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The genetic history of Ice Age Europe

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The genetic history of Ice Age Europe

A full list of authors and affiliations appears at the end of the article.

Abstract

Modern humans arrived in Europe ~45,000 years ago, but little is known about their genetic composition before the start of farming ~8,500 years ago. We analyze genome-wide data from 51 Eurasians from ~45,000–7,000 years ago. Over this time, the proportion of Neanderthal DNA decreased from 3–6% to around 2%, consistent with natural selection against Neanderthal variants in modern humans. Whereas the earliest modern humans in Europe did not contribute substantially to present-day Europeans, all individuals between ~37,000 and ~14,000 years ago descended from a single founder population which forms part of the ancestry of present-day Europeans. A ~35,000 year old individual from northwest Europe represents an early branch of this founder population which was then displaced across a broad region, before reappearing in southwest Europe during the Ice Age ~19,000 years ago. During the major warming period after ~14,000 years ago, a new genetic component related to present-day Near Easterners appears in Europe. These results document how population turnover and migration have been recurring themes of European pre-history.

Modern humans arrived in Europe around 45,000 years ago and have lived there ever since, even during the Last Glacial Maximum 25,000–19,000 years ago when large parts of Europe were covered in ice¹. A major question is how climatic fluctuations influenced the population history of Europe and to what extent changes in material cultures documented by archaeology and correlating to climatic events corresponded to movements of people. To date, it has been difficult to address this question because genome-wide ancient DNA has been retrieved from just five Upper Paleolithic individuals in Eurasia^{2–4}. Here we assemble and analyze genome-wide data from 51 modern humans dating from 45,000 to 7,000 years ago (Table 1; Extended Data Table 1; Supplementary Information section 1).

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Correspondence and requests for materials should be addressed to David Reich (reich@genetics.med.harvard.edu).

*These authors contributed equally

+These authors co-supervised the study

Author Contributions

JKr, SP and DR conceived the idea for the study. QF, CP, MH, WH, MMe, VSlo, RGC, APD, ND, VSla, AT, FM, BG, EV, MRG, LGS, CN-M, MT-N, SC, OTM, SB, MPer, DCo, MLa, SR, AR, FV, CT, KW, DG, HR, IC, DFI, PSe, MAM, CC, HB, NJC, KH, VM, DGD, JS, DCa, RP, JKr, SP and DR assembled archaeological material. QF, CP, MH, DFe, AF, WH, MMe, AM, BN, NR, VSlo, ST, HB, DGD, MPR, RP, JKr, SP and DR performed or supervised wet laboratory work. QF, CP, MH, MPet, SM, AP, IL, MLi, IM, SS, PSk, JKe, NP and DR analyzed data. QF, CP, MH, MPet, JKe, SP and DR wrote the manuscript and supplements.

The aligned sequences are available through the European Nucleotide Archive under accession number PRJEB13123.

The authors declare no competing financial interests.

Readers are welcome to comment on the online version of the paper.

Ancient DNA data

We extracted DNA from human remains in dedicated clean rooms⁵, and transformed the extracts into Illumina sequencing libraries^{6–8}. A major challenge in ancient DNA research is that the vast majority of the DNA extracted from most specimens is of microbial origin, making random shotgun sequencing prohibitively expensive. We addressed this problem by enriching the libraries for between 390,000 and 3.7 million single nucleotide polymorphisms (SNPs) in the nuclear genome via hybridizing to pools of previously synthesized 52-base-pair oligonucleotide probes targeting these positions (this strategy makes it possible to generate genome-wide data from samples with high percentages of microbial DNA that are not practical to study by shotgun sequencing)^{3,9}. We sequenced the isolated DNA fragments from both ends, and mapped the consensus sequences to the human genome (*hg19*), retaining fragments that overlapped the targeted SNPs. After removing fragments with identical start and end positions to eliminate duplicates produced during library amplification, we chose one fragment at random to represent each individual at each SNP.

Contamination from present-day human DNA is a danger in ancient DNA research. To address this we took advantage of three characteristic features of ancient DNA (Supplementary Information section 2). First, for an uncontaminated specimen, we expect only a single mitochondrial DNA sequence to be present, allowing us to detect contamination as a mixture of mitochondrial sequences. Second, because males carry a single X chromosome, we can detect contamination in male specimens as polymorphisms on chromosome X¹⁰. Third, cytosines at the ends of genuine ancient DNA molecules are often deaminated, resulting in apparent cytosine to thymine substitutions¹¹. Thus, restricting analysis to molecules with evidence of such deamination filters out the great majority of contaminating molecules¹². For libraries from males with evidence of mitochondrial DNA contamination or X chromosomal contamination estimates >2.5%—as well as for all libraries from females—we restricted the analyses to sequences with evidence of cytosine deamination (Supplementary Information section 2). After merging libraries from the same individual and limiting to individuals with >4,000 targeted SNPs covered at least once, 38 individuals remained, which we merged with newly generated shotgun sequencing data from the *Karelia* individual⁹ (2.0-fold coverage), and published data from ancient^{2–4,7,13–19} and present-day humans²⁰. The final dataset includes 51 ancient modern humans, of which 16 had at least 790,000 SNPs covered (Figure 1; Table 1; Extended Data Table 1).

Natural selection has reduced Neanderthal ancestry over the last 45,000 years

We used two previously published statistics^{3,7,21} to ask if the proportion of Neanderthal ancestry in Eurasians changed over the last 45,000 years. Whereas on the order of 2% of present-day Eurasian DNA is of Neanderthal origin (Extended Data Table 2), the ancient modern human genomes carry significantly more Neanderthal DNA (Figure 2) ($P \ll 10^{-12}$). Using one statistic, we estimate a decline from 4.3–5.7% from a time shortly after introgression to 1.1–2.2% in Eurasians today (Figure 2). Using the other statistic, we estimate a decline from 3.2–4.2% to 1.8–2.3% (Extended Data Figure 1, Extended Data

Table 3). Because all the European samples we analyzed dating to between 37,000 and 14,000 years ago are consistent with descent from a single founding population, admixture with populations with lower Neanderthal ancestry cannot explain the steady decrease in Neanderthal-derived DNA that we detect during this period, showing that natural selection against Neanderthal DNA must have driven this phenomenon (Figure 2). We also obtain an independent line of evidence for selection from our observation that the decrease in Neanderthal-derived alleles is more marked near genes than in less constrained regions of the genome ($P=0.010$) (Supplementary Information section 3; Extended Data Table 3)^{22–25}.

Y chromosomes, mitochondrial DNA and phenotypically important mutations

We used the proportion of sequences mapping to the Y chromosome to infer sex (Extended Data Table 4; Supplementary Information section 4), and determined Y chromosome haplogroups for the males. We were surprised to find haplogroup R1b in the ~14,000-year-old *Villabruna* individual from Italy. While the predominance of R1b in western Europe today is owes its origin to Bronze Age migrations from the eastern European steppe⁹, its presence in *Villabruna* and in a ~7,000-year-old farmer from Iberia⁹ document a deeper history of this haplotype in more western parts of Europe. Additional evidence of an early link between west and east comes from the *HERC2* locus, where a derived allele that is the primary driver of light eye color in Europeans appears nearly simultaneously in specimens from Italy and the Caucasus ~14,000-13,000 years ago. Extended Data Table 5 presents results for additional alleles of known phenotypic importance. When analyzing the mitochondrial genomes we note the presence of haplogroup M in a ~27,000-year-old individual from southern Italy (*Ostuni1*) in agreement with the observation that this haplogroup, which today occurs in Asia and is absent in Europe, was present in pre-Last Glacial Maximum Europe and became lost during the Ice Age²⁶. We also find that the ~33,000 year old *Muierii2* from Romania carries a basal version of haplogroup U6, in agreement with the hypothesis that the presence of derived versions of this haplogroup in North Africans today is due to back-migration from western Eurasia²⁷.

Genetic clustering of the ancient specimens

This dataset provides an unprecedented opportunity to study the population history of Upper Paleolithic Europe over more than 30,000 years. In order to not prejudice any association between genetic and archaeological groupings among the individuals studied, we first allowed the genetic data alone to drive the groupings of the specimens and only afterward examined their associations with archaeological cultural complexes. We began by computing f_3 -statistics¹⁴ of the form $f_3(X, Y; Mbuti)$, which measure shared genetic drift between a pair of ancient individuals after divergence from an outgroup (here *Mbuti* from sub-Saharan Africa), which allowed us to observe clear clusters of samples (Figure 3A; Extended Data Figure 2). Through Multi-Dimensional Scaling (MDS) analysis of this matrix (Figure 3B), as well as through D -statistic analyses²⁸ (Supplementary Information section 5), we identified five clusters of individuals with substantial shared genetic drift, which we name after the oldest individual with >1.0-fold coverage in each cluster (Supplementary

Information section 5; Table 1; Extended Data Table 1). In contrast, we were not able to identify clear structure among these samples based on model-based clustering^{29,30}, which may reflect the fact that many of the samples are so ancient that present-day patterns of human variation are not very relevant to understanding their patterns of genetic differentiation^{4,13}. The “Vestonice Cluster” is composed of 14 pre-Ice Age individuals from 34,000-26,000 years ago, who are all associated with the archaeologically defined Gravettian culture. The “Mal’ta Cluster” is composed of three individuals from the Glacial Maximum 24,000-17,000 years ago from the Lake Baikal region of Siberia. The “El Mirón Cluster” is composed of 6 Late Glacial individuals from 19,000-14,000 years ago, who are all associated with the Magdalenian culture. The “Villabruna Cluster” is composed of 13 post-Ice Age individuals from 14,000-7,000 years ago, associated with the Azilian, Epipaleolithic and Mesolithic cultures. The “Satsurblia Cluster” is composed of two individuals from 13,000-10,000 years ago from the northern Caucasus². There were ten samples that we did not assign to any cluster, either because of evidence of representing distinct early lineages, (*Ust’-Ishim*, *Oase1*, *Kostenki14*, *GoyetQ116-1*, *Muierii2*, *Cioclovina1*, *Kostenki12*), or because they were admixed between major clusters (*Karelia* or *Motala12*), or of very different ancestry (*Stuttgart*). To classify the ancestry of additional low coverage samples, we built an admixture graph that fits the allele frequency correlation patterns among high coverage samples²⁸ (Supplementary Information section 6; Figure 4a). We fit each low coverage sample into the graph in turn, including all fragments from every individual rather than just ones with evidence of cytosine deamination, accounting for contamination bias by modeling (Supplementary Information section 7).

