the skeletal material for ancient DNA  
488 analysis, and generation of radiocarbon dates and associated isotopic information. We  
489 handled remains with respect, seeking to minimize damage to them for example by  
490 prioritizing analysis of disarticulated ossicles or petrous bones wherever possible, and using  
491 other minimally-invasive sampling techniques such as drilling from the cranial base or  
492 soaking teeth in extraction buffer <sup>47</sup>. Additionally, we employed a standardized in-solution  
493 capture method, which maximizes DNA recovery while minimizing the required input  
494 material.

495

496 DNA was extracted from powder using an automated protocol with silica-coated magnetic  
497 beads and binding buffer <sup>48</sup>. DNA extracts were converted to double-stranded libraries using  
498 a partial UDG treatment <sup>49</sup>. Amplified libraries were enriched using two rounds of  
499 consecutive hybridisation capture enrichment 1240k strategy <sup>50,51</sup>). Captured libraries were  
500 sequenced on an Illumina NextSeq500 instrument with 2 × 76 cycles (2 × 7 cycles for the  
501 indices) or an Illumina HiSeq X10 with 2 × 101 cycles (2 × 7 for the index). We trimmed  
502 adapters, merged paired-end sequences, and aligned to the human genome (hg19) and  
503 mitochondrial genome (RSRS) using BWA 0.6.1 <sup>52</sup>. The computational pipelines are available

504 on GitHub (<https://github.com/DReichLab/ADNA-Tools>,  
505 <https://github.com/DReichLab/adna-workflow>).

506  
507 We evaluated ancient DNA authenticity using several criteria: a rate of cytosine deamination  
508 at the terminal nucleotide above 3%; a ratio of Y to combined X + Y chromosome sequences  
509 below 0.03 or above 0.35 <sup>53</sup>(intermediate values are indicative of the presence of DNA from  
510 at least two individuals of different sex); for male individuals with sufficient coverage, an X  
511 chromosome contamination estimate whose lower bound of the 95% confidence interval is  
512 <1.1% (all but one below 0.5%); and an upper-bound rate for the 95% confidence interval  
513 for the rate to the consensus mitochondrial sequence that exceeds 95%, as computed using  
514 contamMix-1.0.10 <sup>54</sup>. We added tags to samples that gave evidence of contamination by any  
515 of these criteria and discarded samples with at least two signals of contamination.

516

### 517 **Genetic sex, mitochondrial and Y chromosome haplogroup determination**

518 To determine genetic sex, we searched for evidence of a Y chromosome by computing the  
519 ratio of Y-chromosomal 1240k positions with available data divided by the number of X-  
520 chromosomal and Y-chromosomal 1240k positions with available data. Individuals with a  
521 ratio of more than 0.35 were considered genetic males, and individuals with less than 0.03  
522 were considered genetic females. To check for sex chromosome aneuploidies, we computed  
523 the mean coverage on X-chromosomal and Y-chromosomal 1240k positions. We normalised  
524 these values by autosomal coverage on 1240k positions for each individual. We did not find  
525 any evidence of sex chromosome aneuploidies. To determine mitochondrial haplogroups  
526 (Supplementary Table 1), we constructed a consensus sequence using RSRS sequence with  
527 samtools and bcftools <sup>55</sup>, restricting to sequences with a mapping quality of >30 and a base  
528 quality of >30. We called haplogroups with Haplogrep2.1.1 <sup>56</sup>. We determined Y chromosome  
529 haplogroups (Supplementary Table 1) based on the nomenclature of the International  
530 Society of Genetic Genealogy (<http://www.isogg.org>) version 14.76 (25 April 2019),  
531 restricting to sequences with a mapping quality of 30 or more and a base quality of 30 or  
532 more. For determining chromosome Y, we analysed not only targeted SNPs but also off-  
533 target SNPs, and determined allelic status by majority rule as discussed in detail in

534 Supplementary Table 3, following the methodology described in <sup>57</sup>. For the statistical tests,  
535 we used all the available individuals from the relevant periods as well as all the produced  
536 individuals with enough available positions. We met the assumptions of the statistical tests  
537 used. We have not assumed normality in the statistical tests.

538

### 539 **Biological kinship estimation and family reconstruction**

540 We followed the same approach described by <sup>58</sup>. We focused on 1st, 2nd, and 3rd-degree  
541 relatives for family reconstruction but also noted individuals detected as relatives up to the  
542 4<sup>th</sup> degree. The complete list is reported in Supplementary Table 7.

543

### 544 **Principal component analysis and f-statistics analyses**

545 We used Western Eurasian populations genotyped on the Affymetrix Human Origins SNP  
546 array to perform Principal Components Analysis with *smartPCA* <sup>24</sup>. In this PCA, we projected  
547 all the samples we report in this paper as well as other relevant ancient DNA data  
548 (Supplementary Table 1). We used same dataset to perform f-statistics-based analyses using  
549 admixtools 7.0.2 <sup>24</sup>. (Supplementary Section 5)

550 We performed *qpAdm* analyses following the same strategy as in Patterson et al. 2022 <sup>25</sup>.  
551 Individuals labelled as Ancient\_Africa, WHGB, and Turkey\_N were used as right outgroup  
552 populations and WHGA and Balkan\_N as left sources. qpWave was performed using the same  
553 strategy.

554

### 555 **ROH**

556 We called ROH with the methodology described in Ringbauer et al.<sup>35</sup> optimised for the study  
557 of ancient individuals, restricting to individuals with more than 400,000 SNPs. We plotted  
558 the results with the python scripts at (<https://github.com/hringbauer/hapROH>).

559

### 560 **Imputation and IBD**

561 We imputed and phased with GLIMPSE <sup>259</sup> following the methodology in <sup>60</sup>. We called IBD  
562 using ancIBD<sup>60</sup>. We filtered for IBD >12 cM and plotted the connections using Rstudio 4.3.2.  
563 Further details are provided in Supplementary Section 5.

564

### 565 **Local ancestry maps**

566 We used diploid imputed genotypes to perform analyses. We ran RFMix v2.03-r0<sup>27</sup> using  
567 Balkan\_N and WHGA as reference populations. We plotted results with Python 3.7.6 and  
568 Rstudio 4.3.2.

569

### 570 **Selection**

571 The selection analysis is detailed in Supplementary Section 5

572

573 We can provide the full code used in this project upon request.

574

### 575 **Data availability**

576 All sequencing data are freely available at the European Nucleotide Archive (ENA) with the  
577 accession number PRJEB64177. All the data used to compare the data produced in this study  
578 is available in the Allen Ancient DNA Resource (AADR) <sup>61</sup>

579

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604

605 **Author contributions**

606 P. G, R. P, P. B, D. H, M. T-N, A. A., and D. R conceived the study. M. T-N, A. A, F. P, A. S, M. D, J.  
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 613 P. B, and D. R wrote the manuscript with inputs from all co-authors.

614

615 **Competing Interests Statement**

616

617 The authors declare no competing interests.

618

619 **Tables**

620

621 **Table 1: Patterns of relationships at three LBK sites with substantial new data (\* $p < 0.05$ )**

	Polgár-Ferenci-hát (n=45)	Nitra Horné Krškany (n=47)	Asparn-Schletz (n=92)
Ratio related/unrelated	0.83	0.65	0.15
Ratio of males/females related	0.94/0.69*	0.66/0.65	0.1/0.05

Average no. of relatives	1.1	0.60	0.1
--------------------------	-----	------	-----

622

623 **Figure legends**

624

625 **Figure 1: The LBK and ALPC extension:** A) Map of the extent of the LBK and ALPC cultures  
 626 in Central Europe. Generated with Illustrator. The extent of the LBK and ALPC cultures was  
 627 obtained from Gronenborn and Horejs 2023 <sup>62</sup>. B) Location of the studied sites, the symbols  
 628 depict the cultural attribution.

