

# 1 Socio-cultural practices may have affected sexual 2 dimorphism in stature in Early Neolithic Europe

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15

## Abstract

16 The rules and structure of human culture impact health and disease as much as genetics  
17 or the natural environment. To study the origin and evolution of these patterns, we take a  
18 multidisciplinary approach combining ancient DNA, skeletal metrics, paleopathology, and stable  
19 isotopes. Our analysis focuses on cultural, environmental, and genetic contributions to variation  
20 in stature in four populations of Early Neolithic Europe. In Central Europe, low female stature  
21 is likely due to male preference in resource allocation under conditions of stress. In contrast,  
22 shorter male stature in Mediterranean populations may reflect a lack of preference. Our analysis  
23 suggests that biological consequences of sex-specific inequities can be linked to culture as early  
24 as 7000 years before present. Understanding these patterns is key to interpreting the evolution  
25 of genetic and socio-cultural determinants of health, and our results show that culture, more  
26 than environment or genetics, drove height disparities in Early Neolithic Europe.

## 27 1 Introduction

28 Human skeletal variation reflects varying combinations of genetic, cultural, and environmental fac-  
29 tors. While there are many links between culture and health in the modern world, the history and  
30 evolution of these relationships are not always well established. Due to the entanglement of these  
31 factors, our ability to draw conclusions about their effects has been limited in archaeological data.  
32 With the recent advent of ancient DNA sequencing technology, genetic information from ancient  
33 populations has become increasingly available. However, attempting to analyze changing patterns of

34 variation based solely on genetic data is difficult—genotypes do not necessarily equate to phenotypes  
35 due to the effect of the environment.<sup>1</sup> Similarly, while it is tempting to predict phenotypic changes  
36 in ancient people based on their genetic variation, this is currently challenging as genetic effects are  
37 not always transferable across populations.<sup>2</sup> Our solution is to integrate these complementary fields  
38 to construct multidisciplinary analyses with phenotype, genotype, culture, and environment data  
39 from ancient human populations. This approach allows us to begin to separate the effects of these  
40 variables and reveal the interactions between genes, environment, and culture which are critical in  
41 shaping human health and variation.

42 Many traits of interest, including height, are highly polygenic, with thousands of independent  
43 genetic variants contributing significantly to heritability. One common approach to addressing the  
44 role of genetics in morphological change is to compare patterns of phenotypic variation with genetic  
45 ancestry or genome-wide patterns of genetic variation.<sup>3–5</sup> However, even for highly polygenic traits  
46 like height, genome-wide variation may not be directly relevant, leading to spurious associations  
47 between genetic effects, ancestry, and environmental confounds. For example, if a population is tall  
48 and has a high proportion of ancestry from Neolithic sources, it could be concluded that Neolithic  
49 ancestry is associated with “genetic tallness”; however, the effects could equally be non-genetic  
50 and related to lifestyle changes associated with agriculture. An alternative approach is to focus  
51 only on genetic variation that is known to be associated with a specific trait.<sup>6,7</sup> Effect sizes for  
52 these trait-related variants estimated from genome-wide association studies (GWAS) of present-day  
53 individuals can be combined with genetic data from ancient individuals to calculate polygenic risk  
54 scores (PRS), which can be thought of as estimated genetic values for the phenotype. In European  
55 ancestry populations, polygenic scores for height can explain up to 25% of phenotypic variation  
56 in present-day individuals,<sup>8</sup> and 6–8% of variation in ancient individuals.<sup>9,10</sup> On a broad scale,  
57 temporal changes in polygenic score over time in Europe are qualitatively consistent with changes  
58 in stature as inferred from the skeletal record,<sup>11</sup> while local deviations from this pattern provide  
59 evidence of environmental effects.<sup>10,11</sup>

60 Analyses of human populations over tens of thousands of years involve individuals that are  
61 diverse in genetic ancestry, environment, and culture and it is challenging to exclude the possibility  
62 of confounding by unmeasured variables. We therefore focus specifically on the European Early  
63 Neolithic. One of the most studied periods in prehistory, it represents a fundamental shift in  
64 technology, culture, and genetics. In particular, the *Linearbandkeramik* (LBK) culture of Central  
65 Europe is one of the most comprehensively documented Early Neolithic cultures, with an abundance  
66 of excavated settlements and cemeteries.<sup>12</sup> LBK groups tended to choose settlement locations based  
67 on the presence of rich loess soils for farming, and the northern edge of these soils appears to  
68 delineate the northern limit of LBK sites.<sup>13,14</sup> Bioarchaeological evidence indicates broad regional  
69 differences between individuals from northern settlements in this agricultural boundary zone vs  
70 southern settlements in a climate zone that was more comfortable for Neolithic crops.<sup>15,16</sup> Based  
71 on this, we divided our Central European group into Northern (above 50°N latitude) and Southern

72 (below 50°N) populations. The Mesolithic hunter-gatherer population in Central Europe made a  
73 limited genetic contribution to the LBK population, whose members harbor only traces of hunter-  
74 gatherer admixture.<sup>7,17,18</sup> Contemporary populations from southeastern Europe have similarly low  
75 levels of hunter-gatherer ancestry.<sup>19</sup> In contrast, Neolithic southern European populations associated  
76 with the Cardial and Impressed Ware cultures followed a separate migration route (Figure 1),  
77 occupied a milder climate zone, and carried more Mesolithic ancestry.<sup>17,20</sup> Individuals in this region  
78 tend to be shorter than those from Central Europe and combined with their admixed ancestry this  
79 has led to suggestions of a genetic basis for decreased statures in this region.<sup>7,21</sup>

80 By comparing and contrasting these four closely related archaeological populations, we aim to  
81 investigate how differences in environment and genetics combine to produce observed phenotypes.  
82 We collected genetic data, skeletal metrics, paleopathology, and dietary stable isotopes to begin  
83 separating the effects of each on Neolithic stature trends. By specifically investigating and con-  
84 trolling for the effects of genetics in these samples, we are able to provide nuanced interpretations  
85 of height variation, gain a better understanding of the aspects of height which are controlled by  
86 genetics or environment, and show evidence for sex-specific cultural effects which modify the ge-  
87 netically predicted patterns. We illustrate the strengths of leveraging multidisciplinary datasets,  
88 and indicate caution when analyzing genotype-phenotype relationships without complete data, es-  
89 pecially for traits which are not preserved in the archaeological record and cannot be directly tested.  
90 This integrated analysis highlights the role of plasticity in morphology, and establishes culturally  
91 mediated disparities at least as early as the European Neolithic.

## 92 2 Results

### 93 2.1 Distribution of stature, polygenic scores, and stable isotope values

94 We collected either genetic, dietary stable isotope, paleopathology, or skeletal metric data from  
95 1282 individuals associated with the archaeological LBK culture in Northern and Southern Central  
96 Europe dated to between 7700-6900 years before present (BP), as well as 135 individuals from  
97 the southeastern (Balkan) and 160 individuals from the southern (Mediterranean) regions dated  
98 between 8000-6000 BP. All individuals included in the analysis have at least one of the four data  
99 types available (Materials and Methods; Figure 2; Supplementary Figure 1).