A single founding population during most of the Upper Paleolithic period in Europe

Prior to this work, the most ambitious genetic analysis of early modern humans in Europe was based on the ~37,000-year-old *Kostenki14*⁴. That analysis suggested that the population to which *Kostenki14* belonged harbored within it the three major lineages that exist in mixed form in Europe today¹⁵: (1) a lineage related to all later pre-Neolithic Europeans, (2) a “Basal Eurasian” lineage that split from the ancestors of Europeans and East Asians before they separated from each other; and (3) a lineage related to the ~24,000-year-old *Mal’ta1* from Siberia. With our more extensive sampling of Ice Age Europe, we find no support for this model. When we test whether the ~45,000-year-old *Ust’-Ishim* – an early Eurasian without any evidence of Basal Eurasian ancestry – shares more alleles with one test individual or another by computing statistics of the form $D(\text{Test1}, \text{Test2}; \text{Ust’-Ishim}, \text{Mbuti})$, we find that the statistic is consistent with zero when the *Test* populations are any pre-Neolithic Europeans or present-day East Asians^{3,13,31}. This would not be expected if some of the pre-Neolithic Europeans, including *Kostenki14*, had Basal Eurasian ancestry (Supplementary Information section 8). We also find no evidence for the suggestion that the *Mal’ta1* lineage contributed to Upper Paleolithic Europeans⁴, because when we compute the statistic $D(\text{Test1}, \text{Test2}; \text{Mal’ta1}, \text{Mbuti})$, we find that the statistic is consistent with zero when the *Test* populations are any pre-Neolithic Europeans beginning with *Kostenki14*, implying descent from a single founder population since separation from the lineage leading to *Mal’ta1* (Supplementary Information section 9). A corollary of this finding is that the

widespread presence of *Mal'ta1*-related ancestry in present-day Europeans¹⁵ is due to migrations from the Eurasian steppe in the Neolithic and Bronze Age periods⁹; it is not due to population structure within pre-Neolithic Europe as proposed in the initial analysis of the *Kostenki14* genome⁴.

Resurgence of an early branching European lineage during the Last Glacial Maximum

Among the newly reported individuals, *GoyetQ116-1* from present-day Belgium is the oldest at ~35,000 years ago. It is similar to the ~37,000 year old *Kostenki14* and all later samples in that it shares more alleles with present-day Europeans (e.g. *French*) than with East Asians (e.g. *Han*). In contrast, *Ust'-Ishim* and *Oase1*, which predate *GoyetQ116-1* and *Kostenki14*, do not show any distinctive affinity to later Europeans (Extended Data Table 6). Thus, from at least about 37,000 years ago, populations in Europe shared at least some ancestry with present Europeans. However, *GoyetQ116-1* differs from *Kostenki14* and from all individuals of the succeeding Vestonice Cluster in that both f_3 -statistics (Figure 3; Extended Data Figure 2) and D -statistics show that it shares more alleles with members of the El Mirón Cluster who lived 19,000-14,000 years ago than with other pre-Neolithic Europeans (Supplementary Information section 10). Thus, *GoyetQ116-1* has affinity to individuals who lived more than fifteen thousand years later. While at least half of the ancestry of all El Mirón Cluster individuals comes from the *GoyetQ116-1* cluster, this proportion varies, with the largest amount in individuals outside Iberia ($Z=-4.8$) (Supplementary Information section 10).

A drawing together of the ancestry of Europe and the Near East after ~14,000 years ago

Beginning around 14,000 years ago with the Villabruna Cluster, the strong affinity to *GoyetQ116-1* seen in El Mirón Cluster individuals who belong the Late Glacial Magdalenian Culture is greatly attenuated (Supplementary Information section 10). To test if this change might reflect gene flow from populations that did not descend from the >37,000 year old European founder population, we computed statistics of the form $D(\text{Early European}, \text{Later European}; Y, \text{Mbuti})$ where Y are various present-day non-Africans. If no gene flow from exogenous populations occurred, this statistic is expected to be zero. Figure 4b shows that it is consistent with zero ($|Z|<3$) for nearly all individuals dating to between about 37,000 and 14,000 years ago. However, beginning with the Villabruna Cluster, it becomes highly significantly negative in comparisons where the non-European population (Y) is Near Easterners (Figure 4b; Extended Data Figure 3; Supplementary Information section 11). This must reflect gene flow into the Villabruna Cluster from a population related to present-day Near Easterners rather than gene flow in the reverse direction, because we do not see similar patterns in earlier Europeans although they share substantial amounts of their ancestry with the Villabruna Cluster (Figure 4b). The “Satsurblia Cluster” individuals from the Caucasus dating to ~13,000-10,000 years ago² share more alleles with the Villabruna Cluster individuals than they do with earlier Europeans, indicating that they are related to the population that contributed new alleles to people in the Villabruna Cluster, although they

cannot be the direct source of the gene flow, among other reasons because they have large amounts of Basal Eurasian ancestry while Villabruna Cluster individuals do not² (Supplementary Information section 12; Extended Data Figure 4). One possible explanation for the sudden drawing together of the ancestry of Europe and the Near East at this time is long-distance migrations from the Near East into Europe. However, a plausible alternative is population structure, whereby Upper Paleolithic Europe harbored multiple groups that differed in their relationship to the Near East, with the balance shifting among groups as a result of demographic changes after the Ice Age.

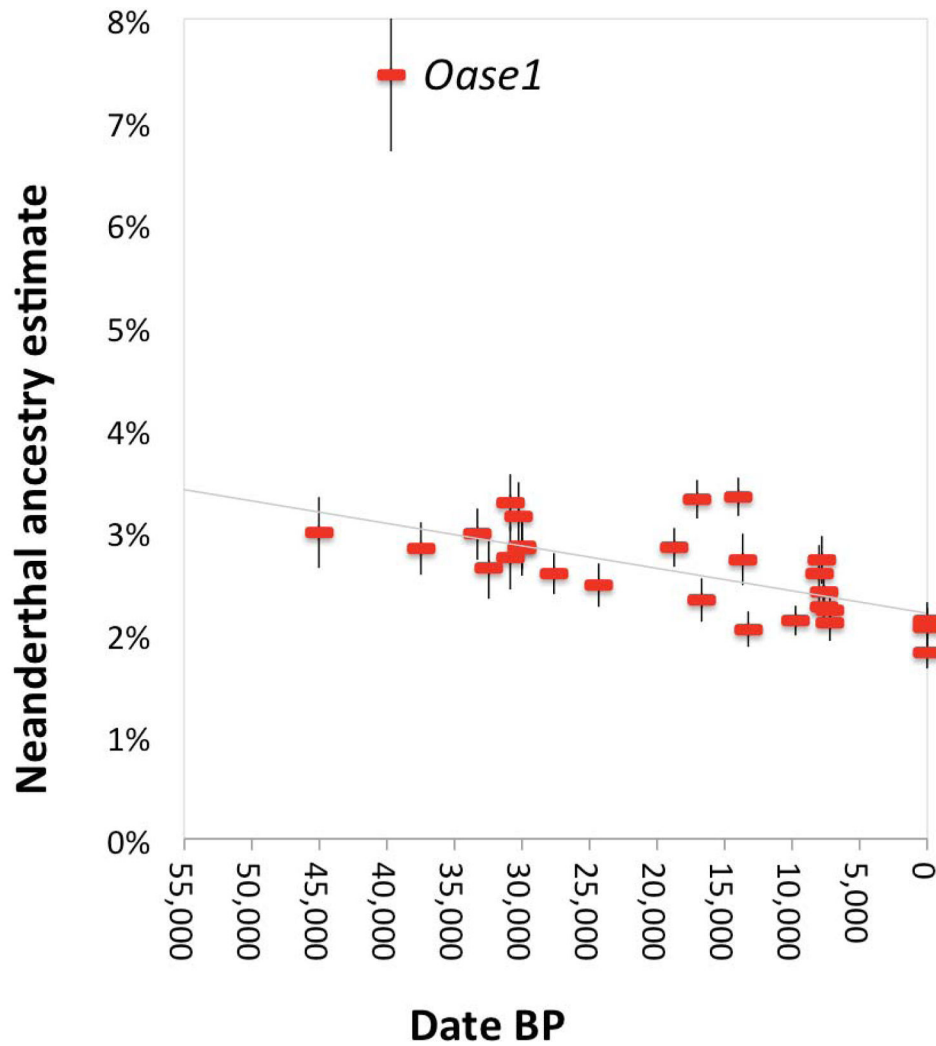
The Villabruna Cluster includes the largest group of samples in this study. This allows us to study heterogeneity within this cluster (Supplementary Information section 13). First, we detect differences in the degree of allele sharing with members of the El Mirón Cluster, as revealed by significant statistics of the form $D(\text{Test1}, \text{Test2}; \text{El Mirón Cluster}, \text{Mbuti})$. Second, we detect an excess of allele sharing with East Asians in a subset of Villabruna Cluster individuals - beginning with a ~13,000 year old sample from Switzerland - as revealed by significant statistics of the form $D(\text{Test1}, \text{Test2}; \text{Han}, \text{Mbuti})$ (Figure 4b and Extended Data Figure 3). For example, *Han* Chinese share more alleles with two Villabruna Cluster individuals (*Loschbour* and *LaBranca1*) than they do with *Kostenki14*, as reflected in significantly negative statistics of the form $D(\text{Kostenki14}, \text{Loschbour/LaBranca1}; \text{Han}, \text{Mbuti})^4$. This statistic was originally interpreted as evidence of Basal Eurasian ancestry in *Kostenki14*. However, because this statistic is consistent with zero when *Han* is replaced with *Ust'-Ishim*, these findings cannot be driven by Basal Eurasian ancestry (as we also discuss above), and must instead be driven by gene flow between populations related to East Asians and the ancestors of some Europeans (Supplementary Information section 8).