629

630 **Figure 2: The genomic ancestry diversity in the LBK/ALPC** A) Histograms of point  
 631 estimates of ancestry proportions of LBK and ALPC individuals for both the autosomes and  
 632 X-chromosome (generated with ggplot2 <sup>63</sup>). B) Range of dates and culture span of the  
 633 individuals included in the study

634

635 **Figure 3: Kinship patterns in LBK sites:** Burial layouts for A) Nitra Horné Krškany (top)  
 636 and B) Polgár-Ferenci-hát (bottom). Each symbol represents one individual: squares males,  
 637 circles females. Red denotes the main genetic cluster, green WHG outliers, and violet EEF  
 638 outliers. Light brown are children. Blue lines or circles are 1<sup>st</sup>-degree relatives and the yellow  
 639 pottery symbol grave goods in burials. Only individuals with ancestry information are  
 640 plotted C) Dietary isotopes at Nitra Horné Krškany coded by families. Families at Nitra Horné  
 641 Krškany do not cluster in dietary-specific groups. All plots are restricted to individuals with  
 642 qpAdm estimates.

643

644 **Figure 4: Asparn-Schletz population size:** Inferred population size trajectory of Asparn-  
 645 Schletz and Nitra based on HapNe-LD. The recent contraction in Nitra Horné Krškany likely  
 646 reflects undetected families in the sample, while the Asparn-Schletz individuals have no  
 647 evidence of being more closely related to each other than they are to the more widely  
 648 sampled LBK. Error bars represent one (dark) and two (light) standard deviations.

649

650 **Figure 5: The LBK/ALPC networks:** I: A) A heatmap showing the intensity of IBD,  
651 presenting the average total length of IBD segments > 12cM shared between all possible  
652 pairs by area or period. The numbers after the site names show the number of individuals  
653 per site included in these analyses. B) Regression of summed IBD >12cM shared between  
654 individuals of each pair of sites (averaged over all pairs), and geographic distance. Polgár-  
655 Ferenci-hat has more connections with closer sites supporting a localised ALPC community,  
656 while Asparn-Schletz and Nitra Horné Krškany do not show a clear association with distance,  
657 as would be expected if the western LBK expansion was so rapid that nearby groups were  
658 hardly more closely related than groups far apart.

659  
660 **Figure 6: Selection in Neolithic genomes:** (A) B1 scores in the ALPC. (B) B1 scores in the  
661 LBK. B1 shows regions with balancing selection, the highest signal on chromosome 6 at HLA.

662 **Extended Figure 1: Principal Component Analysis (PCA):** PCA performed with 879  
663 modern Eurasian individuals in which the ancient individuals were projected. The modern  
664 individuals have been removed from the image. The PCA shows the clustering of the LBK and  
665 the position of individuals along the X axis, indicating differential WHG affinities and showing  
666 that WHG (represented by two Körös culture outliers with entirely WHG ancestry) are more  
667 closely related to ALPC. Three individuals: I6914 (Austria\_LBK) and I1507, I497 (Körös) are  
668 outliers.

669  
670 **Extended Figure 2: qpWave plots:** qpWave plots to test for individual differentiation, with  
671 each population represented in one plot. Grey colour means results were highly significant  
672 (little genetically related). The number after the name of each individual relates is the point  
673 estimate of WHG ancestry from qpAdm. A) ALPC individuals. Individuals I21898, I10349,  
674 I21902, I18660, I10350, I18656, I18695, I4186, I1499, I21714, and I2377 are labelled in our  
675 analysis as ALPC outliers with high WHG ancestry. Individuals: I21828, I21830, I10351,  
676 I10352, I10353, I18657, I21767, 17933, I1500, I2380, I3537, I17455, I18636, I29883,  
677 I18641 and I4187 are labelled in our analysis as ALPC outliers with low WHG ancestry. B)  
678 Austria LBK Individuals: Individuals I27785, I25349, I6913, I6912 and I24028 are labelled  
679 in our analysis as outliers with high WHG ancestry. C) Germany LBK Individuals, D) Slovakia

680 LBK Individuals: Individual I18144 is labelled in our analysis as an outlier with high WHG  
681 ancestry. E) Transdanubia\_Hungary LBK Individuals: individuals I1882 and I1883 are  
682 labelled in our analysis as outliers with high WHG ancestry. We used qpWave from  
683 admixtools to perform the plots, each square represents the two-sided p-value of every  
684 single test.

685  
686 **Extended Figure 3: Parental haplogroups:** Distribution of the Y chromosome and mtDNA  
687 haplogroups per population. The Y-axis represents the number of individuals.

688  
689 **Extended Figure 4: Isotopic data:** Isotope data from Pólgar-Ferenci-hát. Here we plot the  
690 ratio  $\delta^{13}\text{C}/\delta^{15}\text{N}$ . Each dot represents one individual and the colour denotes the family.

691  
692 **Extended Figure 5: Rund of Homozigosity:** ROH distribution in the dataset. A) LBK  
693 individuals, B) ALPC individuals, C) Koros and Starcevo individuals. Individuals with more  
694 than 400,000 SNPs and the assessed ROH. Individuals in the ALPC group show a higher rate  
695 of close-kin unions (as reflected in the presence of ROH segments  $>20\text{cM}$ ) than the rest of  
696 the dataset.

697  
698 **Extended Figure 6: Natural selection in Neolithics:** *Tests for positive selection and long-*  
699 *term balancing selection in the ALPC and LBK population, made with the qqman<sup>64</sup>. The red*  
700 *lines indicate the top 0.05% cutoff. (A) Normalized iHS scores in ALPC. (B) Normalized iHS*  
701 *scores in LBK. (C) Normalized unphased iHS scores in ALPC. (D) Normalized unphased iHS*  
702 *scores in LBK. (E) Normalized nSL scores in ALPC. (F) Normalized nSL scores in LBK. (G)*  
703 *Normalized unphased nSL scores in ALPC. (H) Normalized unphased nSL scores in LBK.*

704  
705 **Extended Figure 7:** Correspondence between the ancestry in ALPC and LBK segments with  
706 the selection scan values. Each dot represents a region of 0.2 cM of the genome, in the Y-axis  
707 we display the average WHG ancestry of the region, and in the X-axis the average selection  
708 scores from candidate SNPs within the region (Supplementary Table S11). We show the two-  
709 sided Spearman correlation coefficients and p-value.