100 Observed patterns of femur length vary between sexes and populations. Male femora show no  
101 apparent difference between the Central and Balkan regions ( $p=0.56$ ), but Mediterranean males are  
102 significantly shorter ( $p=5.5 \times 10^{-7}$ ,  $\beta=-1.44\text{cm}$ ). Conversely, female femora show a different pattern,  
103 with no significant difference between Mediterranean, South Central ( $p=0.97$ ), and Balkan ( $p=0.54$ )  
104 populations, but substantially shorter values in the North Central population ( $p=9 \times 10^{-07}$ ,  $\beta=-$   
105  $2.0\text{cm}$ ) (Figure 3A). Differences between male and female femur lengths are highly significant in all  
106 populations ( $p<2 \times 10^{-16}$ ). In contrast to the differences in femoral lengths, polygenic scores for height  
107 are very similar between all populations (pairwise t-tests  $p > 0.9$ ) using the clumping/thresholding

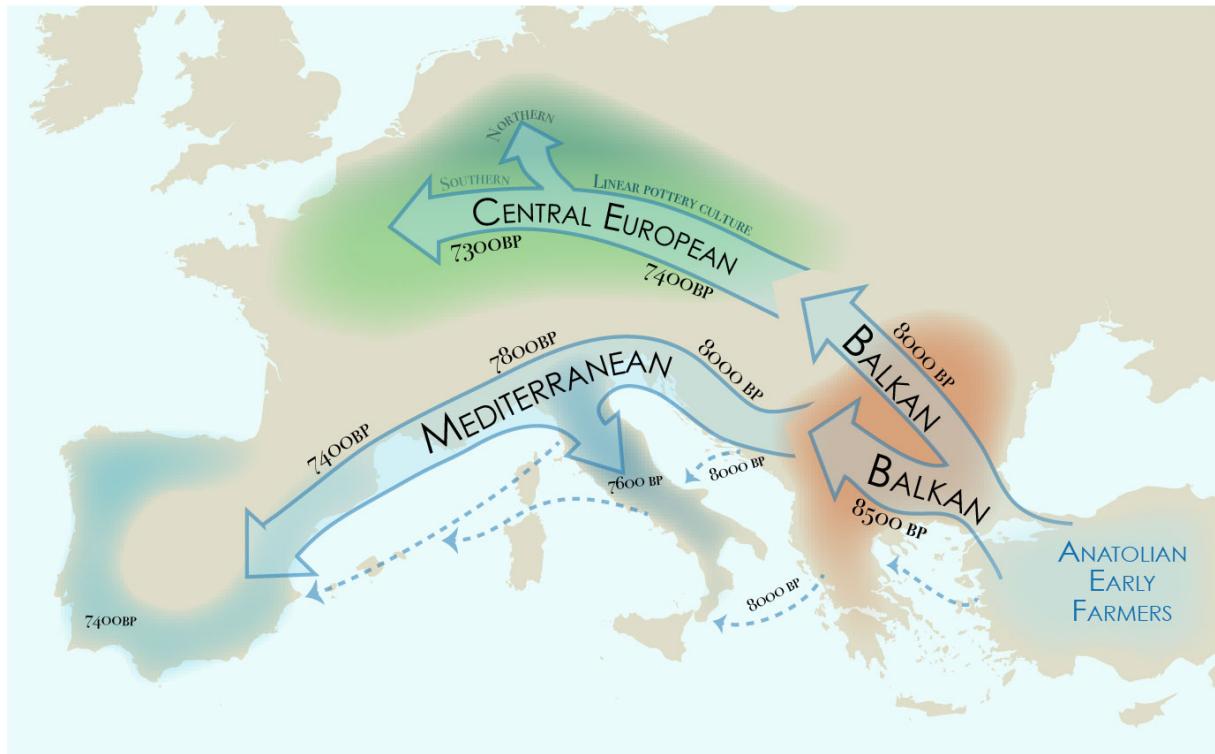


Figure 1: This schematic illustration highlights the two main migration routes from Anatolia to Europe during the Early Neolithic period.<sup>22</sup> Populations followed two routes: southern, along the Mediterranean coast (including sea routes, generalized here by dashed blue lines) where they admixed with existing hunter-gatherer populations; or northern, through the Balkans and into Central Europe, with only limited hunter-gatherer admixture. We analyze patterns within the Linearbandkeramik culture, dividing it into Northern and Southern Central European groups.

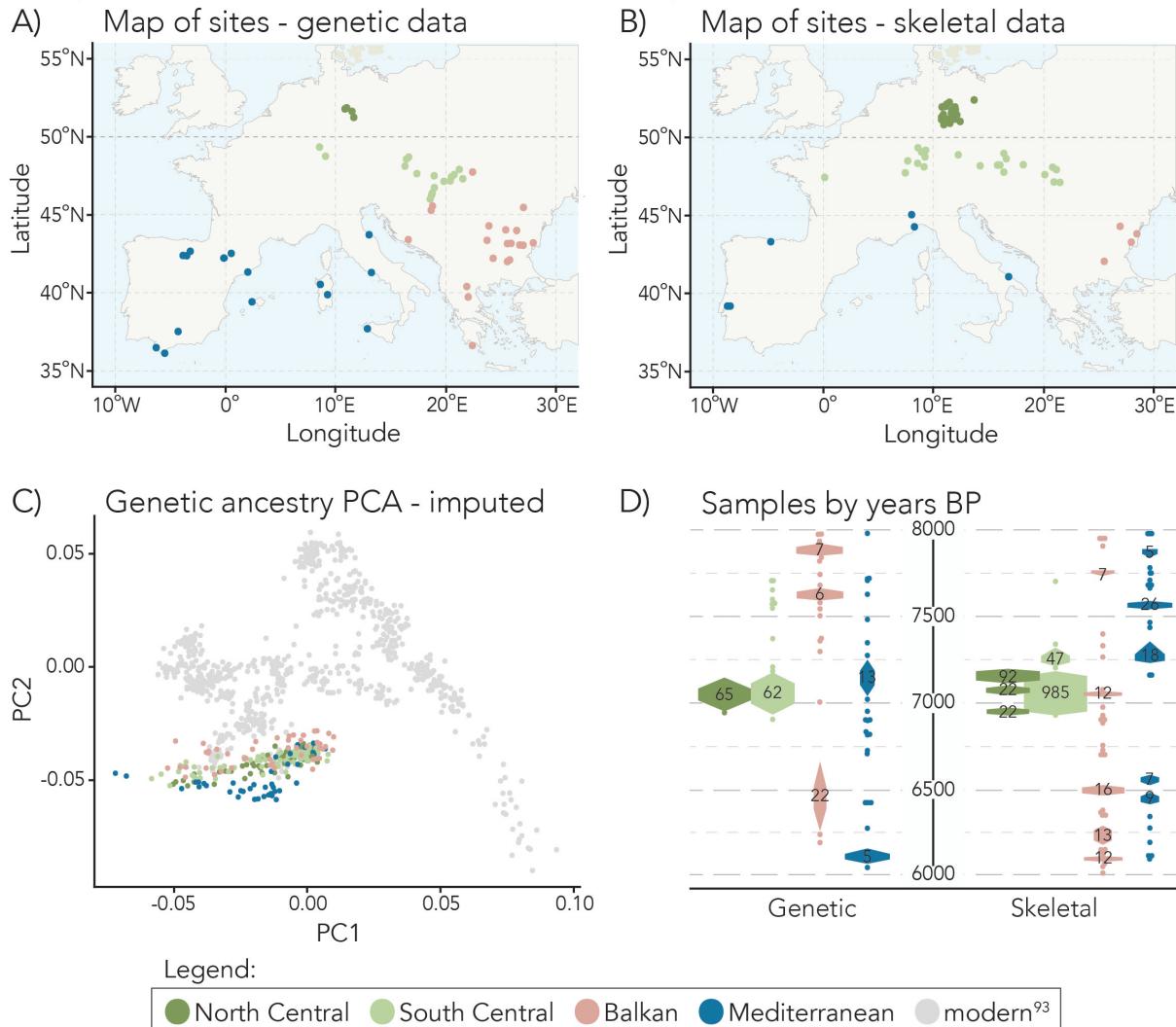


Figure 2: *Upper row: Sites used for genetic (A) and skeletal (B) data in the analysis. The Central European population is split into Northern and Southern groups at 50°N latitude (emphasized). Lower row: (C) imputed genetic data projected into the PCA space of 777 modern Eurasian individuals (grey points). (D) sample numbers by years before present (years BP) for skeletal (right) and genetic (left) data.*

108 PRS construction (Figure 3B). PRS constructed with LDpred show Mediterranean individuals to be  
109 shorter than the other populations ( $p=0.002$ ; Supplementary Figure 5). However, PRS constructed  
110 using summary statistics derived from between-sibling analysis finds similar genetic values in all  
111 populations with both PRS construction methods, so we conclude that apparent lower Mediter-  
112 ranean PRS may be due to population stratification in the GWAS data and may not reflect a  
113 true genetic difference. There are no significant differences between male and female PRS in any  
114 population (Figure 3B), providing no evidence for a genetic basis to this dimorphism.

