Supporting Information

Supporting methods

Estimating ancestry components in Admixture and Structure: Estimated individual ancestry from supervised and unsupervised clustering in *Admixture* are highly concordant for the autosomes for both migration events, and for the X chromosome for the Neolithic migration scenario. For X-chromosomal ancestry estimated for the steppe migration, however, reference individuals do not emerge as clusters in unsupervised *Admixture,* therefore results cannot be used in this framework. For both the X chromosome and autosomes, unsupervised *Structure* estimates are similar to those from *Admixture*. Perhaps surprisingly, supervised *Structure* produces different estimates for both individual and population-level ancestry estimates; however, as we show below, this deviation is likely an effect of a small sample size, as increasing the sample size for supervised *Structure* leads to results similar to *Admixture* and unsupervised *Structure* for an example case with high coverage data. In all *Structure* analyses, we use 10,000 burn-in iterations, followed by 50,000 iterations.

As our inference methods rely on the mean ancestry in the population (and are supported by the variance), we focus on comparisons of these summary statistics. The population mean and variance of ancestry for supervised *Structure,* supervised and unsupervised *Admixture* are highly concordant (no data for BA X chromosome in unsupervised *Admixture*). However, the mean ancestry estimated in supervised *Structure* is qualitatively different, and the variance is substantially lower. That is, downstream analyses using the first three methods would produce similar estimates of the levels of sex bias during the two migrations, while inference from supervised *Structure* would be qualitatively different, though notably, still in the same direction we observe for both migration events. Below, we investigated various factors, particularly sample size, that can cause the deviation in supervised *Structure* results by investigating HapMap data. We conclude that the differing finding using supervised *Structure* for the ancient individuals is due to sensitivity to low sample sizes for the supervised *Structure* algorithm and we use estimates from supervised *Admixture* for inference in the main text, and reiterate that inference using unsupervised *Structure* would be highly similar because of the similar mean and variance of ancestry estimated. Ancestry for each individual is presented as the average estimated individual ancestry using ten independent seeds, considering the X chromosome and autosomes separately.

Comparing ancestry estimation methods using HapMap data: As three methods produce concordant results, with only supervised *Structure* differing, we investigated various factors, particularly sample size, that can cause the deviation in supervised *Structure* results. We investigated the effect of sample size by reproducing the analysis on a larger dataset from the HapMap Phase 3 Project (*44*). We considered the recent admixture between Africans and Europeans, using YRI and CEU as reference populations and estimating ancestry in ASW individuals (Fig. S5). To examine the impact of sample size on accuracy of ancestry inference, we down sampled the reference populations considering sample sizes ranging from 4 diploid individuals in each reference population to 112 (the maximum number of CEU individuals). The set of individuals in smaller reference panels are subsets of the larger panels.

For each sample size of reference populations, we estimated ancestry in 16 ASW individuals using each of the four methods (supervised and unsupervised settings in each *Admixture* and *Structure*), based on the sample size of BA. Figure S5 plots the mean and variance of ancestry in the ASW population for each sample size and method. Each point is based on 10 replicates, first averaging ancestry by individual. For computational speed, we estimated ancestry from 15,000 randomly drawn autosomal SNPs, after LD pruning using the same method as for the ancient DNA (see Genetic samples and populations). For each replicate, we resampled 15,000 SNPs and use a new seed. As in our data, supervised *Structure* is an outlier in its behavior. Indeed, as the ancient samples are largely haploid, the corresponding sample sizes most representative are in the range of four to ten diploid CEU/YRI individuals. Particularly in this range of the plot, the mean and variance of ancestry estimated using supervised *Structure* differ greatly from those using all other methods, and are further from corresponding estimates at large sample sizes under all methods (Fig. S5). As the sample sizes increase for the reference populations, the results from supervised *Structure* approach the results of the three other methods (Fig. S5). We conclude that the differing finding using supervised *Structure* for the ancient individuals is due to sensitivity to low sample sizes for the supervised *Structure* algorithm. Therefore, we use estimates from supervised *Admixture* for inference in the main text, and reiterate that inference using unsupervised *Structure* would be highly similar because of the similar mean and variance of ancestry estimated. Ancestry for each individual is presented as the average estimated individual ancestry using ten independent seeds, considering the X chromosome and autosomes separately.