710

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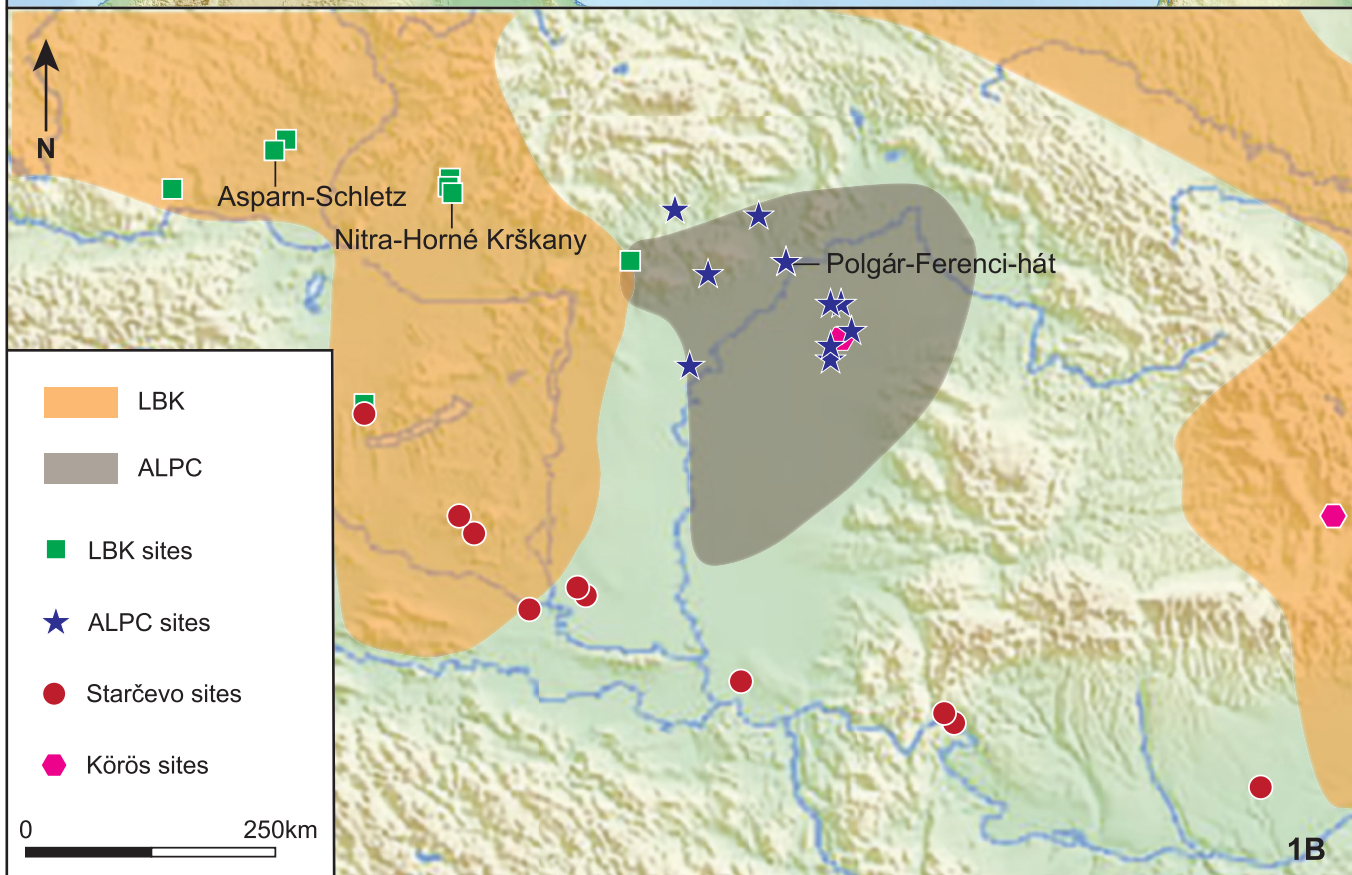
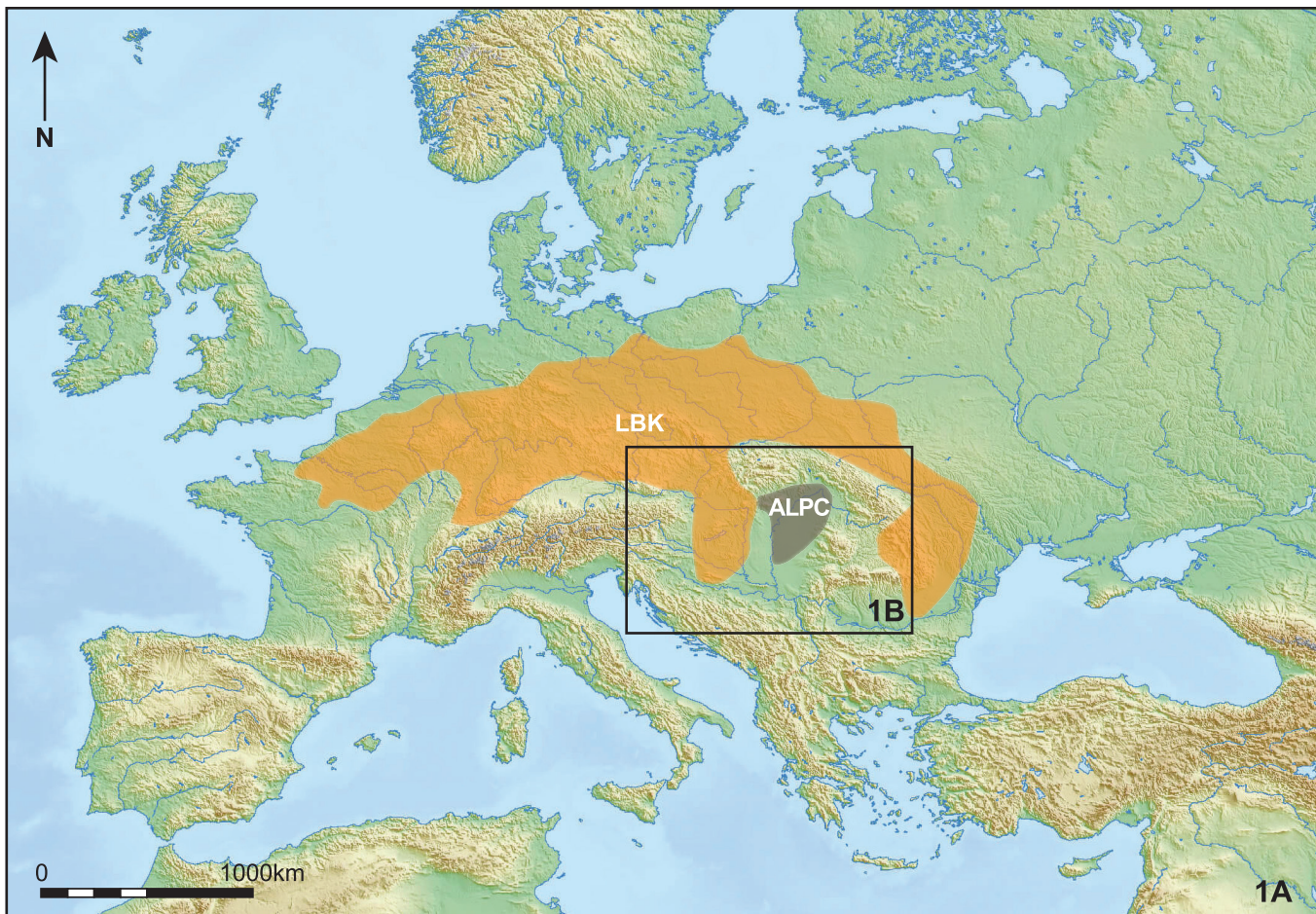
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