Conclusions

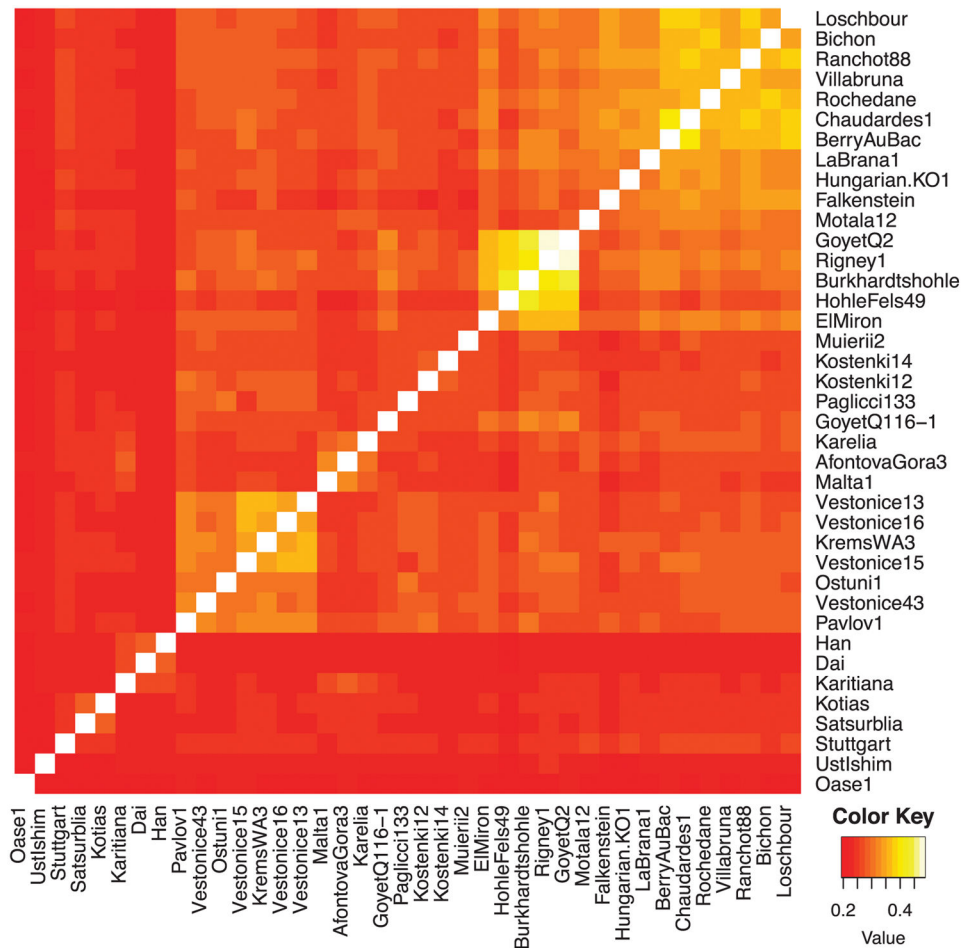
We have shown that the population history of pre-Neolithic Europe was complex in several respects. First, at least some of the initial modern humans to appear in Europe, exemplified by *Ust'-Ishim* and *Oase1*, failed to contribute appreciably to the current European gene pool. Only from around 37,000 years ago do all the European individuals analyzed share ancestry with present-day Europeans³. Second, from the time of *Kostenki14* about 37,000 years ago until the time of the Villabruna Cluster about 14,000 years ago, all individuals seem to derive from a single ancestral population with no evidence of substantial genetic influx from elsewhere. It is interesting that during this time, the *Mal'ta* Cluster is not represented in any of the individuals we sampled from Europe. Thus, while individuals assigned to the Gravettian cultural complex in Europe are associated with the Vestonice Cluster, there is no genetic connection between them and the *Mal'ta1* individual in Siberia despite the fact that Venus figurines are associated with both. This suggests that if this similarity is not a coincidence³², it reflects diffusion of ideas rather than movements of people. Third, we find that *GoyetQ116-1* derives from a different deep branch of the European founder population than the Vestonice Cluster which became predominant in many places in Europe between 34,000 and 26,000 years ago including at Goyet Cave. *GoyetQ116-1* is chronologically associated with the Aurignacian cultural complex. Thus, the subsequent spread of the Vestonice Cluster, which is associated with the Gravettian cultural complex, shows that the spread of the latter culture was mediated at least in part by population movements. Fourth,

the population represented by *GoyetQ116-1* did not disappear, as its descendants became widespread again after ~19,000 years ago in the El Mirón Cluster when we detect them in Iberia. The El Mirón Cluster is associated with the Magdalenian culture and may represent a post-ice age expansion from southwestern European refugia³³. Fifth, beginning with the Villabruna Cluster at least ~14,000 years ago, all European individuals analyzed show an affinity to the Near East. This correlates in time to the Bølling-Allerød interstadial, the first significant warming period after the Ice Age³⁴. Archaeologically, it correlates with cultural transitions within the Epigravettian in Southern Europe³⁵ and the Magdalenian-to-Azilian transition in Western Europe³⁶. Thus, the appearance of the Villabruna Cluster may reflect migrations or population shifts within Europe at the end of the Ice Age, an observation that is also consistent with the evidence of turnover of mitochondrial DNA sequences at this time^{26,37}. One scenario that could explain these patterns is a population expansion from southeastern European or west Asian refugia after the Ice Age, drawing together the genetic ancestry of Europe and the Near East. Sixth, within the Villabruna Cluster, some, but not all, individuals have affinity to East Asians. An important direction for future work is to generate similar ancient DNA data from southeastern Europe and the Near East to arrive at a more complete picture of the Upper Paleolithic population history of western Eurasia³⁸.

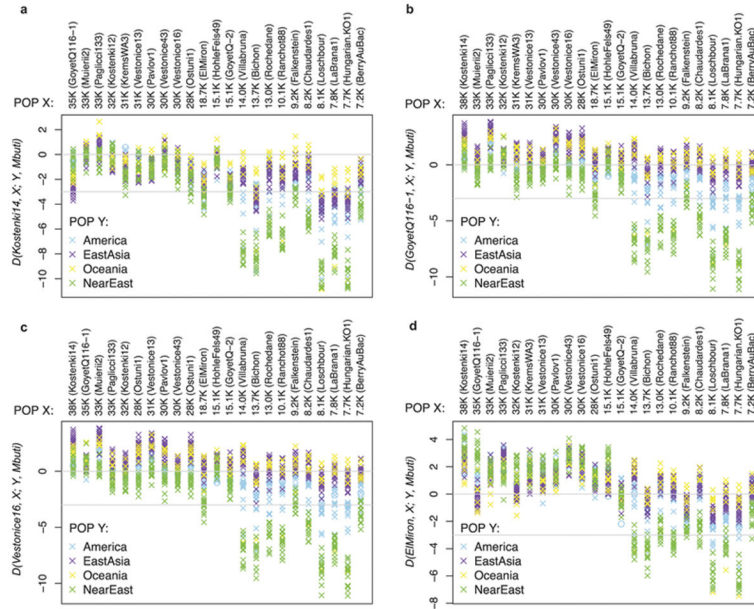
Extended Data

**Extended Data Figure 1. A decrease in Neanderthal ancestry in the last 45,000 years**

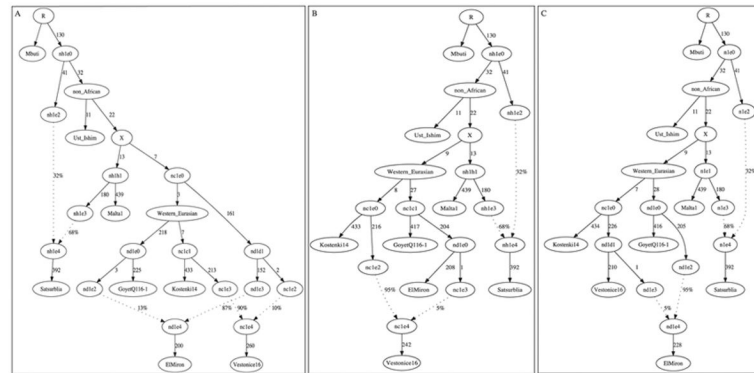
This is similar to Figure 2, except we use ancestry estimates from rates of alleles matching to Neanderthal rather than f_4 -ratios, as described in Supplementary Information section 3). The least squares fit excludes *Oase1* (as an outlier with recent Neanderthal ancestry) and Europeans (known to have reduce Neanderthal ancestry). The regression slope is significantly negative ($P=0.00004$, Extended Data Table 3).



Extended Data Fig. 2. Heat matrix of pairwise $f_3(X, Y; Mbuti)$ for selected ancient samples
 We analyze only samples with at least 30,000 SNPs covered at least once, which pass our quality control.



Extended Data Fig. 3. Studying how the relatedness of non-European populations to pairs of European hunter-gatherers changes over time
 We examine statistics of the form $D(W, X; Y, Mbuti)$, with the Z-score given on the y-axis, where W is an early European hunter-gatherer, X is another European hunter-gatherer (in chronological order on the x-axis), and Y is a non-European population (see legend). **A:** $W=Kostenki14$. **B:** $W=GoyetQ116-1$. **C:** $W=Vestonice16$. **D:** $W=ElMiron$. $|Z| > 3$ scores are considered statistically significant (horizontal line). The similar Figure 4b gives absolute D -statistic values rather than Z-scores (for $W=Kostenki14$) and uses pooled regions rather than individual populations Y.



Extended Data Figure 4. An Admixture Graph model that fits the data for *Satsurblia*, an Upper Paleolithic sample from the Caucasus
 This model uses 127,057 SNPs covered in all populations. Estimated genetic drifts are given along the solid lines in units of f_2 -distance (parts per thousand), and estimated mixture proportions are given along the dotted lines. All three models provide an fit to the allele frequency correlation data among *Mbuti*, *UstIshim*, *Kostenki14*, *Vestonice16*, *Malta1*, *ElMiron* and *Satsurblia* within the limits of our resolution, in the sense that all empirical

f_2 -, f_3 - and f_4 -statistics relating the samples are within three standard errors of the expectation of the model. Models in which *Satsurblia* is modeled as unadmixed cannot be fit.