Simulations to estimate range of sex bias during Neolithic Transition: For a constant admixed population of size N, with $N \epsilon \{1{,}000; 5{,}000; 10{,}000\}$, we simulated the ancestry proportion of individuals in the admixed population recursively for 40 generations, or approximately 1,000 years, assuming a single admixture event followed by no further migration (Fig. 1). For a generation time of ~25 years, this number of generations approximately corresponds to the difference in time between the onset of migration and the radiocarbon ages of sampled admixed individuals for each migration (*6*).

We set the total contributions from each population based on their autosomal ancestry levels (*17*,*35*,*36*), with HG as 0.087, and AF to be 0.913. We define the level of sex bias as the ratio of male to female contributions from a given source population, B, considering $B \epsilon \left\{\frac{1}{40},\dots,\frac{i}{40},\dots,1,\dots,\frac{40}{i},\dots,\frac{40}{1}\right\}$. Given the overall contribution from each source population, as well as a specified value of male to female contributions, B, the female and male contribution parameters can be exactly solved. That is, adapting eq. 1 from (*35*), for male contribution from population α given by $m_\alpha,$ the probability of a randomly chosen individual in the first generation of the admixed population having a male parent from each source population is $m_{HG} = 2 * 0.087\left(\frac{B}{B+1}\right)$, $m_{AF} = 2 * 0.913\left(\frac{B}{B+1}\right)$. Similarly, the female contributions (f_α) can be written as: $f_{HG} = 2 * 0.087 - m_{HG}$, $f_{AF} = 2 * 0.913 - m_{AF}$. Given the sex-specific contribution parameters, we then did 1,000 replicate simulations for different levels of specified sex bias.

***Simulating autosomal ancestry*:** For the first generation, $g = 1$, we randomly chose and matched $2N$ parents, making N parental pairs. Each parent is drawn with probability given by their sex-specific contribution level. An individual's autosomal ancestry is calculated as the average of its parent's autosomal ancestries. Then, for $g \geq 2$, we calculated ancestry in N

individuals at generation g by randomly choosing and pairing $2N$ parents from the population in the previous generation, $g-1$.

Simulating X-chromosomal ancestry: we followed the same procedure as for the autosomes, but instead considered separate populations of males and females, each with $N/2$ individuals. For the female population, we generated N parental pairs by drawing an individual from each the male and female population from the previous generation. For the male population, we drew $N/2$ mothers from the female population in the previous generation. Ancestry of females was calculated as the average of the parental ancestries, while ancestry for males is equal to the ancestry of the mother.

At $g=40$, we randomly sampled 20 individuals, and calculated the mean autosomal ancestry and mean X-chromosomal ancestry in the sample. The mean X-chromosomal ancestry is calculated as a weighted mean of the female and male X-chromosomal ancestries, based on the proportion of females in the data set (75%). Figure 3A shows the values of sex bias, B, for which the observed X to autosomal ancestry ratio is within the middle 50% and 80% of ratios calculated from the 1,000 simulated populations with that level of specified sex bias.

The effect of drift on admixture fractions is larger in smaller populations (*45*); we therefore expect a larger possible range of sex bias values to produce values of X-to-autosomal ancestry ratios similar to those estimates from the data for smaller population sizes. Yet, even simulations with an admixed population size of 1,000 suggest less than 1.2 males migrating for every female from AF to CE.

Supplemental references

44. International HapMap 3 Consortium, et al. (2010). Integrating common and rare genetic variation in diverse human populations. *Nature* 467, 52–58.
45. Long, J. C. (1991). The genetic structure of admixed populations. *Genetics*, *127*(2), 417-428.

