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# Ancient human genomes suggest three ancestral populations for present-day Europeans

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

# **Abstract**

We sequenced the genomes of a ~7,000 year old farmer from Germany and eight ~8,000 year old hunter-gatherers from Luxembourg and Sweden. We analyzed these and other ancient genomes 1-4 with 2,345 contemporary humans to show that most present Europeans derive from at least three highly differentiated populations: West European Hunter-Gatherers (WHG), who contributed ancestry to all Europeans but not to Near Easterners; Ancient North Eurasians (ANE) related to Upper Paleolithic Siberians<sup>3</sup>, who contributed to both Europeans and Near Easterners; and Early European Farmers (EEF), who were mainly of Near Eastern origin but also harbored WHG-related ancestry. We model these populations' deep relationships and show that EEF had ~44% ancestry from a "Basal Eurasian" population that split prior to the diversification of other non-African lineages.

> Near Eastern migrants played a major role in the introduction of agriculture to Europe, as ancient DNA indicates that early European farmers were distinct from European huntergatherers<sup>4,5</sup> and close to present-day Near Easterners<sup>4,6</sup>. However, modelling present-day Europeans as a mixture of these two ancestral populations<sup>4</sup> does not account for the fact that they are also admixed with a population related to Native Americans<sup>7,8</sup>. To clarify the prehistory of Europe, we sequenced nine ancient genomes (Fig. 1A; Extended Data Fig. 1): "Stuttgart" (19-fold coverage), a ~7,000 year old skeleton found in Germany in the context

Supplementary Information is linked to the online version of the paper at www.nature.com/nature. The fully public version of the Human Origins dataset can be found at http://genetics.med.harvard.edu/reichlab/Reich Lab/Datasets.html. The full version of the dataset (including additional samples) is available to researchers who send a signed letter to DR indicating that they will abide by specified usage conditions (SI9).

#### Author contributions

BB, EEE, JBu, MS, SP, JKe, DR and JKr supervised the study. IL, NP, AM, GR, SM, KK, PHS, JGS, SC, ML, QF, HL, CdF, KP, WH, MMet, MMey and DR analyzed genetic data. FH, EF, DD, MF, J-MG, JW, AC and JKr obtained human remains. AM, CE, RBo, KB, SS, CP, NR and JKr processed ancient DNA. IL, NP, SN, NR, GA, HAB, GBa, EB, OB, RBa, GBe, HB-A, JBe, FBe, CMB, FBr, GBJB, FC, MC, DECC, DCor, LD, GvD, SD, J-MD, SAF, IGR, MG, MH, BH, TH, UH, ARJ, SK-Y, RKh, EK, RKi, TK, WK, VK, AK, LL, SL, TL, RWM, BM, EM, JMol, JMou, KN, DN, TN, LO, JP, FP, OLP, VR, FR, IR, RR, HS, ASaj, ASal, EBS, ATar, DT, ST, IU, OU, RVa, MVi, MVo, CW, LY, PZ, TZ, CC, MGT, AR-L, SAT, LS, KT, RVi, DCom, RS, MMet, SP and DR assembled the genotyping dataset. IL, NP, DR and JKr wrote the manuscript with help from all co-authors.

The aligned sequences are available through the European Nucleotide Archive under accession number PRJEB6272.

The authors declare competing financial interests: UH is an employee of Illumina, TL is an employee of AMGEN, and JM is an employee of 23andMe.

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<sup>&</sup>lt;sup>†</sup>Correspondence and requests for materials should be addressed to David Reich (reich@genetics.med.harvard.edu) or Johannes Krause (johannes.krause@uni-tuebingen.de).

53Currently employed by AMGEN; 33 Kazantzaki Str, Ilioupolis 16342, Athens, Greece

<sup>83</sup> Present address: Banaras Hindu University, Varanasi, 221 005, India

of artifacts from the first widespread farming culture of central Europe, the *Linearbandkeramik*; "Loschbour" (22-fold), an ~8,000 year old skeleton from the Loschbour rock shelter in Luxembourg, discovered in the context of hunter-gatherer artifacts (SI1; SI2); and seven ~8,000 year old samples (0.01–2.4-fold) from a hunter-gatherer burial in Motala, Sweden (the highest coverage individual was "Motala12").

Sequence reads from all samples revealed >20% C $\rightarrow$ T and G $\rightarrow$ A deamination-derived mismatches at the ends of the molecules that are characteristic of ancient DNA<sup>9,10</sup> (SI3). We estimate nuclear contamination rates to be 0.3% for Stuttgart and 0.4% for Loschbour (SI3), and mitochondrial (mtDNA) contamination rates to be 0.3% for Stuttgart, 0.4% for Loschbour, and 0.01–5% for the Motala individuals (SI3). Stuttgart has mtDNA haplogroup T2, typical of Neolithic Europeans<sup>11</sup>, and Loschbour and all Motala individuals have the U5 or U2 haplogroups, typical of hunter-gatherers<sup>5,9</sup> (SI4). Stuttgart is female, while Loschbour and five Motala individuals are male (SI5) and belong to Y-chromosome haplogroup I, suggesting that this was common in pre-agricultural Europeans (SI5).

We carried out large-scale sequencing of libraries prepared with uracil DNA glycosylase (UDG), which removes deaminated cytosines, thus reducing errors arising from ancient DNA damage (SI3). The ancient individuals had indistinguishable levels of Neanderthal ancestry when compared to each other (~2%) and to present-day Eurasians (SI6). The heterozygosity of Stuttgart (0.00074) is at the high end of present-day Europeans, while that of Loschbour (0.00048) is lower than in any present humans (SI2), reflecting a strong bottleneck in Loschbour's ancestors as the genetic data show that he was not recently inbred (Extended Data Fig. 2). High copy numbers for the salivary amylase gene (AMY1) have been associated with a high starch diet<sup>12</sup>; our data are consistent with this finding in that the ancient hunter gatherers La Braña (from Iberia)<sup>2</sup>, Motala 12, and Loschbour had 5, 6 and 13 copies respectively, whereas the Stuttgart farmer had 16 (SI7). Both Loschbour and Stuttgart had dark hair (>99% probability); and Loschbour, like La Braña and Motala12, likely had blue or intermediate-colored eyes (>75%) while Stuttgart likely had brown eyes (>99%) (SI8). Neither Loschbour nor La Braña carries the skin-lightening allele in SLC24A5 that is homozygous in Stuttgart and nearly fixed in Europeans today<sup>2</sup>, but Motala12 carries at least one copy of the derived allele, showing that this allele was present in Europe prior to the advent of agriculture.

We compared the ancient genomes to 2,345 present-day humans from 203 populations genotyped at 594,924 autosomal single nucleotide polymorphisms (SNPs) with the Human Origins array<sup>8</sup> (SI9) (Extended Data Table 1). We used ADMIXTURE<sup>13</sup> to identify 59 "West Eurasian" populations that cluster with Europe and the Near East (SI9 and Extended Data Fig. 3). Principal component analysis (PCA)<sup>14</sup> (SI10) (Fig. 1B) indicates a discontinuity between the Near East and Europe, with each showing north-south clines bridged only by a few populations of mainly Mediterranean origin. We projected<sup>15</sup> the newly sequenced and previously published<sup>1–4</sup> ancient genomes onto the first two principal components (PCs) (Fig. 1B). Upper Paleolithic hunter-gatherers<sup>3</sup> from Siberia like the MA1 (Mal'ta) individual project at the northern end of the PCA, suggesting an "Ancient North Eurasian" meta-population (ANE). European hunter-gatherers from Spain<sup>2</sup>, Luxembourg, and Sweden<sup>4</sup> fall beyond present-day Europeans in the direction of European differentiation

from the Near East, and form a "West European Hunter-Gatherer" (WHG) cluster including Loschbour and La Braña², and a "Scandinavian Hunter-Gatherer" (SHG) cluster including the Motala individuals and  $\sim 5,000$  year old hunter-gatherers from the Pitted Ware Culture⁴. An "Early European Farmer" (EEF) cluster includes Stuttgart, the  $\sim 5,300$  year old Tyrolean Iceman¹ and a  $\sim 5,000$  year old Swedish farmer⁴.

Patterns observed in PCA may be affected by sample composition (SI10) and their interpretation in terms of admixture events is not straightforward, so we rely on formal analysis of f-statistics<sup>8</sup> to document mixture of at least three source populations in the ancestry of present Europeans. We began by computing all possible statistics of the form  $f_3(Test; Ref_1, Ref_2)$  (SI11), which if significantly negative show unambiguously<sup>8</sup> that Test is admixed between populations anciently related to Ref<sub>1</sub> and Ref<sub>2</sub> (we choose Ref<sub>1</sub> and Ref<sub>2</sub> from 5 ancient and 192 present populations). The lowest  $f_3$ -statistics for Europeans are negative (93% are >4 standard errors below 0), with most showing strong support for at least one ancient individual being one of the references (SI11). Europeans almost always have their lowest  $f_3$  with either (EEF, ANE) or (WHG, Near East) (SI11, Table 1, Extended Data Table 1), which would not be expected if there were just two ancient sources of ancestry (in which case the best references for all Europeans would be similar). The lowest  $f_3$ -statistic for Near Easterners always takes Stuttgart as one of the reference populations, consistent with a Near Eastern origin for Stuttgart's ancestors (Table 1). We also computed the statistic  $f_4(Test, Stuttgart; MAI, Chimp)$ , which measures whether MAI shares more alleles with a Test population or with Stuttgart. This statistic is significantly positive (Extended Data Fig. 4, Extended Data Table 1) if *Test* is nearly any present-day West Eurasian population, showing that MA1-related ancestry has increased since the time of early farmers like Stuttgart (the analogous statistic using Native Americans instead of MA1 is correlated but smaller in magnitude (Extended Data Fig. 5), indicating that MA1 is a better surrogate than the Native Americans who were first used to document ANE ancestry in Europe<sup>7,8</sup>). The analogous statistic  $f_4(Test, Stuttgart; Loschbour, Chimp)$  is nearly always positive in Europeans and negative in Near Easterners, indicating that Europeans have more ancestry from populations related to Loschbour than do Near Easterners (Extended Data Fig. 4, Extended Data Table 1). Extended Data Table 2 documents the robustness of key f<sub>4</sub>statistics by recomputing them using transversion polymorphisms not affected by ancient DNA damage, and also using whole-genome sequencing data not affected by SNP ascertainment bias. Extended Data Fig. 6 shows the geographic gradients in the degree of allele sharing of present-day West Eurasians (as measured by f<sub>4</sub>-statistics) with Stuttgart (EEF), Loschbour (WHG) and MA1 (ANE).

To determine the minimum number of source populations needed to explain the data for many European populations taken together, we studied the matrix of all possible statistics of the form  $f_4(Test_{base}, Test_i; O_{base}, O_j)$  (SI12).  $Test_{base}$  is a reference European population,  $Test_i$  is the set of all other European Test populations,  $O_{base}$  is a reference outgroup, and  $O_j$  is the set of other outgroups (ancient DNA samples, Onge, Karitiana, and Mbuti). The rank of the (i, j) matrix reflects the minimum number of sources that contributed to the Test populations  $Test_0$  populations representing most present-day European groups, this analysis rejects descent from just two sources (P<10<sup>-12</sup>

by a Hotelling T-test<sup>17</sup>). However, three source populations are consistent with the data after excluding the Spanish who have evidence for African admixture<sup>18–20</sup> (P=0.019, not significant after multiple-hypothesis correction), consistent with the results from ADMIXTURE (SI9), PCA (Fig. 1B, SI10) and f-statistics (Extended Data Table 1, Extended Data Fig. 6, SI11, SI12). We caution that the finding of three sources could be consistent with a larger number of mixture events. Moreover, the source populations may themselves have been mixed. Indeed, the positive  $f_4(Stuttgart, Test; Loschbour, Chimp)$  statistics obtained when Test is Near Eastern (Extended Data Table 1) imply that the EEF had some WHG-related ancestry, which was greater than 0% and as high as 45% (SI13).