Extended Data Table 1

The 51 ancient modern humans analyzed in this study

| Sample Code | Data source | Country | Lat. | Long. | Cal BP 95.4% | Date type (ref.) | Culture | Remain | SNP Panel |
|-----------------|-------------|-------------|-------|-------|---------------|-------------------|----------------|----------|-----------|
| UstIshim | 1 | Russia | 57.43 | 71.10 | 47,480-42,560 | Direct-UF (1) | Unassigned | Femur | Shotgun |
| Oase1 | 2 | Romania | 45.12 | 21.90 | 41,640-37,580 | Direct-UF (3) | Unassigned | Mandible | Shotgun |
| Kostenki14* | New | Russia | 51.23 | 39.30 | 38,680-36,260 | Direct-UF (4) | Unassigned | Tibia | 3.7M |
| GoyetQ116-1 | New | Belgium | 50.26 | 4.28 | 35,160-34,430 | Direct-NotUF (5) | Aurignacian | Humerus | 1240k |
| Muierii2 | New | Romania | 45.11 | 23.46 | 33,760-32,840 | Direct-UF (6) | Unassigned | Temporal | 3.7M |
| Paglicci133 | New | Italy | 41.65 | 15.61 | 34,580-31,210 | Layer (7) | Gravettian | Tooth | 1240k |
| Cioclovina1 | New | Romania | 45.35 | 23.84 | 33,090-31,780 | Direct-UF (8) | Unassigned | Cranium | 1240k |
| Kostenki12 | New | Russia | 51.23 | 39.30 | 32,990-31,840 | Layer (9) | Unassigned | Cranium | 3.7M |
| KremsWA3 | New | Austria | 48.41 | 15.59 | 31,250-30,690 | Layer (10) | Gravettian | Cranium | 1240K |
| Vestonice13 | New | Czech | 48.53 | 16.39 | 31,070-30,670 | Layer (9) | Gravettian | Femur | 3.7M |
| Vestonice15 | New | Czech | 48.53 | 16.39 | 31,070-30,670 | Layer (9) | Gravettian | Femur | 3.7M |
| Vestonice14 | New | Czech | 48.53 | 16.39 | 31,070-30,670 | Layer (9) | Gravettian | Femur | 390k |
| Pavlov1 | New | Czech | 48.53 | 16.39 | 31,110-29,410 | Layer (9) | Gravettian | Femur | 3.7M |
| Vestonice43 | New | Czech | 48.53 | 16.39 | 30,710-29,310 | Layer (9) | Gravettian | Femur | 3.7M |
| Vestonice16 | New | Czech | 48.53 | 16.39 | 30,710-29,310 | Layer (9) | Gravettian | Femur | 3.7M |
| Ostuni2 | New | Italy | 40.73 | 17.57 | 29,310-28,640 | Direct-UF (New) | Gravettian | Femur | 3.7M |
| GoyetQ53-1 | New | Belgium | 50.26 | 4.28 | 28,230-27,720 | Direct-NotUF (5) | Gravettian | Fibula | 1240k |
| Paglicci108 | New | Italy | 41.65 | 15.61 | 28,430-27,070 | Layer (5) | Gravettian | Phalanx | 1240k |
| Ostuni1 | New | Italy | 40.73 | 17.57 | 27,810-27,430 | Direct-UF (New) | Gravettian | Tibia | 3.7M |
| GoyetQ376-19 | New | Belgium | 50.26 | 4.28 | 27,720-27,310 | Direct-NotUF (5) | Gravettian | Humerus | 1240k |
| GoyetQ56-16 | New | Belgium | 50.26 | 4.28 | 26,600-26,040 | Direct-NotUF (5) | Gravettian | Fibula | 1240k |
| Malta1 | 11 | Russia | 52.9 | 103.5 | 24,520-24,090 | Direct-UF (11) | Unassigned | Humerus | Shotgun |
| ElMiron | New | Spain | 43.26 | -3.45 | 18,830-18,610 | Direct-UF (5) | Magdalenian | Toe | 3.7M |
| AfontovaGora3 | New | Russia | 56.05 | 92.87 | 16,930-16,490 | Layer (5) | Unassigned | Tooth | 3.7M |
| AfontovaGora2 | 11 | Russia | 56.05 | 92.87 | 16,930-16,490 | Direct-UF (11) | Unassigned | Humerus | Shotgun |
| Rigney1 | New | France | 47.23 | 6.10 | 15,690-15,240 | Direct-NotUF (12) | Magdalenian | Mandible | 1240k |
| HohleFels49 | New | Germany | 48.22 | 9.45 | 16,000-14,260 | Layer (13) | Magdalenian | Femur | 390k |
| GoyetQ-2 | New | Belgium | 50.26 | 4.28 | 15,230-14,780 | Direct-NotUF (5) | Magdalenian | Humerus | 1240k |
| Brillenhohle | New | Germany | 48.24 | 9.46 | 15,120-14,440 | Direct-UF (14) | Magdalenian | Cranium | 390k |
| HohleFels79 | New | Germany | 48.22 | 9.45 | 15,070-14,270 | Direct-UF (5) | Magdalenian | Cranium | 390k |
| Burkhardtshohle | New | Germany | 48.32 | 9.35 | 15,080-14,150 | Direct-UF (15) | Magdalenian | Cranium | 1240k |
| Villabruna | New | Italy | 46.15 | 12.21 | 14,180-13,780 | Direct-UF (16) | Epigravettian | Femur | 3.7M |
| Bichon | 17 | Switzerland | 47.01 | 6.79 | 13,770-13,560 | Direct-UF (17) | Azilian | Petrous | Shotgun |
| Satsurblia | 17 | Georgia | 42.24 | 42.92 | 13,380-13,130 | Direct-UF (17) | Epigravettian | Petrous | Shotgun |
| Rochedane | New | France | 47.21 | 6.45 | 13,090-12,830 | Direct-NotUF (5) | Epipaleolithic | Mandible | 1240k |
| Ibousseries39 | New | France | 44.29 | 4.46 | 12,040-11,410 | Direct-NotUF (5) | Epipaleolithic | Femur | 390k |

| Sample Code | Data source | Country | Lat. | Long. | Cal BP 95.4% | Date type (ref.) | Culture | Remain | SNP Panel |
|---------------|---------------|------------|-------|-------|---------------|------------------------------|-----------------|---------|-----------|
| Continenza | New | Italy | 41.96 | 13.54 | 11,200-10,510 | Layer (New) | Mesolithic | Cranium | 3.7M |
| Ranchot88 | New | France | 47.91 | 5.43 | 10,240-9,930 | Direct-NotUF ⁽⁵⁾ | Mesolithic | Cranium | 1240k |
| LesCloseaux13 | New | France | 48.52 | 2.11 | 10,240-9,560 | Direct-NotUF ⁽¹⁸⁾ | Mesolithic | Femur | 1240k |
| Kotias | ¹⁷ | Georgia | 42.13 | 43.12 | 9,890-9,550 | Direct-UF ⁽¹⁷⁾ | Mesolithic | Tooth | Shotgun |
| Falkenstein | New | Germany | 48.06 | 9.04 | 9,410-8,990 | Direct-UF ⁽¹⁹⁾ | Mesolithic | Fibula | 390k |
| Karelia | ²⁰ | Russia | 61.65 | 35.65 | 8,800-7,950 | Layer ⁽²¹⁾ | Mesolithic | Tooth | Shotgun |
| Bockstein | New | Germany | 48.33 | 10.09 | 8,370-8,160 | Layer ⁽²²⁾ | Mesolithic | Tooth | 390k |
| Ofnet | New | Germany | 48.49 | 10.27 | 8,430-8,060 | Layer ⁽²³⁾ | Mesolithic | Tooth | 390k |
| Chaudardes1 | New | France | 49.24 | 3.46 | 8,360-8,050 | Direct-NotUF ⁽⁵⁾ | Mesolithic | Tibia | 1240k |
| Loschbour | ²⁴ | Luxembourg | 49.70 | 6.24 | 8,160-7,940 | Direct-UF ⁽²⁴⁾ | Mesolithic | Tooth | Shotgun |
| LaBrana1 | ²⁵ | Spain | 42.93 | -5.35 | 7,940-7,690 | Direct-UF ⁽²⁶⁾ | Mesolithic | Tooth | Shotgun |
| Hungarian.KO1 | ²⁷ | Hungarian | 47.93 | 21.20 | 7,730-7,590 | Direct-UF ⁽²⁷⁾ | Neolithic | Petrous | Shotgun |
| Motala12 | ²⁴ | Sweden | 58.54 | 15.05 | 7,670-7,580 | Direct-UF (New) | Mesolithic | Tooth | Shotgun |
| BerryAuBac | New | France | 49.24 | 3.54 | 7,320-7,170 | Direct-NotUF ⁽⁵⁾ | Mesolithic | Radius | 1240k |
| Stuttgart | ²⁴ | Germany | 48.78 | 9.18 | 7,260-7,020 | Direct-UF (New) | Early Neolithic | Tooth | Shotgun |

Note: All dates are obtained as described in Supplementary Information section 1. When an individual has a direct date from an element from the same skeleton it is marked "Direct", followed by a hyphen to indicate whether the date is obtained by ultrafiltration ("UF") or without ("NotUF"). If the date is from the archaeological layers, we mark the date type as "Layer". All the dates were calibrated using IntCal13²⁸ and the OxCal4.2 program²⁹.

* We represent Kostenki14 in most analyses by our newly reported 16.1x capture data, but repeat key analyses on the previously reported 2.8x shotgun data³⁰.

[†] Mean coverage is computed on the 3.7M SNP targets.