115 Signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  suggest different dietary patterns in each of the analyzed groups  
116 (Figure 3C, D). Both the Mediterranean and Balkan groups are significantly distinct from the  
117 Central in  $\delta^{13}\text{C}$  (multiple comparisons, maximum  $p=4.0 \times 10^{-16}$ ) and  $\delta^{15}\text{N}$  (multiple comparisons,  
118 maximum  $p=7.7 \times 10^{-13}$ ) values. Generally, the Balkan population is characterized by high  $\delta^{15}\text{N}$   
119 values, while Mediterranean populations show high  $\delta^{13}\text{C}$  relative to the Central Europeans (Figure  
120 3C). The exception to this pattern is a cluster of individuals, classified as Balkan in our analysis,  
121 which overlaps with the North Central population as well as some of the Mediterranean. These  
122 points represent individuals from present-day Greece and indicate that the diets of these peoples  
123 might better be classified as Mediterranean than Balkan. Nitrogen values are generally elevated in  
124 males compared to females (Figure 3D), but this difference is only significant in the Mediterranean  
125 ( $p=0.035$ ).

## 126 2.2 Patterns of non-genetic factors in Central Europe

127 The most dramatic observation is the difference in female stature and consequent sexual dim-  
128 porphism in Northern compared to Southern Central Europe. Female femora in the North are  
129 significantly shorter than female femora in the South ( $p=2.7 \times 10^{-6}$ ,  $\beta=1.7\text{cm}$ ), while male femora  
130 are highly similar ( $p=0.35$ ) (Figure 3A). On average, male femora from the North are about 13%  
131 longer than female femora, Southern Central and Balkan male femora are about 9% and 11% longer  
132 respectively, and Mediterranean male femora are only 5% longer (Figure 3A). These values are  
133 reduced slightly when calculated using estimated statures instead of femora (North Central: 10%,  
134 South Central: 7%, Balkans: 8%, Mediterranean: 4%), possibly due to error associated with stature  
135 estimation (see Ref. 9) and body proportions, or because the relationship between femur length and  
136 stature is different between males and females. Where we have both genetic and metric data for  
137 the same individuals, there is a qualitative relationship between femur length and PRS; PRS tends  
138 to increase as femur lengths increase (Supplementary Figure 3B). However, the effect of PRS on  
139 femur length is only marginally significant ( $p=0.05$ ), likely due to the small number of individuals  
140 with both types of data available in the sample ( $n=55$ ).

141 Overall, trends in dietary stable isotopes show that both males and females in Southern Cen-  
142 tral Europe have significantly higher  $\delta^{15}\text{N}$  (male  $p=1.3 \times 10^{-9}$ ,  $\beta=0.83\text{\textperthousand}$ ; female  $p=5.3 \times 10^{-9}$ ,  
143  $\beta=0.87\text{\textperthousand}$ ) and lower  $\delta^{13}\text{C}$  (male  $p=3 \times 10^{-4}$ ,  $\beta=-0.30\text{\textperthousand}$ ; female  $p=8.1 \times 10^{-7}$ ,  $\beta=-0.38\text{\textperthousand}$ ) as  
144 compared to the North. However, while males in both regions qualitatively have higher nitrogen,

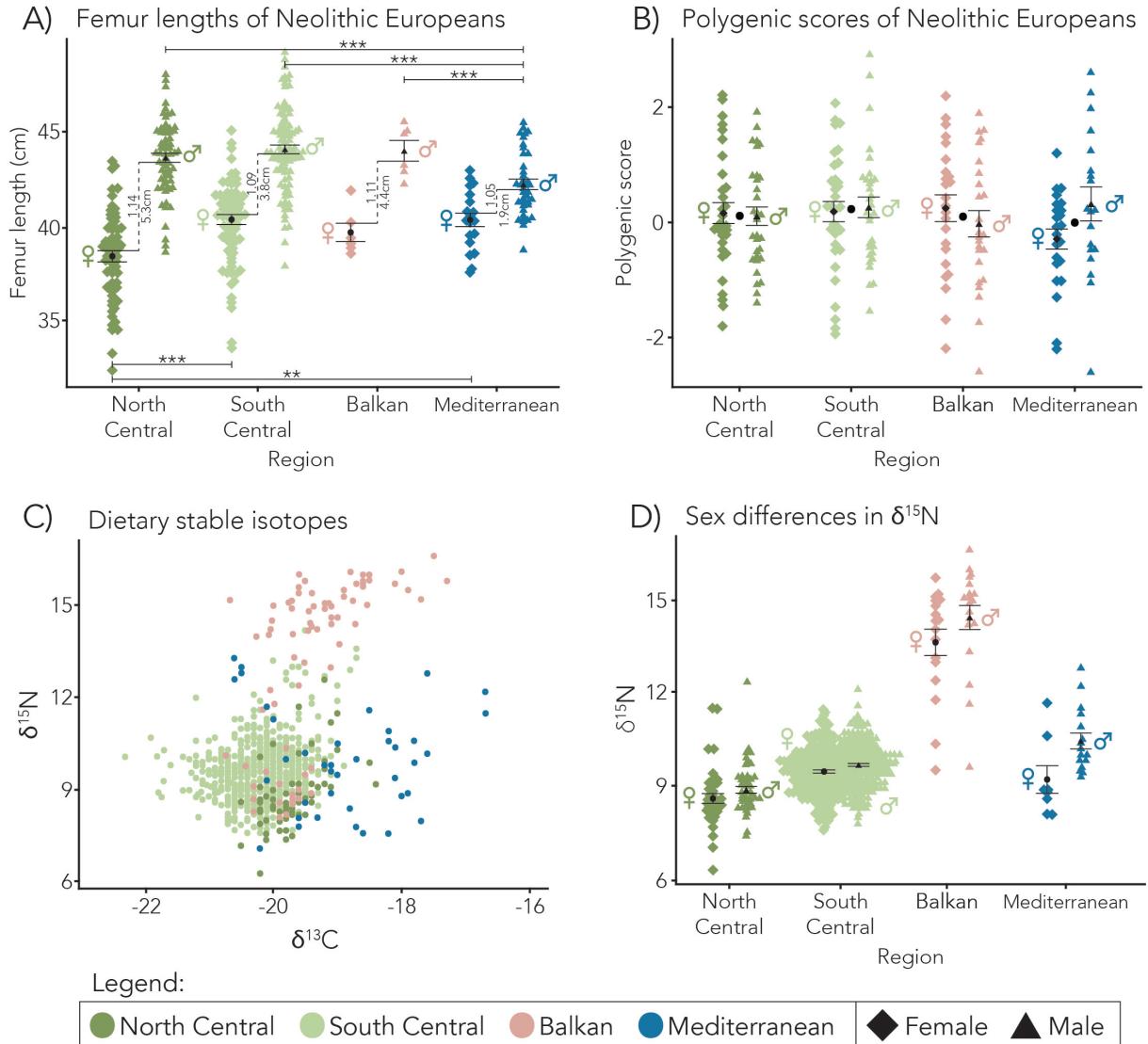


Figure 3: Solid bars across the tops of plots indicate significant differences between male populations by pairwise t-test; solid bars below plots indicate significant differences between female populations by pairwise t-test; p-values  $< 0.05$  (\*),  $< 0.01$  (\*\*) and  $< 0.001$  (\*\*\*) $; black points indicate the mean of each group; and vertical bars show mean standard error. A) Femur length in the four population: values to the right of the vertical dotted line are the difference between the mean male and female femora; values to the left are the sexual dimorphism ratios of male/female femur lengths for each population. B) Polygenic scores for the four populations show similar scores for individuals across all regions. Differences between male and female PRS are not significant. C) Plot of  $\delta^{13}\text{C}$  (x-axis) and  $\delta^{15}\text{N}$  (y-axis) dietary stable isotopes for the four populations: individuals from the Balkans are distinguished by high nitrogen values, while those in the Mediterranean generally have higher carbon. D) Sex differences in  $\delta^{15}\text{N}$  values by sex for each population:  $\delta^{15}\text{N}$  values are slightly higher for males in all populations, but this difference is only significant in the Mediterranean ( $p=0.035$ ).$

