100 ANCIENT GENOMES SHOW REPEATED POPULATION TURNOVERS IN NEOLITHIC DENMARK

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Summary

68   Major migration events in Holocene Eurasia have been characterised genetically at broad
69   regional scales[1–4]. However, insights on the population dynamics in the contact zones are
70   hampered by a lack of ancient genomic data sampled at high spatiotemporal resolution[5–7]. To
71   address this we analysed shotgun-sequenced genomes from 100 skeletons spanning 7,300
72   years of the Mesolithic, Neolithic and early Bronze Age in Denmark and integrated these
73   with proxies for diet (δ^{13}C & δ^{15}N isotopes), mobility (^{87}Sr/^{86}Sr isotopes), and vegetation
74   cover (pollen). We observe that Danish Mesolithic individuals of the Maglemose,
75   Kongemose and Ertebølle cultures form a distinct genetic cluster related to other Western
76   European Hunter-Gatherers (HGs). Despite shifts in material culture they displayed genetic
77   homogeneity from ~10,500 cal. BP until 5,900 cal. BP when Neolithic farmers with
78   Anatolian-derived ancestry arrived. Although this process was delayed by more than a
79   millennium relative to Central Europe it was very abrupt and resulted in a population
80   turnover with limited genetic contribution from local HGs. The succeeding Neolithic
81   population, associated with the Funnel Beaker Culture, persisted for just ~1000 years before
82   immigrants with eastern Steppe-derived ancestry arrived. This second and equally rapid
83   population replacement gave rise to the Single Grave Culture with an ancestry profile more
84   similar to present-day Danes. In our multiproxy dataset these major demographic events are
85   manifested as parallel shifts in genotype, phenotype, diet, and land use.
86

Introduction

88   The Mesolithic and Neolithic periods in southern Scandinavia are marked by a number of
89   pivotal and well-described cultural transitions[8]. However, the genetic and demographic
90   impacts of these events remain largely uncharacterised. The early postglacial human
91   colonisation of the Scandinavian Peninsula (Sweden and Norway) is believed to comprise at
92   least two distinct migration waves: a source related to western European hunter-gatherers
93   (WHG) from the south, and an eastern European hunter-gatherer (EHG) source into the far
94   north, before venturing south along the Atlantic coast of Norway[9,10]. However, insight into
95   the fine-scale structure and mobility of Scandinavian Mesolithic populations is limited,
96   including an almost complete absence of genetic data from southern Scandinavian
97   populations associated with the consecutive Maglemose, Kongemose and Ertebølle cultures
98   in Denmark.
99   The Neolithic transition represents a watershed event in European prehistory, marked by the
100   spread of domesticated crops and livestock from Southwest Asia, starting around 11,000 BP.

101 While migrations and population turnovers associated with this transition have been
102 demonstrated at broad geographical and chronological scales[1–4], coarse sampling and a one-
103 sided focus on genetics have hindered insights on social interaction and detailed demographic
104 processes in the contact zones between locals and newcomers[5–7]. Southern Scandinavia
105 occupies an enigmatic position in this discussion. The Neolithic transition was delayed here
106 by a millennium compared to Central Europe, during which hunter-gatherer societies
107 continued to flourish until c. 5,900 cal. BP, only marginally affected by farmer populations to
108 the south[11]. The significant delay could suggest that the transition to farming in Denmark
109 occurred by a different mechanism involving a stronger element of cultural diffusion[12] than
110 the migration of people (demic diffusion) observed in the rest of Europe[13–15].
111 An extensive archaeological record has indicated that the Funnel Beaker Culture (FBC)
112 thrived for the first millennium of the Neolithic in Denmark, before an apparent decline[16] was
113 followed by the appearance of the Single Grave Culture (SGC). Owing to a lack of genetic
114 data and a robust absolute chronology, the relation between the FBC and the SGC has been
115 extensively debated[17–19]. As such, population dynamics associated with this second cultural
116 transition in Neolithic Denmark are similarly unresolved, including its possible link to the
117 'steppe migrations' that transformed the gene pools elsewhere in Europe around the same
118 time[1,2].
119 To investigate these defining events at high temporal and spatial resolution we analyse a
120 detailed and continuous dataset of 100 ancient Danish shotgun-sequenced genomes (0.01X to
121 7.1X[3]), spanning c. 7,300 years from the Early Mesolithic Maglemose, the Kongemose and
122 Late Mesolithic Ertebølle epochs, the Early and Middle Neolithic FBC and the SGC, up until
123 the Bronze Age (Fig. 1, Supplementary Data I). The archaeological record in Denmark
124 represents a very large assemblage of well-documented Mesolithic and Neolithic human
125 skeletal remains, from a wide range of chronological, topographical and socio-cultural
126 contexts. This is a result of an environment and climate that was amenable to both Mesolithic
127 fisher-hunter-gatherer lifeways[20] and the later Neolithic farming practices, combined with
128 taphonomically favourable preservation conditions for skeletal remains, and a long, prolific
129 history of archaeological research. We employed a multiproxy approach, combining
130 autosomal imputed genomes[3,21] with Y-chromosomal and mitochondrial haplogroups, [14]C-
131 dating, genetic phenotype predictions, as well as $^{87}Sr/^{86}Sr$, $\delta^{13}C$ and $\delta^{15}N$ isotope data as
132 proxies for mobility and diet. Moreover, to investigate a direct link between demographic and
133 environmental processes we align the genetic changes observed in the Danish population over
134 time with changes in local vegetation, based on pollen analyses and quantitative vegetation
135 cover reconstruction.

136 **Results and Discussion**
137
138 ***The Mesolithic***
139 It is not known if shifts in southern Scandinavian Mesolithic material culture occurred in a
140 population continuum or were facilitated by incoming migrants. The Early Mesolithic
141 settlement in Denmark is associated with the Maglemose Culture (c. 11,000-8,400 cal. BP),
142 characterised archaeologically by small flint projectiles in geometric shapes. Until the recent
143 development of underwater archaeology this culture was mainly known from inland locations
144 along lakes and rivers[22]. During the succeeding Kongemose Culture (c. 8,400-7,400 cal. BP),
145 trapeze-shaped flint points dominate the assemblages of arrowheads[23] along with high quality
146 long blades. Most of the larger settlements cluster at good fishing locations along the coasts[24]
147 but there are also specialised hunting camps in the interior[25]. The Late Mesolithic Ertebølle
148 Culture (c. 7,400-5,900 cal. BP), is characterised by flint points with transverse edges.