We used the ADMIXTUREGRAPH software<sup>8,15</sup> to fit a model (a tree structure augmented by admixture events) to the data, exploring models relating the three ancient populations (Stuttgart, Loschbour, and MA1) to two eastern non-Africans (Onge and Karitiana) and sub-Saharan Africans (Mbuti). We found no models that fit the data with 0 or 1 admixture events, but did find a model that fit with 2 admixture events (SI14). The successful model (Fig. 2A) confirms the existence of MA1-related admixture in Native Americans<sup>3</sup>, but includes the novel inference that Stuttgart is partially  $(44 \pm 10\%)$  derived from a lineage that split prior to the separation of eastern non-Africans from the common ancestor of WHG and ANE. The existence of such "Basal Eurasian" admixture into Stuttgart provides a simple explanation for our finding that diverse eastern non-African populations share significantly more alleles with ancient European and Upper Paleolithic Siberian hunter-gatherers than with Stuttgart (that is,  $f_4$ (Eastern non-African, Chimp; Hunter-gatherer, Stuttgart) is significantly positive), but that hunter-gatherers appear to be equally related to most eastern groups (SI14). We verified the robustness of the model by reanalyzing the data using the unsupervised MixMapper<sup>7</sup> (SI15) and TreeMix<sup>21</sup> software (SI16), which both identified the same admixture events. The ANE/WHG split must have occurred >24,000 years ago (as it must predate the age of MA13), and the WHG/Eastern non-African split must have occurred >40,000 years ago (as it must predate the Tianyuan<sup>22</sup> individual from China which clusters with Asians to the exclusion of Europeans). The Basal Eurasian split must be even older, and might be related to early settlement of the Levant<sup>23</sup> or Arabia<sup>24,25</sup> prior to the diversification of most Eurasians, or more recent gene flow from Africa<sup>26</sup>. However, the Basal Eurasian population shares much of the genetic drift common to non-African populations after their separation from Africans, and thus does not appear to represent gene flow between sub-Saharan Africans and the ancestors of non-Africans after the out-of-Africa bottleneck (SI14).

Fitting present-day Europeans into the model, we find that few populations can be fit as 2-way mixtures, but nearly all are compatible with 3-way mixtures of ANE/EEF/WHG (SI14). The mixture proportions from the fitted model (Fig. 2B; Extended Data Table 3) are encouragingly consistent with those obtained from a separate method that relates European populations to diverse outgroups using  $f_4$ -statistics, assuming only that MA1 is an unmixed descendent of ANE, Loschbour of WHG, and Stuttgart of EEF (SI17). We infer that EEF ancestry in Europe today ranges from ~30% in the Baltic region to ~90% in the Mediterranean, consistent with patterns of identity-by-descent (IBD) sharing  $^{27,28}$  (SI18) and shared haplotype analysis (chromosome painting) $^{29}$  (SI19) in which Loschbour shares more

segments with northern Europeans and Stuttgart with southern Europeans. Southern Europeans inherited their European hunter-gatherer ancestry mostly via EEF ancestors (Extended Data Fig. 6), while Northern Europeans acquired up to 50% of WHG ancestry above and beyond the WHG-related ancestry which they received through their EEF ancestors. Europeans have a larger proportion of WHG than ANE ancestry in general. By contrast, in the Near East there is no detectable WHG ancestry, but up to ~29% ANE in the Caucasus (SI14). A striking feature of these findings is that ANE ancestry is inferred to be present in nearly all Europeans today (with a maximum of ~20%), but was absent in both farmers and hunter-gatherers from central/western Europe during the Neolithic transition. At the same time, we infer that ANE ancestry was not completely absent from the larger European region at that time: we find that it was present in ~8,000 years old Scandinavian hunter-gatherers, since MA1 shares more alleles with Motala12 (SHG) than with Loschbour, and Motala12 fits as a mixture of 81% WHG and 19% ANE (SI14).

Two sets of European populations are poor fits for the model. Sicilians, Maltese, and Ashkenazi Jews have EEF estimates of >100% consistent with their having more Near Eastern ancestry than can be explained via EEF admixture (SI17). They also cannot be jointly fit with other Europeans (SI14), and they fall in the gap between European and Near Easterners (Fig. 1B). Finns, Mordovians and Russians (from the northwest of Russia) also do not fit (SI14; Extended Data Table 3) due to East Eurasian gene flow into the ancestors of these northeastern European populations. These populations (and Chuvash and Saami) are more related to East Asians than can be explained by ANE admixture (Extended Data Fig. 7), likely reflecting a separate stream of Siberian gene flow into northeastern Europe (SI14).

Several questions will be important to address in future ancient DNA work. Where and when did the Near Eastern farmers admix with European hunter-gatherers to produce the EEF? How did the ancestors of present-day Europeans first acquire their ANE ancestry? Discontinuity in central Europe during the late Neolithic (~4,500 years ago) associated with the appearance of mtDNA types absent in earlier farmers and hunter-gatherers<sup>30</sup> raises the possibility that ANE ancestry may have also appeared at this time. Finally, it is important to study ancient genome sequences from the Near East to provide insights into the history of the Basal Eurasians.

#### **Online Methods**

### Archeological context, sampling and DNA extraction

The Loschbour sample stems from a male skeleton excavated in 1935 at the Loschbour rock shelter in Heffingen, Luxembourg. The skeleton was AMS radiocarbon dated to  $7,205 \pm 50$  years before present (OxA-7738; 6,220-5,990 cal BC)<sup>31</sup>. At the Palaeogenetics Laboratory in Mainz, material for DNA extraction was sampled from tooth 16 (an upper right M1 molar) after irradiation with UV-light, surface removal, and pulverization in a mixer mill. DNA extraction took place in the palaeogenetics facilities in the Institute for Archaeological Sciences at the University of Tübingen. Three extracts were made in total, one from 80 mg of powder using an established silica based protocol<sup>32</sup> and two additional extracts from 90 mg of powder each with a protocol optimized for the recovery of short DNA molecules<sup>33</sup>.

The Stuttgart sample was taken from a female skeleton excavated in 1982 at the site Viesenhäuser Hof, Stuttgart-Mühlhausen, Germany. It was attributed to the Linearbandkeramik (5,500-4,800 BC) through associated pottery artifacts and the chronology was corroborated by radiocarbon dating of the stratigraphy<sup>34</sup>. Both sampling and DNA extraction took place in the Institute for Archaeological Sciences at the University of Tübingen. Tooth 47 (a lower right M2 molar) was removed and material from the inner part was sampled with a sterile dentistry drill. An extract was made using 40 mg of bone powder<sup>33</sup>.

The Motala individuals were recovered from the site of Kanaljorden in the town of Motala, Östergötland, Sweden, excavated between 2009 and 2013. The human remains at this site are represented by several adult skulls and one infant skeleton. All individuals are part of a ritual deposition at the bottom of a small lake. Direct radiocarbon dates on the remains range between  $7.013 \pm 76$  and  $6.701 \pm 64$  BP (6.361-5.516 cal BC), corresponding to the late Middle Mesolithic of Scandinavia. Samples were taken from the teeth of the nine best preserved skulls, as well as a femur and tibia. Bone powder was removed from the inner parts of the teeth or bones with a sterile dentistry drill. DNA from 100 mg of bone powder was extracted  $^{35}$  in the ancient DNA laboratory of the Archaeological Research Laboratory, Stockholm.

# Library preparation

Illumina sequencing libraries were prepared using either double- or single-stranded library preparation protocols<sup>36,37</sup> (SI1). For high-coverage shotgun sequencing libraries, a DNA repair step with Uracil-DNA-glycosylase (UDG) and endonuclease VIII (endo VIII) treatment was included in order to remove uracil residues<sup>38</sup>. Size fractionation on a PAGE gel was also performed in order to remove longer DNA molecules that are more likely to be contaminants<sup>37</sup>. Positive and blank controls were carried along during every step of library preparation.

## Shotgun sequencing and read processing

All non-UDG-treated libraries were sequenced either on an Illumina Genome Analyzer IIx with 2×76 + 7 cycles for the Loschbour and Motala libraries, or on an Illumina MiSeq with 2×150 + 8 + 8 cycles for the Stuttgart library. We followed the manufacturer's protocol for multiplex sequencing. Raw overlapping forward and reverse reads were merged and filtered for quality<sup>39</sup> and mapped to the human reference genome (hg19/GRCh37/1000Genomes) using the Burrows-Wheeler Aligner (BWA)<sup>40</sup> (SI2). For deeper sequencing, UDG-treated libraries of Loschbour were sequenced on 3 Illumina HiSeq 2000 lanes with 50-bp single-end reads, 8 Illumina HiSeq 2000 lanes of 100-bp paired-end reads and 8 Illumina HiSeq 2500 lanes of 101-bp paired-end reads. The UDG-treated libraries for Motala were sequenced on 8 HiSeq 2000 lanes and 101-bp paired-end reads. The UDG-treated libraries for Motala were sequenced on 8 HiSeq 2000 lanes of 100-bp paired-end reads, with 4 lanes each for two pools (one of 3 individuals and one of 4 individuals). We also sequenced an additional 8 HiSeq 2000 lanes for Motala12, the Motala sample with the highest percentage of endogenous human DNA. For the Loschbour and Stuttgart high coverage individuals, diploid genotype calls were obtained using the Genome Analysis Toolkit (GATK)<sup>41</sup>.

# **Enrichment of mitochondrial DNA and sequencing**

To test for DNA preservation and mtDNA contamination non-UDG-treated libraries of Loschbour and all Motala samples were enriched for human mitochondrial DNA using a bead-based capture approach with present-day human DNA as bait  $^{42}$ . UDG-treatment was omitted in order to allow characterization of damage patterns typical for ancient DNA  $^{10}$ . The captured libraries were sequenced on an Illumina Genome Analyzer IIx platform with 2  $\times$  76 + 7 cycles and the resulting reads were merged and quality filtered  $^{39}$ . The sequences were mapped to the Reconstructed Sapiens Reference Sequence, RSRS  $^{43}$ , using a custom iterative mapping assembler, MIA  $^{44}$  (SI4).

#### **Contamination estimates**

We assessed if the sequences had the characteristics of authentic ancient DNA using four approaches. First we searched for evidence of contamination by determining whether the sequences mapping to the mitochondrial genome were consistent with deriving from more than one individual  $^{44,45}$ . Second, for the high-coverage Loschbour and Stuttgart genomes, we used a maximum-likelihood-based estimate of autosomal contamination that uses variation at sites that are fixed in the 1000 Genomes data to estimate error, heterozygosity and contamination  $^{46}$  simultaneously. Third, we estimated contamination based on the rate of polymorphic sites on the X chromosome of the male Loschbour individual  $^{47}$  (SI3) Fourth, we analyzed non-UDG treated reads mapping to the RSRS to search for aDNA-typical damage patterns resulting in C $\rightarrow$ T changes at the 5'-end of the molecule  $^{10}$  (SI3).

# Phylogenetic analysis of the mitochondrial genomes

All nine complete mitochondrial genomes that fulfilled the criteria of authenticity were assigned to haplogroups using Haplofind  $^{48}$ . A Maximum Parsimony tree including present day humans and previously published ancient mtDNA sequences was generated with MEGA  $^{49}$ . The effect of branch shortening due to a lower number of substitutions in ancient lineages was studied by calculating the nucleotide edit distance to the root for all haplogroup R sequences (SI4).

#### Sex determination and Y-chromosome analysis

We assessed the sex of all sequenced individuals by using the ratio of (chrY) to (chrY +chrX) aligned reads<sup>50</sup>. We downloaded a list of Y-chromosome SNPs curated by the International Society of Genetic Genealogy (ISOGG, http://www.isogg.org) v. 9.22 (accessed Feb. 18, 2014) and determined the state of the ancient individuals at positions where a single allele was observed and MAPQ 30. We excluded C/G or A/T SNPs due to uncertainty about the polarity of the mutation in the database. The ancient individuals were assigned haplogroups based on their derived state (SI5). We also used BEAST v1.7.51<sup>51</sup> to assess the phylogenetic position of Loschbour using 623 males from around the world with 2,799 variant sites across 500kb of non-recombining Y-chromosome sequence<sup>52</sup> (SI5).

# **Estimation of Neanderthal admixture**

We estimate Neanderthal admixture in ancient individuals with the  $f_4$ -ratio or S-statistic  $\hat{a} = f_4(Altai, Denisova; Test, Yoruba)/f_4(Altai, Denisova; Vindija, Yoruba)$ 

which uses whole genome data from Altai, a high coverage ( $52\times$ ) Neanderthal genome sequence<sup>55</sup>, Denisova, a high coverage sequence<sup>37</sup> from another archaic human population ( $31\times$ ), and Vindija, a low coverage ( $1.3\times$ ) Neanderthal genome from a mixture of three Neanderthal individuals from Vindija Cave in Croatia<sup>53</sup>.