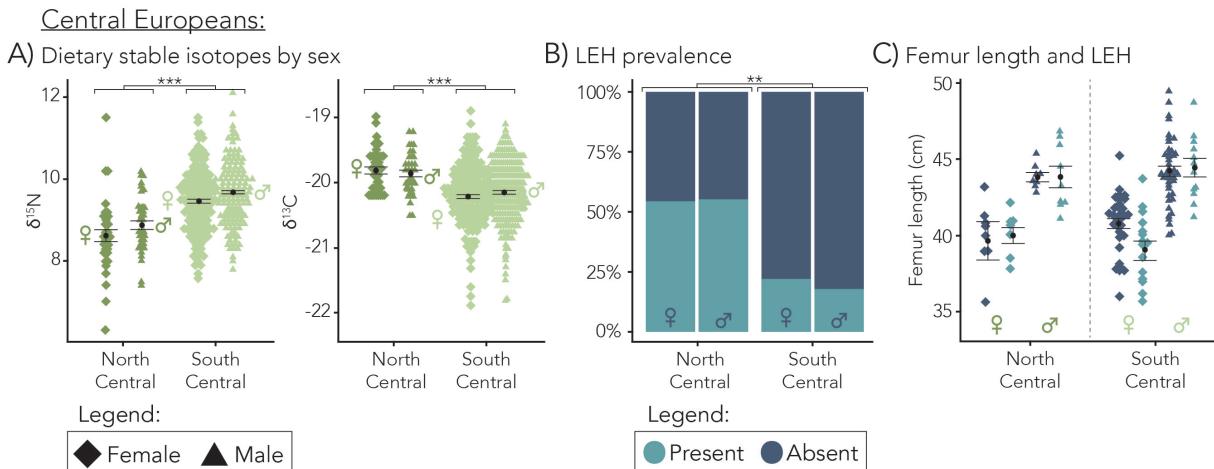


Figure 4: *Evidence of environmental stress in Northern Central Europe. A) Differences in  $\delta^{13}\text{C}$  (right) and  $\delta^{15}\text{N}$  (left) values. Overall, the South has higher nitrogen values than the North ( $p=6.8 \times 10^{-13}$ ), and lower carbon ( $p=5.3 \times 10^{-15}$ ); within each population, the difference in isotopes between sexes is not significant. B) Proportion of linear enamel hypoplasias. The South has significantly less than the North ( $p=0.001$ ). C) Presence of linear enamel hypoplasia is significantly associated with shorter femora ( $p=0.02$ ); differences in prevalence between sexes are not significant.*

145 the interaction effect between sexes is not significant in either North or South (Figure 4A), indicating  
 146 that the difference between male and female values in each region is not significant. There is  
 147 no difference in carbon values between sexes. For individuals with both stature and stable isotope  
 148 values, we find no statistically significant relationship between femur length and  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  in  
 149 either Central group, separately or combined, but the sample is small.

150 We do, however, find a statistically significant relationship between presence of linear enamel  
 151 hypoplasias (LEH) and shorter femora, suggesting that LEH may reflect an underlying variable in  
 152 childhood that also affects stature ( $p=0.021$ ,  $\beta=-1.0\text{cm}$ ) (Figure 4C). Both males and females from  
 153 the North are more likely to have LEH than individuals living in the South ( $p=0.002$ ). Indeed, over  
 154 50% of the Northern sample have LEH while they are only present in about 20% of the Southern  
 155 (Figure 4B). There is no significant difference between the number of males and females with LEH in  
 156 either region. Though the interaction effect between sex and LEH on femur length is not significant,  
 157 qualitatively the effect of LEH on femur length appears greater in females than in males (Figure  
 158 4C). When the sexes are analyzed separately, females with LEH do have significantly shorter femora  
 159 than those without ( $p=0.018$ ,  $\beta=-1.46$ ), which is not the case for males ( $p=0.479$ ). We hypothesize  
 160 that the relationship between LEH and femur length is driven by females, but we lack an adequate  
 161 sample size to detect the interaction effect in the full model. Incidence of cribra orbitalia is also  
 162 significantly higher in the Northern region than in the Southern ( $p=1.8 \times 10^{-6}$ ), though there is no  
 163 relationship with femur length. There are no significant trends related to the presence of porotic  
 164 hyperostosis.

165 In summary, comparison of Northern and Southern Central Europe identifies no predicted genetic  
 166 difference in stature, which is consistent with male but not female femur length. This suggests a

167 non-genetic basis for reduced female stature. Stable isotope data and skeletal stress indicators  
168 suggest lower protein intake and more general stress in the North; however, males and females  
169 overall appear equally affected by these variables. Despite a similar number of hypoplasias in both  
170 sexes, shorter femora in females suggest that increased general stress, due to other unmeasured  
171 environmental or cultural factors, leads to a female-specific reduction in stature.

## 172 2.3 Patterns of genetic ancestry in the Mediterranean

173 In contrast to Northern Central Europe, Mediterranean Neolithic males are shorter than other  
174 groups, but females are not. PCA indicates that individuals from the Central regions and the  
175 Balkans share similar genetic ancestry while those from the Mediterranean are distinct (Figure 2C;  
176 unimputed PCA in Supplementary Figure 4A), a difference known to be due to higher levels of  
177 hunter-gatherer ancestry in the Mediterranean.<sup>17</sup> We therefore additionally compared our samples  
178 to Mesolithic individuals of Western Hunter-Gatherer (WHG) ancestry, as well as individuals from  
179 early Neolithic Anatolia. These two groups represent source populations for the two largest ancestry  
180 components in Europe at this time.<sup>7,17</sup>

181 On the PCA plots of these extended data, Neolithic Anatolians cluster with the Central and  
182 Balkan groups. While Mediterranean are near the farmer cluster, they are shifted towards the  
183 WHG (Figure 5A; unimputed PCA in Supplementary Figure 4B). ADMIXTURE analysis of all six  
184 populations supports this conclusion, showing significantly increased proportions of WHG ancestry  
185 in the Neolithic Mediterranean as compared with the other groups (maximum p=0.002 vs the  
186 Balkans, Fig. 5C). The average proportion of WHG ancestry in the Mediterranean is 11.4%; in the  
187 Balkans, 5.3%; in the South Central, 4.1%; and in the North Central, 1.1%. If there are significant  
188 PRS differences between Mediterranean and other populations, they are likely linked to this greater  
189 WHG ancestry and reflect genetic differences between WHG and other populations.