149 Pottery was introduced from other hunter-gatherer groups to the east and perhaps the
150 southwest[26] and 'exotic' shaft-hole axes suggest exchange with farming societies south of the
151 Baltic Sea[27]. The larger habitation sites, densely scattered along the coasts, probably
152 represent multi-family, year-round occupation[24,28] and they have provided important insights
153 into the physical anthropology and spiritual culture of the period.
154 By analysing genomes from 38 Danish HGs and inferring their ancestry, we examine if
155 cultural transitions observed in the Danish archaeological record are associated with any
156 genetic changes in the population. Model-based clustering (ADMIXTURE), PCA and IBD-
157 sharing analyses show that throughout the Maglemose (n=4), Kongemose (n=8) and Ertebølle
158 (n=27) epochs the region displayed a remarkable genetic homogeneity across a 4,500-year
159 transect (Figs. 1-3; Extended Data Figs. 1-3), supporting interpretations of demographic
160 continuity favoured by some archaeologists[23–25]. From the earliest known skeleton in
161 Denmark, 'Koelbjerg Man' (NEO254, 10,648-10,282 cal. BP[29]) to the most recent Mesolithic
162 skeleton included here, 'Rødhals Man' (NEO645, 5,916-5,795 cal. BP), the individuals
163 derive their ancestry almost exclusively from the same southern European source
164 (Italy_15000BP_9000BP) that predominated in WHG ancestry in Mesolithic Western
165 Europe[3].
166 In the IBD-based PCA the Danish Mesolithic individuals cluster closely together (Extended
167 Data Fig. 4a), but beyond this tight local genetic connection they share most recent ancestry
168 with the geographically and temporally proximate hunter-gatherer individuals from Western
169 Europe (e.g. Cheddar Man, Loschbour and Bichon, commonly referred to as WHG; genetic
170 cluster EuropeW_13500BP_8000BP; Fig. 2). A subtle shift of the earliest Danish individuals
171 towards these western individuals likely reflects their closer temporal proximity captured
172 through IBD sharing (Extended Data Fig. 4a). Although pressure-debitage of blades in the
173 Maglemosian culture and pottery in the Ertebølle culture are both argued to have an eastern
174 origin[9,10,30,31], our data show no evidence for admixture with more eastern hunter-gatherers
175 during those times. This points to cultural diffusion as the source of these introductions in
176 Denmark. When tested with D-statistics, all Danish Mesolithic individuals form a clade with
177 the earliest individual (NEO254), to the exclusion of Swedish Mesolithic hunter-gatherers
178 (Sweden_10000BP_7500BP; Extended Data Fig. 2a) despite the close proximity to Sweden.
179 However, a weak signal of gene flow with eastern European hunter-gatherers was shared
180 across the whole Danish Mesolithic transect (Extended Data Fig. 2b), suggesting contact with
181 communities further to the east prior to their expansion into Denmark before or during the
182 earliest Mesolithic.
183 Genetic phenotype predictions (Supplementary Note 2) indicate a high probability of blue eye
184 pigmentation throughout the Mesolithic, consistent with previous findings[1,15,32], showing that
185 this feature was present already in the early Mesolithic but not fixed in the population. The
186 Mesolithic HGs from Denmark all display high probability of brown/black hair and height
187 predictions generally suggest slightly lower and/or less variable stature than in the succeeding
188 Neolithic period. We caution, however, that the relatively large genetic distance to modern
189 individuals included in the GWAS panel produces scores that are less applicable to
190 Mesolithic individuals than to more recent groups[33].
191 Stable isotope $\delta^{13}C$ values in collagen can inform on the proportion of marine versus
192 terrestrially-derived protein, while $\delta^{15}N$ values reflect the trophic level of the protein
193 sources[34]. The earliest skeleton (NEO254) shows depleted dietary isotopic values (Fig. 3)
194 representing a lifestyle of inland hunter-gatherers of the Early Mesolithic. This result is
195 mirrored in the second earliest known skeleton from Denmark (Tømmerupgårds Mose, see[34]).
196 From later Maglemose (c. 9,500 cal. BP) and throughout the Kongemose and Ertebølle
197 epochs we observe gradually increased $\delta^{13}C$ and $\delta^{15}N$ values (Extended Data Fig. 5 and Figs.
198 S4.1-2). This implies that marine foods progressed to constitute the major supply of proteins,

199 as suggested previously based on data from >30 Mesolithic humans and dogs, from both
200 coastal and inland sites in Denmark[34,35]. During this period global sea-level rise gradually
201 transformed present-day Denmark into an archipelago, where all human groups had ample
202 access to coastal resources within their annual territories[24]. The local Mesolithic population
203 adapted their diet and culture over time to the changing landscape and our data show that this
204 occurred in a continuous population, without any detectable influx of migrants over a 4,500-
205 year period. Low variability in $^{87}Sr/^{86}Sr$ isotope ratios throughout the Mesolithic (Fig. 3,
206 Supplementary Note 5) could indicate limited long-range mobility and/or deriving dietary
207 sources from more homogeneous environments (e.g., marine) than in the succeeding
208 Neolithic periods.
209 Notably, some of the Danish Mesolithic individuals proved to be closely related[3]. Close
210 kinship is demonstrated in the case of two individuals (NEO568/NEO569), father and son,
211 interred next to each other in the *locus classicus* shell midden site of Ertebølle, and in the
212 case of two individuals (NEO732/NEO733), mother and daughter, that were buried together
213 at Dragsholm. The Ertebølle grave was the first discovered human skeleton in Denmark
214 (excavated in the 1890s) that indisputably represented HGs. After the excavation of this site,
215 academic reasoning rooted in Biblical narration about early prehistory in Scandinavia lost
216 momentum. The excavation data cannot reveal if they were buried simultaneously; it can only
217 be ascertained that the boy (infant, <2 years) was positioned less than one metre from his
218 father (the 'Ertebølle Man'). Excavations at Dragsholm in 1973 uncovered a well-preserved
219 double burial containing a grave with two Mesolithic women as well as a male grave with
220 grave goods suggesting an Early Neolithic date for the latter[36]. A close kin relationship was
221 suggested for the two Dragsholm women based on physical anthropological observations[37]. It
222 was suggested that they were sisters, but this can now be corrected to a co-burial of a mother
223 and daughter. Our data also show that the male in the adjacent burial ('Dragsholm Man',
224 NEO962) was not related to the two women. These cases show that close biological kinship
225 was socially relevant to Late Mesolithic groups in Northern Europe and affected the mortuary
226 treatment of dead members of their society.
227
228
229 ***Early Neolithic transition***
230 The emergence of the Neolithic Funnel Beaker Culture (FBC) in Denmark has occupied a
231 central position in archaeological research and debate throughout the last 175 years[8,38,39]. The
232 defining element of the Neolithic, a food-producing economy based on domesticates of
233 southwest Asian origin, was indisputably present in Denmark from c. 5,900 cal. BP[11,38]. The
234 neolithisation saw a boom of new shapes and types introduced in Danish material culture,
235 including funnel-shaped beakers and polished flint axes. From c. 5,800 cal. BP monumental
236 long barrows of wood and earth were added to the repertoire, and c. 200 years later burials
237 built of soil, surrounded by raised stones and including stone-built chambers, were erected as
238 dominant landmarks in the farmland[40]. After 5,300 cal. BP larger and more complex stone-
239 constructed passage graves in large earthen tumuli emerged[41]. Meanwhile, simple, non-
240 monumental burials continued along with the megalithic tombs all through the FBC epoch[42].
241 Habitation deposits, dating to the earliest centuries of the Neolithic, on top of many
242 Mesolithic Ertebølle coastal shell middens may be interpreted as a local continuation of
243 marine gathering and fishing. In contrast, other settlements with regular long houses on easily
244 farmed soils further inland are associated with remains of domestic plants and animals
245 suggesting a very clear distinction from the previous Mesolithic Ertebølle period[39,43].
246 Regardless of these nuances, at c. 5,900 cal. BP our multiproxy dataset documents a dramatic
247 and abrupt concomitant shift in genetic, phenotypic, dietary, and vegetation parameters (Fig.
248 3). This is robust evidence for demic diffusion, settling a long-standing debate[8,38]. As