# Inference of demographic history and inbreeding

We used the Pairwise Sequentially Markovian Coalescent (PSMC)<sup>56</sup> to infer the size of the ancestral population of Stuttgart and Loschbour. This analysis requires high quality diploid genotype calls and cannot be performed in the low-coverage Motala samples. To determine whether the low effective population size inferred for Loschbour is due to recent inbreeding, we plotted the time-to-most-recent common ancestor (TMRCA) along each of chr1-22 to detect runs of low TMRCA.

## Analysis of segmental duplications and copy number variants

We built read-depth based copy number maps for the Loschbour, Stuttgart and Motala12 genomes in addition to the Denisova and Altai Neanderthal genome and 25 deeply sequenced modern genomes<sup>55</sup> (SI7). We built these maps by aligning reads, subdivided into their non-overlapping 36-bp constituents, against the reference genome using the mrsFAST aligner<sup>57</sup>, and renormalizing read-depth for local GC content. We estimated copy numbers in windows of 500 unmasked base pairs slid at 100 bp intervals across the genome. We called copy number variants using a scale space filter algorithm. We genotyped variants of interest and compared the genotypes to those from individuals sequenced as part of the 1000 Genomes Project<sup>58</sup>.

# Phenotypic inference

We inferred likely phenotypes (SI8) by analyzing DNA polymorphism data in the VCF format<sup>59</sup> using VCFtools (http://vcftoools.sourceforge.net/). For the Loschbour and Stuttgart individuals, we included data from sites not flagged as LowQuality, with genotype quality (GQ) of 30, and SNP quality (QUAL) of 50. For Motala12, which is of lower coverage, we included sites having at least 2× coverage and that passed visual inspection of the local alignment using samtools tview (http://samtools.sourceforge.net)<sup>60</sup>

## **Human Origins dataset curation**

The Human Origins array consists of 14 panels of SNPs for which the ascertainment is well known<sup>8,61</sup>. All population genetics analysis were carried out on a set of 594,924 autosomal SNPs, after restricting to sites that had >90% completeness across 7 different batches of sequencing, and that had >97.5% concordance with at least one of two subsets of samples for which whole genome sequencing data was also available. The total dataset consists of 2,722 individuals, which we filtered to 2,345 individuals (203 populations) after removing outlier individuals or relatives based on visual inspection of PCA plots<sup>14,62</sup> or model-based clustering analysis<sup>13</sup>. Whole genome amplified (WGA) individuals were not used in analysis, except for a Saami individual who we included because of the special interest of this population for Northeastern European population history (Extended Data Fig. 7).

# **ADMIXTURE** analysis

We merged all Human Origins genotype data with whole genome sequencing data from Loschbour, Stuttgart, MA1, Motala12, Motala\_merge, and LaBrana. We then thinned the resulting dataset to remove SNPs in linkage-disequilibrium with PLINK 1.07<sup>63</sup>, using a window size of 200 SNPs advanced by 25 SNPs and an r<sup>2</sup> threshold of 0.4. We ran ADMIXTURE 1.23<sup>13,64</sup> for 100 replicates with different starting random seeds, default 5-fold cross-validation, and varying the number of ancestral populations K between 2 and 20. We assessed clustering quality using CLUMPP<sup>65</sup>. We used the ADMIXTURE results to identify a set of 59 "West Eurasian" (European/Near Eastern) populations based on values of a "West Eurasian" ancestral population at K=3 (SI9). We also identified 15 populations for use as "non-West Eurasian outgroups" based on their having at least 10 individuals and no evidence of European or Near Eastern admixture at K=11, the lowest K for which Near Eastern/European-maximized ancestral populations appeared consistently across all 100 replicates.

# **Principal Components Analysis**

We used *smartpca*<sup>14</sup> (version: 10210) from EIGENSOFT<sup>62,66</sup> 5.0.1 to carry out Principal Components Analysis (PCA) (SI10). We performed PCA on a subset on individuals and then projected others using the *lsqproject*: *YES* option that gives an unbiased inference of the position of samples even in the presence of missing data (especially important for ancient DNA).

#### f3-statistics

We use the  $f_3$ -statistic  $f_3(Test;Ref_1Ref_2) = \frac{1}{N} \sum_{i=1}^{N} (t_i - r_{1,i}) (t_i - r_{2,i})$ , where  $t_i$ ,  $r_{1,i}$  and  $r_{2,i}$  are the allele frequencies for the  $i^{th}$  SNP in populations Test,  $Ref_1$ ,  $Ref_2$ , respectively, to determine if there is evidence that the Test population is derived from admixture of populations related to  $Ref_1$  and  $Ref_2$  (SI11). A significantly negative statistic provides unambiguous evidence of mixture in the Test population. We allow  $Ref_1$  and  $Ref_2$  to be any Human Origins population with 4 or more individuals, or Loschbour, Stuttgart, MA1, Motala12, LaBrana. We assess significance of the  $f_3$ -statistics using a block jackknife and a block size of 5cM. We report significance as the number of standard errors by which the statistic differs from zero (Z-score). We also perform an analysis in which we constrain the reference populations to be (i) EEF (Stuttgart) and WHG (Loschbour or LaBrana), (ii) EEF and a Near Eastern population, (iii) EEF and ANE (MA1), or (iv) any two present-day populations, and compute a  $Z_{diff}$  score between the lowest  $f_3$ -statistic observed in the dataset, and the  $f_3$ -statistic observed for the specified pair.

#### f<sub>4</sub>-statistics

We analyze  $f_4$ -statistics of the form  $f_4(A,B;C,D) = \frac{1}{N} \sum_{i=1}^{N} (a_i - b_i) (c_i - d_i)$  to assess if populations A, B are consistent with forming a clade in an unrooted tree with respect to C, D. If they form a clade, the allele frequency differences between the two pairs should be uncorrelated and the statistic has an expected value of 0. We set the outgroup D to be a sub-Saharan African population or Chimpanzee. We systematically tried all possible

combinations of the ancient samples or 15 "non-West Eurasian outgroups" identified by ADMIXTURE analysis as A, B, C to determine their genetic affinities (SI14). Setting A as a present-day test population and B as either Stuttgart or BedouinB, we documented relatedness to C=(Loschbour or MA1) or C=(MA1 and Karitiana) or C=(MA1 or Han) (Extended Data Figs. 4, 5, 7). Setting C as a test population and (A, B) a pair from (Loschbour, Stuttgart, MA1) we documented differential relatedness to ancient populations (Extended Data Fig. 6). We computed *D*-statistics<sup>53</sup> using transversion polymorphisms in whole genome sequence data<sup>55</sup> to confirm robustness to ascertainment and ancient DNA damage (Extended Data Table 2).

# Minimum number of source populations for Europeans

We used  $qpWave^{16,17}$  to study the minimum number of source populations for a designated set of Europeans (SI12). We use  $f_4$ -statistics of the form  $X(l, r) = f_4(l_0, l; r_0, r)$  where  $l_0, r_0$  are arbitrarily chosen "base" populations, and l, r are other populations from two sets L and R respectively. If X(l, r) has rank r and there were n waves of immigration into R with no back-migration from R to L, then r+1 n. We set L to include Stuttgart, Loschbour, MA1, Onge, Karitiana, Mbuti and R to include 23 modern European populations who fit the model of SI14 and had admixture proportions within the interval [0,1] for the method with minimal modeling assumptions (SI17).

## Admixture proportions for Stuttgart in the absence of a Near Eastern ancient genome

We used Loschbour and BedouinB as surrogates for "Unknown hunter-gatherer" and Near Eastern (NE) farmer populations that contributed to Stuttgart (SI13). Ancient Near Eastern ancestry in Stuttgart is estimated by the  $f_4$ -ratio<sup>8,15</sup>  $f_4$ (Outgroup, X; Loschbour, Stuttgart)/ $f_4$ (Outgroup, X; Loschbour, NE). A complication is that BedouinB is a mixture of NE and African ancestry. We therefore subtracted<sup>17</sup> the effects of African ancestry using estimates of the BedouinB African admixture proportion from ADMIXTURE (SI9) or ALDER<sup>68</sup>.

# Admixture graph modeling

We used ADMIXTUREGRAPH<sup>8</sup> (version 3110) to model population relationships between Loschbour, Stuttgart, Onge, and Karitiana using Mbuti as an African outgroup. We assessed model fit using a block jackknife of differences between estimated and fitted f-statistics for the set of included populations (we expressed the fit as a Z score). We determined that a model failed if |Z|>3 for at least one f-statistic. A basic tree model failed and we manually amended the model to test all possible models with a single admixture event, which also failed. Further manual amendment to include 2 admixture events resulted in 8 successful models, only one of which could be amended to also fit MA1 as an additional constraint. We successfully fit both the Iceman and LaBrana into this model as simple clades and Motala12 as a 2-way mixture. We also fit present-day West Eurasians as clades, 2-way mixtures, or 3-way mixtures in this basic model, achieving a successful fit for a larger number of European populations (n=26) as 3-way mixtures. We estimated the individual admixture proportions from the fitted model parameters. To test if fitted parameters for different populations are consistent with each other, we jointly fit all pairs of populations A and B by modifying

ADMIXTUREGRAPH to add a large constant (10,000) to the variance term  $f_3(A_0, A, B)$ . By doing this, we can safely ignore recent gene flow within Europe that affects statistics that include both A and B.

### Ancestry estimates from $f_4$ -ratios

We estimate EEF ancestry using the  $f_4$ -ratio<sup>8,15</sup>  $f_4$ (Mbuti, Onge; Loschbour, European)/  $f_4$ (Mbuti, Onge; Loschbour, Stuttgart), which produces consistent results with ADMIXTUREGRAPH (SI14). We use  $f_4$ (Stuttgart, Loschbour;  $Onge\ MA1$ )/ $f_4$ (Mbuti, MA1; Onge, Loschbour) to estimate Basal Eurasian admixture into Stuttgart. We use  $f_4$ (Stuttgart, Loschbour;  $Onge\ Karitiana$ )/ $f_4$ (Stuttgart, Loschbour;  $Onge\ MA1$ ) to estimate ANE mixture in Karitiana (Fig. 2B). We use  $f_4$ (Test, Stuttgart; Karitiana, Onge)/ $f_4$ (MA1, Stuttgart; Karitiana, Onge) to lower bound ANE mixture into North Caucasian populations.

# MixMapper analysis

We carried out  $MixMapper\ 2.0^7$  analysis, a semi-supervised admixture graph fitting technique. First, we infer a scaffold tree of populations without strong evidence of mixture relative to each other (Mbuti, Onge, Loschbour and MA1). We do not include European populations in the scaffold as all had significantly negative  $f_3$ -statistics indicating admixture. We then ran MixMapper to infer the relatedness of the other ancient and present-day samples, fitting them onto the scaffold as 2- or 3-way mixtures. The uncertainty in all parameter estimates is measured by block bootstrap resampling of the SNP set (100 replicates with 50 blocks).

#### TreeMix analysis

We applied  $TreeMix^{21}$  to Loschbour, Stuttgart, Motala12, and MA1<sup>3</sup>, LaBrana<sup>2</sup> and the Iceman<sup>1</sup>, along with the present-day samples of Karitiana, Onge and Mbuti. We restricted the analysis to 265,521 Human Origins array sites after excluding any SNPs where there were no-calls in any of the studied individuals. The tree was rooted with Mbuti and standard errors were estimated using blocks of 500 SNPs. We repeated the analysis on whole-genome sequence data, rooting with Chimp and replacing Onge with Dai since we did not have Onge whole genome sequence data<sup>55</sup>. We varied the number of migration events (m) between 0 and 5.