190 Computing PRS using clumping/thresholding, we find that the WHG have the lowest PRS of  
191 any population in our data (maximum p=0.002 vs Mediterranean), while Anatolians are similar to  
192 the Balkan and Central Europeans. Among individuals, proportions of WHG ancestry are strongly  
193 associated with decreased PRS ( $p=1.6 \times 10^{-6}$ ,  $\beta=-0.08\text{cm}/\%$ ). However, when we compute PRS  
194 with an infinitesimal LDpred2 model, Mediterranean PRS is intermediate between Neolithic Euro-  
195 peans and WHG. When we repeat the LDpred analysis using summary statistics computed from  
196 between-sibling GWAS,<sup>23</sup> we find that the direction of Hunter-Gatherer PRS flips, and they have  
197 significantly greater PRS than the other groups ( $p=0.002$ ) (Supplementary Figure 3A). The in-  
198 consistency of these results shows that the apparent PRS difference between WHG and Neolithic  
199 populations is highly sensitive to the PRS construction and summary statistics. This may indicate  
200 uncorrected population stratification in the non-sibling GWAS.<sup>24,25</sup> We therefore conclude that  
201 there is no strong evidence for a genetic difference in stature between Mediterranean and other  
202 Neolithic populations.

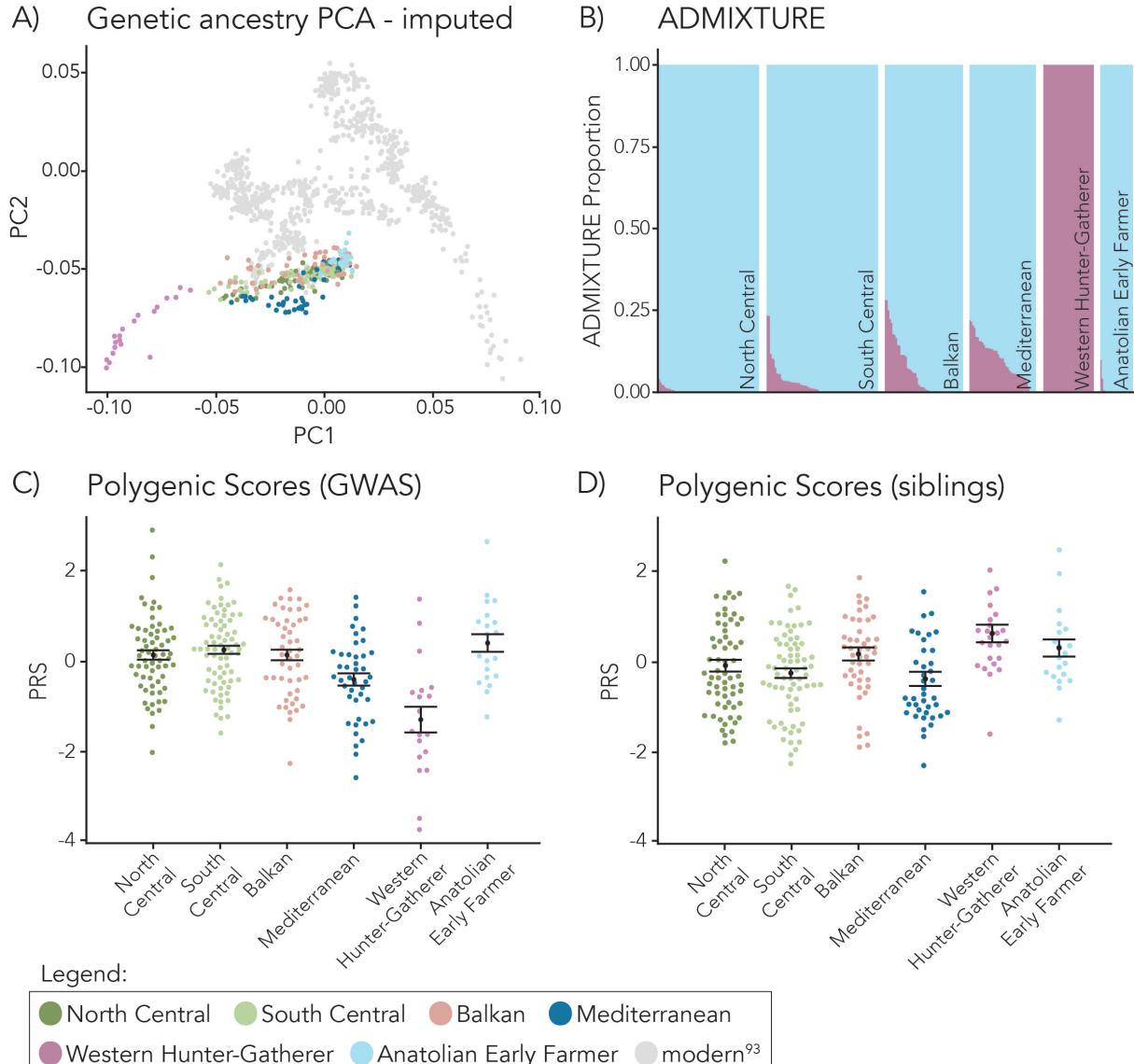


Figure 5: A) Ancient individuals projected into modern PCA space, including those of Mesolithic Western Hunter-Gatherer (WHG) and Anatolian Early Farmer ancestry. B) ADMIXTURE plot of  $K=2$  ancestry groups showing the increased proportion of WHG ancestry in Mediterranean individuals. C) Polygenic scores for each region including Mesolithic Hunter-Gatherers and Anatolian Early Farmers. D) Polygenic scores calculated from between-sibling summary statistics.

## 203 3 Discussion

204 Understanding the causes of past stature variation not only allows us to understand ancient com-  
205 munities, but may also provide us with insights into the origin and evolution of modern health  
206 patterns. However, interpretations of human stature variation through time remain confounded by  
207 the difficulty of separating genetic and environmental effects, obscuring trends. Recently, several  
208 researchers have begun to compile multivariate datasets for the purposes of understanding human  
209 stature.<sup>10,26–28</sup>; however, many of these analyses do not directly take genetic effects into account,<sup>26,28</sup>  
210 or cover very broad temporal or geographic ranges.<sup>10,27</sup> In contrast, we aim to understand these  
211 processes on a finer scale to better interpret the outcomes of biological and environmental/cultural  
212 interactions. For instance, previous studies of stature variation from the Mesolithic to Neolithic  
213 indicated that Neolithic individuals were not achieving their genetic height potential,<sup>10</sup> but our  
214 analyses suggest that this effect might be heterogeneous, pertaining more to some locations and  
215 portions of society than others. Finally, while interesting in its own right, height can also serve  
216 as a model trait for how to incorporate genetics and anthropological data into studies of human  
217 morphology and variation. Here, by integrating genetic, cultural, and environmental data, we are  
218 able to begin teasing apart the contributions of genetic and non-genetic factors in producing the  
219 observed phenotypic variation. We also illustrate the existing limitations of interpreting genetic  
220 data.

221 Overall, the Central and Balkan groups are genetically homogenous with similar levels of hunter-  
222 gatherer admixture and polygenic scores, while Mediterranean individuals have more hunter-gatherer  
223 ancestry (consistent with previous observations<sup>7,17,18</sup>). This may be associated with lower PRS,  
224 though this relationship is not robust and may simply reflect residual population stratification in  
225 the GWAS. None of our populations show evidence for substantial genetic differences in height be-  
226 tween sexes (Supplementary Figure 2), which is expected given that there is little evidence for sex  
227 differences in ancestry, or of sex-specific genetic effects on stature.<sup>29–31</sup> We can therefore largely  
228 exclude a genetic contribution to differences in stature between North Central individuals and other  
229 groups, while we find no strong evidence for a genetic contribution to shorter Mediterranean stature.