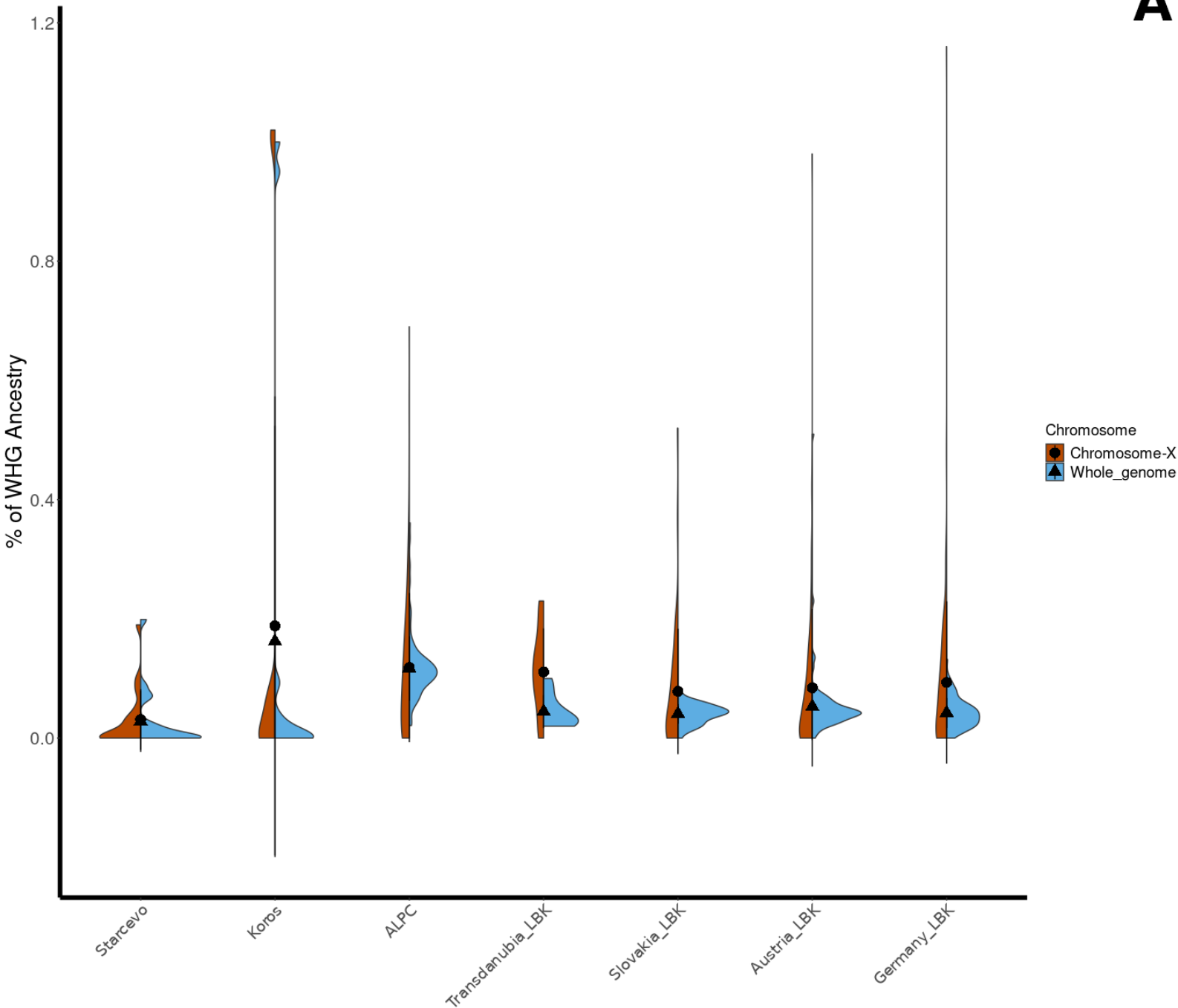
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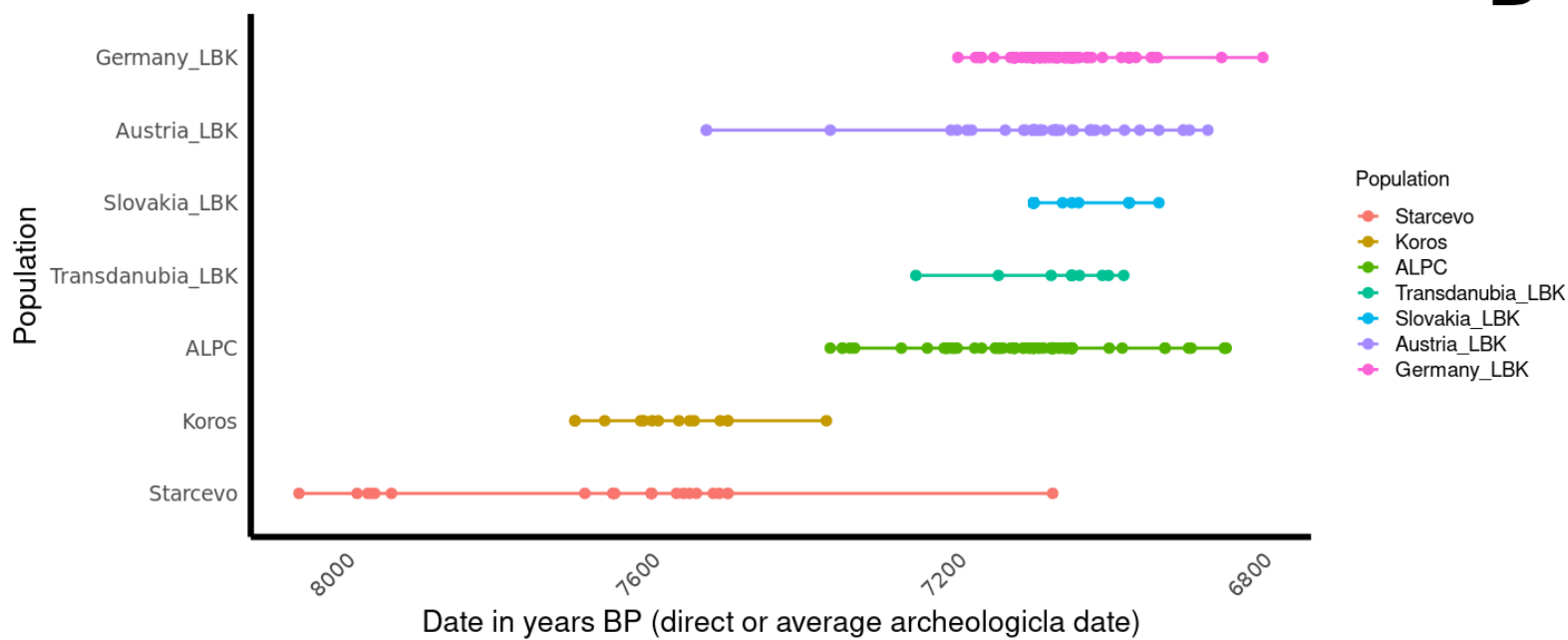
# WHG Ancestry in the studied populations from the individual qpAdm analyses