Extended Data Table 2

Estimated proportion of Neanderthal ancestry

| Sample Code | Age BP | SNPs | f_4 -ratios | | Archaic Ancestry Informative SNPs | | | | S.E. |
|-------------|--------|-----------|---------------|---------------|-----------------------------------|------|-------------|---|------|
| | | | Est. | 95% CI | SNPs | Est. | 95% CI | Increase in Neanderthal ancestry with B | |
| UstIshim | 45,020 | 2,137,615 | 4.4% | 3.6% – 5.3% | 778,774 | 3.0% | 2.3% – 3.7% | -0.9% | 1.3% |
| Oase1 | 39,610 | 285,076 | 9.9% | 8.4% – 11.4% | 59,854 | 7.5% | 6.0% – 8.9% | 2.5% | 1.8% |
| Kostenki14 | 37,470 | 1,774,156 | 3.6% | 2.7% – 4.4% | 632,748 | 2.8% | 2.3% – 3.3% | -1.0% | 1.0% |
| GoyetQ116-1 | 34,795 | 846,983 | 3.4% | 2.4% – 4.3% | | | | | |
| Muierii2 | 33,300 | 98,618 | 5.2% | 3.0% – 7.4% | 22,189 | 3.0% | 2.5% – 3.5% | 0.6% | 1.1% |
| Paglicci133 | 32,895 | 82,330 | 4.1% | 2.1% – 6.0% | | | | | |
| Cioclovina1 | 32,435 | 12,784 | 4.1% | -1.1% – 9.3% | | | | | |
| Kostenki12 | 32,415 | 61,228 | 1.9% | -0.7% – 4.4% | 13,385 | 2.6% | 2.1% – 3.2% | 1.7% | 1.5% |
| KremsWA3 | 30,970 | 203,986 | 3.9% | 2.6% – 5.2% | | | - | | |
| Vestonice13 | 30,870 | 139,568 | 4.6% | 2.6% – 6.5% | 35,983 | 3.3% | 2.7% – 3.8% | 0.3% | 1.3% |
| Vestonice15 | 30,870 | 30,900 | 4.3% | 0.6% – 7.9% | 5,855 | 2.7% | 2.1% – 3.4% | -1.5% | 1.3% |
| Vestonice14 | 30,870 | 5,677 | 2.6% | -5.9% – 11.0% | | | | | |
| Pavlov1 | 30,260 | 57,005 | 4.4% | 1.6% – 7.1% | 9,327 | 3.1% | 2.5% – 3.8% | 0.7% | 1.2% |
| Vestonice43 | 30,010 | 163,946 | 6.9% | 5.2% – 8.5% | 38,749 | 2.9% | 2.4% – 3.3% | 0.9% | 0.9% |

| Sample Code | Age BP | SNPs | f_4 -ratios | | Archaic Ancestry Informative SNPs | | | Increase in Neanderthal ancestry with B | S.E. |
|-----------------|--------|-----------|---------------|---------------|-----------------------------------|------|-------------|---|------|
| | | | Est. | 95% CI | SNPs | Est. | 95% CI | | |
| Vestonice16 | 30,010 | 945,292 | 4.1% | 3.1% – 5.1% | 268,157 | 2.8% | 2.3% – 3.3% | -0.1% | 1.0% |
| Ostuni2 | 28,975 | 17,017 | 1.6% | -3.2% – 6.3% | 2,746 | 2.3% | 1.4% – 3.1% | 1.3% | 1.6% |
| GoyetQ53-1 | 27,975 | 12,567 | 4.8% | -0.7% – 10.3% | | | | | |
| Paglicci108 | 27,750 | 4,330 | 3.4% | -6.0% – 12.7% | | | | | |
| Ostuni1 | 27,620 | 369,313 | 4.2% | 3.0% – 5.4% | 88,449 | 2.6% | 2.2% – 3.0% | 0.1% | 0.9% |
| GoyetQ376-19 | 27,515 | 25,400 | 6.5% | 2.7% – 10.2% | | | | | |
| GoyetQ56-16 | 26,320 | 9,988 | 3.6% | -1.9% – 9.1% | | | | | |
| Malta1 | 24,305 | 1,439,501 | 2.9% | 1.9% – 3.8% | 437,187 | 2.5% | 2.1% – 2.9% | 1.0% | 0.8% |
| ElMiron | 18,720 | 797,714 | 3.6% | 2.6% – 4.5% | 250,071 | 2.8% | 2.5% – 3.2% | 0.6% | 0.9% |
| AfontovaGora3 | 16,710 | 286,355 | 3.0% | 1.8% – 4.2% | 96,237 | 3.3% | 2.9% – 3.7% | -1.5% | 1.0% |
| AfontovaGora2 | 16,710 | 143,751 | 2.2% | 0.4% – 4.0% | 37,280 | 2.3% | 1.9% – 2.7% | -0.3% | 0.9% |
| Rigney1 | 15,465 | 35,600 | 0.8% | -2.6% – 4.2% | | | | | |
| HohleFels49 | 15,130 | 63,151 | 2.3% | -0.6% – 5.2% | | | | | |
| GoyetQ-2 | 15,005 | 72,263 | 1.7% | -0.6% – 4.0% | | | | | |
| Brillenhohle | 14780 | 13,459 | 2.5% | -3.0% – 8.1% | | | | | |
| HohleFels79 | 14,670 | 11,211 | 1.7% | -5.1% – 8.5% | | | | | |
| Burkhardtshohle | 14,615 | 38,376 | 1.7% | -1.6% – 5.0% | | | | | |
| Villabruna | 13,980 | 1,215,433 | 2.7% | 1.8% – 3.5% | 425,148 | 3.3% | 3.0% – 3.7% | 1.1% | 0.9% |
| Bichon | 13,665 | 2,116,782 | 2.9% | 1.9% – 3.8% | 769,422 | 2.7% | 2.2% – 3.2% | 0.7% | 1.3% |
| Satsurbliia | 13,255 | 1,460,368 | 1.5% | 0.6% – 2.4% | 542,561 | 2.0% | 1.7% – 2.4% | 0.9% | 0.6% |
| Rochedane | 12,960 | 237,390 | 1.9% | 0.5% – 3.3% | | | | | |
| Ibousseries39 | 11,725 | 9,659 | 6.4% | -0.8% – 13.7% | | | | | |
| Continenza | 10,855 | 11,717 | 4.1% | -1.4% – 9.6% | 1,733 | 2.9% | 1.8% – 4.0% | -10.6% | 4.4% |
| Ranchot88 | 10,085 | 414,863 | 2.9% | 1.8% – 4.0% | | | | | |
| LesCloseaux13 | 9,900 | 8,635 | -3.0% | -9.7% – 3.8% | | | | | |
| Kotias | 9,720 | 2,133,968 | 1.8% | 1.0% – 2.7% | 779,146 | 2.1% | 1.8% – 2.4% | 0.7% | 0.5% |
| Falkenstein | 9,200 | 64,428 | 4.8% | 1.7% – 7.8% | | | | | |
| Karelia | 8,375 | 1,754,410 | 1.9% | 1.1% – 2.7% | 582,444 | 2.2% | 1.9% – 2.6% | -0.2% | 0.7% |
| Bockstein | 8,265 | 21,977 | 5.7% | 1.0% – 10.5% | | | | | |
| Ofnet | 8,245 | 6,263 | 9.8% | 1.4% – 18.1% | | | | | |
| Chaudardes1 | 8,205 | 92,657 | 1.9% | -0.2% – 3.9% | | | | | |
| Loschbour | 8,050 | 2,091,584 | 2.5% | 1.6% – 3.3% | 774,139 | 2.6% | 2.0% – 3.1% | 2.7% | 1.7% |
| LaBrana1 | 7,815 | 1,884,745 | 1.9% | 1.1% – 2.8% | 642,231 | 2.7% | 2.3% – 3.2% | 0.4% | 0.8% |
| Hungarian.KO1 | 7,660 | 1,410,303 | 2.1% | 1.2% – 3.0% | 439,408 | 2.4% | 2.0% – 2.8% | -0.1% | 1.2% |
| Motala12 | 7,625 | 1,874,519 | 2.5% | 1.6% – 3.3% | 655,685 | 2.3% | 1.9% – 2.7% | -0.1% | 0.7% |
| BerryAuBac | 7,245 | 54,690 | 2.5% | -0.2% – 5.1% | | | | | |
| Stuttgart | 7,140 | 2,078,724 | 1.9% | 1.1% – 2.7% | 767,813 | 2.1% | 1.8% – 2.5% | 0.0% | 0.7% |
| Dai | 0 | 2,144,502 | 1.4% | 0.7% – 2.1% | 782,066 | 1.8% | 1.5% – 2.1% | 1.4% | 0.4% |
| Han | 0 | 2,144,502 | 1.8% | 1.1% – 2.5% | 782,164 | 2.1% | 1.8% – 2.5% | 1.9% | 0.7% |

| Sample Code | Age BP | SNPs | f_4 -ratios | | Archaic Ancestry Informative SNPs | | | Increase in Neanderthal ancestry with B | S.E. |
|-------------|--------|-----------|---------------|-------------|-----------------------------------|------|-------------|---|------|
| | | | Est. | 95% CI | SNPs | Est. | 95% CI | | |
| English | 0 | 2,144,502 | 1.5% | 0.8% – 2.2% | | | | | |
| French | 0 | 2,144,502 | 1.5% | 0.9% – 2.1% | 782,386 | 1.7% | 1.4% – 1.9% | 1.4% | 0.6% |
| Sardinian | 0 | 2,144,502 | 1.2% | 0.6% – 1.9% | 782,351 | 1.7% | 1.4% – 2.0% | 0.7% | 0.5% |
| Karitiana | 0 | | | | 782,037 | 2.1% | 1.7% – 2.4% | 1.5% | 1.0% |

