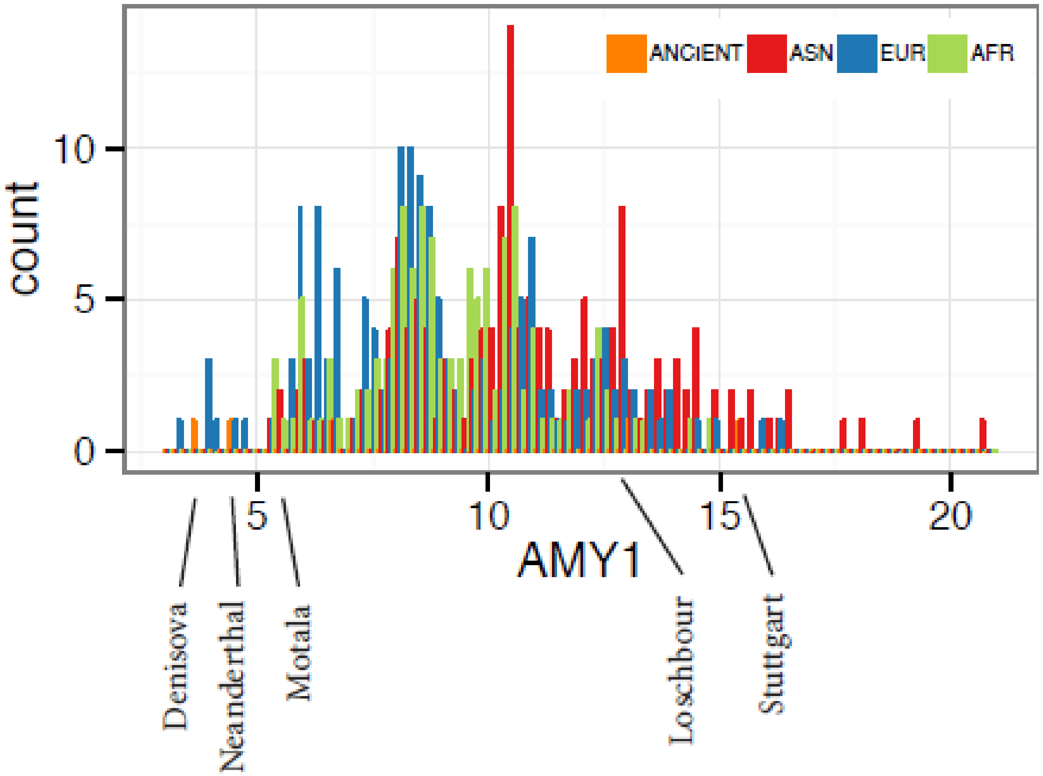
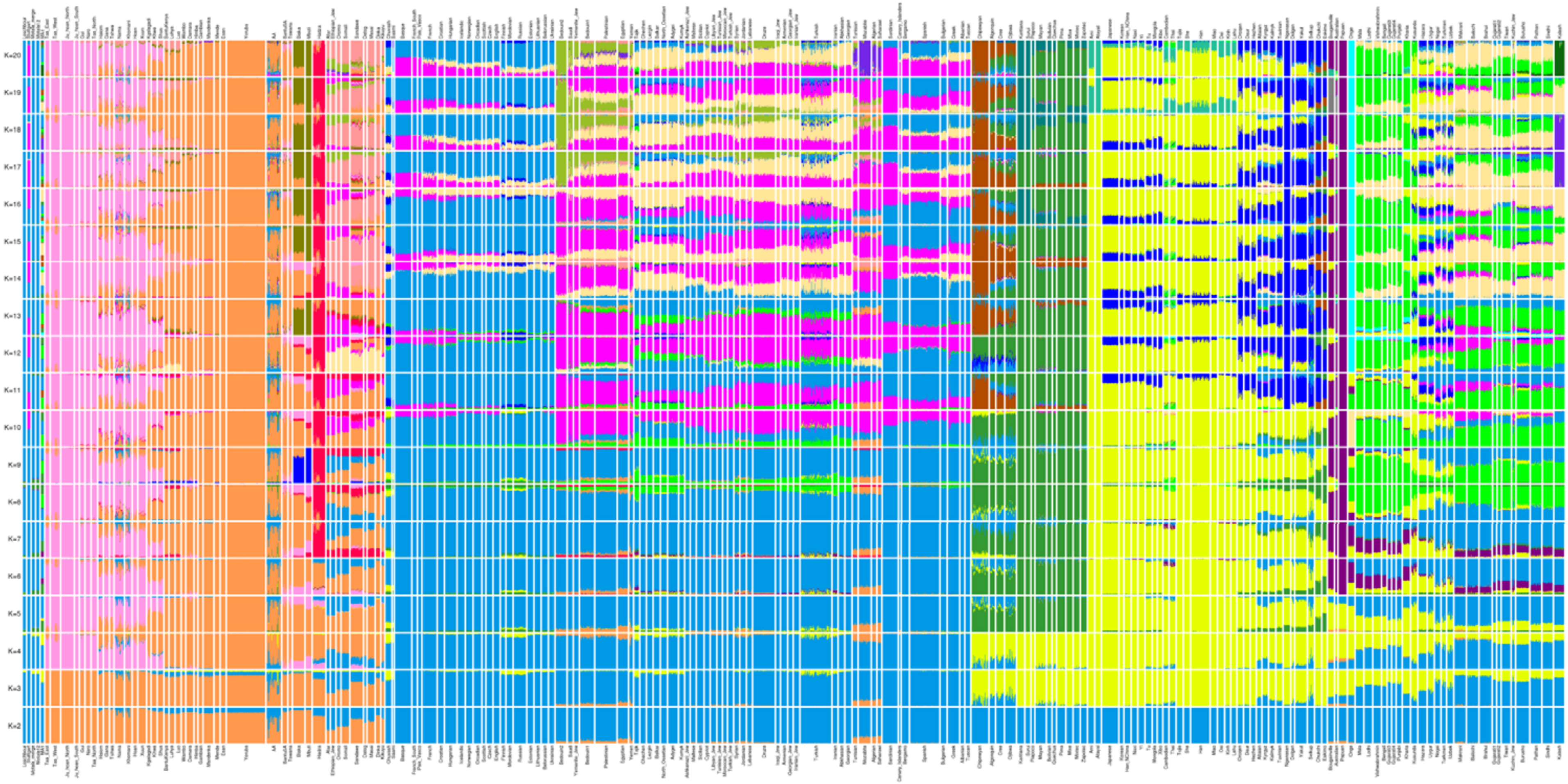
Extended Data

Extended Data Figure 1: Photographs of analyzed ancient DNA 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.

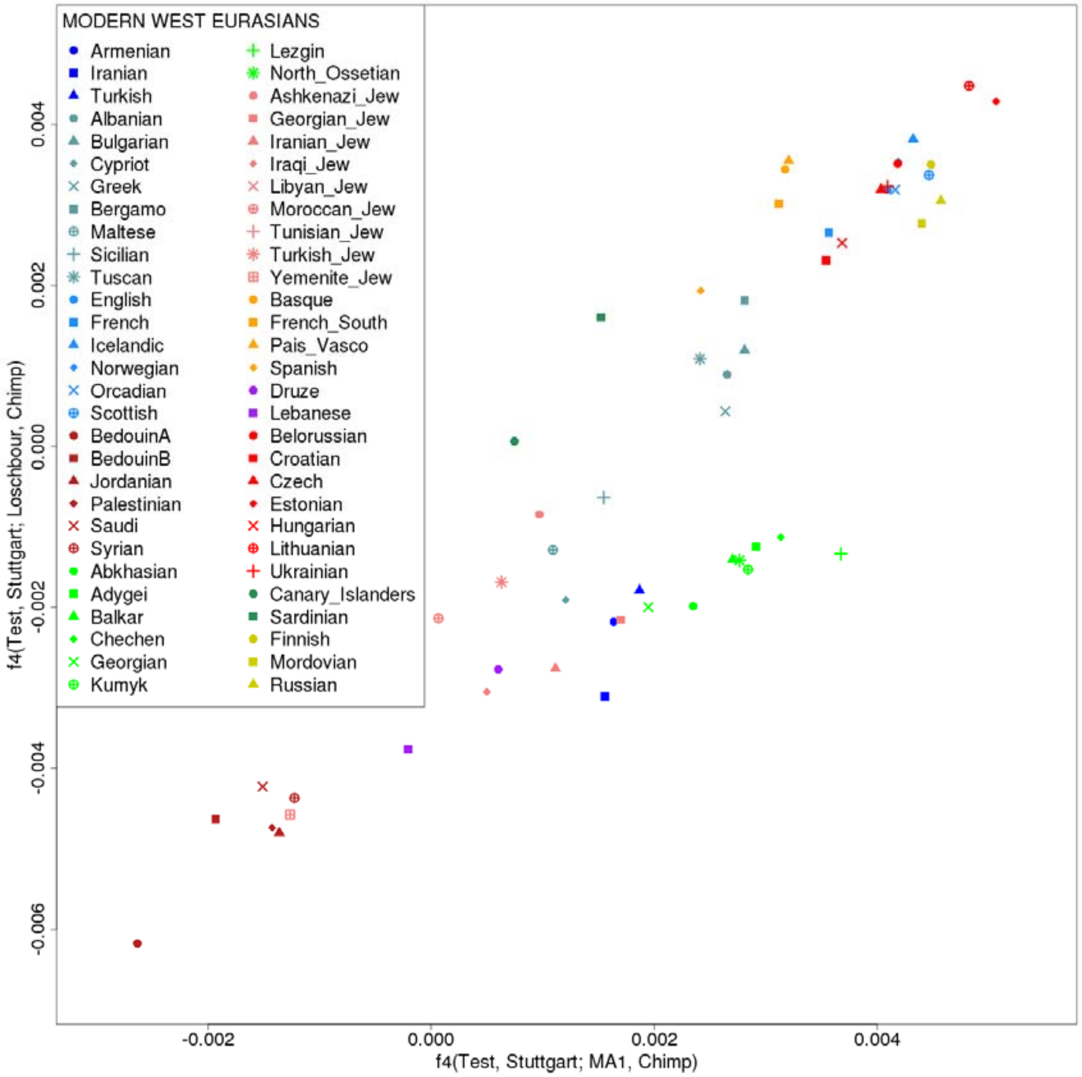
Extended Data Figure 2: Amylase CNV analysis. Distribution of amylase (*AMY1*) copy number inferred for samples from the 1000 Genomes Project compared with ancient genomes: Neanderthal, Denisova, Motala12, Loschbour, and Stuttgart. The relatively high copy number for the pre-agricultural Loschbour sample suggests that high *AMY1* copy number in humans may not entirely be due to selection since the switch to agriculture.