# Inferring admixture proportions with minimal modeling assumptions

We devised a method to infer ancestry proportions from three ancestral populations (EEF, WHG, and ANE) without strong phylogenetic assumptions (SI17). We rely on 15 "non-West Eurasian" outgroups and study  $f_4$ (European, Stuttgart;  $O_1$ ,  $O_2$ ) which equals  $\alpha\beta$   $f_4$ (Loschbour, Stuttgart;  $O_1$ ,  $O_2$ ) +  $\alpha(1-\beta)$   $f_4$ (MA1, Stuttgart;  $O_1$ ,  $O_2$ ) if European has  $1-\alpha$  ancestry from EEF and  $\beta$ ,  $1-\beta$  ancestry from WHG and ANE respectively. This defines a

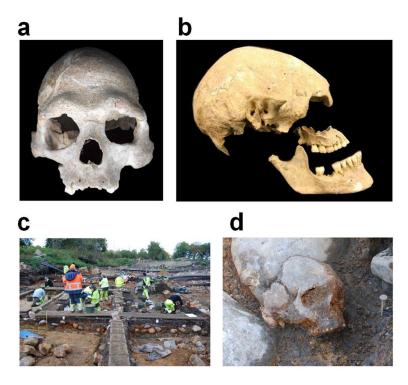
system of  $\binom{15}{2}$  =105 equations with unknowns  $\alpha\beta$ ,  $\alpha(1-\beta)$ , which we solve with least squares implemented in the function *lsfit* in *R* to obtain estimates of  $\alpha$  and  $\beta$ . We repeated this computation 22 times dropping one chromosome at a time<sup>20</sup> to obtain block jackknife<sup>67</sup> estimates of the ancestry proportions and standard errors, with block size equal to the

number of SNPs per chromosome. We assessed consistency of the inferred admixture proportions with those derived from the ADMIXTUREGRAPH model based on the number of standard errors between the two (Extended Data Table 1).

# Haplotype-based analyses

We used RefinedIBD from BEAGLE 4<sup>27</sup> with the settings *ibdtrim*=20 and *ibdwindow*=25 to study IBD sharing between Loschbour and Stuttgart and populations from the POPRES dataset<sup>69</sup>. We kept all IBD tracts spanning at least 0.5 centimorgans (cM) and with a LOD score >3 (SI18). We also used ChromoPainter<sup>29</sup> to study haplotype sharing between Loschbour and Stuttgart and present-day West Eurasian populations (SI19). We identified 495,357 SNPs that were complete in all individuals and phased the data using Beagle 4<sup>27</sup> with parameters *phase-its*=50 and *impute-its*=10. We did not keep sites with missing data to avoid imputing modern alleles into the ancient individuals. We used both unlinked (-k 1000) and linked modes (estimating -n and -M by sampling 10% of individuals). We combined ChromoPainter output for chromosomes 1-22 using ChromoCombine<sup>29</sup>. We carried out a PCA of the co-ancestry matrix using fineSTRUCTURE<sup>29</sup>.

# **Extended Data**

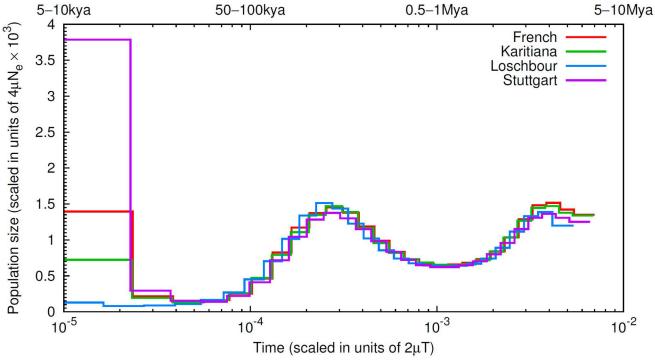


Extended Data Figure 1.

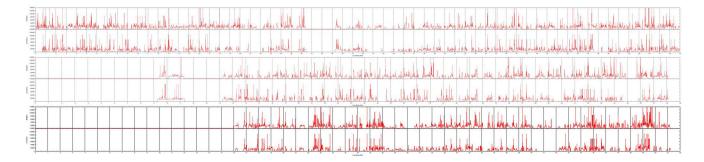
Photographs of analyzed ancient samples.

(A) Loschbour skull; (B) Stuttgart skull, missing the lower right M2 we sampled; (C) excavation at Kanaljorden in Motala, Sweden; (D) Motala 1 in situ.





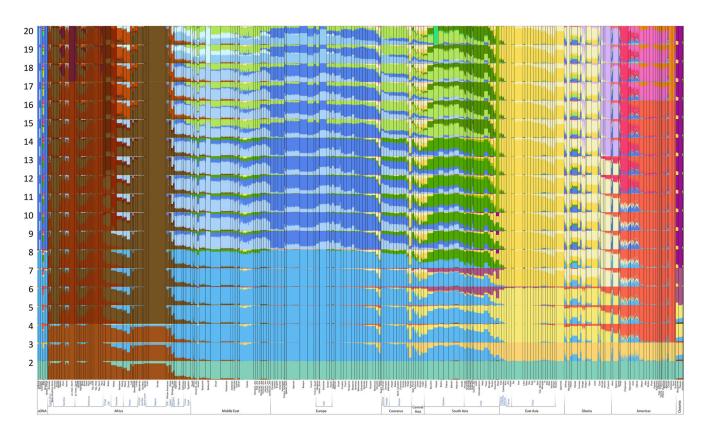
# b



# Extended Data Figure 2.

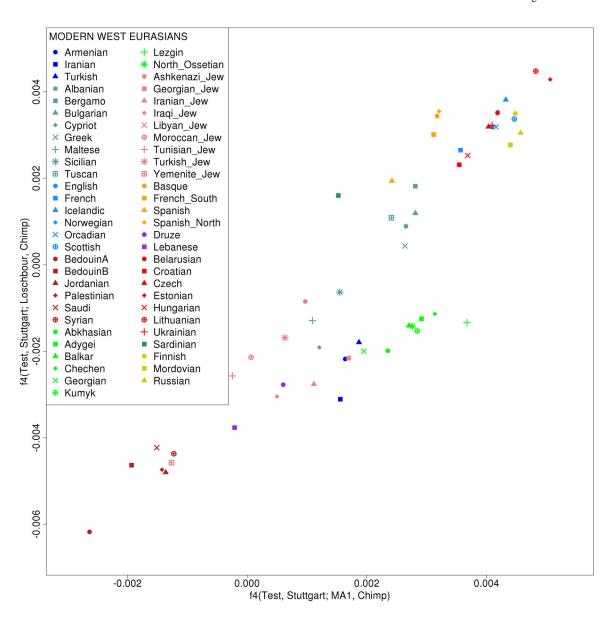
Pairwise Sequential Markovian Coalescent (PSMC) analysis.

(A) Inference of population size as a function of time, showing a very small recent population size over the most recent period in the ancestry of Loschbour (at least the last 5–10 thousand years). (B) Inferred time since the most recent common ancestor from the PSMC for chromosomes 20, 21, 22 (top to bottom); Stuttgart is plotted on top and Loschbour at bottom.



Extended Data Figure 3.
ADMIXTURE analysis (K=2 to K=20).

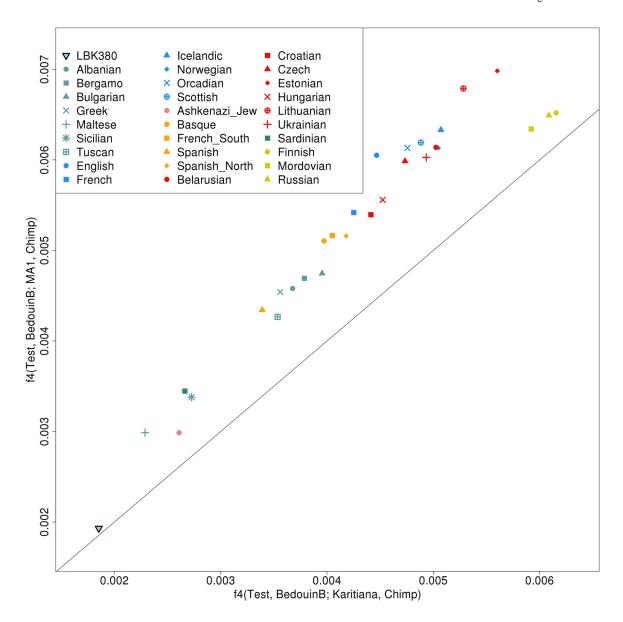
Ancient samples (Loschbour, Stuttgart, Motala\_merge, Motala12, MA1, and LaBrana) are at left.



#### **Extended Data Figure 4.**

ANE ancestry is present in both Europe and the Near East but WHG ancestry is restricted to Europe, which cannot be due to a single admixture event.

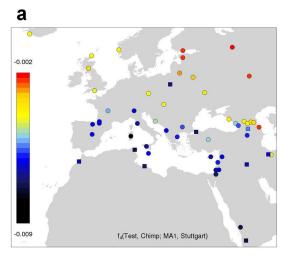
(x-axis) We computed the statistic  $f_4(Test, Stuttgart; MA1, Chimp)$ , which measures where MA1 shares more alleles with a test population than with Stuttgart. It is positive for most European and Near Eastern populations, consistent with ANE (MA1-related) gene flow into both regions. (y-axis) We computed the statistic  $f_4(Test, Stuttgart; Loschbour, Chimp)$ , which measures whether Loschbour shares more alleles with a test sample than with Stuttgart. Only European populations show positive values of this statistic, providing evidence of WHG (Loschbour-related) admixture only in Europeans.

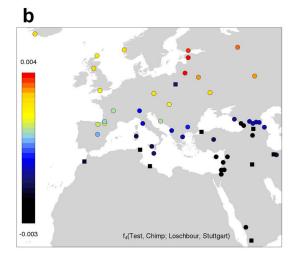


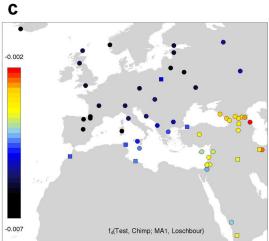
#### **Extended Data Figure 5.**

MA1 is the best surrogate for ANE for which we have data.

Europeans share more alleles with MA1 than with Karitiana, as we see from the fact that in a plot of  $f_4(Test, BedouinB; MA1, Chimp)$  and  $f_4(Test, BedouinB; Karitiana, Chimp)$ , the European cline deviates in the direction of MA1, rather than Karitiana (the slope is >1 and European populations are above the line indicating equality of these two statistics).



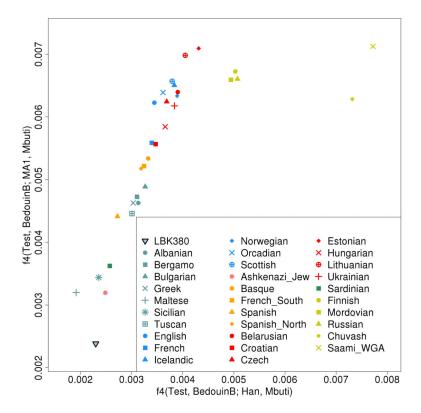




#### Extended Data Figure 6.

The differential relatedness of West Eurasians to Stuttgart (EEF), Loschbour (WHG), and MA1 (ANE) cannot be explained by two-way mixture.

We plot on a West Eurasian map the statistic  $f_4(Test, Chimp; A_1, A_2)$ , where  $A_1$  and  $A_2$  are a pair of the three ancient samples representing the three ancestral populations of Europe. (A) In both Europe and the Near East/Caucasus, populations from the south have more relatedness to Stuttgart than those from the north where ANE influence is also important. (B) Northern European populations share more alleles with Loschbour than with Stuttgart, as they have additional WHG ancestry beyond what was already present in EEF. (C) We observe a striking contrast between Europe west of the Caucasus and the Near East in degree of relatedness to WHG. In Europe, there is a much higher degree of allele sharing with Loschbour than with MA1, which we ascribe to the 60–80% WHG/(WHG+ANE) ratio in most Europeans that we report in S114. In contrast, the Near East has no appreciable WHG ancestry but some ANE ancestry, especially in the northern Caucasus. (Jewish populations are marked with a square in this figure to assist in interpretation as their ancestry is often anomalous for their geographic regions.)



# Extended Data Figure 7.

Evidence for Siberian gene flow into far northeastern Europe.

Some northeastern European populations (Chuvash, Finnish, Russian, Mordovian, Saami) share more alleles with Han Chinese than with other Europeans who are arrayed in a cline from Stuttgart to Lithuanians/Estonians in a plot of  $f_4(Test, BedouinB; Han, Mbuti)$  against  $f_4(Test, BedouinB; MA1, Mbuti)$ .

#### **Extended Data Table 1**

West Eurasians genotyped on the Human Origins array and key f-statistics.