249 observed elsewhere in Europe[13–15] the introduction of farming in Denmark was unequivocally
250 associated with the arrival of people with Anatolian farmer-related ancestry. This resulted in
251 a population replacement with limited genetic contribution from the local HGs. The earliest
252 example of this typical Neolithic ancestry in our Danish dataset is observed in a bog skeleton
253 of a female from Viksø Mose (NEO601) dated to 5,896-5,718 cal. BP (95%). In the PCA all
254 Danish Early Neolithic individuals cluster at the 'late' end of the European Neolithic farmer
255 cline and consistently show some of the largest amounts of HG ancestry (10-35%) among all
256 European Neolithic farmer genomes included (Figs. 1, 3; Extended Data Figs. 1, 5a;
257 Supplementary Data IV). In IBD clustering analyses, the Danish individuals form part of a
258 genetic cluster (Scandinavia_5600BP_4600BP) together with FBC-associated individuals
259 from Sweden and Poland, and also show close affinity with Polish individuals from the
260 Globular Amphora Culture (GAC) (Extended Data Fig. 4b). This could suggest an eastern
261 European proximate origin of the Early Neolithic farmers in Denmark. Using more proximate
262 ancestry modelling, we find that Neolithic FBC-associated individuals across Denmark,
263 Sweden and Poland derived their HG ancestry component predominantly from a source
264 related to western European HGs (EuropeW_13500BP_8000BP). Ancestry related to Danish
265 Mesolithic HGs (Denmark_10500BP_6000BP) is found in smaller proportions (less than
266 ~10%) and only in a subset of the FBC individuals from Denmark (Extended Data Fig. 6).
267 Moreover, this tends to occur in more recent individuals (dated to ~5,400 cal. BP onwards)
268 who are also showing the overall largest amount of total HG ancestry (e.g. NEO945,
269 NEO886; Fig. 3; Extended Data Figs. 3, 6a,b). Using DATES[44], we found that admixture
270 times for a large proportion of Danish Neolithic individuals predates 5,900 cal. BP when FBC
271 emerged in Denmark, particularly for the earliest individuals (Extended Data Fig. 7). More
272 recent admixture times (post dating the arrival of FBC in Denmark) were mainly observed in
273 individuals dated to after ~5,400 cal. BP, and were associated with overall higher HG
274 proportions. These observations were in marked contrast to FBC-associated individuals from
275 Sweden, where admixture times and hunter-gatherer ancestry did not change over time, and
276 no admixture with local Swedish hunter-gatherers was detected.
277 Our results demonstrate a population turnover in Denmark at the onset of the neolithisation
278 by incomers who displayed a mix of Anatolian Neolithic farmer ancestry and non-local HG
279 ancestry. Ancestry related to the local Danish HGs could only be detected late in the Danish
280 Neolithic gene pool, suggesting gene-flow with groups of late surviving HGs, as also
281 documented in other European regions (Iron Gates[45], Central Europe[13], Spain[46]). We do not
282 know how the Mesolithic Ertebølle population disappeared. Some may have been isolated in
283 small 'pockets' of brief existence and/or adapted to a Neolithic lifestyle. The most recent
284 individual in our Danish dataset with HG ancestry is the aforementioned 'Dragsholm Man'
285 (NEO962), dated to 5,947-5,664 cal. BP (95%) and archaeologically assigned to the FBC
286 based on his grave goods[37]. Our data confirm a typical Neolithic diet matching the cultural
287 affinity but contrasting his HG ancestry. He clearly represents a local person of Mesolithic
288 ancestry who lived in the short Mesolithic-Neolithic transition and adopted the culture and
289 diet of the immigrant farmers. A similar case of late HG ancestry in Denmark was observed
290 when analysing human DNA obtained from a piece of chewed birch pitch from the site of
291 Syltholm on Lolland, dated to 5,858–5,661 cal. BP (95%)[47]. Thus, individuals with HG
292 ancestry persisted for decades and perhaps centuries after the arrival of farming groups in
293 Denmark, although they have left only a minor genomic imprint on the population of the
294 subsequent centuries. Similar 'relic' HG ancestry is also found in the Evensås individual
295 (NEO260) from west-coast Sweden, dated to 5913 - 5731 cal. BP[3].
296 From the onset of the Neolithic in Denmark, diet shifted abruptly to a dominance of terrestrial
297 sources as evidenced by δ^{13}C values around -20 ‰ and δ^{15}N values around 10 ‰ (Fig. 3,
298 Extended Data Fig. 5). In line with archaeological evidence these isotopic data show that

299   domesticated crops and animals provided the main supply of proteins from this point
300   onwards. Isotope values remained stable at these levels throughout the following periods,
301   although with somewhat greater variation after c. 4,500 cal. BP (Fig. 3). Five Neolithic and
302   Early Bronze Age individuals have $\delta^{13}C$ and $\delta^{15}N$ values indicating a significant intake of
303   high trophic marine food. This is especially pronounced for the individual NEO898
304   (Svinninge Vejle) which is one of two Danish Neolithic individuals displaying ancestry
305   related to Swedish late HGs (see below). A significantly higher variability in individual
306   $^{87}Sr/^{86}Sr$ values can be seen with the start of the Neolithic. This continues in the later periods
307   (Supplementary Note 5) and is not easily explained by biases in sampling as most of our
308   samples, regardless of ancestry and time period, are concentrated in the more easterly parts of
309   Denmark where bone preservation conditions are generally good (Figs. 1 and S5.3). This
310   pattern could suggest that the Neolithic farmers in Denmark occupied and/or consumed food
311   from more diverse landscapes, or were more mobile than the preceding HGs. The Neolithic
312   transition also marks a considerable rise in frequency of major effect alleles associated with
313   light hair pigmentation[48], whereas predictions throughout the first millennium of the
314   Neolithic (FBC epoch) mostly indicate a lower stature than present day, echoing previous
315   findings[32,49].
316   Pitted Ware Culture (PWC) originated on the Scandinavian Peninsula and the Baltic islands
317   east of the Swedish mainland but emerged around 5,100-4,700 cal. BP in the northern and
318   eastern part of Denmark, where it coexisted with the FBC[50,51]. It is characterised by coarse
319   pottery that is often decorated with pits and subsistence based on a combination of marine
320   species and agricultural products. No burials associated with the PWC have been discovered
321   in Denmark. Interestingly, however, the genomes of two ~5,200-year-old male individuals
322   (NEO33, NEO898) found in Danish wetland deposits proved to be of HG ancestry related to
323   that of PWC individuals from Ajvide on the Baltic island of Gotland (Sweden)[52] (Fig. 4,
324   Extended Data Fig. 4a). Of the two individuals, NEO033 (Vittrup, Northern Jutland) also
325   displays an outlier Sr-signature (Fig. 3), perhaps suggesting a non-local origin that matches
326   his unusual ancestry. Overall, our results demonstrate direct contact across the sea between
327   Denmark and the Scandinavian Peninsula during this period which is in line with
328   archaeological findings[50,51].
329
330   ***Later Neolithic and Bronze Age***
331   Europe was transformed by large-scale migrations from the Pontic-Caspian Steppe around
332   5,000-4,800 cal. BP. This introduced steppe-related ancestry to most parts of the continent
333   within a 1000-years span and gave rise to the Corded Ware Culture (CWC) complex[1,2]. In
334   Denmark, this coincided with the transition from the FBC to Single Grave Culture (SGC), the
335   regional manifestation of the CWC complex. The transition to single graves in round tumuli
336   has been characterised archaeologically by two expansion phases: a primary and rapid
337   occupation of central, western and northern Jutland (west Denmark) starting around 4,800
338   cal. BP and a later and slower expansion across the Eastern Danish Islands starting around
339   4,600 cal. BP[53,54]. In the eastern parts of the country SGC traits are less visible, while FBC
340   traditions such as burial in megalithic grave chambers persisted[55]. This cultural shift
341   represents another classical archaeological enigma, with explanations favouring immigration
342   versus cultural acculturation competing for generations[19,56].
343   Insights from a few low coverage genomes [1,57] have indeed shown a link to the Steppe
344   expansions but by mapping out ancestry components in the 100 ancient genomes we now
345   uncover the full impact of this event and demonstrate a second near-complete population
346   turnover in Denmark within just 1,000 years. This genetic shift was evident from PCA and
347   ADMIXTURE analyses, where Danish individuals dating to the SGC and Late Neolithic and
348   Bronze Age (LNBA) cluster with other European LNBA individuals and show large