### 230 3.1 Sexual dimorphism in Central Europe reflects the effects of culture

231 Dietary differences between Southern and Northern Central European populations may indicate  
232 environmental stress in the North. In the early European Neolithic, the expansion of agriculture is  
233 thought to have been largely limited by poor soils and climate, as colder temperatures and decreased  
234 daylight made it increasingly difficult to grow early cereals (wheat, barley) and pulses (peas).<sup>32,33</sup>  
235 The boundary to which these plants could be grown has been estimated to coincide with the northern  
236 limits of the LBK culture,<sup>13,34</sup> and the majority of our Northern sites are concentrated near this  
237 climate edge in areas of fertile loess soils.<sup>14</sup> However, as there are many nuances which affect  
238 the interpretation of stable isotope values, especially between populations, differences between our

239 Northern and Southern groups may not be as dramatic as they appear. An examination of isotope  
240 values from herbivorous animals in our study regions (using data from Refs. 16,35–39) indicates  
241 that baseline values of  $\delta^{15}\text{N}$  are elevated in the South Central region as compared to the North,  
242 potentially due to differences in climate and the use of manure as fertilizer. Therefore some portion  
243 of the difference between Northern and Southern nitrogen values might be attributable to variation  
244 in climate and farming practices rather than diet. Differences in carbon values between populations  
245 can be similarly sensitive to environment and we feel that interpreting the carbon results would  
246 be difficult without a more in-depth isotopic analysis. However, isotopic values from other studies  
247 show a higher proportion of plant vs animal foods in the North, particularly domesticated cereal  
248 grains.<sup>15,36,40</sup> Additionally, the available archaeological evidence supports some level of dietary  
249 difference between the Northern and Southern regions. While remains of cattle and dairy production  
250 are documented in Northern LBK sites,<sup>41,42</sup> there is less archaeological evidence for the presence of  
251 other wild or domesticated animals that are seen in the South, indicating the people of this region  
252 were highly reliant on plant foods.<sup>40,43</sup> We conclude that our observed differences in Northern and  
253 Southern stable isotope values probably reflect both dietary factors and differences in climate or  
254 farming practices.

255 It is therefore not surprising that people of the Northern Central region exhibit evidence of  
256 increased stress potentially due to unreliable and lower quality food resources. Lower protein con-  
257 sumption could be an indicator of dietary stress and has been linked to decreased stature.<sup>44</sup> Diet  
258 can affect dimorphism in some cases,<sup>45</sup> but the isotopic signatures of males and females in the North  
259 Central, South Central, and Balkan regions are very similar, suggesting that this factor alone does  
260 not explain reduced female stature in the North. Femur length and isotope values for individuals are  
261 not significantly associated in our data, an indication that either diet has little effect on Neolithic  
262 stature or stable isotopes do not capture the elements of diet relevant to height. Alternatively, it is  
263 possible the range of variation in our data is too small to see this effect, or our sample of individuals  
264 with both metric and isotopic data is not large enough. In addition, we only analyzed adult samples  
265 and while the isotopic values of weaned children in the LBK fall within the range of adults,<sup>43</sup> it  
266 is possible that there could be sex differences in childhood diets affecting femur growth. Future  
267 studies incorporating collagen from long bones or teeth, rather than from ribs as we have here,  
268 would give dietary evidence with greater time depth, and might be able to provide more nuanced  
269 interpretations in the absence of a known-sex sub-adult population.

270 Paleopathological analysis also indicates increased stress in the Northern population in the form  
271 of increased incidence of linear enamel hypoplasia and cribra orbitalia. The causes of LEH formation  
272 are varied and their appearance in the bioarchaeological record is generally interpreted as a non-  
273 specific indication of childhood stress.<sup>46</sup> Other archaeological sites have reported a high instance of  
274 LEH with high sexual dimorphism ratios in areas of Neolithic Europe, though the cause and meaning  
275 of these patterns was not explored (e.g. Ref. 47 and references therein). It has been suggested that  
276 cribra orbitalia might also reflect childhood stress, specifically anaemias, even when seen in adults.<sup>48</sup>

277 Our results are consistent with others who have considered the same paleopathologies and found  
278 a qualitative relationship between presence of paleopathology and shorter femora.<sup>10</sup> In our data,  
279 incidence of both LEH and cribra orbitalia are higher in Northern compared to Southern Central  
280 Europe, but are not different between sexes in either group. The association between shorter femora  
281 and presence of LEH appears to be driven by females, suggesting a moderating factor causing a  
282 female-specific effect despite equal incidence of LEH in both sexes.

283 While we see a general increase in stress shared between sexes in North Central Europe, typical  
284 population-level stress responses usually show male vulnerability and female buffering effects.<sup>49–51</sup>  
285 Though the exact causes and mechanisms are not well understood, female biology tends to have a  
286 less extreme response, or is “buffered”, to many diseases<sup>52–54</sup> and environmental changes<sup>55</sup> compared  
287 to males. Our data indicate an opposite pattern in Central Europe, and no evidence of a variable  
288 which acts upon females alone. However, the Northern population shows sexual dimorphism that  
289 is extreme by present-day standards. In most modern global populations the ratio of male to  
290 female height is 1.06-1.08<sup>56</sup> (ratios in Ref. 56 range up to 1.12, but population locations or cultural  
291 affiliations are not given, see Ref. 45), though it is difficult to know how to compare height versus  
292 femur length ratios as the transformation from metrics to stature scales differently in males and  
293 females. Based on 147 European individuals from the past 100 years (using data from Ref. 57),  
294 we find that the height ratio is very similar to the ratio of femur length—typically within 1%. We  
295 therefore conclude that dimorphism ratios in Southern Central (1.09) and Balkan (1.11) Europeans  
296 are elevated and the ratio in the North Central region is exceptionally high (1.14). Few modern  
297 populations have height dimorphism ratios as high as 1.10, and those that we could find in the  
298 literature come from India<sup>58</sup> and the United Arab Emirates,<sup>59</sup> both countries known for their  
299 cultural preferences and biases for male children.<sup>60</sup>

300 We therefore hypothesize that the effects of high environmental stress in the North were mod-  
301 ulated by culture. Other researchers have noted specific situations in which culture buffers males  
302 against environmental effects and creates vulnerability in females: there is an association between  
303 decreased female stature and polygyny in cultures around the globe;<sup>61</sup> female height was more  
304 influenced by economic conditions during infancy and early childhood than males in lower-class  
305 19th-century Europe;<sup>62</sup> sexual dimorphism ratios in modern Chile decreased after the institution  
306 of social and government programs to combat gender inequality;<sup>63</sup> and 20th-century female stature  
307 decreased in India during times of environmental stress due to sexually disproportionate investment  
308 of scarce resources.<sup>60</sup> In LBK sites, strontium isotope values show that females are more likely to  
309 be non-local compared males, suggesting patrilocality and potential differences in cultural treat-  
310 ment of females.<sup>14,64</sup> In parallel to our evidence for higher biological variation in females, ongoing  
311 discussion about the relationship between biological sex and the formation of gendered identities  
312 in the LBK suggests more variation in the roles and identities of females compared to males.<sup>65</sup> We  
313 therefore suggest that culturally mediated differences led to sex-specific stress responses in Neolithic  
314 Central Europe *via* cultural practices which either directly decreased female stature or, more likely,

315 supported catch-up growth preferentially in males. Though dimorphism ratios in the South Central  
316 and Balkan regions are not as extreme as in the North, they are elevated and also consistent with  
317 this pattern of male bias, but response is likely less exaggerated due to lower environmental stress  
318 conditions.

### 319 3.2 Mediterranean differences may have both genetic and environmental bases

320 In the Early Neolithic Mediterranean population we see decreased male stature and low dimorphism  
321 ratios (1.05) relative to other Neolithic populations. Mediterranean populations are genetically dis-  
322 tinct from other Early Neolithic groups with a higher proportion of WHG ancestry. In some analyses,  
323 WHG ancestry proportion correlates with lower PRS for height. However, PRS in the Mediterranean  
324 and WHG populations are sensitive to PRS construction method likely due to residual population  
325 stratification in the GWAS. These inconsistent results mean that we can neither confirm nor exclude  
326 the possibility of a genetic contribution to differences in stature between the Mediterranean and  
327 other Early Neolithic populations, though on balance we find the likelihood for a substantial genetic  
328 contribution to be low. Even if it were not, the genetic effects alone could not explain the reduced  
329 dimorphism ratio, emphasizing the need to also consider cultural/environmental effects.