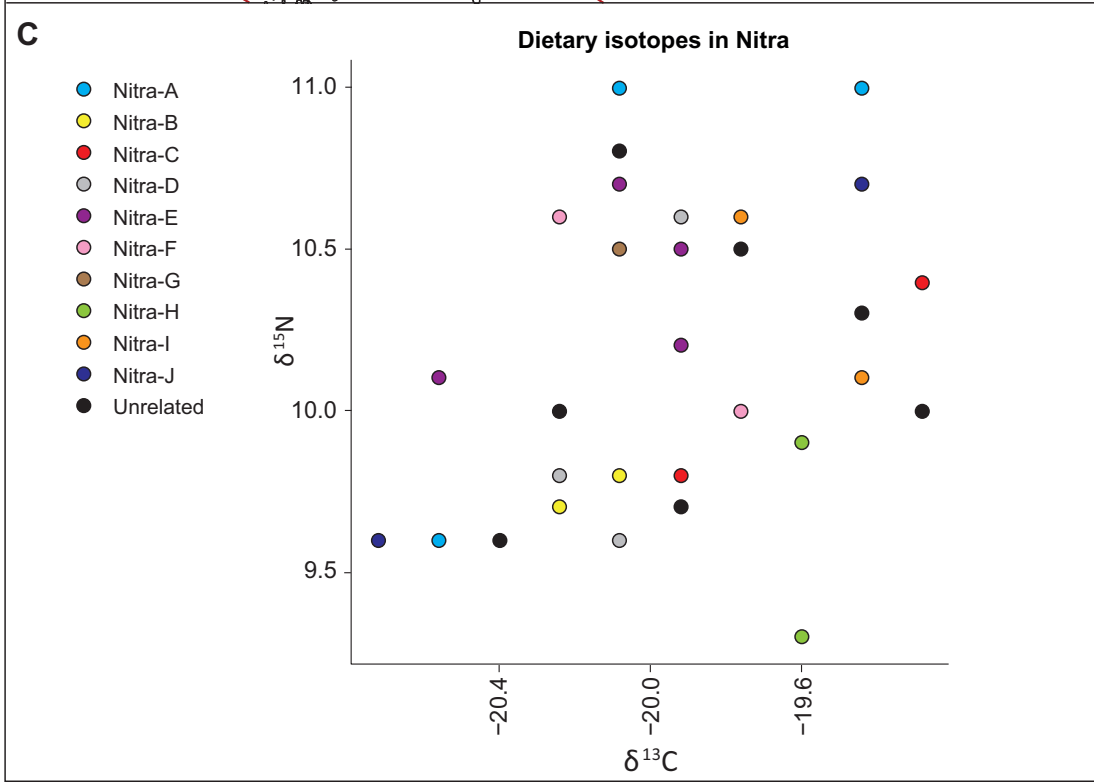
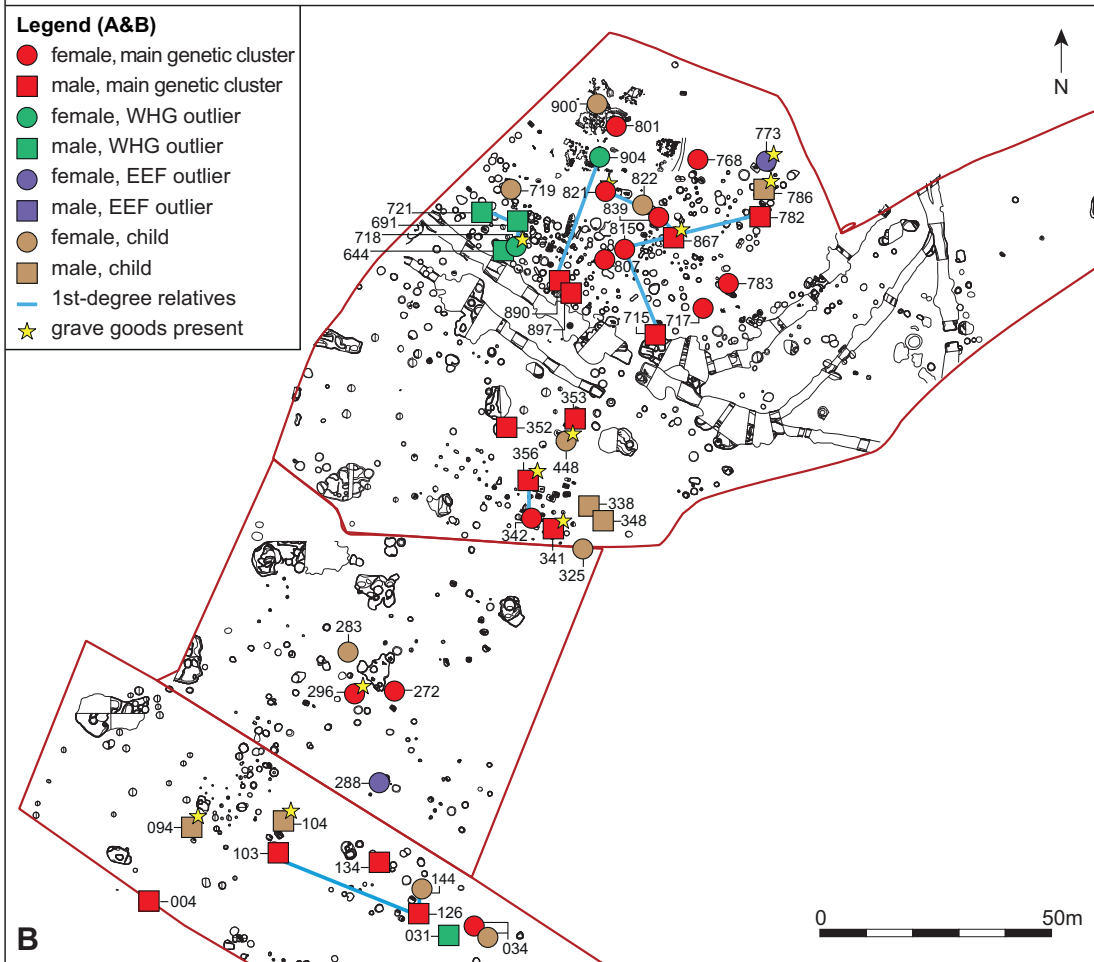
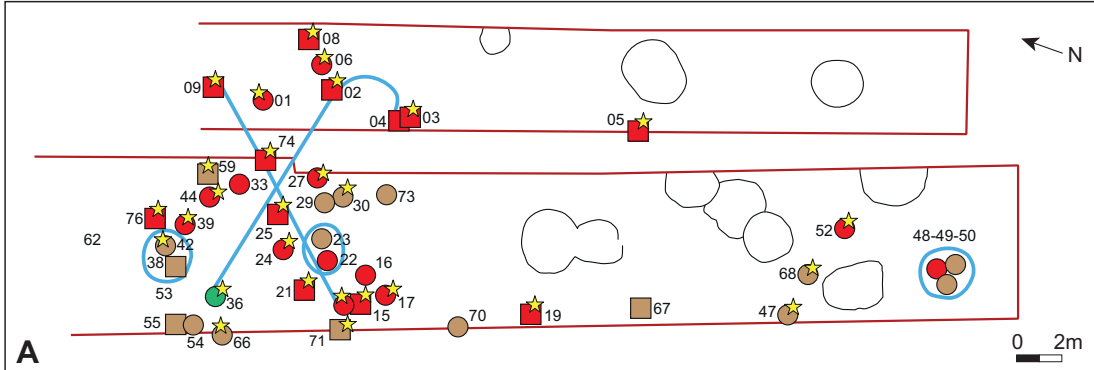
**A**