proportions of ancestry components associated with Yamnaya groups from the Steppe (Figs. 1 and 3, Extended Data Fig. 1). We estimate between ~60-85% ancestry related to Steppe groups (Steppe_5000BP_4300BP), with the remainder contributed from farmer-ancestry individuals associated with Eastern European GAC (Poland_5000BP_4700BP; ~10-23%) and to a lesser extent from local Neolithic Scandinavian farmers (Scandinavia_5600BP_4600BP; ~3-18%) (Extended Data Figs. 6a,b). While the emergence of SGC introduced a major new ancestry component in the Danish gene pool, it was not accompanied by apparent shifts in dietary isotopic ratios, or Sr isotope ratios (Fig. 3). Our complex trait predictions, however, indicate an increase in height (Fig. 3; Supplementary Note 2), which is consistent with ancient Steppe individuals being predicted taller than average European Neolithic individuals before the steppe migrations[32,49,58].

Because of poor preservation conditions in most of western Denmark, we do not have skeletons from the earliest phase of the SGC (c. 4,800 cal. BP) so we cannot unequivocally demonstrate that these people carried steppe-related ancestry. SGC burial customs were implemented in different ways in the southern and the GAC-related northern parts of the peninsula, respectively[18] and considering recent genetic results in other regions[59], it is plausible that differing demographic processes unfolded within Denmark. However, we know that steppe ancestry was present 200 years later in SGC-associated skeletons from the Gjerrild grave[57]. The age of the Gjerrild skeletons (c. 4,600 cal. BP) matches the earliest example of steppe-related ancestry in our current study, identified in a skeleton from a megalithic tomb at Næs (NEO792). We estimated ~85% of Steppe-related ancestry in this individual, the highest amount among all Danish LNBA individuals (Extended Data Fig. 6a). Interestingly, NEO792 is also contemporaneous with the two most recent individuals in our dataset showing Anatolian farmer-related ancestry without any steppe-related ancestry (NEO580, Klokkehøj and NEO943, Stenderup Hage) testifying to a short period of ancestry co-existence before the FBC disappeared – similar to the Mesolithic Ertebølle people of HG ancestry disappearing a thousand years earlier. Using Bayesian modelling we estimate the duration between the first appearance of Anatolian farmer-related ancestry to the first appearance of Steppe-related ancestry in Denmark to be between 876 and 1100 years (95% prob. interval, Supplementary Note 3) implying that the former type of ancestry was dominant for less than 50 generations.

The following Late Neolithic 'Dagger' epoch (c. 4,300-3,700 cal. BP) in Denmark has been described as a time of integration of culturally and genetically distinct groups[54]. Bronze became dominant in the local production of weapons while elegantly surface-flaked daggers in flint were still the dominant male burial gift. Unlike the SGC epoch, this period is richly represented by human skeletal material. Although broad population genomic signatures suggest genetic stability in the LNBA (Fig. 1, Fig. 3), patterns of pairwise IBD-sharing and Y-chromosome haplogroup distributions in a temporal transect of 38 LNBA Danish and southern Swedish individuals indicate at least three distinct ancestry phases during this ~1000-year time span (Extended Data Figs. 4c, 8):

LNBA phase I) An early stage between ~4,600 and 4,300 cal. BP, where Scandinavians cluster with early CWC individuals from Eastern Europe, rich in Steppe-related ancestry and males with an R1a Y-chromosomal haplotype (Extended Data Fig. 8a,b). Archaeologically, these individuals are associated with the later stages of the Danish SGC and the Swedish Battle Axe Culture.

LNBA phase II) An intermediate stage largely coinciding with the Dagger epoch (c. 4,300-3,700 cal. BP), where Danish individuals cluster with central and western European LNBA individuals dominated by males with distinct sub-lineages of R1b-L51 (Extended Data Fig. 8c,d)[3]. Among them are individuals from Borreby (NEO735, 737) and Madesø (NEO752).

398 *LNBA phase III)* A final stage from c. 4,000 cal. BP onwards, where a distinct cluster of
399 Scandinavian individuals dominated by males with I1 Y-haplogroups appears (Extended Data
400 Fig. 8e). Y-chromosome haplogroup I1 is one of the dominant haplogroups in present-day
401 Scandinavians, and we here document its earliest occurrence in a ~4,000-year-old individual
402 from Falköping in southern Sweden (NEO220). The rapid increase in frequency of this
403 haplogroup and associated genome-wide ancestry coincides with increase in human mobility
404 seen in Swedish Sr isotope data, suggesting an influx of people from eastern or north-eastern
405 regions of Scandinavia, and the emergence of stone cist burials in Southern Sweden[60], which
406 were also introduced in eastern Denmark during that period[54,61].

408 Using genomes from LNBA phase III (Scandinavia_4000BP_3000BP) in supervised ancestry
409 modelling we find that they form the predominant ancestry source for later Iron and Viking
410 Age Scandinavians (Extended Data Fig. 6d), and other ancient European groups with a
411 documented Scandinavian or Germanic association (e.g., Anglo-Saxons, Goths; Extended
412 Data Fig. 6e). When projecting 2,000 modern Danish genomes[62] on a PCA of ancient
413 Eurasians the modern individuals occupy an intermediate space on a cline between the LNBA
414 and Viking Age individuals (Fig. 4). This result shows that the foundation for the present day
415 gene pool was in place in LNBA groups already 3000 years ago, but the genetic structure of
416 the Danish population was continually reshaped during succeeding millenia.

418 ### Environmental impact
419 The two documented major population turnovers were accompanied by significant changes in
420 land-use, as apparent from the high-resolution pollen diagram from Lake Højby in Northwest
421 Zealand (Fig. 3) reconstructed using the Landscape Reconstruction Algorithm (LRA,
422 Supplementary Note 6). We have uncovered a direct synchronic link between shifts in a
423 populations' ancestry profile and land-use. During the Mesolithic the landscape was
424 dominated by primary forest trees (*Tilia, Ulmus, Quercus, Fraxinus, Alnus* etc.). At the onset
425 of the Neolithic, the primary forest diminished, cleared by FBC farmers. A new type of forest
426 with more secondary and early successional trees (*Betula* and then *Corylus*) appeared,
427 whereas the proportion between forest and open land remained almost unaltered. From c.
428 5,650 cal. BP deforestation intensified, resulting in an open grassland-dominated landscape.
429 This open phase was short-lived, and the secondary forest expanded again from c. 5,500 to
430 5,000 cal. BP, until another episode of forest clearance occurred during the last part of the
431 FBC epoch. We conclude that the agricultural practice during the FBC was characterised by
432 repeated clearing of the forest followed by regrowth. After c. 4,600 cal BP this strategy
433 changed with the emergence of the SGC and the arrival of Steppe-related ancestry in
434 Denmark. In Western Denmark (Jutland) the arrival of the SGC was characterised by
435 permanent large-scale opening of the landscape to create pastureland[63,64] and we observe here
436 a similar increase of grassland and cropland at Højby Sø in Eastern Denmark around 4,600
437 cal. BP (Fig. 3). Interestingly, this was accompanied by an increase in primary forest cover,
438 especially *Tilia* and *Ulmus* likely reflecting a development of a more permanent division of
439 the landscape into open grazing areas and primary forests.