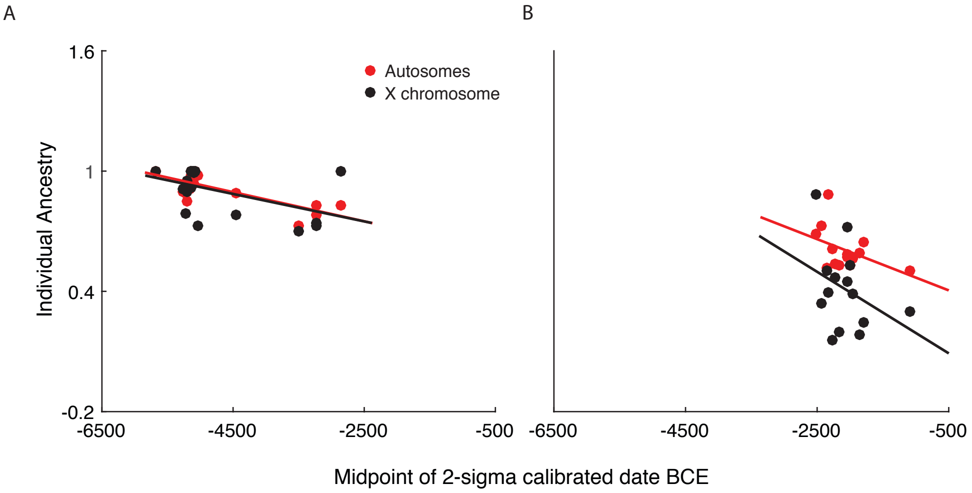

Figure S1: Relationship between sampling age and ancestry. The linear model shows a significant relatinoship between sampling age, measured as the midpoint of the 2-sigma range of calibrated dates BCE, and ancestry for (A) the Neolithic migration ($p=0.02$ & $R^2=0.21$, $p<0.001$ & $R^2=0.59$ for X and autosomes, respectively), but not for (B) the Pontic-Steppe migration ($p=0.21$ & $R^2=0.05$, $p=0.11$ & $R^2=0.13$ for X and autosomes, respectively). Ancestry is relative to the migrating popultion: AF for the Neolithic, and SP for the steppe.