		Sampling Locat	iea		Lowest of X; Ref.	1. a(2)			Lower (Z-s) :	a J. <b>3</b> (X; E.E.F., W.II) and Z.Ellft-3 reports	G) d)			Lowest £3: (Z-9 and	X; Near East, WHC Zdiff<3 reported)	Đ			Lowest 3 (X; EEF, ANE) (Z-3 and ZdEC-3 reported)				A (Soung Lauchbour,	art, X; Chinp)	A(Stanger, X; MA, Ching)	
x	N	Lat.	Long.	Ref 1	Ref 2	etatistic	z	ът1	<sub>ви</sub> 2	statistic	z	zer	Ref 1	<sub>ви</sub> 2	etatietic	z	zer	Ref 1	Ref 2	statistic	z	Z48T	statistic	z	statistic	z
Abbasian	9	45	41.02	Stu	MAI	-0.0053	-2.9						Georgian	Lall	-0.0004	-0.5	2.6	Stu	MAI	-0.0053	-2.9	0.0	0.0020	4.2	-0.0023	-4.7
Adygei	17	44	39	Papaco	Stu	-0.0073	-5.9											Stra	MAI	-0.0067	-4.1	0.3	0.0013	2.6	-0.0029	-6.0
Albanian	6	41.33	19.83	Stra	MAI	-0.0121	-7.0						lragi_lew	Los	-0.0090	-9.1	1.7	Stra	MAI	-0.0121	-7.0	0.0	-0.0009	-1.8	-0.0027	-5.4
Amenian	10	40.29	44.55	GujaratiC	Stu	-0.0070	-8.2											Stra	MAI	-0.0068	-4.1	0.1	0.0022	4.5	-0.0016	-3.3
Ashkonari_lew	7	52.23	21.02	Stra	MAI	-0.0057	-3.4						lragi_lew	Los	-0.0042	-4.7	1.0	Stra	MAI	-0.0057	-3.4	0.0	0.0008	1.7	-0.0010	-2.0
Balkar	10	43.48	43.62	Papaco	Stu	-0.0113	-8.9											Stra	MAI	-0.0092	-5.5	1.1	0.0014	2.9	-0.0027	-5.6
Ranque	29	43.04	-0.65	kaqi_kw	Los	-0.0083	-10.3	Stu	Los	-0.0061	-3.8	1.3	lragi_lew	Los	-0.0083	-10.3	0.0	Stra	MAI	-0.0041	-2.4	2.2	-0.0034	-7.2	-0.0032	-6.7
BodosinA	25	31	35	Eun	Stu	-0.0162	-18.2																0.0062	13.0	0.0026	5.4
BedouisB	19	31	35	Eun	Stu	0.0089	7.8																0.0046	9.3	0.0019	3.9
Belarusian	10	53.92	28.00	Georgian	Los	-0.0133	-17.6						Georgian	Los	-0.0133	-17.6	0.0	Stra	MAI	-0.0002	-6.1	1.9	-0.0035	-6.9	-0.0042	-8.6
Bergamo	12	46	10	Stra	MAI	-0.0106	-6.2	Stu	Los	-0.0068	-4.2	1.7	lragi_lew	Los	-0.0100	-11.9	0.3	Stra	MAI	-0.0006	-6.2	0.0	-0.0018	-3.9	-0.0028	-5.8
Bulgarian	10	42.16	24.74	Stra	MAI	-0.0130	-8.2	Stu	LaB	-0.0074	-4.5	2.8	lragi_lew	Los	-0.0106	-12.4	1.5	Stra	MAI	-0.0130	-6.2	0.0	-0.0012	-2.5	-0.0028	-5.9
Chechen	9	43.33	45.65	Stra	MAI	-0.0056	-3.2						Georgian	Los	-0.0002	-0.3	2.8	Stra	MAI	-0.0056	-3.2	0.0	0.0011	2.3	-0.0031	-6.2
Crossian	10	43.51	16.45	Stra	MAI	-0.0114	-6.7	Stu	Los	-0.0065	-3.8	2.1	lragi_lew	Los	-0.0112	-13.0	0.2	Stra	MAI	-0.0114	-6.7	0.0	-0.0023	-4.7	-0.0035	-7.4
Cyprion		35.13	33.43	Stra	MAI	-0.0057	-3.2						Yessenite_low	Los	-0.0013	-1.5	2.5	Stra	MAI	-0.0057	-3.2	0.0	0.0019	3.9	-0.0012	-2.5
Cauch	10	50.1	14.4	Googian	Los	-0.0137	-17.9	Stra	Los	-0.0088	-5.3	3.0	Georgian	Los	-0.0137	-17.9	0.0	Stra	MAI	-0.0121	-7.2	0.9	-0.0032	-6.6	-0.0040	-6.2
Dezas	39	32	38	Stra	MAI	-0.0024	-1.5											Stra	MAI	-0.0024	-1.5	0.0	0.0028	5.9	-0.0006	-1.3
English	10	50.75	-2.09	kaqi_lew	Los	-0.0129	-14.8	Stra	Los	-0.0090	-5.5	2.2	Imqi_lew	Los	-0.0129	-14.8	0.0	Stra	MAI	-0.0125	-7.4	0.1	-0.0032	-6.5	-0.0041	-8.5
Demiss	10	58.54	24.89	Abkhasian	Los	-0.0124	-15.1						Abkhasian	Los	-0.0124	-15.1	0.0	Stu	MAI	-0.0094	-5.6	1.9	-0.0043	-8.5	-0.0051	-99.1

		Sampling Local	iea .		Lowest of X; Re	1. 2/2)			Lowe (Z-s)	a f <b>3</b> (X; EEF, WII and ZEIII-3 reports	IG) od)			Lowest /3	X; Near East, WBC Zdiff<3 reported)	Đ			Low (Z-0	et 3 (X; EEF, ANI and ZdBC3 reporte	t) d)		A(Sout)	part, X; Chings)	A <sub>CState</sub> Mil, C	part, X; kinp)
x	N	Lat.	Long.	Ref 1	Ref 2	etatletic	z	ът1	Ref 2	statistic	z	2.68	Ref 1	12 гм	etatletic	z	zer	Ref 1	Ref 2	statistic	z	z.ear	statistic	z	statistic	z
Finnish	7	60.2	24.9	Abkhesian	Los	-0.0102	-11.3						Abkhasian	Los	-0.0102	-11.3	0.0	Stu	MAI	-0.0078	-4.4	1.4	-0.0035	-6.9	-0.0045	-9.1
French	25	46	2	Stra	MAI	-0.0131	-8.4	Stra	Los	-0.0098	-6.3	1.5	Iraqi_lov	Los	-0.0129	-16.8	0.2	Stu	MAI	-0.0131	-8.4	0.0	-0.0027	-5.6	-0.0036	-7.7
French_South	7	43.44	-0.62	kaqi_lew	Los	-0.0095	-9.5	Stra	LaB	-0.0089	-5.0	0.3	Iraqi_lew	Los	-0.0095	-9.5	0.0	Stra	MAI	-0.0086	-4.8	0.4	-0.0030	-62	-0.0031	-6.2
Georgian	10	42.5	41.85	GujarasiC	Stu	-0.0036	-4.0											Stu	MAI	-0.0036	-2.1	-0.2	0.0020	4.2	-0.0019	-3.9
Georgian, Jow	7	41.72	44.78	GujarasiC	Stu	-0.0009	-0.9											Stu	MAI	-0.0002	-0.1	0.3	0.0022	4.3	-0.0017	-3.4
Gnuk	20	29.54	23.17	Stra	MAI	-0.0118	-7.4						Itaqi_lew	Los	-0.0080	-11.1	2.3	Stu	MAI	-0.0118	-7.4	0.0	-0.0004	-0.9	-0.0026	-5.6
Hungarian	20	47.49	19.08	Stra	MAI	-0.0133	-8.4	Stra	Los	-0.0087	-5.6	2.2	Iraqi_lew	Los	-0.0127	-15.9	0.4	Stra	MAI	-0.0133	-8.4	0.0	-0.0025	-5.3	-0.0037	-7.8
lestandie	12	64.13	-21.93	Abkhasian	Los	-0.0121	-15.6	Stra	Los	-0.007s	-4.8	2.7	Abkhasian	Los	-0.0121	-15.6	0.0	Stu	MAI	-0.0097	-5.9	1.5	-0.0038	-2.2	-0.0043	-8.9
Iranian	8	35.59	51.46	Papaco	Stu	-0.0094	-7.2											Stra	MAI	-0.0087	-5.2	0.4	0.0031	6.3	-0.0016	-3.2
Iranian_Jow	9	35.7	51.42	GujarasiC	Stu	-0.0018	-2.0											Stra	MAI	-0.0012	-0.6	0.2	0.0028	5.7	-0.0011	-2.2
Iraqi_lew	6	33.33	44.42	Vishvahrahmin	Stra	-0.0026	-2.6											Stra	MAI	-0.0009	-0.5	0.9	0.0030	6.1	-0.0005	-1.0
Jordanian	9	32.05	35.90	Eun	Stra	-0.0145	-14.3																0.0048	9.6	0.0014	2.8
Kumpk		43.25	46.58	Plapoco	Stra	-0.0111	-8.2											Stra	MAI	-0.0009	-6.5	0.1	0.0015	3.1	-0.0028	-5.7
Lebatese		33.82	35.57	Eun	Stra	-0.0105	-9.4											Stra	MAI	-0.0068	-3.9	1.9	0.0038	2.2	0.0002	0.4
Lorgin	9	42.12	48.18	Sm	MAI	-0.0100	-6.0											Stu	MAI	-0.0000	-6.0	0.0	0.0013	2.7	-0.0037	-7.5
Libyan, Jon	9	32.92	13.18	Eun	Stra	-0.0051	-4.4											Stra	MAI	0.0000	0.0	2.7	0.0030	62	0.0004	0.9
Lithumian	10	54.9	23.92	Abkbasian	Los	-0.0119	-14.9						Abkhasian	Los	-0.0119	-14.9	0.0	Stu	MAI	-0.0069	-3.9	2.8	-0.0045	-9.0	-0.0048	-9.9
Malusa		35.94	14.38	Sm	MAI	-0.0086	-4.9						Yemenite_low	Los	-0.0051	-6.0	2.0	Stu	MAI	-0.0086	-4.9	0.0	0.0013	2.7	-0.0011	-2.3
Mondovian	10	54.18	45.18	Abkbasian	Los	-0.0115	-14.4						Abkhasian	Los	-0.0115	-14.4	0.0	Stu	MAI	-0.0113	-6.6	0.3	-0.0028	-5.5	-0.0044	-9.0
Monocan_Jew	6	34.02	-6.84	Eun	Stu	-0.0062	-5.2						Yemenite_low	Los	-0.0021	-2.2	2.9	Stra	MAI	-0.0032	-1.7	1.4	0.0021	43	-0.0001	-0.1
North_Ossetian	10	43.02	44.65	Papaco	Stu	-0.0093	-7.2											Stra	MAI	-0.0076	-4.4	1.0	0.0014	2.9	-0.0028	-5.6
Norwogian	11	60.36	5.36	Goorgian	Los	-0.0120	-14.8						Georgian	Los	-0.0120	-14.8	0.0	Stra	MAI	-0.0093	-5.4	1.4	-0.0035	-7.3	-0.0042	-6.7
Ocadian	13	59	-3	Armenian	Los	-0.0102	-13.4	Stra	Los	-0.0059	-3.6	2.5	Armenian	Los	-0.0102	-13.4	0.0	Stu	MAI	-0.0098	-5.9	0.5	-0.0032	-6.7	-0.0042	-8.6
Palestinian	38	32	35	Esan	Stu	-0.0120	-13.2																0.0047	10.2	0.0014	3.1
Ressian	22	61	40	Chukchi	Los	-0.0119	-11.3						Abkhasian	Los	-0.0119	-17.1	0.0	Stu	MAI	-0.0006	-6.6	0.8	-0.0030	-6.2	-0.0046	-9.4
Sandinian	27	40	*	Sm	LaB	-0.0044	-2.6	Stra	LaB	-0.0044	-2.6	0.0	Itaqi_lew	Los	-0.0033	-4.2	0.0	Stu	MAI	-0.0035	-2.1	0.3	-0.0016	-3.4	-0.0015	-3.3
Sandi	8	18.49	42.52	Kgalagadi	Stu	-0.0042	-3.6																0.0042	8.6	0.0015	3.1
Scottish	4	56.04	-3.94	lraqi_lew	Los	-0.0103	-8.3						Iraqi_lew	Los	-0.0103	-8.3	0.0	Stu	MAI	-0.0090	-4.7	0.7	-0.0034	-6.4	-0.0045	-8.7
Sicilian	11	37.59	13.77	Stra	MAI	-0.0108	-6.5						Yemenite_low	Los	-0.0066	-8.1	2.4	Stra	MAI	-0.0008	-6.5	0.0	0.0006	1.3	-0.0015	-3.2
Spanish	53	40.43	-2.83	kuqi_kre	Los	-0.0126	-17.8	Stu	Los	-0.0104	-6.8	1.4	Itaqi_lew	Los	-0.0126	-17.8	0.0	Stra	MAI	-0.0120	-7.6	0.3	-0.0019	-4.2	-0.0024	-5.2
Spanish_North	5	42.8	-27	Braqi_Sew	Los	-0.0112	-9.9	Stu	Los	-0.0102	-5.4	0.5	Iraqi_lew	Los	-0.0112	-9.9	0.0	Stu	MAI	-0.0082	-4.4	1.3	-0.0035	-6.9	-0.0032	-6.4
Syrian	8	35.13	36.87	Esan	Stu	-0.0101	-8.7																0.0044	8.6	0.0012	2.4
Tunician_lew	7	36.8	10.18	Gambian	Stu	-0.0026	-2.0																0.0026	5.2	0.0002	0.5
Turkish	56	39.22	32.66	Piapoco	Stu	-0.0129	-113											Stu	MAI	-0.0006	-6.9	1.3	0.0018	3.8	-0.0019	-4.0
Turkish_Jew	8	41.02	28.95	Sm	MAI	-0.0075	-43						Yemeniu_low	Los	-0.0049	-5.8	1.4	Stu	MAI	-0.0075	-4.3	0.0	0.0017	1.6	-0.0006	-1.3
Toscan	8	43	11	Stea	MAI	-0.0109	-6.4	Stu	Las	-0.0055	-3.2	2.3	Iraqi_lew	Los	-0.0092	-10.1	0.9	Stu	MAI	-0.0009	-6.4	0.0	-0.0011	-2.2	-0.0024	-5.0
Ukrainian	9	50.29	31.56	Goorgian	Los	-0.0134	-16.7						Georgian	Los	-0.0134	-16.7	0.0	Stu	MAI	-00114	-6.6	1.3	-0.0032	-6.4	-0.0041	-8.5
Yemeniu_Jew		15.35	442	Eun	Stu	-0.0027	-2.4																0.0046	9.1	0.0013	2.6