441 ### Drivers of change
442 We have demonstrated examples of both cultural and demic diffusion during the Mesolithic
443 and Neolithic periods in Denmark. Shifts in the Mesolithic material culture appeared without
444 any detectable levels of changes in ancestry, whereas the two cultural shifts in the Neolithic
445 period were clearly driven by new people coming in. Accordingly, groupings of artefacts and
446 monuments into archaeological cultures do not always represent genetically distinct

447 populations and the underlying mechanisms responsible for prehistoric cultural shifts must be
448 examined on a case-by-case basis.
449 It remains a mystery why the Neolithic farming expansion came to a 1000-year standstill
450 before entering Southern Scandinavia. It may be that it was complicated by a high Mesolithic
451 HG population density owing to a very productive marine and coastal environment[20,65].
452 Further, the Danish Ertebølle population may have been acquainted with armed conflict[11,66]
453 enabling territorial defence against intruders. Alternatively, it has been argued that changing
454 climatic conditions around 6,000 cal. BP became a driver since it enhanced the potential for
455 farming further north[67] but other studies have not confirmed this[68]. The second population
456 turnover in the late Neolithic resulted in a short period of three competing cultural complexes
457 in Denmark, namely the FBC, the PWC, and the SGC. The latter introduced the steppe-
458 related ancestry which has prevailed to this day. There is archaeological evidence that this
459 was a violent time, both in Denmark[69] and elsewhere[70,71]. Additionally, ancient DNA
460 evidence has demonstrated that plague was widespread during this period[72,73]. In tandem with
461 other indicators of population declines[74], and widespread reforestation after 5,000 cal. BP[75], it
462 suggests that the local populations of Central and Northern Europe may have been severely
463 impacted prior to the arrival of newcomers with Steppe-related ancestry. This could explain
464 the rapid population turnover and limited admixture with locals we observe.
465 While the two major shifts in Danish Mesolithic and Neolithic material culture may have had
466 different drivers and causes, the outcomes were ultimately the same: new people arrived and
467 rapidly took over the territory. With this arrival, the local landscape was modified to fit the
468 lifestyle and culture of the immigrants. This is the hallmark of the Anthropocene, observed
469 here in high resolution in prehistoric Denmark.
470

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631

Figure Legends

Fig. 1. Overview of dataset. a, Geographic locations and age ranges relating to the 100 sequenced genomes from Denmark. We define the 'Late Neolithic to Early Bronze Age' category by a combination of age and presence of steppe-related ancestry, see 1c. **b,** PCA of 179 ancient Danish individuals (Supplement Data Table III) ranging from the Mesolithic to the Viking Age, including previously published ones [1,47,57,76], in the context of broader West Eurasian genetic diversity (n=983 modern individuals, open grey circles; n=1,105 ancient individuals, filled grey circles). Ancient individuals from Denmark are coloured according to the period as defined in 1a and 1c. **c,** Unsupervised model-based clustering (ADMIXTURE) for K=8 ancestry components in Danish individuals, as well as contextual data from selected groups (left panels) that represent relevant ancestry components. See Extended Data Fig. 1 for individual labels. Black crosses indicate low coverage genomes represented by pseudo-haploid genotypes.

Fig. 2. Identity-by-descent sharing patterns in ancient Danish individuals, c. 10,500-3000 cal. BP. Heatmap showing relative IBD-sharing rate of 72 imputed ancient individuals from Denmark (n=67 herein reported NEO#, n=5 previously published in[1,47,57,76], from the Mesolithic to the Bronze Age with selected genetic clusters. Individuals are grouped by their genetic cluster membership. See Supplementary Data III for dataset and ancestry category definition.

Fig 3. Genetic, phenotypic, dietary, and environmental shifts in Denmark through time. Evidence of two population turnovers in chronologically sorted multiproxy data from 100 Danish Mesolithic, Neolithic and early Bronze Age skeletons (Supplement Data Table I). The figure shows concomitant changes in (from the top) admixture proportions in non-imputed genome-wide data, Y-chromosomal and mitochondrial haplogroups, genetic phenotype predictions (based on imputed data) and $^{87}Sr/^{86}Sr$ and $\delta^{13}C$ and $\delta^{15}N$ isotope data as proxies for mobility and diet, respectively. Predicted height values represent differences (in cm) from the average height of the present-day Danish population; probabilities for the hair colours (blond, brown, black, red) and eye colours (blue, brown) are shown, with grey denoting probability of intermediate eye colour (including grey, green and hazel). Lower panel shows the quantitative changes in vegetation cover, based on pollen analyses at Lake Højby in Zealand. Note that the vegetation panel covers a shorter time interval than the other panels. Black vertical lines mark the first presence of Anatolian Neolithic farmer ancestry and Steppe-related ancestry, respectively. Individuals with low genomic coverage, signs of possible contamination, and/or low genotype prediction score (GP) are indicated (see Methods).

Fig. 4. Genetic legacy of ancient Danish individuals. Principal component analysis of 2000 modern Danish genomes from the iPSYCH study[77] in the context of ancient western Eurasian individuals. Coloured symbols indicate sample age for ancient Danish individuals, whereas grey symbols indicate 1,145 ancient imputed individuals from across Western Eurasia[3]. Modern Danish individuals are indicated by black filled circles and are shown on the right panel. The inset on the right panel shows a zoomed view of the cluster with modern Danes. The colour scale within the inset represents the age range of the ancient samples within the zoomed region only.

Methods

Ancient genomic analyses

The 100 ancient Danish genomes analysed here contribute to the 317 shotgun-sequenced genomes in Allentoft et al.[3]. All details concerning sampling, DNA extraction, library preparation, sequencing, basic bioinformatics, authentication, and dataset construction are found in the mentioned paper together with all site descriptions and sample metadata. A condensed list of metainformation on the 100 Danish individuals is released here (Supplementary Data I) together with a text summarising the study sites and skeletons (Supplementary Note 1). In short, laboratory work was carried out in dedicated aDNA cleanlab facilities (University of Copenhagen) using optimised aDNA methods[1,78]. Double-stranded blunt-end libraries were sequenced (80bp and 100bp single-end reads) on Illumina HiSeq 2500 and 4000 platforms. Initial shallow shotgun-screening was used to identify samples with sufficient DNA preservation for deeper genomic sequencing. Of the 100 Danish samples that qualified for this, 65 were from tooth cementum, 29 were petrous bones, and six were obtained from other bones (Supplementary Data 1). Sequence reads were bioinformatically mapped to the human reference genome (build 37), filtered and merged to sample level followed by estimates of genomic overage, post-mortem DNA damage, contamination, and genetic sex ID (see[3]). For these 100 samples we observed C-to-T deamination fractions ranging from 12.2% to 66.7%, with an average of 34.9% across all samples (Supplementary Data 1), consistent with highly degraded ancient DNA. We genetically identified 67 males, 32 females and one undetermined in our dataset (Supplement Data Table I).