330 While the dimorphism ratio in the Mediterranean Neolithic is low, it is not outside the range of  
331 present-day populations.<sup>56</sup> In fact, while males are relatively short, the longest average female femur  
332 lengths of our data are in the Mediterranean. This reduction in dimorphism is commonly seen in  
333 populations where the sexes experience an equal stress burden: as males tend to be more sensitive,  
334 decreasing their height, females are biologically buffered and stature remains consistent.<sup>49–51,66</sup>  
335 Although we do not have paleopathological stress data for the Mediterranean individuals in our  
336 sample, published values for other Neolithic Mediterranean populations are generally similar to those  
337 for South Central Europe,<sup>67–69</sup> with exceptions.<sup>70</sup> Dietary isotopes indicate that the Mediterranean  
338 diet differs in some aspects, with increased  $\delta^{13}\text{C}$  values compared to the other Neolithic populations,  
339 but similar  $\delta^{15}\text{N}$  values. Our data indicates similar protein intake and low-level stress as other  
340 Neolithic populations, but do not suggest any clear hypothesis for the difference in male stature  
341 between the Mediterranean and other Neolithic groups. Possible differences in Mediterranean body  
342 proportions which are not captured by femur length should be mentioned as a caveat, though  
343 this likely would not be enough to account for the differences in stature compared to the rest of  
344 Europe, and would not affect observed dimorphism within the population. Our hypothesis is that  
345 the Mediterranean experienced similar levels of environmental stress as other Neolithic groups, but  
346 that they did not share the cultural practices which preferentially supported males and increased  
347 female vulnerability.

### 348 3.3 Conclusion

349 By integrating genetic and anthropological data, we are able to begin to understand the contribu-  
350 tions of genetics and environment to human variation, allowing us to better interpret the genetic,

351 environmental, and cultural landscapes of Neolithic Europe. Our results are consistent with a model  
352 in which sexually dimorphic differences in femur length are culturally and environmentally driven:  
353 relatively low dimorphism in the Mediterranean caused by female buffering to environmental stress  
354 and less cultural male preference, and high dimorphism in Northern Central Europe caused by the  
355 interaction of relatively high environmental stress and strong cultural male preference. Some analy-  
356 ses suggest that differences in average femur length between Central/Southeastern Europe and the  
357 Mediterranean are associated with differing genetic ancestries, but lack of robustness, uncertainty  
358 about the transferrability of polygenic scores, and questions of residual population stratification  
359 prevent us from interpreting this conclusively. In this study we focused on the European Early  
360 Neolithic because of its relative genetic, cultural, and environmental homogeneity, but, with more  
361 data, these methods could be extended to other populations, traits, and timescales to further ex-  
362 plore the effects of human culture on biological variation. Using this approach, we gain a deeper  
363 understanding of the relationship between phenotypic plasticity, culture and genetic architecture,  
364 which constrain the mechanisms by which human biology adapts to environment.

## 365 4 Materials and Methods

366 We collected a combination of genetic, dietary stable isotope, skeletal metric, and paleopathological  
367 (stress) data from 1282 individuals from the Central European Early Neolithic associated with the  
368 archaeological LBK culture, approximately 7700-6900 BP (Figure 2, Supplementary Table 1). As  
369 there is archaeological evidence for broad regional variation within the LBK and our sampled sites  
370 form clear geographic groups, we divided these individuals into two regions based on geographical  
371 location, those to the north of 50°N latitude (North Central) and those to the south (South Central)  
372 (Figure 2A-B; North Central n=203, n femur length=133, n isotopes=100, n aDNA=67, n stress=83;  
373 South Central n=1067, n femur length=187, n isotopes=670, n aDNA=72, n stress=523). Each  
374 individual has at least one of the data types, and while some individuals have multiple data types,  
375 the overlaps are small (Supplementary Figure 1).

376 To provide wider context, we also compared Central individuals to other Neolithic populations  
377 from southern European (Mediterranean) and southeastern European (Balkan) regions, and re-  
378 stricted to individuals dated to 8000-6000 BP. We chose these regions as the Neolithic transition  
379 occurs at similar times and is associated with populations closely related to Central Europe. The  
380 acceptable date range for inclusion in the study was expanded from that which defines the LBK as  
381 these dates encompass comparable Early Neolithic phases in other parts of Europe while maximizing  
382 the number of eligible individuals. There could be a possibility that the later Balkan and Mediter-  
383 ranean individuals were more adapted to Neolithic life than the Central European groups, as these  
384 samples cover a longer time period, but we found no statistical within-population differences in  
385 our variables between the early and late ranges of our time span (minimum p=0.08). We excluded  
386 areas such as Scandinavia and Britain, where Neolithic technologies were not generally adopted

387 until a later date. For the final analysis, we included 127 Mediterranean (n femur length=60, n  
388 isotopes=25, n aDNA=42) and 139 Balkan (n femur length=12, n isotopes=78, n aDNA=49) indi-  
389 viduals (Figure 2). Unfortunately, there is a wide range of recording and reporting used for skeletal  
390 stress indicators, and it was not possible to build a statistically powerful dataset in these two popu-  
391 lations for comparison; as a result, we did not analyze paleopathology in these populations. Finally,  
392 we collected genetic data from Mesolithic hunter-gatherer (n=25, 14000-7080BP, south of 48°N)  
393 and Anatolian Neolithic (n=21) individuals for additional comparison.

#### 394 4.1 Genetic data

395 We obtained genetic data for a total of 276 individuals.<sup>7,18-20,71-87</sup> Most data were generated by  
396 targeting a set of 1.24 million SNPs (the “1240k” capture reagent).<sup>17,75</sup> For each individual, we  
397 randomly selected a single allele from each of the 1240k sites. Coverage in our dataset is low  
398 (median coverage=0.33; coverage above 0.60 n=71), and typically, it is not possible to directly infer  
399 diploid genotypes, potentially limiting PRS performance. Imputation of missing genotypes has been  
400 shown to help improve polygenic predictions for low coverage ancient samples,<sup>9</sup> and we therefore  
401 imputed diploid genotypes using the two-stage method described in that paper, restricting to SNPs  
402 in the 1240k set.

403 We calculated polygenic scores as previously described.<sup>9</sup> Briefly, we used standing height sum-  
404 mary statistics generated by *fastGWA* from 456,000 individuals of European ancestry in the UK  
405 Biobank<sup>88</sup> for analyses of combined-sex PRS, and summary statistics from male- and female-only  
406 UK Biobank GWAS generated by the Neale Lab.<sup>89</sup> To test the potential effects of residual pop-  
407 ulation structure in our data, we also computed PRS using additional summary statistics from a  
408 between-sibling GWAS (n=99,997).<sup>23</sup> We intersected the sites from each of these datasets with those  
409 on the 1240k array and then further restricted to HapMap3 SNPs (SNPs n=405,000). We computed  
410 polygenic scores using both a clumping/thresholding approach ( $r^2=0.3$ , p-value cutoff= $10^{-6}$ , 100kb  
411 windows in *plink2*<sup>90</sup>), and an infinitesimal *LDpred2* model using their pre-computed LD reference  
412 panel.<sup>91</sup> Finally, we computed polygenic scores using the --score command in *plink2*. In order to  
413 maximize the possibility of detecting sex-specific effects, we generated sex-specific PRS using three  
414 different approaches: 1) calculating PRS for all individuals using the female summary statistics; 2)  
415 calculating PRS for all individuals using the male summary statistics; and 3) calculating PRS for  
416 males and females separately using their respective summary statistics. While approach 3 seems  
417 at first to be the best for detecting these effects, observed patterns potentially become difficult to  
418 interpret due to differences in scaling between male and female PRS calculated as separate datasets.