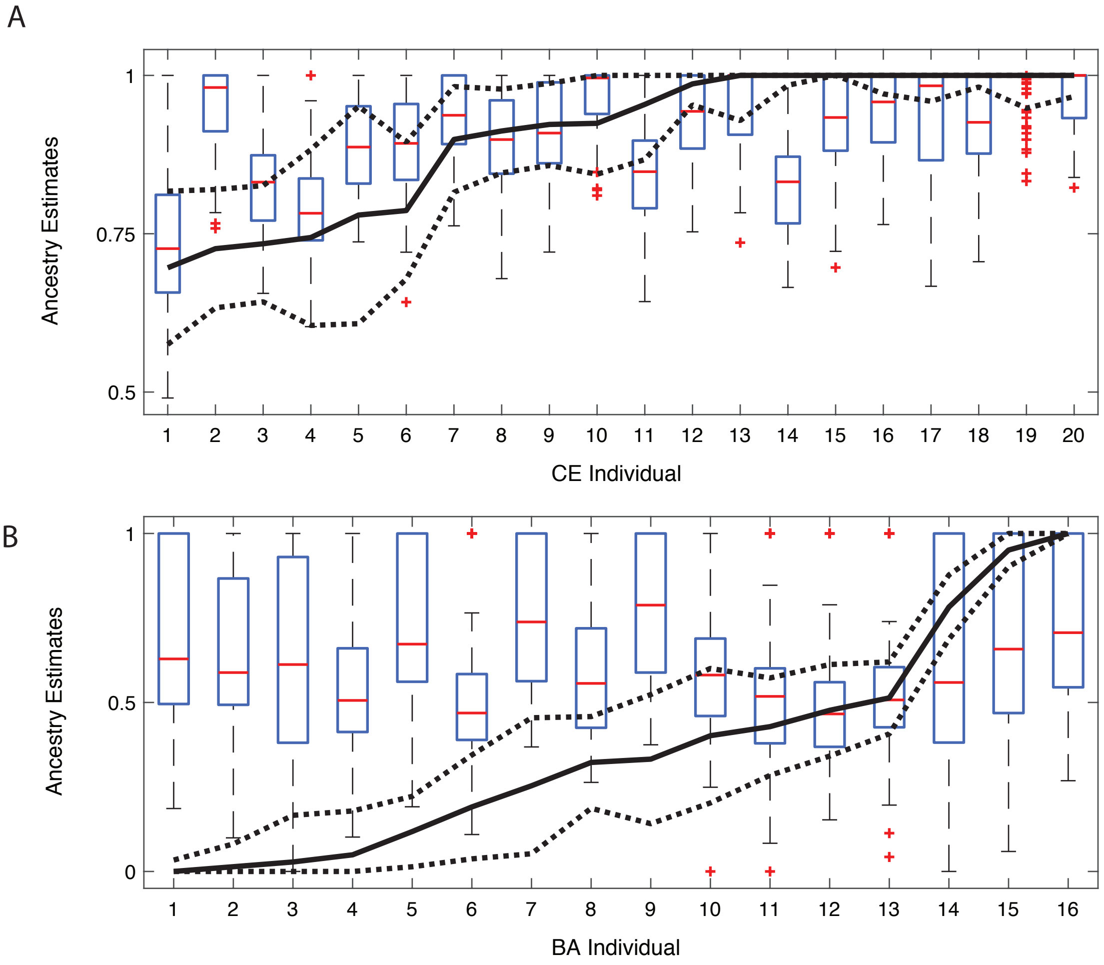

Figure S2: Resampling SNPs to estimate autosomal ancestry. Each boxplot represents the range of estimated autosomal ancestries over 100 resampled sets of randomly drawn autsomal SNPs to match the number of X-chromosomal SNPs. The lines correspond to estimated X-chromosomal ancestry by individual, with dotted lines marking within one standard error. (A) The Neolithic Transition. The number of SNPs is 3,763. The distribution of X and autosomal ancestry largely overlap in most individuals. (B) The Pontic Steppe migration. The number of SNPs is 4,605. Ancestry on the X is either lower or similar for all individuals. For both panels, individuals are presented in the same order as Fig. 2.

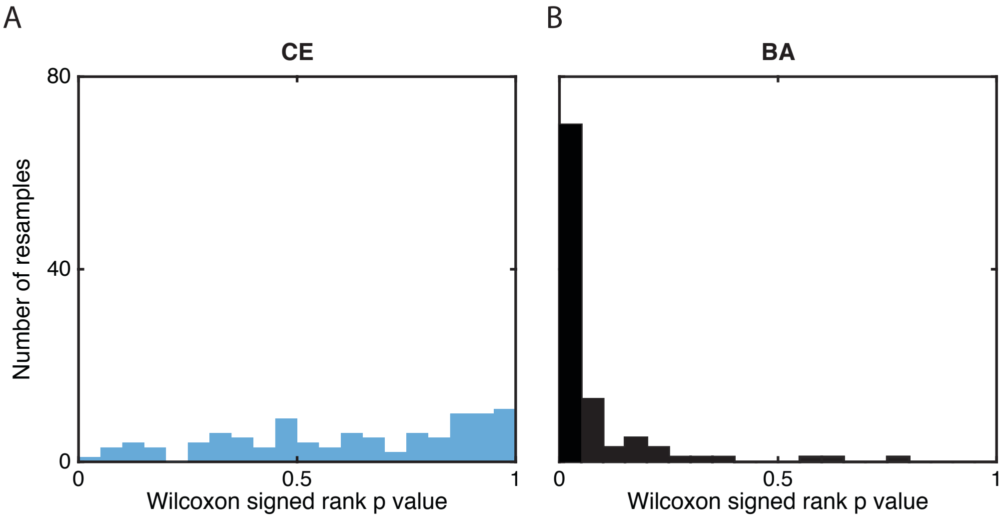

Figure S3: Histogram of p values for the Wilcoxon sign-rank test. In each admixed population, the comparison of the distribution of ancestry on the X chromsome to the 100 autosomal ancestry distributions for (A) the CE population, and (B) the BA population.