We utilised a new computational method optimised for low-coverage data[21], to impute genotypes based on genotype likelihoods of ancient individuals with the samtools/bcftools pipeline, and using the 1000 Genomes phased data[79] as a reference panel. To generate the main dataset in[3] this was jointly applied to 1,664 shotgun-sequenced ancient genomes, including our 100 ancient Danish genomes, and resulted in a dataset of 8.5 million common SNPs (>1% minor allele frequency and imputation info score > 0.5) for imputed diploid ancient genomes. After removing genomes with low coverage (<0.1X), low imputation quality (average genotype probability < 0.98), contamination estimates >5%, or close relatives (1st or 2nd degree, lowest coverage relative removed), 67 of the 100 danish genomes were retained as imputed in downstream analyses. The remaining 33 genomes were analysed as pseudo-haploid genotypes.

For population genetic analyses, we combined ancient samples with two different modern reference panels:

- "1000G" dataset: Whole-genome sequencing data of 2,504 individuals from 26 world-wide populations from the 1000 Genomes project, with genotypes at 7,321,965 autosomal SNPs
- "HO" dataset: SNP array data of 2,180 modern individuals from 213 world-wide populations, with genotypes at 535,880 autosomal SNPs

Analyses were based on the "1000G" dataset unless otherwise noted. Individuals not passing imputation quality control cutoffs mentioned above were included in PCA and ADMIXTURE analyses as pseudo-haploid genotypes. Four Danish individuals showed possible signs of DNA contamination (Fig. 3, Supplementary Data I) and were excluded from most analyses. To take full advantage of the extensive multiproxy data they were, however, included in Fig. 3. Individual metadata for all genetic analyses related to the ancient Danish

individuals as well as selected subset of relevant West Eurasian individuals, are reported in
Supplementary Data III.

For PCA combining ancient and modern Western Eurasians (Fig. 1b), we used the data and
framework from[3] to capture West Eurasian genetic diversity based on n=983 modern
genomes and n=1,105 ancient genomes ("HO" dataset). Data from a total of 179 Ancient
Danish genomes are shown in Fig 1b of which 83 are previously published[1,47,57,76]
(Supplementary Data III) - the latter being primarily from the Bronze Age and Viking
periods. For PCA projecting low coverage individuals, we used smartpca with options
'lsqproject: YES' and 'autoshrink: YES'.

The ADMIXTURE results presented in this study represent subsets of individuals from the
full ADMIXTURE runs in[3] where 1,593 ancient individuals were analysed (n=1,492
imputed, n=101 pseudo-haploid, n=71 excluded as close relatives or with a contamination
estimate >5%; "HO" dataset). Figure 1c represents 176 ancient Danish genomes after
excluding three close relatives (Supplementary Data I and IV).

D-statistics were obtained using pseudo-haploid genotypes at transversion SNPs in the
'1000G' dataset, grouping the non-Danish individuals into populations using their
membership in the genetic clusters inferred from IBD sharing (Supplementary Data IV). We
computed D-statistics from genotypes in PLINK format using the 'qpdstat' function
implemented in the ADMIXTOOLS 2 R package[80].

Analysis of IBD sharing and mixture models were carried out as described in [3] using the
same set of inferred genetic clusters (see Supplementary Data IV). Briefly, we used IBDseq[81]
to detect IBD segments, a carried out genetic clustering of the individuals using hierarchical
community detection on a network of pairwise IBD-sharing similarities. IBD-based PCA was
carried out in R using the 'eigen' function on a covariance matrix of pairwise IBD sharing
between the respective ancient individuals. We estimated ancestry proportion in supervised
modelling of target individuals as mixtures of different sets of putative source groups via
non-negative least squares on relative IBD-sharing rate vectors.

Admixture time inference for FBC-associated individuals was carried out using the linkage-
disequilibrium-based method DATES[44] ("HO" dataset). We estimated admixture time
separately for each target individual from Denmark and Sweden, using hunter-gatherer
individuals (n=58) and early farmer individuals (n=49) as the two source groups.

For the PCAs presented in Fig. 4 including modern Danish samples we projected 2,000
imputed samples[82] of individuals born 1981-2005 from the iPSYCH2012 case-cohort study[62]
onto the PCA space spanned by the 1145 non-low coverage or related european and western
Asian ancient imputed samples[3]. Otherwise the analysis is identical to the one described
above. The modern individuals were selected from a subset of the random population
subcohort component of iPSYCH2012 having all four grandparents born in Denmark, and
being of Danish/European ancestry as determined in a separate already existing PCA of main
modern day ancestry groups[82]. This was done using Eigensoft 7.2.1 on the intersect of
imputed SNPs from the ancient and modern samples, filtered by minor allele frequency 0.05,
pruned using PLINK v1.90b6.21[83] based on source samples (parameters: –indep-pairwise
1000 50 0.25) leaving 146,895 variants.

772 The genetic predictions of eye and hair colour were done based on the HIrisPlex system[84].
773 We used imputed effect allele dosages of 18 out of 24 main effect HIrisPlex variants,
774 available for the ancient samples, to derive probabilities for brown, blue and
775 grey/intermediate eye colour and blond, brown, black and red hair colour, following
776 HIrisPlex formulas (see further details in Supplementary Note 2). We predicted relative
777 'genetic height' using allelic effect estimates from 310 common autosomal SNPs with
778 robustly genome-wide significant allelic effects (P<1e-15) in a recent GWAS of height in the
779 UK Biobank[85]. Per-sample height polygenic score (PGS) was calculated for ancient
780 individuals as well as 3,467 Danish ancestry male conscripts from the random population
781 subcohort of the iPSYCH2012 case-cohort study[62] by summing allelic effect multiplied with
782 the effect allele imputed dosage[82] across the 310 loci. For further details see Supplementary
783 Note 2. Only a fraction of the 100 Danish skeletons were suitable for stature estimation by
784 actual measurement, which is why these values are not reported here.
785
786 **Radiocarbon dates and Bayesian modelling of ancestry chronology**
787 For the 100 sample ages in this study we use midpoint estimates of the calibrated and
788 reservoir corrected probability distribution of the radiocarbon age (Supplementary Data I;
789 further AMS dates, associated isotopic measurements, calibrations and reservoir corrections
790 are accessible in[3]. Focusing on estimating the interval between the two major population
791 turnovers, we established a precise chronology using 81 radiocarbon dates from 64 Danish
792 sites relevant to this particular interval (Supplementary Note 3). A Bayesian approach applied
793 to the radiocarbon dates unifies radiocarbon results, ancestry information, and the high
794 precision curve into one calibration process, thereby gaining greater precision. All models
795 and data calibrations were performed utilising OxCal v4.4[86–89] and the calibration dataset
796 from Reimer et al.[90]. We used a trapezoidal phase prior[91,92] for the calculation of the
797 transitional time interval to determine duration between the first appearance of Anatolian
798 farmer-related ancestry to the first appearance of Steppe-related ancestry in Denmark. We
799 corrected the reservoir effect on bones with significantly elevated isotope values (δ13C -
800 18.00 and δ15N +12.00) directly in the models using previously defined reservoir ages as
801 input and calculated the DRE (diet reconstruction estimates) for the individual in ^{14}C years
802 based on the collagen isotope values (Supplementary Note 3, Figs. S3.1, S3.2); for a similar
803 method see[93,94]. For combining radiocarbon dates related to the same individual we used the
804 R_Combine() function. The resultant final model showed Amodel=104.4 and Aoverall=90.2,
805 a very high agreement.
806
807 **Stable isotope proxies for diet and mobility**
808 Bulk collagen isotope values of carbon (δ13C) and nitrogen (δ15N) represent protein sources
809 consumed over several years before death, depending on the skeletal part and the age at death
810 of the individual[95,96]. Generally, δ13C values inform on the proportion of marine versus
811 terrestrial protein, while δ15N values reflect the trophic level from which the proteins were
812 acquired[97,98]. See Supplementary Note 4 for further discussion. Stable isotope values were
813 measured in collagen from all 100 skeletons and the full assemblage of isotopic
814 measurements is available in Supplementary Data II, and further discussed in Supplementary
815 Note 4. Most of the δ13C and δ15N measurements were conducted at the ^{14}C Centre,
816 University of Belfast according to standard protocols[99], based on a modified Longin method
817 including ultra-filtration[99,100]. Measured uncertainty was within the generally accepted range
818 of ±0.2‰ (1 sd) and all samples were within the acceptable atomic C:N range of 2.9-3.6,
819 showing low likelihood of diagenesis[101,102].
820