419 We computed principal components for both unimputed and imputed data using *smartpca*,<sup>92</sup> pro-  
420 jecting ancient individuals onto principal component axes defined by 777 present-day West Eurasian  
421 individuals.<sup>93</sup> We also estimated K=2 unsupervised ADMIXTURE<sup>94</sup> components for unimputed  
422 ancient individuals after first LD pruning using the command --pairwise-indep 200 25 0.4 in *plink2*.

## 423 4.2 Osteology and stable isotope data

424 We aggregated skeletal metric data from both published<sup>57,95–99</sup> and unpublished (n=28) sources.  
425 Maximum femur lengths were recorded when available, otherwise we estimated femur length from  
426 published stature estimates.<sup>9</sup> Estimated femur lengths correlate highly with stature estimates, but  
427 decrease the error that results from combining different estimations methods. The method from  
428 Ref. 100 provides separate equations for estimating the statures of northern vs. southern Europeans  
429 when using the tibia, due to differences in body proportions between the regions. There are two  
430 Mediterranean samples for which we estimated the length of the femur based on statures which used  
431 the southern tibia equation. Ref. 100 does not provide regional equations for femur estimation, so  
432 for these two individuals, we estimated femur length using the reverse of this region-agnostic femur  
433 equation.

434 For the individuals in this study who do not have genetic data, morphology was used to estimate  
435 sex. The majority of individuals have been taken from previous publications, and we used the sexes  
436 which had been estimated by those authors. For the individuals in our study which have not  
437 been previously published, sex was determined by co-authors using a 5-point scale on the cranium  
438 and pelvis as described by Ref. 101. For all individuals, sexes determined as probable male or  
439 probable female were coded in our study as either male or female as appropriate. Subadults and  
440 those with indeterminate morphologies were coded as NA, resulting in these individuals being  
441 dropped from the sex-specific analyses. The majority of sexes for individuals with metric data were  
442 determined by, or supervised by, co-authors and the remainder (n=13) either have genetic sexes  
443 or come from Ref. 57 which we consider a reliable source. Despite generally high accuracy for  
444 morphological sex determination, some level of uncertainty always remains, mainly due to variation  
445 in sexual dimorphism and preservation of the remains.<sup>102</sup> Sex estimations for our sample have  
446 all been performed in the last 20 years, and the majority within the last 5 years, meaning the  
447 researchers who performed them should be aware of avoiding the biases which can affect sex-ratios  
448 in the estimations of older data. Our dataset is large enough that small errors in classification  
449 of sex should not make substantial differences to results or interpretation, but the potential for  
450 inaccurate morphological sex estimations must always be considered in any osteological analysis. A  
451 large portion of our paleopathology data comes from tables S3 and S6 of Ref. 103, in which there  
452 are many instances of the same individual listed in both tables, but with discordant sex estimations.  
453 As we could not determine the reason for these discrepancies, we used the sex which was reported  
454 in the original publications cited as sources for their data. The few individuals (n=3) for whom this  
455 could not be resolved were treated as indeterminate and coded as NA. Ages were determined based  
456 on the average of the age range reported for each individual in their original publications.

457 For the paleopathological data in Central Europe, we took data from published sources,<sup>95,96,103–106</sup>  
458 as presence/absence of linear enamel hypoplasia (LEH), porotic hyperostosis, and cribra orbitalia.  
459 These three pathologies are often used by anthropologists as indicators of general, non-specific stress  
460 experienced by individuals or populations. While the exact etiologies of these pathologies are gen-

461 erally not known, they have been shown to change through time within and between populations,  
462 and often correlate with environmental, social, or cultural shifts. Linear enamel hypoplasias are  
463 horizontal defects in tooth enamel that form during episodes of childhood stress severe enough to  
464 interrupt growth for some period of time, usually associated with dietary deficiency or infectious  
465 disease.<sup>46</sup> Individuals can exhibit one or multiple LEH on single or multiple teeth and in order  
466 to minimize errors from differences in reporting LEH in the literature, we have simply recorded  
467 whether an individual had any LEH (present) or none (absent). Porotic hyperostosis and cribra  
468 orbitalia are both porous lesions that are distinguished by their appearance on either the cranial  
469 vault or roof of the eye orbit respectively. The etiologies of these are mostly unknown and though  
470 they are traditionally associated with anaemias, there are also a number of other conditions that  
471 can produce the same type of lesions. Medically, there is little evidence of these pathological changes  
472 despite their prevalence in the bioarchaeological record.<sup>107</sup> Similar to LEH, we have recorded these  
473 as either present or absent for each individual in order to standardize between reporting conventions  
474 across publications.

475 While sensitive to confounding factors such as climate, vegetation, and individual metabolism,<sup>108</sup>  
476  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope data can be used to reconstruct aspects of diet.<sup>109</sup> Here, carbon values  
477 are indicative of dietary plant resources and of the terrestrial vs marine vs limnic provenance of  
478 food, while nitrogen values are mainly associated with dietary protein intake and generally indi-  
479 cate proportions of plant- vs animal-based diets.<sup>108,109</sup> We collected dietary stable isotopes  $\delta^{13}\text{C}$   
480 and  $\delta^{15}\text{N}$  from published<sup>16,35–37,43,95,97,103,105,106,108,110–113</sup> and unpublished (n=38) reports. We  
481 excluded atomic mass spectrometer (AMS) values derived from radiocarbon dating, as they may  
482 not be comparable to isotope-ratio mass spectrometer (IRMS) measurements, as well as values from  
483 children below the age of three, due to increased nitrogen values from breastfeeding. Stable isotope  
484 values from older children were included in population-wide diet analyses as the isotope ranges fall  
485 within those of adults; however, we only included adults with estimated sexes in the sex-based diet  
486 analyses. If information on the sampled material was available, we chose values measured from  
487 rib collagen, as these samples are most plentiful, though they only reflect the last few years of the  
488 individual's life.

489 All previously unpublished osteological data was collected and analyzed by co-authors with  
490 permission from the necessary regulating organizations and in accordance with German laws and  
491 policies.

## 492 4.3 Statistical models

493 We tested the effects of PRS, femur length, and isotope data on stature using linear regression  
494 models including sex and geographic region as covariates in combination with other variables as  
495 appropriate (e.g., femur ~ sex + region + PRS;  $\delta^{15}\text{N}$  ~ sex + region + femur). We included  
496 interaction terms to test the relationships between geographic regions and sex (e.g., femur ~ region  
497 \* sex) and used t-tests to test within-sex differences between regions. We used logistic regression

498 with the same covariates to test for factors affecting presence/absence of paleopathologies. We  
499 carried out all statistical tests using the base functions in R version 4.0.<sup>114</sup>

#### 500 4.4 Data Availability

501 All non-genetic data and polygenic scores used in this analysis are provided in Supplementary Table  
502 1. Original ancient DNA data files can be downloaded from the resources provided in their cited  
503 publications. Previously published osteological data can be found in their cited sources (Supple-  
504 mentary Table 1).

#### 505 4.5 Code Availability

506 R code used in this analysis is available at [https://github.com/mathilab/Neolithic\\_height.git](https://github.com/mathilab/Neolithic_height.git).

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#### 514 4.7 Author Contributions

515 S.L.C. designed the study, collected data, performed analysis, and wrote the manuscript; I.M.  
516 designed the study and wrote the manuscript; N.N. and K.W.A., contributed data and archaeological  
517 background; E.R. contributed data and performed analysis; M.F., J.W., H.M., and W.H. contributed  
518 data. All authors edited and approved the final version.

#### 519 4.8 Competing Interests

520 The authors declare no competing interests.

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