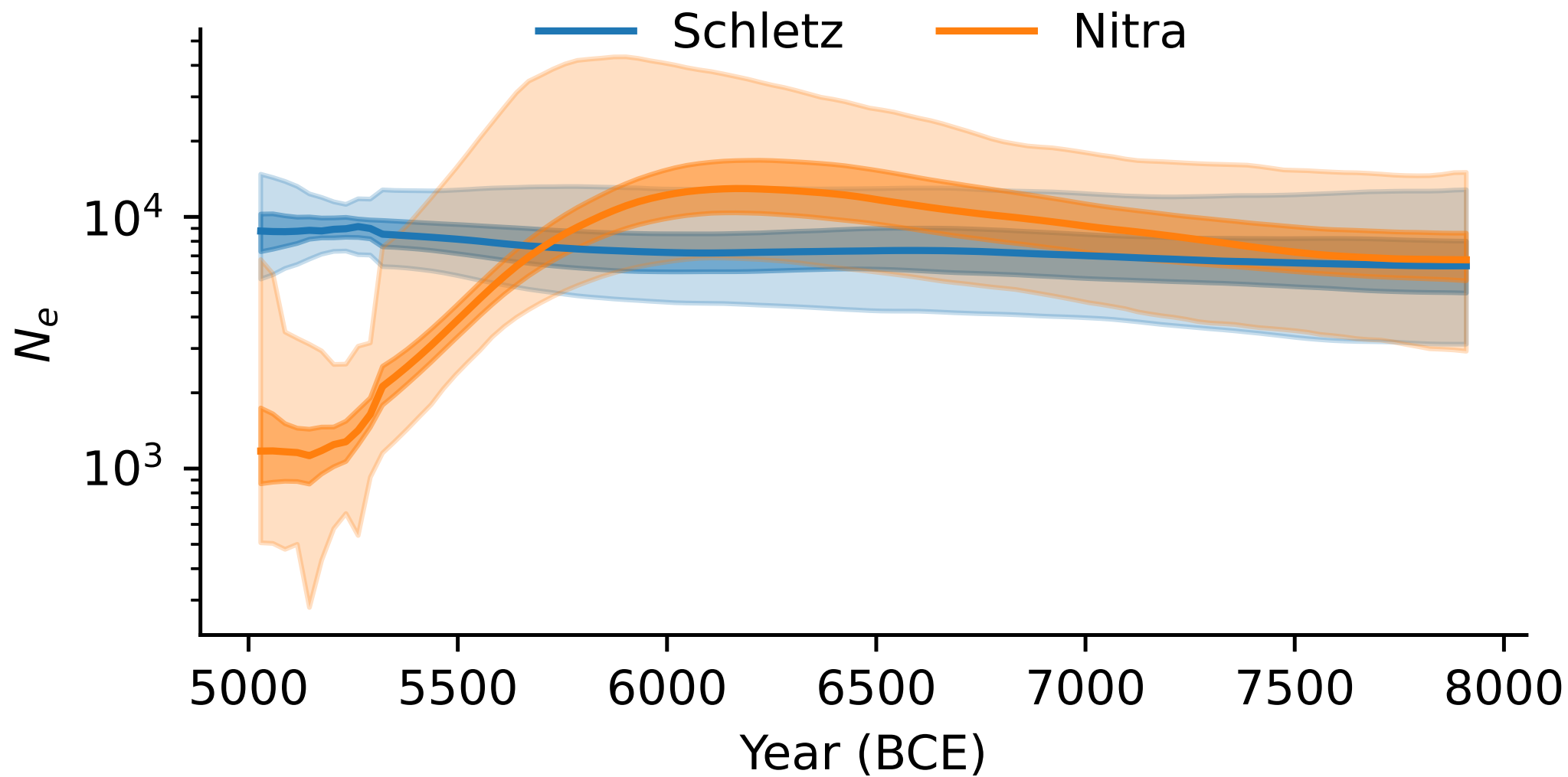


## Temporal range of the studied individuals

**B**