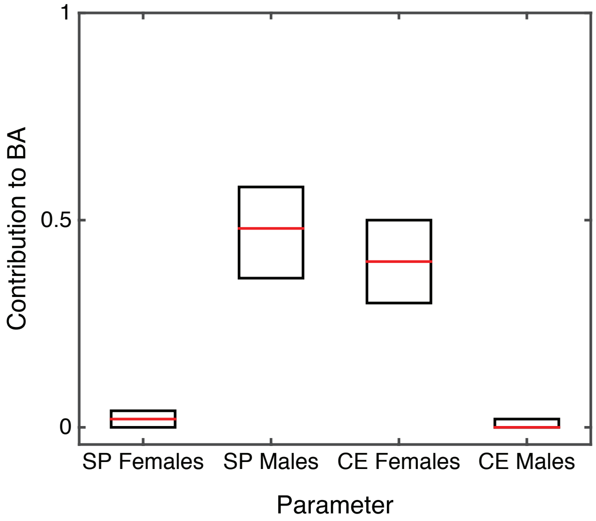

Figure S4: Estimated sex-specific contributions from Pontic Steppe (SP) and early Central Europeans (CE) to LNBA Europeans (BA) . Under a model of constant contributions over time, each box represents the middle fifty percent of parameter sets for the smallest 0.1% of Euclidean distances between the model-predicted and observed X and autosomal ancestry from a grid of possible parameter values. Red line is the median of plotted values.

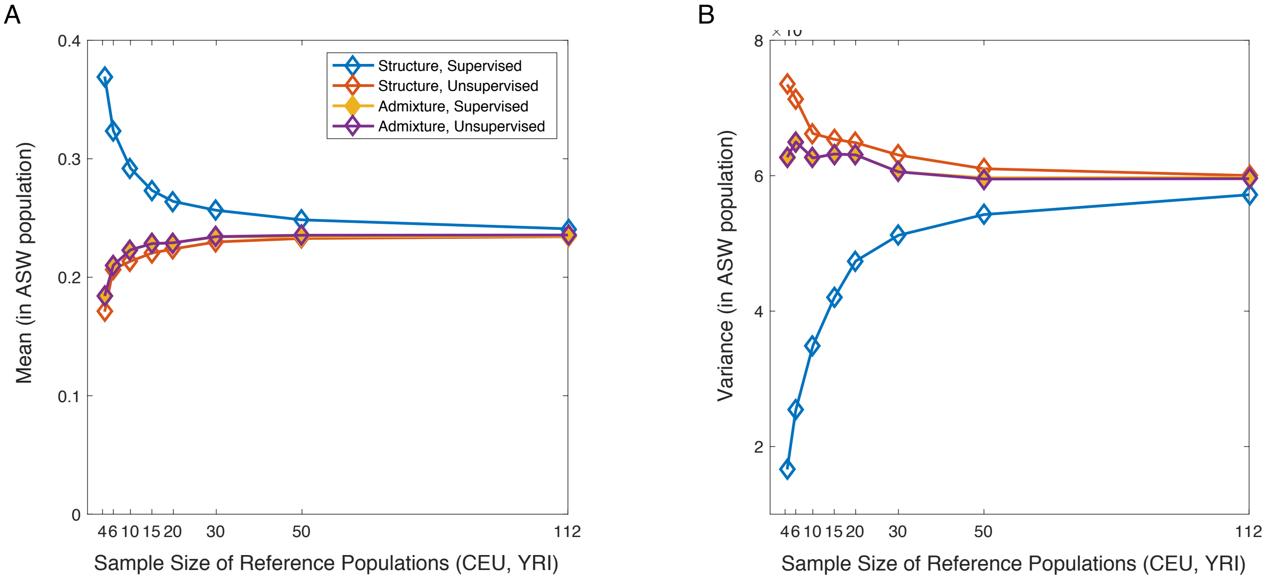

Figure S5: Comparing ancestry estimation methods using HapMap data. The (A) variance and (B) mean of CEU-related ancestry in the ASW (African American) population by sample size for four clustering methods. Each point is based on 10 replicates, first averaging ancestry by individual. For each replicate, we resample 15,000 SNPs and use a new seed. Supervised clustering in *Structure* produces qualitatively different results than the other methods for small sample sizes.