821 Strontium isotope analyses can provide a proxy for individual mobility[103–105]. The ^{87}Sr/^{86}Sr
822 ratio in specific skeletal elements may reflect the local geological signature obtained through
823 diet by the individual during early childhood and it will usually remain unchanged during life
824 and after death[106]. Ongoing controversies exist over the exact use of geographically-defined
825 baseline values[107,108] why we here restrict our observations and interpretations of Sr-variation
826 to patterns that are only relative to our own data. Measurements of ^{87}Sr/^{86}Sr ratios in teeth
827 and petrous bones were conducted at the Geochronology and Isotope Geochemistry
828 Laboratory (Dept. of Geological Sciences, University of North Carolina- Chapel Hill) and
829 data are found in Supplementary Data II. For further details see Supplementary Note 5.
830
831 **Vegetation modelling**
832 Using a high-resolution pollen diagram from Lake Højby, Northwest Zealand[109], we
833 reconstruct the changes in vegetation cover during the period 5,000-2,400 cal BC using the
834 Landscape-Reconstruction Algorithm (LRA[110,111]). While the LRA has previously been
835 applied at low temporal resolution regional scale (e.g.[112,113]), and to Iron Age (and later)
836 pollen diagrams[114,115], it is the first time that this quantitative method is applied at local scale
837 to a pollen record spanning the Mesolithic and Neolithic periods in Denmark. In total 60
838 pollen samples between 6,900 and 4,400 cal. BP were included and the temporal resolution
839 between samples is approximately 40 years. Regional vegetation was estimated with the
840 model REVEALS[110] based on pollen data from six other lakes on Zealand (see Fig. S6.1).
841 From this, regional pollen rain is calculated and local scale vegetation around Højby Sø
842 calculated using the LOVE model[111]. Average pollen productivity estimates for Europe[116] for
843 25 wind pollinated species were applied. The reconstructed cover for plant species were then
844 combined into four land cover categories, crops (only cereals), grassland (all other herbs),
845 secondary forest (*Betula* and *Corylus*) and primary forest (all other trees). The vegetation
846 reconstruction from Højby Sø is used to illustrate the vegetation development at the
847 Mesolithic/Neolithic transition in eastern Denmark. For more details see Supplementary Note
848 6.
849

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942 **Extended Data Figures**

943
944 **Extended Data Fig. 1. Model-based clustering.** Unsupervised model-based clustering results (ADMIXTURE)
945 for K=2 to K=15 assumed components for all published shotgun-sequenced ancient individuals from Denmark -
946 including the herein presented 93 genomes (contamination <5% and close relative pairs excluded). Imputed

947   genomes were used where available[3]. For low-coverage individuals (indicated with black cross) pseudo-haploid
948   genotypes were used.
949   **Extended Data Fig. 2. Allele sharing of Danish Mesolithic individuals. a,** D-statistic testing whether Danish
950   Mesolithic individuals form a clade with the earliest Danish Mesolithic individual in the dataset (NEO254,
951   Koelbjerg Man) to the exclusion of a genetic cluster of Mesolithic hunter-gatherer individuals from Sweden
952   (Sweden_10000BP_7500BP). **b,** D-statistic testing whether Danish Mesolithic individuals form a clade with a
953   genetic cluster of Western European hunter-gatherer individuals (EuropeW_13500BP_8000BP) to the exclusion
954   of a genetic cluster of Eastern European hunter-gatherer individuals (RussiaNW_11000BP_8000BP). Error bars
955   indicate three standard errors.
956
957   **Extended Data Fig. 3. IBD sharing among ancient individuals from Denmark.** Heatmap showing pairwise
958   amount of total length of IBD shared between 72 ancient Danish individuals dated to older than 3,000 cal. BP.
959   Colours in border and text indicate genetic cluster membership, and dendrograms show clustering hierarchy.
960
961   **Extended Data Fig. 4. Genetic affinities of ancient individuals from Denmark.** Panels show principal
962   component analyses based on pairwise IBD-sharing of **a,** 30 imputed Danish Mesolithic individuals in context
963   of 105 European hunter-gatherers (right panel shows Danish individuals coloured by age); **b,** 22 imputed Danish
964   early Neolithic individuals within the context of 170 Anatolian and European Neolithic farmers **c,** 21 imputed
965   Danish LNBA individuals within the context of 127 European LNBA individuals. Symbol colour and shape
966   indicate the genetic cluster of an individual (Supplementary Data III). The extent of PCA positions of
967   individuals from Denmark are indicated with a dotted line hull. Ancestry cluster categories defined in[3].
968
969   **Extended data Fig. 5. Dietary isotopic signatures.** $\delta^{13}C$ and $\delta^{15}N$ values in bone/dentine samples from 100
970   ancient Danish individuals, coloured according to their main genetic ancestry group. A fundamental dietary and
971   genetic shift is observed at the transition from the Mesolithic to the Neolithic c. 5,900 cal. BP (dashed line).
972   Four anomalous individuals are highlighted. Data from[3] and Supplementary Data II.
973
974   **Extended Data Fig. 6. Ancestry modelling of ancient individuals. a,** Heatmap of ancestry proportions for 72
975   ancient individuals from Denmark dated to older than 3,000 cal. BP estimated from supervised mixture models.
976   Results for three different sets of ancestry source groups (deep, fEur, postNeol, Supplementary Data IV) are
977   distinguished in facet rows. Genetic cluster membership for Danish target individuals is indicated by column
978   facets. **b,** Spatial distribution of estimated ancestry proportions of three different HG sources for Neolithic
979   farmer individuals from Scandinavia and Poland. **c,** Spatial distribution of estimated ancestry proportions of two
980   different farmer sources for LNBA individuals from Scandinavia and Poland. **d,** Ancestry proportions for
981   Scandinavian Iron Age and Viking Age individuals (postBA reference set). **e,** Ancestry proportions for selected
982   ancient European individuals with ancestry related to Scandinavian LNBA individuals (source
983   Scandinavia_4000BP_3000BP, postBA reference set, Supplementary Data IV).
984
985   **Extended Data Fig. 7. Contrasting hunter-gatherer admixture dynamics in Neolithic farmer individuals**
986   **from Denmark and Sweden. a,** Admixture time estimated using DATES[44] as a function of age for Neolithic
987   farmer individuals from Denmark (left) and Sweden (right). Pie charts indicate ancestry composition (light grey
988   - farmer ancestry; dark grey - non-local hunter-gatherer ancestry; colour - local hunter-gatherer ancestry). **b,**
989   Total amount of hunter-gatherer ancestry proportion as a function of admixture time for Neolithic farmer
990   individuals from Denmark (left) and Sweden (right). Error bars indicate ± 1 standard error of admixture time
991   estimate.
992
993   **Extended Data Fig. 8. Fine-scale structure in late Neolithic and Early Bronze Age (LNBA) Scandinavians**
994   **c. 4,500-3,000 cal. BP. a-e,** Geographic locations and PCA based on pairwise IBD sharing (middle) of 148
995   European LNBA individuals predating 3,000 cal. BP (Supplementary Data IV). Geographic locations are shown
996   for 65 individuals belonging to the five genetic clusters observed in 38 ancient Scandinavians (**a,b,** LNBA phase
997   I; **c,d,** LNBA phase II; **e,** LNBA phase III; temporal sequence shown in timeline in centre of plot). Individual
998   assignments and frequency distribution of major Y chromosome haplogroups are indicated in maps and
999   timeline. Plot symbols with black circles indicate the 38 Scandinavian individuals in the PCA panels. Ancestry
1000   proportions for the 38 Scandinavian individuals estimated using proximal source groups from outside
1001   Scandinavia (postNeolScand source set) are shown on the right of the respective cluster results.