Note: Zdiff is the number of standard errors of the difference between the lowest f3-statistic over all reference pairs and the lowest f3-statistic for a subset of reference pairs.

Abbreviations used: Stu: Stuttgart; Los: Loschbour; LaB: LaBrana.

# **Extended Data Table 2**

Confirmation of key findings on transversions and on whole genome sequence data.

			D(A, B; C, D)	on Human Ori	gins genotype	data			D(A,B;C,D) on whole genome sequence data transversions								
Interpretation					594,924	SNPs	110,817 trans	versions									
	A	В	c	D	statistic	z	statistic	z	A	В	c	D	statistic	z			
Stuttgart has Near Eastern ancestry	Stuttgart	Armenian	Loschbour	Chimp	0.0219	4.5	0.0189	2.9									
Europeans	Stuttgart	French	Loschbour	Chimp	-0.0266	-5.7	-0.031	-5.0	Stuttgart	French2	Loschbour	Chimp	-0.03	-4.7			
have more WHG-related ancestry than Stuttgart	Lithuanian	Stuttgart	Loschbour	Chimp	0.0446	9.1	0.0477	7.2									
West Eurasians have	French	Stuttgart	MAI	Chimp	0.0367	7.7	0.0386	5.5	French2	Stuttgart	MA1	Chimp	0.037	6.4			
more ANE- related ancestry than Stuttgart	Lezgin	Stuttgart	MA1	Chimp	0.0372	7.6	0.0409	5.6									
MAI is a better surrogate of ANE ancestry than Karitiana	French	Chimp	MAI	Karitiana	0.0207	4.5	0.0214	2.8	French2	Chimp	MAI	Karitiana2	0.026	3.8			

			D(A, B; C, D)	on Human Ori	gins genotype	data			D(A,B;C,D) on whole genome sequence data transversions								
Interpretation					594,924	SNPs	110,817 trans	versions									
	A	В	c	D	statistic	z	statistic	z	A	В	С	D	statistic	z			
	Loschbour	Stuttgart	Onge	Chimp	0.0196	3.5	0.0202	2.5	-								
	Loschbour	Stuttgart	Papuan	Chimp	0.0142	2.6	0.0127	1.5	Loschbour	Stuttgart	Papuan2	Chimp	0.017	2.7			
	Loschbour	Stuttgart	Dai	Chimp	0.0164	3.2	0.021	2.8	Loschbour	Stuttgart	Dai2	Chimp	0.018	2.9			
Eastern non- Africans closer	MAI	Stuttgart	Papuan	Chimp	0.0139	2.2	0.0103	1.0	MAI	Stuttgart	Papuan2	Chimp	0.018	2.8			
to WHG/ANE/S	MAI	Stuttgart	Dai	Chimp	0.0174	3.0	0.016	1.7	MAl	Stuttgart	Dai2	Chimp	0.028	4.3			
HG than to EEF	Motala12	Stuttgart	Papuan	Chimp	0.0182	3.2	0.011	1.1	Motala12	Stuttgart	Papuan2	Chimp	0.023	3.7			
	Motala12	Stuttgart	Dai	Chimp	0.0156	2.8	0.0149	1.6	Motala12	Stuttgart	Dai2	Chimp	0.02	3.2			
	LaBrana	Stuttgart	Papuan	Chimp	0.0123	2.3	0.0101	1.1	LaBrana	Stuttgart	Papuan2	Chimp	0.02	3.2			
	LaBrana	Stuttgart	Dai	Chimp	0.0149	2.9	0.0228	2.5	LaBrana	Stuttgart	Dai2	Chimp	0.024	3.7			
Native Americans closer to ANE than to WHG	Karitiana	Chimp	MAI	Loschbour	0.0467	7.1	0.0467	4.4	Karitiana2	Chimp	MAI	Loschbour	0.052	7.1			
West	Stuttgart	Chimp	Karitiana	Papuan	0.0559	10.9	0.0474	6.6	Stuttgart	Chimp	Karitiana2	Papuan2	0.052	7.6			
Eurasians closer to Native Americans than to other Eastern non- Africans	Stuttgart	Chimp	Karitiana	Onge	0.0237	5.1	0.0179	2.6									
	Loschbour	MA1	Dai	Chimp	-0.0015	-0.2	0.0016	0.2	Loschbour	MA1	Dai2	Chimp	-0.013	-1.9			
Ancient Eurasian	Loschbour	MAl	Papuan	Chimp	0.0002	0.0	0.0012	0.1	Loschbour	MA1	Papuan2	Chimp	-0.003	-0.4			
hunter- gatherers	Loschbour	Motala12	Dai	Chimp	0.0024	0.4	0.009	0.9	Loschbour	Motala12	Dai2	Chimp	-0.002	-0.3			
equally related to Eastern non-	Loschbour	Motala12	Papuan	Chimp	-0.0028	-0.4	0.0046	0.5	Loschbour	Motala12	Papuan2	Chimp	-0.004	-0.6			
Africans other than Native Americans	MAI	Motala12	Dai	Chimp	0.0026	0.4	0.0047	0.4	MAI	Motala12	Dai2	Chimp	0.01	1.5			
Americans	MA1	Motala12	Papuan	Chimp	-0.0047	-0.7	-0.001	-0.1	MA1	Motala12	Papuan2	Chimp	-0.004	-0.5			
	LaBrana	Loschbour	Dai	Chimp	-0.0028	-0.5	0.0024	0.3	LaBrana	Loschbour	Dai2	Chimp	0.007	1.1			
LaBrana and Loschbour are	LaBrana	Loschbour	Papuan	Chimp	-0.0031	-0.5	-0.0012	-0.1	LaBrana	Loschbour	Papuan2	Chimp	0.002	0.3			
a clade	LaBrana	Loschbour	MAI	Chimp	-0.006	-0.8	0.0101	0.7	LaBrana	Loschbour	MA1	Chimp	0.005	0.7			
SHG closer to	Motala12	Loschbour	MAI	Chimp	0.0425	5.3	0.0353	2.6	Motala12	Loschbour	MA1	Chimp	0.042	5.9			
ANE than to WHG	Motala12	LaBrana	MA1	Chimp	0.0465	5.8	0.0347	2.4	Motala12	LaBrana	MA1	Chimp	0.038	5.4			
LaBrana and Loschbour equally related to Stuttgart	LaBrana	Loschbour	Stuttgart	Chimp	-0.0176	-2.6	-0.0106	-1.0	LaBrana	Loschbour	Stuttgart	Chimp	-0.012	-1.8			

# **Extended Data Table 3**

Admixture proportions for European populations. The estimates from the model with minimal assumptions are from SI17. The estimates from the full modeling are from SI14 either by single population analysis or co-fitting population pairs and averaging over fits (these averages are the results plotted in Fig. 2B). Populations that do not fit the models are not reported.

	popula	ll modeling ation relation dividual fit	nships			populatio	nodeling of on relationships raged fits)				odeling of population relationships with inimal assumptions	Model-based (averaged) - Model with minimal assumptions (Z-score)			
	EEF	WHG	ANE		EEF		WHG		ANE	EEF	WHG	ANE	EEF	WHG	ANE
				Mean	Range	Mean	Range	Mean	Range						
Albanian	0.781	0.092	0.127	0.781	0.772-0.819	0.082	0.032-0.098	0.137	0.129-0.158	0.595 ± 0.112	$0.353 \pm 0.150$	$0.052 \pm 0.049$	1.658	-1.807	1.741
Ashkenazi_Jew	0.931	0	0.069							$0.938 \pm 0.146$	$-0.021 \pm 0.185$	$0.083\pm0.049$			
Basque	0.593	0.293	0.114	0.569	0.527-0.616	0.335	0.255-0.392	0.096	0.076-0.129	$0.569 \pm 0.091$	$0.315 \pm 0.124$	$0.115\pm0.041$	-0.001	0.165	-0.472
Belarusian	0.418	0.431	0.151	0.426	0.397-0.464	0.408	0.338-0.443	0.167	0.150-0.199	$0.272 \pm 0.094$	$0.554 \pm 0.131$	$0.174\pm0.047$	1.637	-1.118	-0.158
Bergamo	0.715	0.177	0.108	0.721	0.704-0.793	0.163	0.061-0.189	0.117	0.104-0.147	$0.644 \pm 0.125$	$0.248 \pm 0.170$	$0.108\pm0.053$	0.615	-0.503	0.162