Admixed Population	Individual	Archeological Label	# SNPs (X)	# SNPs (Autosomes)
CE	I0172	Central_MN	3,348	296,827
CE	I0560	Central_MN	1,293	125,647
CE	I1497	Central_MN	2,945	282,608
CE	I1495	Hungary_EN	2,595	282,090
CE	I1496	Hungary_EN	2,504	277,979
CE	I1498	Hungary_EN	2,944	281,643
CE	I1499	Hungary_EN	2,979	282,181
CE	I1500	Hungary_EN	2,540	282,155
CE	I1505	Hungary_EN	2,835	271,675
CE	I1506	Hungary_EN	2,553	250,572
CE	I1508	Hungary_EN	2,118	202,813
CE	Iceman	Iceman (MN)	3,563	329,264
CE	I0022	LBK_EN	1,226	127,899
CE	I0025	LBK_EN	2,838	276,166
CE	I0026	LBK_EN	3,002	287,954
CE	I0046	LBK_EN	3,008	281,010
CE	I0054	LBK_EN	3,269	305,318
CE	I0100	LBK_EN	3,159	309,476
CE	I0659	LBK_EN	1,186	181,302
CE	I1550	LBK_EN	1,827	178,442
	Mean Ancestry:			
BA	I0047	Unetice_EBA	3,377	295,581
BA	I0049	Corded_Ware_Germany	2,314	190,443
BA	I0059	BenzigerodeHeimburg_LN	3,124	273,321
BA	I0099	Halberstadt_LBA	3,081	339,040
BA	I0103	Corded_Ware_Germany	4,273	358,176
BA	I0104	Corded_Ware_Germany	3,223	336,630
BA	I0115	Unetice_EBA	1,513	130,840
BA	I0116	Unetice_EBA	1,353	229,560
BA	I0117	Unetice_EBA	3,520	307,312
BA	I0118	Alberstedt_LN	4,205	364,382
BA	I0164	Unetice_EBA	3,278	301,603
BA	I0803	Unetice_EBA	1,241	84,086
BA	I1532	Corded_Ware_Germany	1,248	171,544
BA	RISE00	Corded_Ware_Estonia	2,538	221,453
BA	RISE150	Unetice_EBA	2,383	248,413
BA	RISE577	Unetice_EBA	2,857	254,460
	Mean Ancestry:			

Table S1: Sample descriptions for admixed populations. Archeological label follows Mathieson et al. (2015). For CE individuals, the archeological label in the archeological culture from Mathieson et al. (2015). For BA individuals, all individuals are ‘Central_LNBA;’ therefore, we use the more detailed information from Lazaridis et al. (2016)

Admixed Population	Individual	Ancestry, X chromosome	Ancestry, Autosomes (All SNPs)	Ancestry, Autosomes (Median of 100 SNP sets)
CE	I0172	0.74	0.80	0.78
CE	I0560	0.70	0.74	0.73
CE	I1497	1.00	0.85	0.83
CE	I1495	0.78	0.89	0.89
CE	I1496	1.00	1.00	1.00
CE	I1498	0.79	0.89	0.89
CE	I1499	0.95	0.86	0.85
CE	I1500	0.90	0.95	0.94
CE	I1505	0.92	0.91	0.91
CE	I1506	0.91	0.91	0.90
CE	I1508	1.00	1.00	1.00
CE	Iceman	0.73	0.82	0.83
CE	I0022	1.00	1.00	0.98
CE	I0025	1.00	0.94	0.93
CE	I0026	0.92	0.99	1.00
CE	I0046	0.99	0.98	0.94
CE	I0054	1.00	0.95	0.96
CE	I0100	0.73	0.96	0.98
CE	I0659	1.00	0.99	1.00
CE	I1550	1.00	0.96	0.93
	Mean Ancestry:	***0.903***	***0.919***	***0.913***
BA	I0047	0.51	0.55	0.511
BA	I0049	0.33	1.00	1.000
BA	I0059	0.43	0.54	0.519
BA	I0099	0.19	0.54	0.479
BA	I0103	1.00	1.00	0.732
BA	I0104	0.25	1.00	0.789
BA	I0115	0.03	0.66	0.599
BA	I0116	0.40	0.63	0.589
BA	I0117	0.05	0.56	0.516
BA	I0118	0.48	0.54	0.493
BA	I0164	0.32	0.62	0.561
BA	I0803	0.78	0.68	0.573
BA	I1532	0.00	1.00	0.625
BA	RISE00	0.95	0.68	0.642
BA	RISE150	0.12	1.00	0.674
BA	RISE577	0.01	0.61	0.582
	Mean Ancestry:	***0.366***	***0.726***	***0.618***

Table S2: Individual ancestry in admixed populations. Ancestry is estimated in ADMIXTURE v1.3 using supervised clustering, results presented are the component clustering with the migrating population. For the Neolithic Transition with admixed CE individuals, that is AF ancestry, for Pontic Steppe migration with admixed BA individuals, that is SP ancestry.