Data Availability Statement

Sequencing data analysed in this study is released in the accompanying study 'Population Genomics of Late Stone Age Western Eurasia'[3]. These are publicly available on the European Nucleotide Archive under accession PRJEB64656, together with sequence alignment map files, aligned using human build GRCh37. The full analysis dataset including both imputed and pseudohaploid genotypes for all ancient individuals used in this study is available at https://doi.org/10.17894/ucph.d71a6a5a-8107-4fd9-9440-bdafdfe81455. Aggregated IBD-sharing data as well as hi-resolution versions of supplementary figures are available at Zenodo under accession doi.org/10.5281/zenodo.8196989. Maps were created in R using public domain Natural Earth map data.

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Author Contributions

M.E.A., M.S., and A.F. contributed equally to this work. E.W. initiated the study. M.E.A., M.S., A.F., T.W., K.K. and E.W. led the study. M.E.A., M.S., A.F., M.M, R.N., T.W., K.K. and E.W. conceptualised the study. M.E.A., M.S., T.S.K., R.D., R.N., O.D., T.W., K.K. and E.W. supervised the research. M.E.A., R.D., R.N., T.W., K.K. and E.W. acquired funding for research. A.F., J.S., K.G.S., M.L.S.J., M.U.H., B.H.N, E.K., J.H., K.B.P., L.P., L.K., P. Lotz., P. Lysdahl, P.B., P.V.P., R. Maring, S.W., S.A.S, S.H.A, T.J. and N.L. were involved in sample collection. M.E.A., M.S., A.I., J.S., A.P., B.S.d.M., L.V., A.J. Stern, D.J.L., T.S.K., R.D., R.N., O.D., F. R., K.K. and E.W. were involved in developing and applying methodology. M.E.A., J.S. and L.V. led the DNA laboratory work research component. K.G.S. led bioarchaeological data curation. M.E.A., M.S., A.R.-M., E.K.I.-P., W.B., A.I., A.P., B.S.d.M., B.S.P., R.A.H., T.V., H.M., A.V., A.B.N., P. Rasmussen, G.R., A. Ramsøe, A.J. Schork, A. Rosengren, R.Maring, S.R., T.S.K., and O.D. undertook formal analyses of data. M.E.A., M.S., A.F., K.G.S., A.I., R. Macleod, A. Rosengren, B.S.P., M.F.M., A.B.N., M.U.H, N.N.J., L.P., N.L., T.W., K.K., and E.W. drafted the main text (M.E.A., M.S. and A.F. led this). M.E.A., M.S., A.F., K.G.S., A.I., R. Macleod., A. Rosengren, B.S.P., M.L.S.J., M.N., J.S., T.D.P., M.F.M., A.B.N., M.U.H., L. Sørensen, P.O.N., P. Rasmussen, A.R-M, E.K.I-P, W.B., A.P., B.S.d.M., F.D., R.A.H, T.V., H.M., A.V., L.V., A.J. Stern, A.J. Schork, A. Ruter, A.B.G, B.H.N., E.B.P., E.K., J.H., K.B.P., L.P., L.K., M.J., O.C.U., P.L., P.B., P.V.P., R. Maring, R.I., S.W., S.A.S., T.J., N.L., D.J.L., S.R., T.S.K., K.H.K., R.D., R.N., O.D., T.W. and K.K. drafted supplementary notes and materials. All authors read, commented on, and agreed upon the submitted manuscript.

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1100   **Ethics declarations**

1101
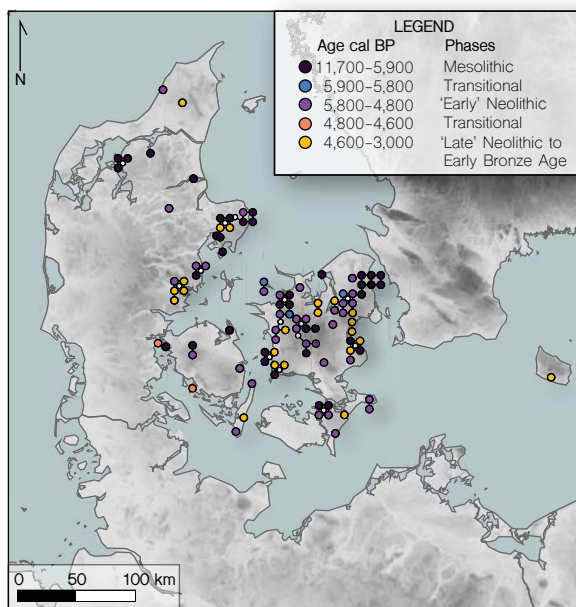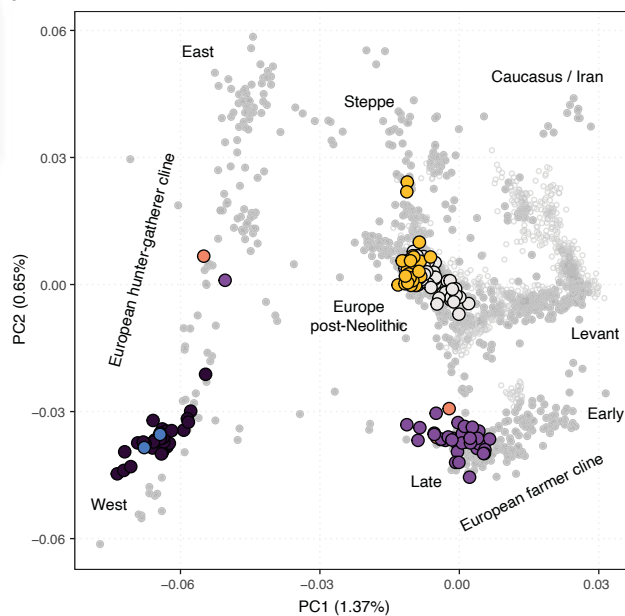
1102   <u>Competing interests</u>

1103

1104   The authors declare no competing interests.

1105

a

LEGEND

Age cal BP	Phases
11,700–5,900	Mesolithic
5,900–5,800	Transitional
5,800–4,800	'Early' Neolithic
4,800–4,600	Transitional
4,600–3,000	'Late' Neolithic to Early Bronze Age

0   50   100 km

b

East

Caucasus / Iran

Steppe

European hunter-gatherer cline

Europe post-Neolithic

Levant

Early

West

Late

European farmer cline

PC2 (0.65%)

PC1 (1.37%)

c

Italy | Mesolithic Sweden | Russia | Anatolia Neolithic Steppe BA Yamnaya | Mesolithic | Transitional | Early Neolithic | Transitional | Late Neolithic Early BA | Iron Age | Viking Age