	Full modeling of population relationships (individual fits)			Full modeling of population relationships (averaged fits)							odeling of population relationships with hinimal assumptions		Model-based (averaged) - Model with minimal assumptions (Z-score)			
	EEF	WHG	ANE		EEF		WHG		ANE	EEF	WHG	ANE	EEF	WHG	ANE	
				Mean	Range	Mean	Range	Mean	Range							
Bulgarian	0.712	0.147	0.141	0.718	0.707-0.778	0.132	0.047-0.151	0.151	0.138-0.175	$0.556 \pm 0.110$	$0.328 \pm 0.143$	$0.116 \pm 0.043$	1.469	-1.372	0.804	
Croatian	0.561	0.293	0.145	0.564	0.548-0.586	0.285	0.242-0.310	0.151	0.137-0.172	$0.453 \pm 0.122$	$0.407 \pm 0.159$	$0.140\pm0.046$	0.911	-0.768	0.238	
Czech	0.495	0.338	0.167	0.489	0.460-0.531	0.348	0.273-0.382	0.163	0.145-0.196	$0.402 \pm 0.117$	$0.400 \pm 0.162$	$0.198\pm0.050$	0.744	-0.322	-0.698	
English	0.495	0.364	0.141	0.503	0.476-0.536	0.353	0.296-0.382	0.144	0.130-0.169	$0.475 \pm 0.091$	$0.357 \pm 0.125$	$0.168 \pm 0.043$	0.304	-0.028	-0.561	
Estonian	0.322	0.495	0.183	0.323	0.293-0.345	0.49	0.451-0.520	0.187	0.172-0.205	$0.072 \pm 0.121$	$0.778 \pm 0.176$	$0.150 \pm 0.064$	2.070	-1.636	0.584	
French	0.554	0.311	0.135	0.563	0.537-0.601	0.297	0.230-0.328	0.14	0.126-0.169	$0.498 \pm 0.097$	$0.359 \pm 0.127$	$0.142 \pm 0.039$	0.672	-0.487	-0.060	
French_South	0.675	0.195	0.13	0.636	0.589-0.738	0.256	0.111-0.323	0.108	0.088-0.151	$0.636\pm0.116$	$0.225 \pm 0.165$	$0.140\pm0.057$	-0.003	0.189	-0.558	
Greek	0.792	0.058	0.151	0.791	0.780-0.816	0.048	0.019-0.060	0.161	0.150-0.171	$0.658 \pm 0.098$	$0.255 \pm 0.127$	$0.086\pm0.039$	1.357	-1.627	1.915	
Hungarian	0.558	0.264	0.179	0.548	0.520-0.590	0.279	0.199-0.313	0.174	0.156-0.210	$0.391 \pm 0.109$	$0.454 \pm 0.153$	$0.155 \pm 0.050$	1.437	-1.145	0.371	
Icelandic	0.394	0.456	0.15	0.409	0.386-0.424	0.448	0.409-0.473	0.143	0.126-0.170	$0.342\pm0.102$	$0.476 \pm 0.137$	$0.182\pm0.045$	0.654	-0.204	-0.861	
Lithuanian	0.364	0.464	0.172	0.352	0.327-0.384	0.488	0.433-0.527	0.16	0.135-0.184	$0.248\pm0.117$	$0.548\pm0.163$	$0.205 \pm 0.052$	0.886	-0.367	-0.864	
Maltese	0.932	0	0.068							$1.298 \pm 0.185$	$-0.509 \pm 0.248$	$0.211 \pm 0.079$				
Norwegian	0.411	0.428	0.161	0.417	0.388-0.438	0.423	0.383-0.450	0.16	0.140-0.181	$0.273 \pm 0.115$	$0.557 \pm 0.161$	$0.170\pm0.055$	1.252	-0.831	-0.185	
Orcadian	0.457	0.385	0.158	0.465	0.439-0.493	0.378	0.329-0.403	0.157	0.140-0.179	$0.395 \pm 0.088$	$0.437 \pm 0.122$	$0.168\pm0.041$	0.798	-0.487	-0.264	
Sardinian	0.817	0.175	0.008	0.818	0.791-0.874	0.141	0.058-0.182	0.041	0.026-0.068	$0.883\pm0.128$	$0.075 \pm 0.166$	$0.042\pm0.048$	-0.510	0.400	-0.024	
Scottish	0.39	0.428	0.182	0.408	0.387-0.424	0.421	0.384-0.448	0.171	0.149-0.201	$0.286\pm0.112$	$0.532 \pm 0.156$	$0.182\pm0.053$	1.091	-0.712	-0.210	
Sicilian	0.903	0	0.097							$1.012 \pm 0.149$	$-0.131 \pm 0.199$	$0.119\pm0.060$				
Spanish	0.809	0.068	0.123	0.759	0.736-0.804	0.126	0.066-0.170	0.115	0.091-0.151	$0.856\pm0.126$	$-0.015 \pm 0.165$	$0.160\pm0.049$	-0.769	0.855	-0.922	
Spanish_North	0.713	0.125	0.163	0.612	0.561-0.660	0.292	0.214-0.365	0.096	0.072-0.126	$0.581 \pm 0.120$	$0.298 \pm 0.158$	$0.121\pm0.046$	0.254	-0.038	-0.533	
Tuscan	0.746	0.136	0.118	0.751	0.737-0.806	0.123	0.047-0.145	0.126	0.114-0.150	$0.734\pm0.118$	$0.153 \pm 0.160$	$0.113 \pm 0.054$	0.141	-0.188	0.249	
Ukrainian	0.462	0.387	0.151	0.463	0.445-0.491	0.376	0.322-0.399	0.16	0.148-0.187	$0.259 \pm 0.123$	$0.596 \pm 0.173$	$0.145 \pm 0.057$	1.661	-1.269	0.269	
Finnish										$-0.299 \pm 0.204$	$1.194\pm0.296$	$0.105\pm0.105$				
Mordovian										$-0.255 \pm 0.173$	$1.151 \pm 0.246$	$0.104\pm0.090$				
Russian										$-0.303 \pm 0.211$	$1.230\pm0.301$	$0.072\pm0.106$				

# **Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

# **Authors**

Iosif Lazaridis<sup>1,2</sup>, Nick Patterson<sup>2</sup>, Alissa Mittnik<sup>3</sup>, Gabriel Renaud<sup>4</sup>, Swapan Mallick<sup>1,2</sup>, Karola Kirsanow<sup>5</sup>, Peter H. Sudmant<sup>6</sup>, Joshua G. Schraiber<sup>7</sup>, Sergi Castellano<sup>4</sup>, Mark Lipson<sup>8</sup>, Bonnie Berger<sup>2,8</sup>, Christos Economou<sup>9</sup>, Ruth Bollongino<sup>5</sup>, Qiaomei Fu<sup>1,4,10</sup>, Kirsten I. Bos<sup>3</sup>, Susanne Nordenfelt<sup>1,2</sup>, Heng Li<sup>1,2</sup>, Cesare de Filippo<sup>4</sup>, Kay Prüfer<sup>4</sup>, Susanna Sawyer<sup>4</sup>, Cosimo Posth<sup>3</sup>, Wolfgang Haak<sup>11</sup>, Fredrik Hallgren<sup>12</sup>, Elin Fornander<sup>12</sup>, Nadin Rohland<sup>1,2</sup>, Dominique Delsate<sup>13,14</sup>, Michael Francken<sup>15</sup>, Jean-Michel Guinet<sup>13</sup>, Joachim Wahl<sup>16</sup>, George Avodo<sup>17</sup>, Hamza A. Babiker<sup>18,19</sup>, Graciela Bailliet<sup>20</sup>, Elena Balanovska<sup>21</sup>, Oleg Balanovsky<sup>21,22</sup>, Ramiro Barrantes<sup>23</sup>, Gabriel Bedoya<sup>24</sup>, Haim Ben-Ami<sup>25</sup>, Judit Bene<sup>26</sup>, Fouad Berrada<sup>27</sup>, Claudio M. Bravi<sup>20</sup>, Francesca Brisighelli<sup>28</sup>, George B. J. Busby<sup>29,30</sup>, Francesco Cali<sup>31</sup>, Mikhail Churnosov<sup>32</sup>, David E. C. Cole<sup>33</sup>, Daniel Corach<sup>34</sup>, Larissa Damba<sup>35</sup>, George van Driem<sup>36</sup>, Stanislav Dryomov<sup>37</sup>, Jean-Michel Dugoujon<sup>38</sup>, Sardana A. Fedorova<sup>39</sup>, Irene Gallego Romero<sup>40</sup>, Marina Gubina<sup>35</sup>, Michael Hammer<sup>41</sup>, Brenna M. Henn<sup>42</sup>, Tor Hervig<sup>43</sup>, Ugur Hodoglugil<sup>44</sup>, Aashish R. Jha<sup>40</sup>, Sena Karachanak-Yankova<sup>45</sup>, Rita Khusainova<sup>46,47</sup>, Elza Khusnutdinova<sup>46,47</sup>, Rick Kittles<sup>48</sup>, Toomas Kivisild<sup>49</sup>, William Klitz<sup>7</sup>, Vaidutis Ku inskas<sup>50</sup>, Alena Kushniarevich<sup>51</sup>, Leila Laredi<sup>52</sup>, Sergey Litvinov<sup>46,47,51</sup>,

Theologos Loukidis<sup>53</sup>, Robert W. Mahley<sup>54</sup>, Béla Melegh<sup>26</sup>, Ene Metspalu<sup>55</sup>, Julio Molina<sup>56</sup>, Joanna Mountain<sup>57</sup>, Klemetti Näkkäläjärvi<sup>58</sup>, Desislava Nesheva<sup>45</sup>, Thomas Nyambo<sup>59</sup>, Ludmila Osipova<sup>35</sup>, Jüri Parik<sup>55</sup>, Fedor Platonov<sup>60</sup>, Olga Posukh<sup>35</sup>, Valentino Romano<sup>61</sup>, Francisco Rothhammer<sup>62,63,64</sup>, Igor Rudan<sup>65</sup>, Ruslan Ruizbakiev<sup>66</sup>, Hovhannes Sahakyan<sup>51,67</sup>, Antti Sajantila<sup>68,69</sup>, Antonio Salas<sup>70</sup>, Elena B. Starikovskaya<sup>37</sup>, Ayele Tarekegn<sup>71</sup>, Draga Toncheva<sup>45</sup>, Shahlo Turdikulova<sup>72</sup>, Ingrida Uktveryte<sup>50</sup>, Olga Utevska<sup>73</sup>, René Vasquez<sup>74</sup>, Mercedes Villena<sup>74</sup>, Mikhail Voevoda<sup>35,75</sup>, Cheryl Winkler<sup>76</sup>, Levon Yepiskoposyan<sup>67</sup>, Pierre Zalloua<sup>77,78</sup>, Tatijana Zemunik<sup>79</sup>, Alan Cooper<sup>11</sup>, Cristian Capelli<sup>29</sup>, Mark G. Thomas<sup>80</sup>, Andres Ruiz-Linares<sup>80</sup>, Sarah A. Tishkoff<sup>81</sup>, Lalji Singh<sup>82,83</sup>, Kumarasamy Thangaraj<sup>82</sup>, Richard Villems<sup>51,55,84</sup>, David Comas<sup>85</sup>, Rem Sukernik<sup>37</sup>, Mait Metspalu<sup>51</sup>, Matthias Meyer<sup>4</sup>, Evan E. Eichler<sup>6,86</sup>, Joachim Burger<sup>5</sup>, Montgomery Slatkin<sup>7</sup>, Svante Pääbo<sup>4</sup>, Janet Kelso<sup>4</sup>, David Reich<sup>1,2,87,†</sup>, and Johannes Krause<sup>3,88,90,†</sup>

# **Affiliations**

<sup>1</sup>Department of Genetics, Harvard Medical School, Boston, MA, 02115, USA <sup>2</sup>Broad Institute of Harvard and MIT, Cambridge, MA, 02142, USA <sup>3</sup>Institute for Archaeological Sciences, University of Tübingen, Tübingen, 72074, Germany <sup>4</sup>Max Planck Institute for Evolutionary Anthropology, Leipzig, 04103, Germany <sup>5</sup>Johannes Gutenberg University Mainz, Institute of Anthropology, Mainz, D-55128, Germany <sup>6</sup>Department of Genome Sciences, University of Washington, Seattle, WA, 98195, USA <sup>7</sup>Department of Integrative Biology, University of California, Berkeley, CA, 94720-3140, USA <sup>8</sup>Department of Mathematics and Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA <sup>9</sup>Archaeological Research Laboratory, Stockholm University, 114 18, Sweden <sup>10</sup>Key Laboratory of Vertebrate Evolution and Human Origins of Chinese Academy of Sciences, IVPP, CAS, Beijing, 100049, China <sup>11</sup>Australian Centre for Ancient DNA, School of Earth and Environmental Sciences, University of Adelaide, Adelaide, South Australia, SA 5005, Australia <sup>12</sup>The Cultural Heritage Foundation, Västerås, 722 12, Sweden <sup>13</sup>National Museum of Natural History, L-2160, Luxembourg <sup>14</sup>National Center of Archaeological Research, National Museum of History and Art, L-2345, Luxembourg <sup>15</sup>Department of Paleoanthropology, Senckenberg Center for Human Evolution and Paleoenvironment, University of Tübingen, Tübingen, D-72070, Germany <sup>16</sup>State Office for Cultural Heritage Management Baden-Württemberg, Osteology, Konstanz, D-78467, Germany <sup>17</sup>Center for Global Health and Child Development, Kisumu, 40100, Kenya <sup>18</sup>Institutes of Evolution, Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JT, UK <sup>19</sup>Biochemistry Department, Faculty of Medicine, Sultan Qaboos University, Alkhod, Muscat, 123, Oman <sup>20</sup>Laboratorio de Genética Molecular Poblacional, Instituto Multidisciplinario de Biología Celular (IMBICE), CCT-CONICET & CICPBA, La Plata, B1906APO, Argentina <sup>21</sup>Research Centre for Medical Genetics, Moscow, 115478, Russia <sup>22</sup>Vavilov Institute for General Genetics, Moscow, 119991, Russia <sup>23</sup>Escuela de Biología, Universidad de Costa Rica, San José, 2060, Costa Rica <sup>24</sup>Institute of