Population	Individual	# SNPs (X)	# SNPs (Autosomes)
HG	I0011	2,597	238,300
HG	I0012	2,248	288,233
HG	I0013	294	114,115
HG	I0014	2,847	277,370
HG	I0015	1,394	224,569
HG	I0017	2,826	326,861
HG	I0585	3,315	331,453
HG	I1507	2,881	304,651
HG	Loschbour	4,483	370,431
AF	I0707	3,928	347,728
AF	I0708	3,120	339,272
AF	I0709	3,196	344,000
AF	*I0723*	*1,069*	*158,218*
AF	*I0724*	*101*	*20,982*
AF	*I0726*	*910*	*84,012*
AF	*I0727*	*95*	*16,374*
AF	I0736	3,090	293,649
AF	I0744	2,665	316,049
AF	I0745	3,290	346,243
AF	I0746	3,310	347,428
AF	I1096	2,658	292,505
AF	I1097	2,532	289,766
AF	I1098	3,401	302,251
AF	I1099	1,579	215,557
AF	I1100	1,338	121,755
AF	I1101	2,176	259,865
AF	I1102	1,087	168,149
AF	I1103	1,900	239,704
AF	I1579	3,525	306,814
AF	I1580	3,853	332,503
AF	I1581	3,578	308,641
AF	I1583	3,665	349,463
AF	I1585	3,621	309,674
SP	I0231	3,695	361,283
SP	I0357	2,298	189,734
SP	I0370	2,047	254,721
SP	I0429	1,600	224,264
SP	I0438	1,353	206,462
SP	*I0439*	*600*	*96,300*
SP	I0441	328	37,237
SP	I0443	3,344	347,381
SP	I0444	1,345	197,552

Table S3: Source population individuals. Ancestry is estimated in *Admixture* using supervised clustering, results presented are the component clustering with the migrating population. Individuals in italicized rows were not included in main text analyses (Materials & Methods). HG—Hunter gatherer, AF—Anatolian Farmer, SP—Pontic-Caspian Steppe.

Admixed Population	Individual	Ancestry, Admixture Unsupervised	Ancestry, Structure Unsupervised	Ancestry, Structure Supervised
CE	I0172	0.83	0.83	0.75
CE	I0560	0.77	0.86	0.76
CE	I1497	0.86	0.89	0.78
CE	I1495	0.90	0.86	0.77
CE	I1496	1.00	1.00	0.83
CE	I1498	0.90	0.96	0.79
CE	I1499	0.87	0.86	0.78
CE	I1500	0.95	1.00	0.83
CE	I1505	0.92	0.93	0.79
CE	I1506	0.92	0.91	0.79
CE	I1508	1.00	1.00	0.85
CE	Iceman	0.85	0.85	0.76
CE	I0022	1.00	1.00	0.84
CE	I0025	0.95	0.99	0.80
CE	I0026	0.99	1.00	0.82
CE	I0046	0.98	0.98	0.80
CE	I0054	0.95	0.95	0.79
CE	I0100	0.97	1.00	0.82
CE	I0659	0.99	1.00	0.82
CE	I1550	0.96	0.96	0.80
	Mean Ancestry:	***0.928***	***0.941***	***0.798***
BA	I0047	0.59	0.65	0.24
BA	I0049	1.00	0.99	0.26
BA	I0059	0.59	0.58	0.23
BA	I0099	0.59	0.67	0.23
BA	I0103	1.00	0.92	0.27
BA	I0104	1.00	0.79	0.28
BA	I0115	0.70	0.69	0.26
BA	I0116	0.68	0.77	0.22
BA	I0117	0.61	0.63	0.22
BA	I0118	0.58	0.68	0.22
BA	I0164	0.67	0.71	0.25
BA	I0803	0.75	0.69	0.26
BA	I1532	1.00	0.81	0.24
BA	RISE00	0.75	0.93	0.24
BA	RISE150	1.00	0.83	0.25
BA	RISE577	0.67	0.80	0.25
	Mean Ancestry:	***0.761***	***0.759***	***0.244***

Table S4: Replication of individual autosomal ancestry in admixed populations. Ancestry is estimated in *Admixture* using unsupervised clustering, and in *Structure* using both supervised and unsupervised clustering. Results presented are the component clustering with the migrating population. For the Neolithic Transition with admixed CE individuals, that is AF ancestry, for Pontic Steppe migration with admixed BA individuals, that is SP ancestry. Results are the mean by individual of ten independent runs. We use ten different subsamples of 25,000 SNPs.