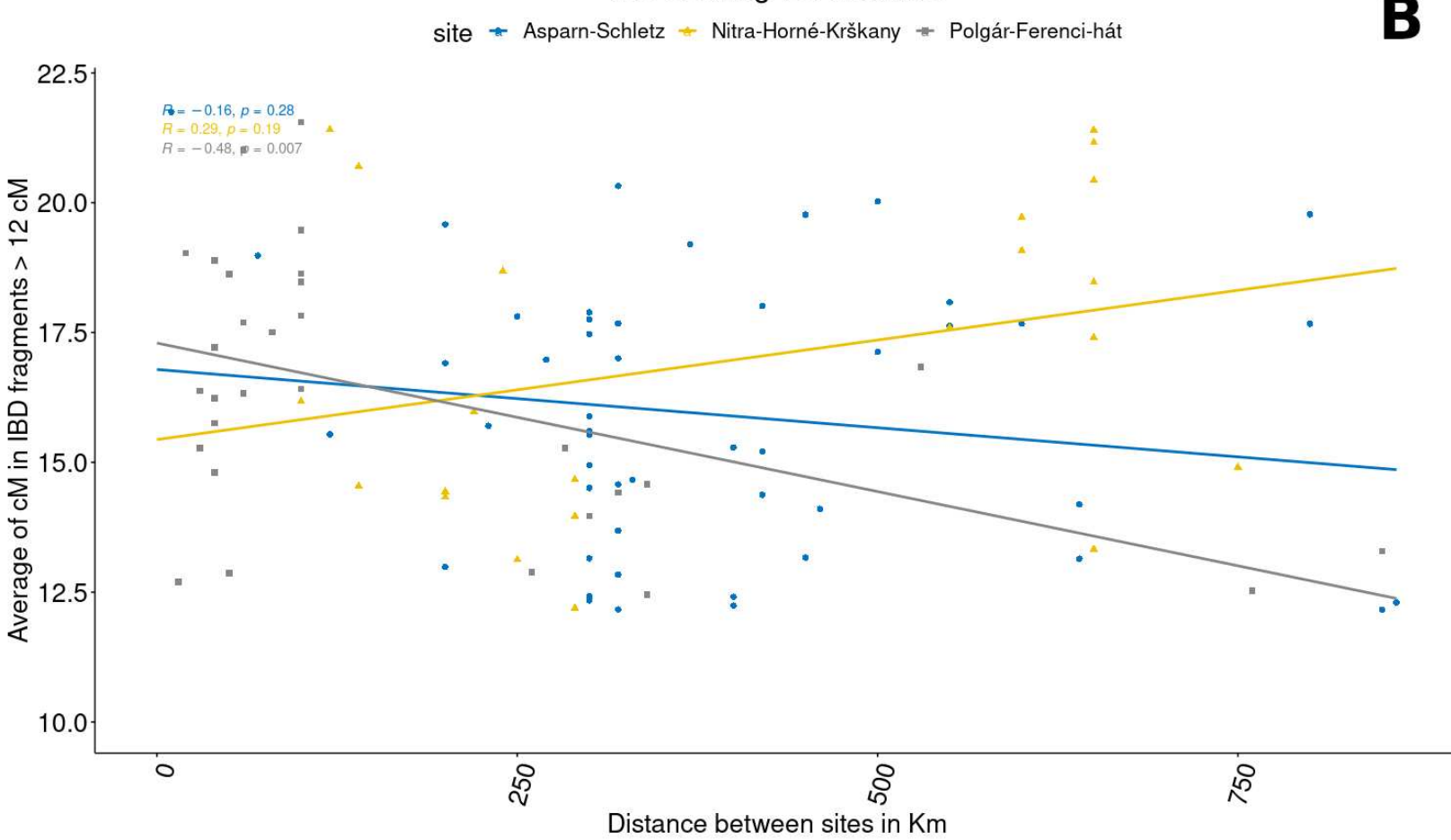


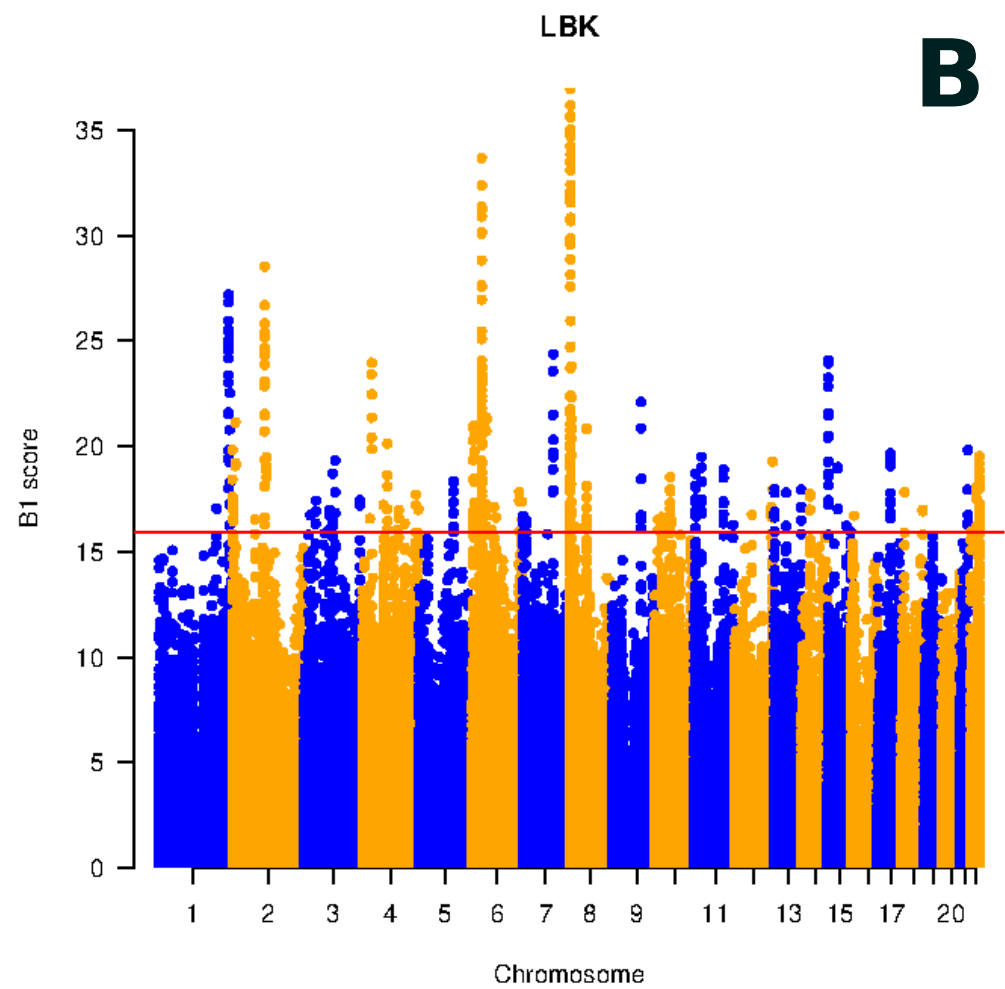
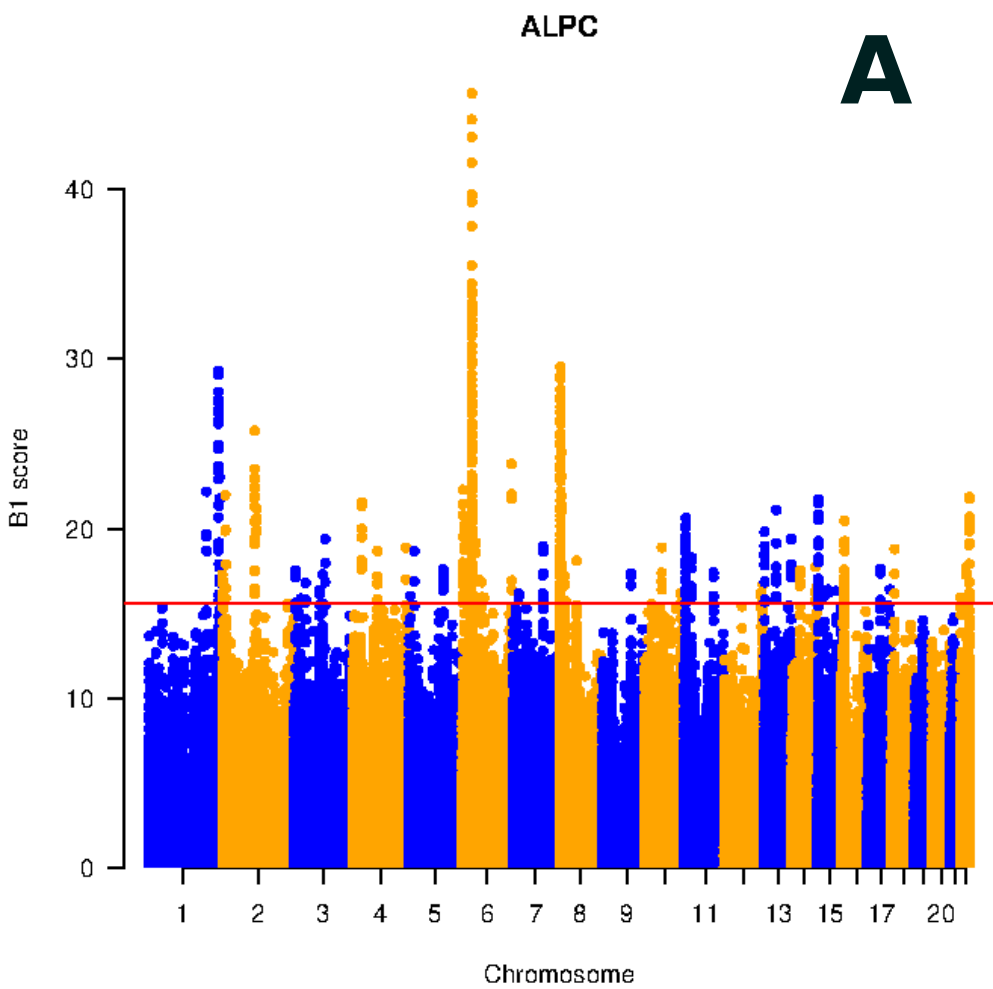