Biology, Research group GENMOL, Universidad de Antioquia, Medellín, Colombia <sup>25</sup>Rambam Health Care Campus, Haifa, 31096, Israel <sup>26</sup>Department of Medical Genetics and Szentagothai Research Center, University of Pécs, Pécs, H-7624 Hungary <sup>27</sup>Al Akhawayn University in Ifrane (AUI), School of Science and Engineering, Ifrane, 53000, Morocco <sup>28</sup>Forensic Genetics Laboratory, Institute of Legal Medicine, Università Cattolica del Sacro Cuore, Rome, 00168, Italy <sup>29</sup>Department of Zoology, University of Oxford, Oxford, OX1 3PS, UK <sup>30</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK <sup>31</sup>Laboratorio di Genetica Molecolare, IRCCS Associazione Oasi Maria SS, Troina, 94018, Italy <sup>32</sup>Belgorod State University, Belgorod, 308015, Russia <sup>33</sup>Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, M5G 1L5, Canada <sup>34</sup>Servicio de Huellas Digitales Genéticas, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, 1113 CABA, Argentina 35 Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, 630090, Russia <sup>36</sup>Institute of Linguistics, University of Bern, Bern, CH-3012, Switzerland <sup>37</sup>Laboratory of Human Molecular Genetics, Institute of Molecular and Cellular Biology, Russian Academy of Science, Siberian Branch, Novosibirsk, 630090, Russia <sup>38</sup>Anthropologie Moléculaire et Imagerie de Synthèse, CNRS UMR 5288, Université Paul Sabatier Toulouse III, Toulouse, 31000, France <sup>39</sup>Yakut Research Center of Complex Medical Problems and North-Eastern Federal University, Yakutsk, 677010, Russia <sup>40</sup>Department of Human Genetics, University of Chicago, Chicago, IL, 60637, USA 41ARL Division of Biotechnology, University of Arizona, Tucson, AZ, 85721, USA <sup>42</sup>Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, 11794, USA <sup>43</sup>Department of Clinical Science, University of Bergen, Bergen, 5021, Norway 44NextBio, part of Illumina, Santa Clara, CA, USA 95050 <sup>45</sup>Dept. of Medical Genetics, National Human Genome Center, Medical University Sofia, Sofia, 1431, Bulgaria 46Institute of Biochemistry and Genetics, Ufa Research Centre, Russian Academy of Sciences, Ufa, 450054, Russia <sup>47</sup>Department of Genetics and Fundamental Medicine, Bashkir State University, Ufa, 450074, Russia <sup>48</sup>College of Medicine, University of Illinois at Chicago, Chicago, IL, 60607, USA <sup>49</sup>Division of Biological Anthropology, University of Cambridge, Cambridge, United Kingdom CB2 1QH 50Department of Human and Medical Genetics, Vilnius University, Vilnius, LT-08661, Lithuania <sup>51</sup>Estonian Biocentre, Evolutionary Biology group, Tartu, 51010, Estonia <sup>52</sup>Translational Medicine and Neurogenetics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, 67404, France <sup>54</sup>Gladstone Institutes, San Francisco, CA, 94158, USA 55 Department of Evolutionary Biology, University of Tartu, Tartu, 51010, Estonia <sup>56</sup>Centro de Investigaciones Biomédicas de Guatemala, Ciudad de Guatemala, Guatemala <sup>57</sup>Research Department, 23andMe, Inc. Mountain View, CA, 94043, USA <sup>58</sup>Cultural Anthropology Program, University of Oulu, Oulu, 90014, Finland <sup>59</sup>Department of Biochemistry, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania <sup>60</sup>Research Institute of Health, North-Eastern Federal University, Yakutsk, 677000, Russia <sup>61</sup>Dipartimento di Fisica e Chimica. Università di Palermo, Palermo, 90128, Italy 62 Instituto de Alta Investigación,

Universidad de Tarapacá, Arica, Chile 63 Programa de Genética Humana ICBM Facultad de Medicina Universidad de Chile, Santiago, Chile <sup>64</sup>Centro de Investigaciones del Hombre en el Desierto, Arica, Chile 65 Centre for Population Health Sciences, The University of Edinburgh Medical School, Edinburgh, Scotland, EH8 9AG, UK 66Deceased: formerly of the Institute of Immunology, Academy of Science, Tashkent, 70000, Uzbekistan 67Laboratory of Ethnogenomics, Institute of Molecular Biology, National Academy of Sciences of Armenia, Yerevan, 0014, Armenia <sup>68</sup>Department of Forensic Medicine, Hjelt Institute, University of Helsinki, Helsinki, 00014, Finland <sup>69</sup>Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center, Fort Worth, Texas 76107, USA 70 Unidade de Xenética, Departamento de Anatomía Patolóxica e Ciencias Forenses, and Instituto de Ciencias Forenses, Grupo de Medicina Xenómica (GMX), Facultade de Medicina, Universidade de Santiago de Compostela, Galcia, 15872, Spain <sup>71</sup>Research Fellow, Henry Stewart Group, Russell House, London WC1A 2HN, UK <sup>72</sup>Institute of Bioorganic Chemistry Academy of Sciences Republic of Uzbekistan, Tashkent, 100125, Uzbekistan <sup>73</sup>Department of Genetics and Cytology, V.N. Karazin Kharkiv National University, Kharkiv, 61077, Ukraine <sup>74</sup>Instituto Boliviano de Biología de la Altura, Universidad Autonoma Tomás Frías, Potosí, Bolivia 75 Inst. of Internal Medicine, Siberian Branch of Russian Acad. of Medical Sciences, Novosibirsk, 630089, Russia <sup>76</sup>Basic Research Laboratory, NCI, NIH, Frederick National Laboratory, Leidos Biomedical, Inc., Frederick, MD 21702, USA 77Lebanese American University, School of Medicine, Beirut, 13-5053, Lebanon <sup>78</sup>Harvard School of Public Health, Boston, 02115, USA 79Department of Medical Biology, University of Split, School of Medicine, Split, 21000, Croatia 80 Department of Genetics, Evolution and Environment, University College London, WC1E 6BT, UK 81Department of Biology and Genetics. University of Pennsylvania, Philadelphia, Pennsylvania, 19104, USA 82CSIR-Centre for Cellular and Molecular Biology, Hyderabad, 500 007, India <sup>84</sup>Estonian Academy of Sciences, Tallinn, 10130, Estonia <sup>85</sup>Institut de Biologia Evolutiva (CSIC-UPF), Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, 08003, Spain 86 Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA 98195 87 Howard Hughes Medical Institute, Harvard Medical School, Boston, MA, 02115, USA 88 Senckenberg Centre for Human Evolution and Palaeoenvironment, University of Tübingen, 72070, Germany 90Max Planck Institut für Geschichte und Naturwissenschaften, Jena, 07745, Germany

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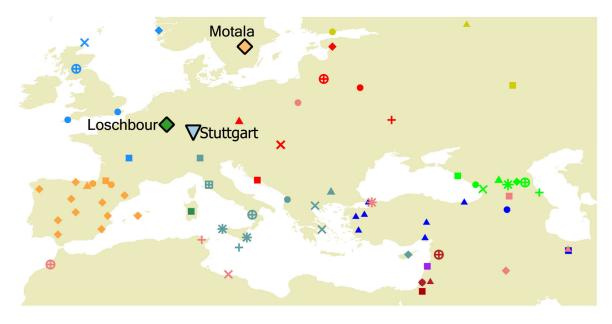
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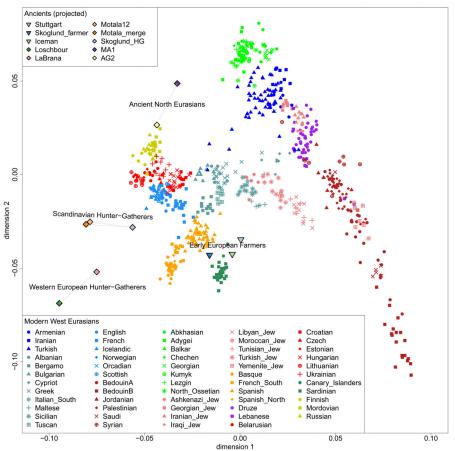
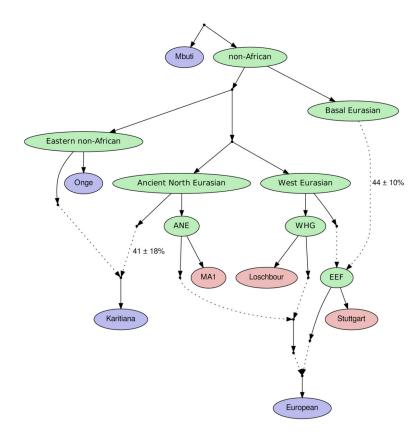


Figure 1. Map of West Eurasian populations and Principal Component Analysis
(a) Geographical locations of analyzed samples, with color coding matching the PCA. We show all sampling locations for each population, which results in multiple points for some

(e.g., Spain). (b) PCA on all present-day West Eurasians, with ancient and selected eastern non-African samples projected. European hunter-gatherers fall beyond present-day Europeans in the direction of European differentiation from the Near East. Stuttgart clusters with other Neolithic Europeans and present-day Sardinians. MA1 falls outside the variation of present-day West Eurasians in the direction of southern-northern differentiation along dimension 2.



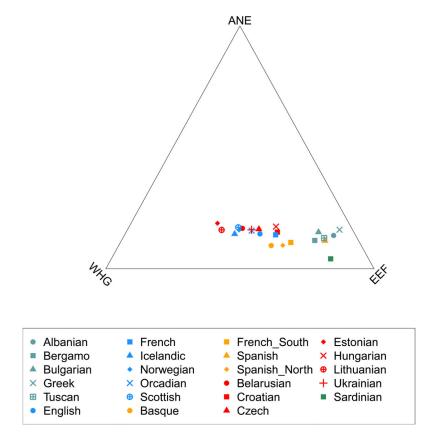


Figure 2. Modeling of West Eurasian population history

(a) A three-way mixture model that is a fit to the data for many populations. Present-day samples are colored in blue, ancient in red, and reconstructed ancestral populations in green. Solid lines represent descent without mixture, and dashed lines represent admixture. We print mixture proportions and one standard error for the two mixtures relating the highly divergent ancestral populations. (We do not print the estimate for the "European" population as it varies depending on the population). (b) We plot the proportions of ancestry from each of three inferred ancestral populations (EEF, ANE and WHG).

Table 1 Lowest  $f_3$ -statistics for each West Eurasian population

Ref <sub>1</sub>	Ref <sub>2</sub>	Target for which these two references give the lowest $f_3(X; Ref_1, Ref_2)$
WHG	EEF	Sardinian***
WHG	Near East	Basque, Belarusian, Czech, English, Estonian, Finnish, French_South, Icelandic, Lithuanian, Mordovian, Norwegian, Orcadian, Scottish, Spanish, Spanish_North, Ukrainian
WHG	Siberian	Russian
EEF	ANE	Abkhasian***, Albanian, Ashkenazi_Jew****, Bergamo, Bulgarian, Chechen****, Croatian, Cypriot****, Druze**, French, Greek, Hungarian, Lezgin, Maltese, Sicilian, Turkish_Jew, Tuscan
EEF	Native American	Adygei, Balkar, Iranian, Kumyk, North_Ossetian, Turkish
EEF	African	BedouinA, BedouinB <sup>†</sup> , Jordanian, Lebanese, Libyan_Jew, Moroccan_Jew, Palestinian, Saudi****, Syrian, Tunisian_Jew***, Yemenite_Jew***
EEF	South Asian	Armenian, Georgian****, Georgian_Jew*, Iranian_Jew***, Iraqi_Jew***

Note: WHG = Loschbour or LaBraña; EEF=Stuttgart; ANE=MA1; Native American=Piapoco; African=Esan, Gambian, or Kgalagadi; South Asian=GujaratiC or Vishwabrahmin. Statistics are negative with Z<-4 unless otherwise noted:  $^{\dagger}$  (positive) or \*, \*\*, \*\*\*, \*\*\*\*, to indicate Z less than 0, -1, -2, and -3 respectively. The complete list of statistics can be found in Extended Data Table 1.