Admixed Population	Individual	Ancestry, Admixture Unsupervised	Ancestry, Structure Unsupervised	Ancestry, Structure Supervised
CE	I0172	0.72	0.86	0.72
CE	I0560	0.72	0.85	0.71
CE	I1497	1.00	1.00	0.80
CE	I1495	0.73	0.93	0.74
CE	I1496	1.00	1.00	0.85
CE	I1498	0.78	0.83	0.72
CE	I1499	0.91	0.98	0.80
CE	I1500	0.90	0.94	0.74
CE	I1505	0.90	0.94	0.76
CE	I1506	0.89	0.97	0.78
CE	I1508	1.00	1.00	0.85
CE	Iceman	0.73	0.77	0.70
CE	I0022	1.00	1.00	0.86
CE	I0025	0.99	1.00	0.78
CE	I0026	0.91	0.97	0.75
CE	I0046	0.98	1.00	0.77
CE	I0054	1.00	1.00	0.79
CE	I0100	0.72	0.73	0.70
CE	I0659	1.00	1.00	0.81
CE	I1550	1.00	1.00	0.80
	Mean Ancestry:	***0.894***	***0.937***	***0.772***
BA	I0047	-	0.46	0.21
BA	I0049	-	0.46	0.20
BA	I0059	-	0.52	0.23
BA	I0099	-	0.18	0.16
BA	I0103	-	0.91	0.26
BA	I0104	-	0.28	0.18
BA	I0115	-	0.04	0.15
BA	I0116	-	0.63	0.20
BA	I0117	-	0.15	0.15
BA	I0118	-	0.53	0.21
BA	I0164	-	0.35	0.19
BA	I0803	-	0.66	0.22
BA	I1532	-	0.00	0.12
BA	RISE00	-	0.72	0.20
BA	RISE150	-	0.15	0.19
BA	RISE577	-	0.01	0.16
	Mean Ancestry:	***NA***	***0.378***	***0.189***

Table S5: Replication of individual X-chromosomal ancestry in admixed populations. Ancestry is estimated in *Admixture* using unsupervised clustering, and in *Structure* using both supervised and unsupervised clustering. Results presented are the component clustering with the migrating population. For the Neolithic Transition with admixed CE individuals, that is AF ancestry. For the Pontic Steppe migration with admixed BA individuals, that is SP ancestry. Results are the mean by individual of ten independent runs.

Individual	Ancestry, X chromosome	Ancestry, Autosomes (All SNPs)
I0172	0.72	0.63
I0560	1.00	0.81
I1497	1.00	0.83
I1495	0.99	0.88
I1496	1.00	1.00
I1498	0.89	0.94
I1499	1.00	0.84
I1500	1.00	0.94
I1505	1.00	0.93
I1506	1.00	0.92
I1508	1.00	1.00
I0022	1.00	1.00
I0025	1.00	0.91
I0026	1.00	0.90
I0046	0.99	0.89
I0054	1.00	0.87
I0100	0.80	0.83
I0659	1.00	1.00
I1550	1.00	1.00
Mean Ancestry:	***0.968***	***0.901***

Table S6: Ancestry estimates using alternative farming source individuals for the Neolithic migration. Ancestry is estimated in ADMIXTURE v1.3 using supervised clustering, results presented are the component clustering with the migrating Near Eastern population. Lazaridis does not include Iceman.

Individual	Ancestry, X chromosome	Ancestry, Autosomes (All SNPs)
I0108	0.34	0.50
I0111	0.11	0.59
I0112	0.42	0.55
I0113	0.16	0.55
I1549	0.18	0.61
RISE569	0.50	0.56
Mean Ancestry:	***0.284***	***0.560***

Table S7: Individual ancestry in admixed Bell Beaker individuals. Ancestry is estimated in ADMIXTURE v1.3 using supervised clustering, results presented are the component clustering with the migrating steppe population.