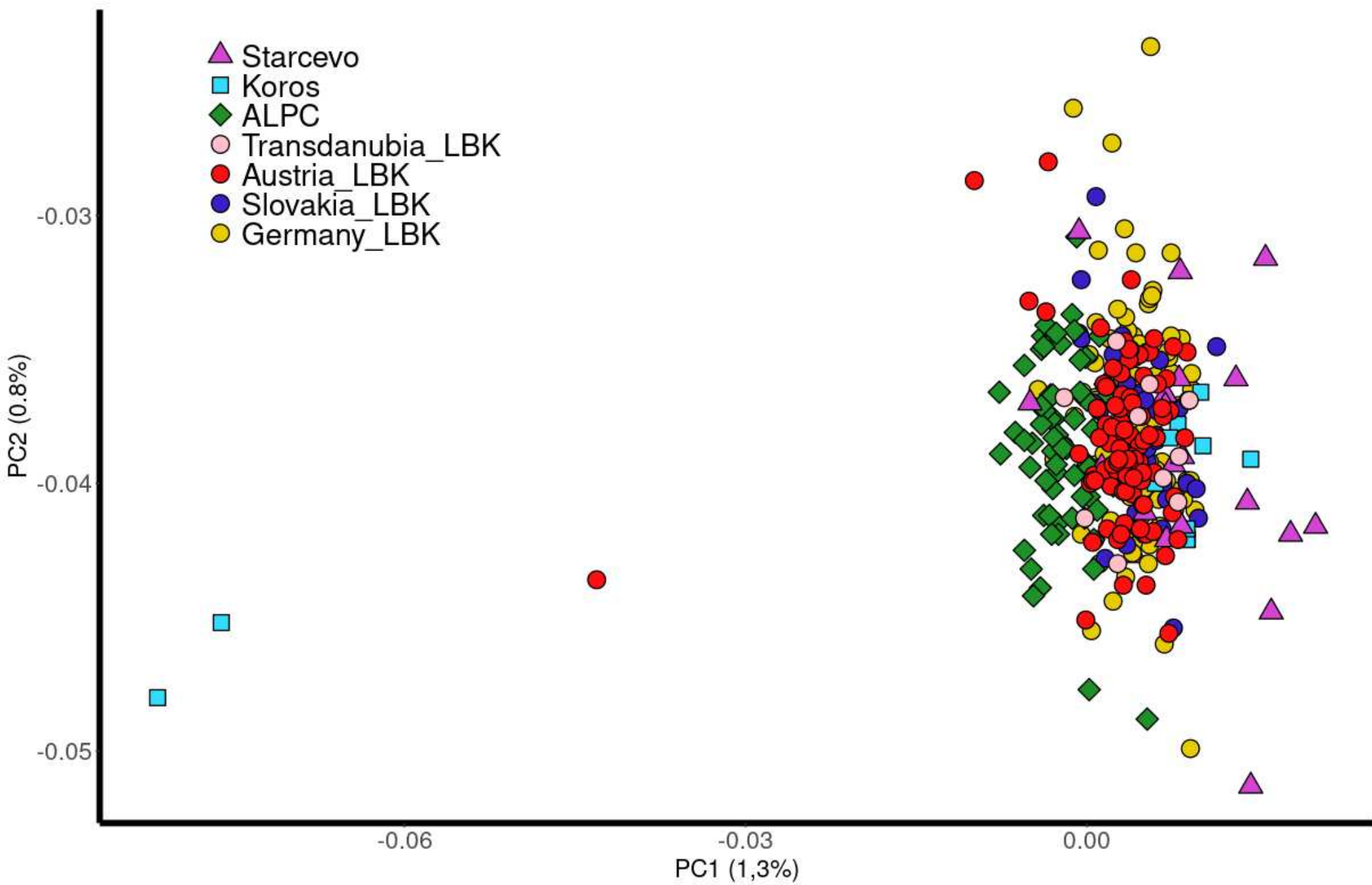
### IBD sharing between sites



### IBD sharing vs. distance

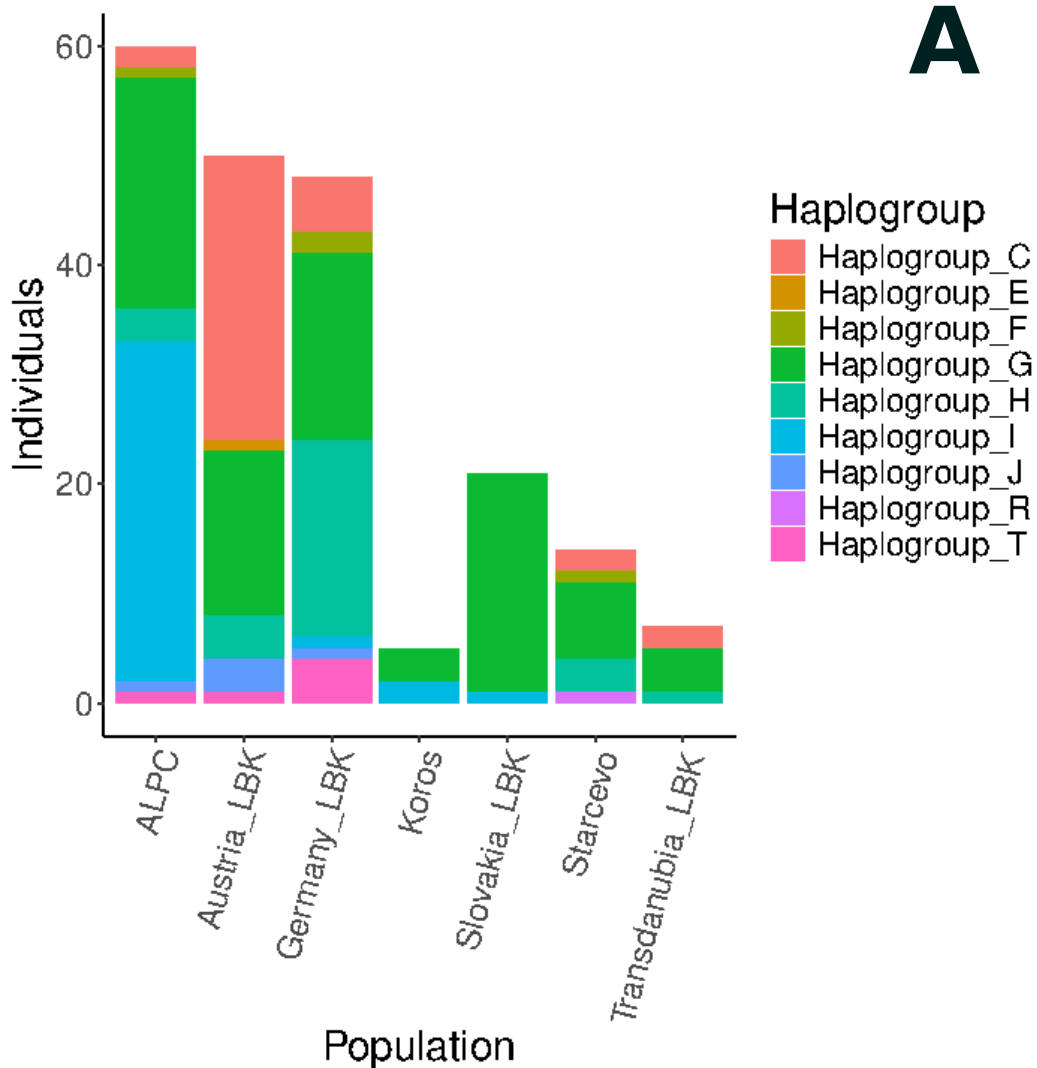




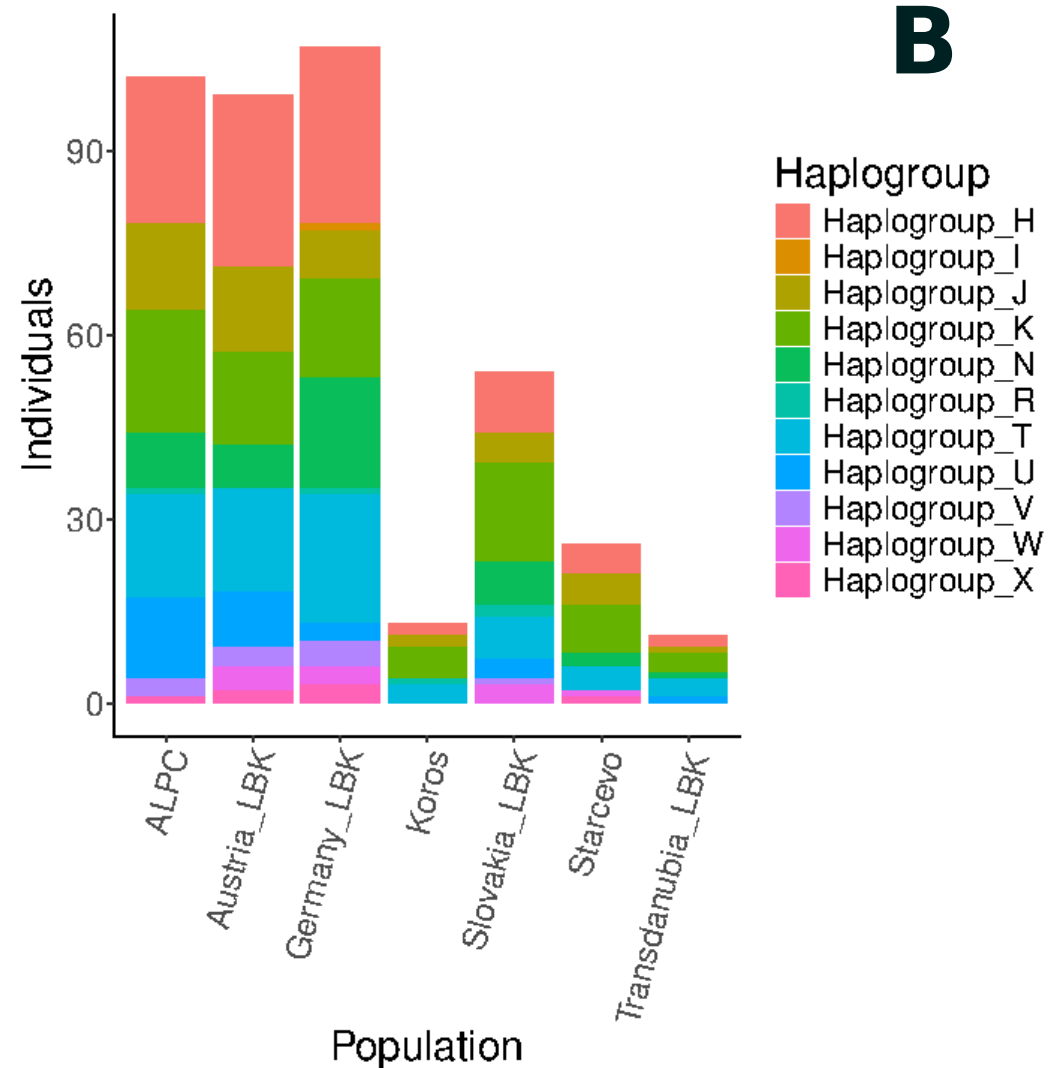




### Chromosome Y



### Mitochondria



# Dietary isotopes in Pólgar-Ferenci-hát