Extended Data Table 3

Significant correlation of Neanderthal ancestry estimate with specimen age

| Subset of samples | N | P-value for date correlation | Decrease in ancestry per 10,000 years | Estimate of Neanderthal ancestry at different time points | | | |
|--|----|------------------------------|---------------------------------------|---|------------------|------------------|------------------|
| | | | | 0 years ago (present) | 50,000 years ago | 55,000 years ago | 60,000 years ago |
| f_4-ratio estimates | | | | | | | |
| Core Set 1 (all ancient samples (except <i>Oase1</i>) + <i>Han</i> + <i>Dai</i>) | 57 | 5×10^{-22} | 0.48–0.73% | 1.1–2.2% | 4.0–5.4% | 4.3–5.7% | 4.5–6.0% |
| Subset of Core Set 1 (<32kya) | 50 | 2×10^{-15} | 0.59–0.98% | 0.9–2.1% | 4.5–6.4% | 4.8–6.9% | 5.1–7.4% |
| Subset of Core Set 1 (>32kya or <25kya) | 44 | 4×10^{-18} | 0.44–0.69% | 1.0–2.2% | 3.7–5.2% | 4.0–5.5% | 4.2–5.8% |
| Subset of Core Set 1 (>25kya or <14kya) | 47 | 5×10^{-21} | 0.48–0.73% | 1.0–2.2% | 3.9–5.3% | 4.2–5.7% | 4.5–6.0% |
| Subset of Core Set 1 (>14kya or present day) | 37 | 2×10^{-18} | 0.47–0.74% | 1.1–2.4% | 4.1–5.5% | 4.3–5.8% | 4.6–6.2% |
| Subset of Core Set 1 (only ancient samples) | 50 | 4×10^{-15} | 0.46–0.76% | 1.0–2.3% | 4.0–5.4% | 4.3–5.8% | 4.5–6.1% |
| Subset of Core Set 1 (individuals with >200,000 SNPs) | 28 | 4×10^{-19} | 0.46–0.71% | 1.1–2.3% | 3.9–5.3% | 4.2–5.7% | 4.4–6.0% |
| Modification of Core Set 1 (replace East Asians with Europeans) | 58 | 2×10^{-23} | 0.49–0.73% | 1.1–2.3% | 4.0–5.4% | 4.3–5.8% | 4.6–6.1% |
| All ancient samples including <i>Oase1</i> + <i>Han</i> + <i>Dai</i> | 58 | 8×10^{-29} | 0.57–0.81% | 1.0–2.2% | 4.3–5.7% | 4.7–6.1% | 5.0–6.5% |
| All ancient samples | 51 | 1×10^{-20} | 0.57–0.86% | 0.9–2.2% | 4.4–5.8% | 4.7–6.2% | 5.0–6.6% |
| All ancient samples except <i>Oase1</i> or <i>UstIshim</i> | 49 | 8×10^{-12} | 0.45–0.81% | 1.0–2.3% | 4.0–5.6% | 4.2–6.0% | 4.5–6.4% |
| Ancestry informative SNPs | | | | | | | |
| Core Set 2 (all ancient samples (except <i>Oase1</i>) + <i>Han</i> + <i>Dai</i> + <i>Karitiana</i>) | 29 | 4×10^{-11} | 0.21–0.39% | 1.8–2.3% | 3.1–4.0% | 3.2–4.2% | 3.3–4.3% |
| Subset of Core Set 2 (no <i>Han</i> , <i>Dai</i> , <i>Karitiana</i> , <i>Stuttgart</i>) | 25 | 1×10^{-4} | 0.11–0.36% | 1.8–2.5% | 2.9–3.8% | 3.0–4.0% | 3.0–4.1% |
| Subset of Core Set 2 (no <i>Han</i> , <i>Dai</i> , <i>Karitiana</i> , <i>Stuttgart</i> , <i>UstIshim</i>) | 24 | 2×10^{-4} | 0.11–0.37% | 1.8–2.5% | 2.9–3.8% | 2.9–4.0% | 3.0–4.2% |

Note: The “Core Set 1,” used for the f_4 -ratio analyses, refers to 50 ancient samples (removing *Oase1* as an outlier) along with 7 East Asians (*Dai* and *Han*). “Core Set 2,” used for the analyses of Neanderthal ancestry informative SNPs, refers to 26 ancient samples (removing *Oase1*) along with *Han*, *Dai*, and *Karitiana*

Extended Data Table 4

Sex determination for newly reported samples. Y-rate is the ratio of N_Y/N_{auto} divided by the same quantity for the genome-wide target set. Female sex (F) is inferred as Y-rate < 0.05 and male sex (M) as Y-rate > 0.

| Sample | Target | Type | N_{auto} | N_X | N_Y | N_X/N_{auto} | N_Y/N_{auto} | X-rate | Y-rate | Sex |
|-----------------|----------------|--------|-------------------|--------|--------|-----------------------|-----------------------|--------|--------|-----|
| | 1240k or 2.2M* | | 1151240 | 49711 | 32681 | 0.0432 | 0.0284 | | | |
| | 390k | | 388745 | 1819 | 2242 | 0.0047 | 0.0058 | | | |
| Kostenki14 | 2.2M | all | 29633405 | 395534 | 262846 | 0.0133 | 0.0089 | 0.309 | 0.312 | M |
| GoyetQ116-1 | 1240k | all | 2122620 | 36391 | 22256 | 0.0171 | 0.0105 | 0.397 | 0.369 | M |
| Cioclovina1 | 1240k | Damage | 11521 | 184 | 125 | 0.0160 | 0.0108 | 0.370 | 0.382 | M |
| Kostenki12 | 2.2M | Subset | 63908 | 856 | 504 | 0.0134 | 0.0079 | 0.310 | 0.278 | M |
| Muierii2 | 2.2M | Damage | 81165 | 2177 | 8 | 0.0268 | 0.0001 | 0.621 | 0.003 | F |
| Vestonice13 | 2.2M | Damage | 119094 | 1578 | 1059 | 0.0133 | 0.0089 | 0.307 | 0.313 | M |
| Vestonice15 | 2.2M | Damage | 28762 | 338 | 227 | 0.0118 | 0.0079 | 0.272 | 0.278 | M |
| Vestonice14 | 390k | Damage | 4846 | 8 | 11 | 0.0017 | 0.0023 | 0.353 | 0.394 | M |
| Vestonice43 | 2.2M | Damage | 136933 | 1826 | 1204 | 0.0133 | 0.0088 | 0.309 | 0.310 | M |
| Pavlov1 | 2.2M | Damage | 54429 | 631 | 404 | 0.0116 | 0.0074 | 0.268 | 0.261 | M |
| Vestonice16 | 2.2M | Subset | 2433741 | 30463 | 20976 | 0.0125 | 0.0086 | 0.290 | 0.304 | M |
| KremsWA3 | 1240k | all | 235069 | 4119 | 2661 | 0.0175 | 0.0113 | 0.406 | 0.399 | M |
| Ostuni2 | 2.2M | Damage | 15749 | 138 | 1 | 0.0088 | 0.0001 | 0.203 | 0.002 | F |
| Ostuni1 | 2.2M | Damage | 427199 | 10868 | 47 | 0.0254 | 0.0001 | 0.589 | 0.004 | F |
| Paglicci108 | 1240k | Damage | 3883 | 124 | 2 | 0.0319 | 0.0005 | 0.740 | 0.018 | F |
| GoyetQ53-1 | 1240k | Damage | 10771 | 311 | 4 | 0.0289 | 0.0004 | 0.669 | 0.013 | F |
| GoyetQ376-19 | 1240k | Damage | 20052 | 680 | 10 | 0.0339 | 0.0005 | 0.785 | 0.018 | F |
| GoyetQ56-16 | 1240k | Damage | 8702 | 304 | 7 | 0.0349 | 0.0008 | 0.809 | 0.028 | F |
| Paglicci133 | 1240k | Subset | 81092 | 1641 | 983 | 0.0202 | 0.0121 | 0.469 | 0.427 | M |
| ElMiron | 2.2M | Damage | 1765696 | 40647 | 196 | 0.0230 | 0.0001 | 0.533 | 0.004 | F |
| HohleFels79 | 390k | Damage | 10188 | 28 | 22 | 0.0027 | 0.0022 | 0.587 | 0.374 | M |
| AfontovaGora3 | 2.2M | Damage | 291798 | 8705 | 37 | 0.0298 | 0.0001 | 0.691 | 0.004 | F |
| HohleFels49 | 390k | Damage | 61051 | 113 | 111 | 0.0019 | 0.0018 | 0.396 | 0.315 | M |
| Rigney1 | 1240k | Damage | 32797 | 1131 | 9 | 0.0345 | 0.0003 | 0.799 | 0.010 | F |
| GoyetQ-2 | 1240k | Damage | 65563 | 1123 | 706 | 0.0171 | 0.0108 | 0.397 | 0.379 | M |
| Brillenhohle | 390k | Damage | 12603 | 22 | 22 | 0.0017 | 0.0017 | 0.373 | 0.303 | M |
| Burkhardtshohle | 1240k | Damage | 34207 | 563 | 407 | 0.0165 | 0.0119 | 0.381 | 0.419 | M |
| Villabruna | 2.2M | Subset | 5505838 | 72055 | 52110 | 0.0131 | 0.0095 | 0.303 | 0.333 | M |
| Rochedane | 1240k | Subset | 256325 | 4780 | 2830 | 0.0186 | 0.0110 | 0.432 | 0.389 | M |
| Continenza | 2.2M | Damage | 10647 | 208 | 2 | 0.0195 | 0.0002 | 0.452 | 0.007 | F |
| Ibousseries39 | 390k | Damage | 8246 | 12 | 22 | 0.0015 | 0.0027 | 0.311 | 0.463 | M |
| Ranchot88 | 1240k | Damage | 594962 | 18520 | 119 | 0.0311 | 0.0002 | 0.721 | 0.007 | F |
| LesCloseaux13 | 1240k | Damage | 7326 | 275 | 2 | 0.0375 | 0.0003 | 0.869 | 0.010 | F |
| Falkenstein | 390k | Damage | 58970 | 113 | 102 | 0.0019 | 0.0017 | 0.410 | 0.300 | M |

| Sample | Target | Type | N _{auto} | N _X | N _Y | N _X /N _{auto} | N _Y /N _{auto} | X-rate | Y-rate | Sex |
|-------------|--------|--------|-------------------|----------------|----------------|-----------------------------------|-----------------------------------|--------|--------|-----|
| Bockstein | 390k | Damage | 20214 | 62 | 0 | 0.0031 | 0.0000 | 0.655 | 0.000 | F |
| Ofnet | 390k | Damage | 5294 | 13 | 1 | 0.0025 | 0.0002 | 0.525 | 0.033 | F |
| Chaudardes1 | 1240k | Damage | 84052 | 1429 | 865 | 0.0170 | 0.0103 | 0.394 | 0.363 | M |
| BerryAuBac | 1240k | All | 49670 | 902 | 554 | 0.0182 | 0.0112 | 0.421 | 0.393 | M |

*We restrict analysis to the 1240k target set for study of the 2.2M capture datasets.

Extended Data Table 5

Allele counts at SNPs thought to be affected by selection in samples that have at least 1.0-fold coverage. rs4988235 is responsible for lactase persistence in Europe^{59,60}. The SNPs at *SLC24A5* and *SLC45A2* are responsible for light skin pigmentation. The SNP at *EDAR*^{61,62} affects tooth morphology and hair thickness. The SNP at *HERC2*^{63,64} is the primary determinant of light eye color in present-day Europeans. We present the fraction of fragments overlapping each SNP that are derived; the observation of a low rate of derived alleles does not prove that the individual carried the allele, and instead may reflect sequencing error or ancient DNA damage. We highlight in light gray sites that we judge (based on the derived allele count) are likely to be heterozygous for the derived allele, and in dark gray sites that are likely to be homozygous.

| SNP | | <i>LCT</i> | <i>SLC45A2</i> | <i>SLC24A5</i> | <i>EDAR</i> | <i>HERC2</i> |
|-------------|--------------------------|------------|----------------|----------------|-------------|--------------|
| | | rs4988235 | rs16891982 | rs1426654 | rs3827760 | rs12913832 |
| UstIshim | Ancestral | G | C | G | A | A |
| | Derived | A | G | A | G | G |
| | Coverage | 31 | 46 | 52 | 42 | 50 |
| | Derived allele frequency | 0% | 0% | 2% | 0% | 0% |
| Kostenki14 | Coverage | 140 | 113 | 6 | 45 | 52 |
| | Derived allele frequency | 0% | 2% | 17% | 0% | 0% |
| GoyetQ116-1 | Coverage | 8 | 6 | 0 | 9 | 1 |
| | Derived allele frequency | 0% | 0% | n/a | 0% | 0% |
| Vestonice16 | Coverage | 13 | 18 | 0 | 4 | 5 |
| | Derived allele frequency | 0% | 6% | | 0% | 0% |
| Malta1 | Coverage | 1 | 0 | 2 | 2 | 2 |
| | Derived allele frequency | 0% | | 0% | 0% | 0% |
| ElMiron | Coverage | 2 | 10 | 0 | 7 | 5 |
| | Derived allele frequency | 0% | 0% | | 0% | 0% |
| Villabruna | Coverage | 17 | 52 | 5 | 19 | 10 |
| | Derived allele frequency | 0% | 0% | 0% | 0% | 100% |
| Bichon | Coverage | 11 | 4 | 25 | 16 | 9 |
| | Derived allele frequency | 0% | 0% | 0% | 0% | 33% |
| Satsurbliia | Coverage | 1 | 2 | 4 | 1 | 4 |

| | | <i>LCT</i> | <i>SLC45A2</i> | <i>SLC24A5</i> | <i>EDAR</i> | <i>HERC2</i> |
|---------------|--------------------------|------------|----------------|----------------|-------------|--------------|
| | SNP | rs4988235 | rs16891982 | rs1426654 | rs3827760 | rs12913832 |
| | Derived allele frequency | 0% | 0% | 100% | 0% | 50% |
| Kotias | Coverage | 16 | 22 | 13 | 20 | 15 |
| | Derived allele frequency | 0% | 0% | 100% | 0% | 0% |
| Loschbour | Coverage | 19 | 18 | 20 | 17 | 21 |
| | Derived allele frequency | 0% | 0% | 0% | 0% | 100% |
| LaBraná1 | Coverage | 8 | 6 | 2 | 11 | 3 |
| | Derived allele frequency | 12% | 0% | 0% | 0% | 100% |
| Hungarian.KO1 | Coverage | 1 | 2 | 2 | 1 | 2 |
| | Derived allele frequency | 0% | 0% | 50% | 0% | 100% |
| Motala12 | Coverage | 2 | 0 | 3 | 3 | 1 |
| | Derived allele frequency | 0% | | 0% | 33% | 100% |
| Karelia | Coverage | 1 | 9 | 4 | 0 | 1 |
| | Derived allele frequency | 0% | 67% | 0% | | 0% |
| Stuttgart | Coverage | 25 | 21 | 15 | 29 | 21 |
| | Derived allele frequency | 0% | 0% | 100% | 0% | 0% |

Extended Data Table 6

All European hunter-gatherers after Kostenki14 share genetic drift with present-day Europeans. We compute the statistic $D(\text{Han}, \text{Test}; \text{French}, \text{Mbuti})$. Measuring whether present-day *French* share more alleles with *Han* or with a *Test* population (restricting to ancient samples with at least 30,000 SNPs covered at least once). Present-day Europeans share significantly more genetic drift with European hunter-gatherers from *Kostenki14* onward than they do with *Han*. Thus, by the date of *Kostenki14*, there was already West Eurasian-specific genetic drift.

| Test | SNPs used | D-value | Z score |
|-------------|-----------|---------|---------|
| Ust'-Ishim | 2,050,358 | 0.003 | 6.6 |
| Oase1 | 278,785 | 0.005 | 10.6 |
| Kostenki14 | 1,676,253 | -0.002 | -5.5 |
| Muierii2 | 95,787 | -0.004 | -6.3 |
| GoyetQ116-1 | 811,756 | -0.004 | -8.0 |
| Kostenki12 | 59,850 | -0.004 | -5.1 |
| Paglicci133 | 79,624 | -0.004 | -5.5 |
| Vestonice13 | 136,598 | -0.004 | -7.1 |
| Vestonice15 | 30,252 | -0.006 | -6.4 |
| Vestonice16 | 914,141 | -0.004 | -9.1 |
| Pavlov1 | 55,835 | -0.005 | -6.3 |

| Test | SNPs used | D-value | Z score |
|-----------------|-----------|---------|---------|
| Vestonice43 | 160,463 | -0.004 | -6.9 |
| KremsWA3 | 229,187 | -0.005 | -10.2 |
| Ostuni1 | 360,347 | -0.004 | -8.6 |
| Malta1 | 1,401,718 | -0.005 | -11.3 |
| ElMiron | 777,654 | -0.007 | -14.7 |
| AfontovaGora2 | 141,073 | -0.007 | -13.6 |
| AfontovaGora3 | 707,617 | -0.006 | -13.6 |
| HohleFels49 | 62,816 | -0.004 | -5.2 |
| Rigney1 | 34,445 | -0.006 | -6.1 |
| GoyetQ-2 | 70,210 | -0.006 | -8.8 |
| Burkhardtshohle | 37,234 | -0.006 | -6.2 |
| Villabruna | 1,170,777 | -0.010 | -24.7 |
| Bichon | 2,034,069 | -0.009 | -23.6 |
| Satsurbliia | 1,419,824 | -0.005 | -13.1 |
| Rochedane | 229,806 | -0.011 | -20.8 |
| Ranchot88 | 402,274 | -0.010 | -21.8 |
| Kotias | 2,047,856 | -0.006 | -15.8 |
| Falkenstein | 64,043 | -0.008 | -11.6 |
| Chaudardes1 | 90,047 | -0.011 | -16.0 |
| Loschbour | 2,037,082 | -0.011 | -25.4 |
| LaBran1 | 1,824,307 | -0.009 | -23.0 |
| Motala12 | 1,816,201 | -0.009 | -23.8 |
| Hungarian.KO1 | 1,372,801 | -0.010 | -26.5 |
| Karelia | 1,701,664 | -0.009 | -21.9 |
| Stuttgart | 2,023,939 | -0.009 | -23.9 |
| BerryAuBac | 53,028 | -0.011 | -14.0 |

Supplementary Material

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Authors

Qiaomei Fu^{1,2,3}, Cosimo Posth^{4,5,*}, Mateja Hajdinjak^{3,*}, Martin Petr³, Swapan Mallick^{2,6,7}, Daniel Fernandes^{8,9}, Anja Furtwängler⁴, Wolfgang Haak^{5,10}, Matthias Meyer³, Alissa Mittnik^{4,5}, Birgit Nickel³, Alexander Peltzer⁴, Nadin Rohland², Viviane Slon³, Sahra Talamo¹¹, Iosif Lazaridis², Mark Lipson², Iain Mathieson², Stephan Schiffels⁵, Pontus Skoglund², Anatoly P. Derevianko^{12,13}, Nikolai Drozdov¹², Vyacheslav Slavinsky¹², Alexander Tsybankov¹², Renata Grifoni Cremonesi¹⁴, Francesco Mallegni¹⁵, Bernard Gély¹⁶, Eligio Vacca¹⁷, Manuel R. González Morales¹⁸, Lawrence G. Straus^{18,19}, Christine Neugebauer-Maresch²⁰, Maria Teschler-Nicola^{21,22}, Silviu Constantin²³, Oana Teodora Moldovan²⁴, Stefano Benazzi^{11,25}, Marco Peresani²⁶, Donato Coppola^{27,28}, Martina Lari²⁹, Stefano

Ricci³⁰, Annamaria Ronchitelli³⁰, Frédérique Valentin³¹, Corinne Thevenet³², Kurt Wehrberger³³, Dan Grigorescu³⁴, Hélène Rougier³⁵, Isabelle Crevecoeur³⁶, Damien Flas³⁷, Patrick Semal³⁸, Marcello A. Mannino^{11,39}, Christophe Cupillard^{40,41}, Hervé Bocherens^{42,43}, Nicholas J. Conard^{43,44}, Katerina Harvati^{43,45}, Vyacheslav Moiseyev⁴⁶, Dorothee G. Drucker⁴², Ji í Svoboda^{47,48}, Michael P. Richards^{11,49}, David Caramelli²⁹, Ron Pinhasi⁸, Janet Kelso³, Nick Patterson⁶, Johannes Krause^{4,5,43,+}, Svante Pääbo^{3,+}, and David Reich^{2,6,7,+}

Affiliations

¹Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, IVPP, CAS, Beijing 100044, China ²Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA ³Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig 04103, Germany ⁴Institute for Archaeological Sciences, Archaeo- and Palaeogenetics, University of Tübingen, Tübingen 72070, Germany ⁵Max Planck Institute for the Science of Human History, 07745 Jena, Germany ⁶Broad Institute of MIT and Harvard, Cambridge Massachusetts 02142, USA ⁷Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts 02115, USA ⁸School of Archaeology and Earth Institute, Belfield, University College Dublin, Dublin 4, Ireland ⁹CIAS, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal ¹⁰Australian Centre for Ancient DNA, School of Biological Sciences, The University of Adelaide, SA-5005 Adelaide, Australia ¹¹Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany ¹²Institute of Archaeology and Ethnography, Russian Academy of Sciences, Siberian Branch, 17 Novosibirsk, RU-630090, Russia ¹³Altai State University, Barnaul, RU-656049, Russia ¹⁴Dipartimento di Civiltà e Forme del Sapere, Università di Pisa, 56126 Pisa, Italy ¹⁵Department of Biology, University of Pisa 56126 Pisa, Italy ¹⁶Direction régionale des affaires culturelles Rhône-Alpes, 69283 Lyon cedex 01, France ¹⁷Dipartimento di Biologia, Università degli Studi di Bari 'Aldo Moro', 70125 Bari, Italy ¹⁸Instituto Internacional de Investigaciones Prehistoricas, Universidad de Cantabria, 39005 Santander, Spain ¹⁹Department of Anthropology MSC01 1040, University of New Mexico, Albuquerque, NM 87131-0001, USA ²⁰Quaternary Archaeology, Institute for Institute for Oriental and European Archaeology, Austrian Academy of Sciences, 1010 Vienna, Austria ²¹Department of Anthropology, Natural History Museum Vienna, 1010 Vienna, Austria ²²Department of Anthropology, University of Vienna, 1090 Vienna, Austria ²³"Emil Racovita" Institute of Speleology, 010986 Bucharest 12, Romania ²⁴"Emil Racovita" Institute of Speleology, Cluj Branch, 400006 Cluj, Romania ²⁵Department of Cultural Heritage, University of Bologna, Ravenna, 48121, Italy ²⁶Sezione di Scienze Preistoriche e Antropologiche, Dipartimento di Studi Umanistici, Università di Ferrara, 44100 Ferrara, Italy ²⁷Università degli Studi di Bari 'Aldo Moro', 70125 Bari, Italy ²⁸Museo di "Civiltà preclassiche della Murgia meridionale", 72017 Ostuni, Italy ²⁹Dipartimento di Biologia, Università di Firenze, 50122 Florence, Italy ³⁰Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente, U.R. Preistoria e Antropologia, Università degli Studi di Siena, 53100 Siena, Italy ³¹CNRS/ UMR

7041 ArScAn MAE, 92023 Nanterre, France ³²INRAP/ UMR 8215 Trajectoires 21, 92023 Nanterre, France ³³Ulmer Museum, 89073 Ulm, Germany ³⁴University of Bucharest, Faculty of Geology and Geophysics, Department of Geology, 01041 Bucharest, Romania ³⁵Department of Anthropology, California State University Northridge, Northridge, CA 91330-8244, USA ³⁶Université de Bordeaux, CNRS, UMR 5199-PACEA, 33615 Pessac Cedex, France ³⁷TRACES – UMR 5608, Université Toulouse Jean Jaurès, Maison de la Recherche, 31058 Toulouse Cedex 9, France ³⁸Royal Belgian Institute of Natural Sciences, 1000 Brussels, Belgium ³⁹Department of Archaeology, School of Culture and Society, Aarhus University, 8270 Højbjerg, Denmark ⁴⁰Service Régional d'Archéologie de Franche-Comté, 25043 Besançon Cedex, France ⁴¹Laboratoire de Chrono-Environnement, UMR 6249 du CNRS, UFR des Sciences et Techniques, 25030 Besançon Cedex, France ⁴²Department of Geosciences, Biogeology, University of Tübingen, 72074 Tübingen, Germany ⁴³Senckenberg Centre for Human Evolution and Palaeoenvironment, University of Tübingen, 72072 Tübingen, Germany ⁴⁴Department of Early Prehistory and Quaternary Ecology, University of Tübingen, 72070 Tübingen, Germany ⁴⁵Institute for Archaeological Sciences, Paleoanthropology, University of Tübingen, 72070 Tübingen, Germany ⁴⁶Museum of Anthropology and Ethnography, Saint Petersburg 34, Russia ⁴⁷Department of Anthropology, Faculty of Science, Masaryk University, 611 37 Brno, Czech Republic ⁴⁸Institute of Archaeology at Brno, Academy of Science of the Czech Republic, 69129 Dolní Věstonice, Czech Republic ⁴⁹Department of Anthropology, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada

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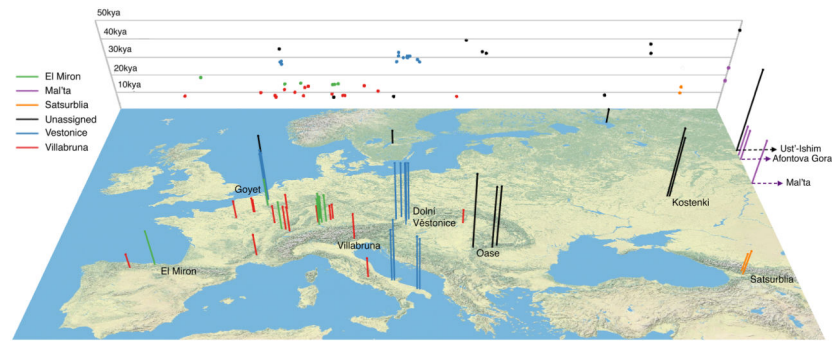


Figure 1. Location and age of 51 ancient samples

Each bar corresponds to a sample, the color code designates the genetically defined sample cluster, and the height is proportional to sample age (the background grid shows a projection of longitude against sample age). To help in visualization, we add jitter for sites with multiple samples from nearby locations. Four samples that are from Siberia are plotted at the far eastern edge of the map.

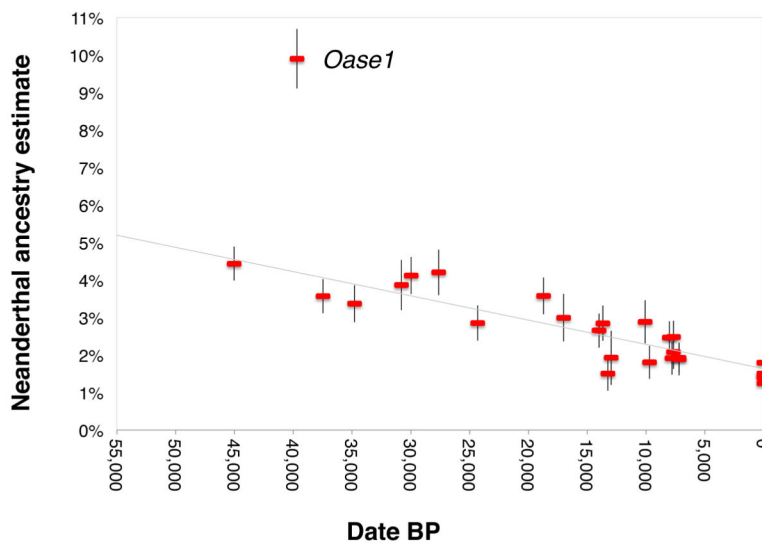


Figure 2. Decrease of Neanderthal ancestry over time

Plot of radiocarbon date against Neanderthal ancestry for samples with at least >200,000 SNPs covered, along with present-day Eurasians (standard errors are from a Block Jackknife). The least squares fit (gray) excludes the data from *Oase1* (an outlier with recent Neanderthal ancestry) and three present-day European populations (known to have less Neanderthal ancestry than East Asians). The slope is significantly negative for all eleven subsets of samples we analyzed ($10^{-29} < P < 10^{-11}$ based on a Block Jackknife) (Extended Data Table 3).

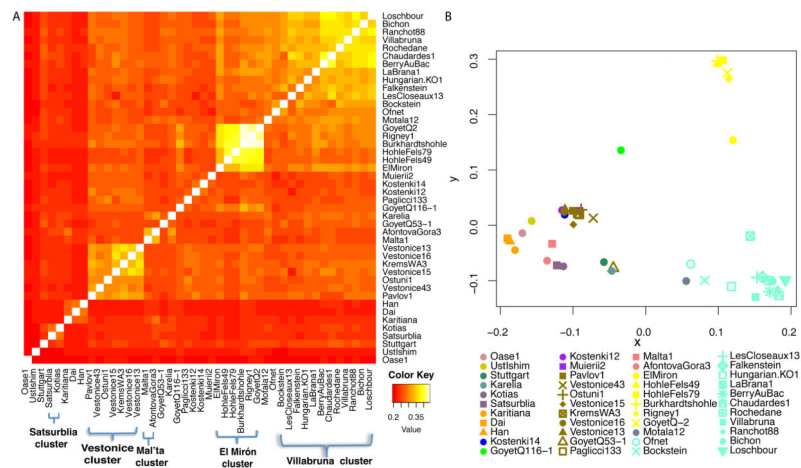


Figure 3. Genetic clustering

(A) Shared genetic drift measured by $f_3(X, Y; \text{Mbuti})$ among samples with at least 30,000 SNPs covered (for AfontovaGora3, EIMiron, Falkenstein, GoyetQ-2, GoyetQ53-1, HohleFels49, HohleFels79, LesCloseaux13, Ofnet, Ranchot88 and Rigney1, we use all sequences for higher resolution). Lighter colors indicate more shared drift. (B) Multidimensional Dimensional Scaling (MDS) analysis, computed using the R software cmdscale package, highlights the main genetic groupings analyzed in this study: Vestonice Cluster (brown), Mal'ta Cluster (pink), El Mirón Cluster (yellow), Villabruna Cluster (light blue), and Satsurbliia Cluster (dark purple). The affinity of GoyetQ116-1 (green) to the El Mirón Cluster is evident in both views of the data.

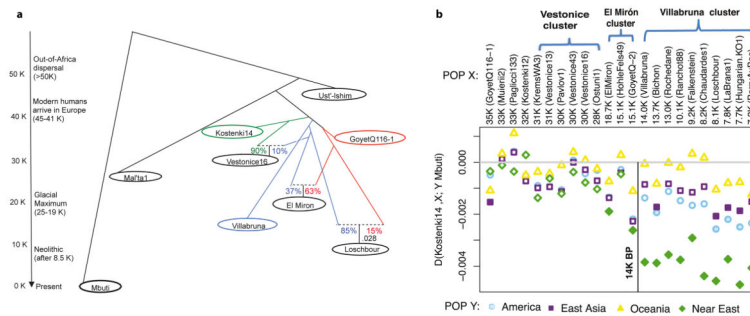


Figure 4. Population history inferences

(A) Admixture Graph relating selected high coverage samples. Dashed lines show inferred admixture events; the estimated mixture proportions fitted using the ADMIXTUREGRAPH software are labeled²⁸ (the estimated genetic drift on each branch is given in a version of this graph shown in Supplementary Information section 6). The samples are positioned vertically based on their radiocarbon date, but we caution that the population split times are not accurately known. We use color to highlight important early branches of the European founder population: the *Kostenki14* lineage is modeled as the predominant contributor to the Vestonice Cluster (green); the *GoyetQ116-1* lineage as the predominant contributor to the El Mirón Cluster (red); and the *Villabruna* lineage as broadly represented across many clusters. (B) Drawing together of European and Near Eastern populations ~14,000 years ago. Plot of affinity of each pre-Neolithic European population X to non-Africans outside Europe Y moving forward in time, comparing to *Kostenki14* as a baseline; values $Z < -3$ standard errors below zero are indicated with filled symbols (we restricted to individuals with $>50,000$ SNPs). We observe an affinity to Near Easterners beginning with the Villabruna Cluster, and another to East Asians that affects a subset of the Villabruna Cluster.