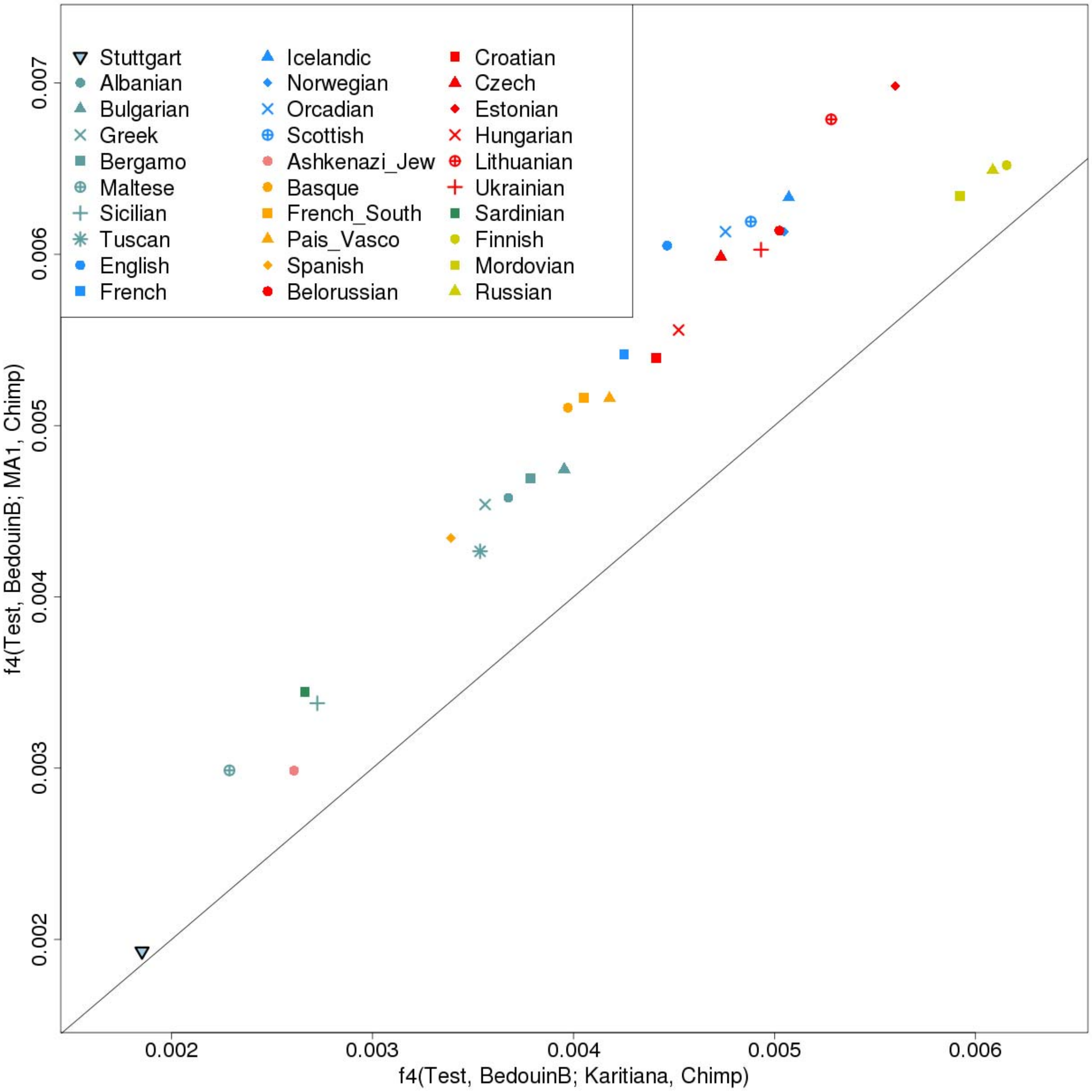
Extended Data Figure 3: ADMIXTURE analysis (K=2 to K=20). Ancient samples (Loschbour, Stuttgart, Motala_merge, Motala12, and MA1) have been placed on the left. The order of the modern populations has been determined by applying hierarchical clustering (as implemented in the function "hclust" of R) over the set of admixture coefficients over all K=2 to K=20 runs, with the goal of bringing populations with similar admixture coefficients visually close.



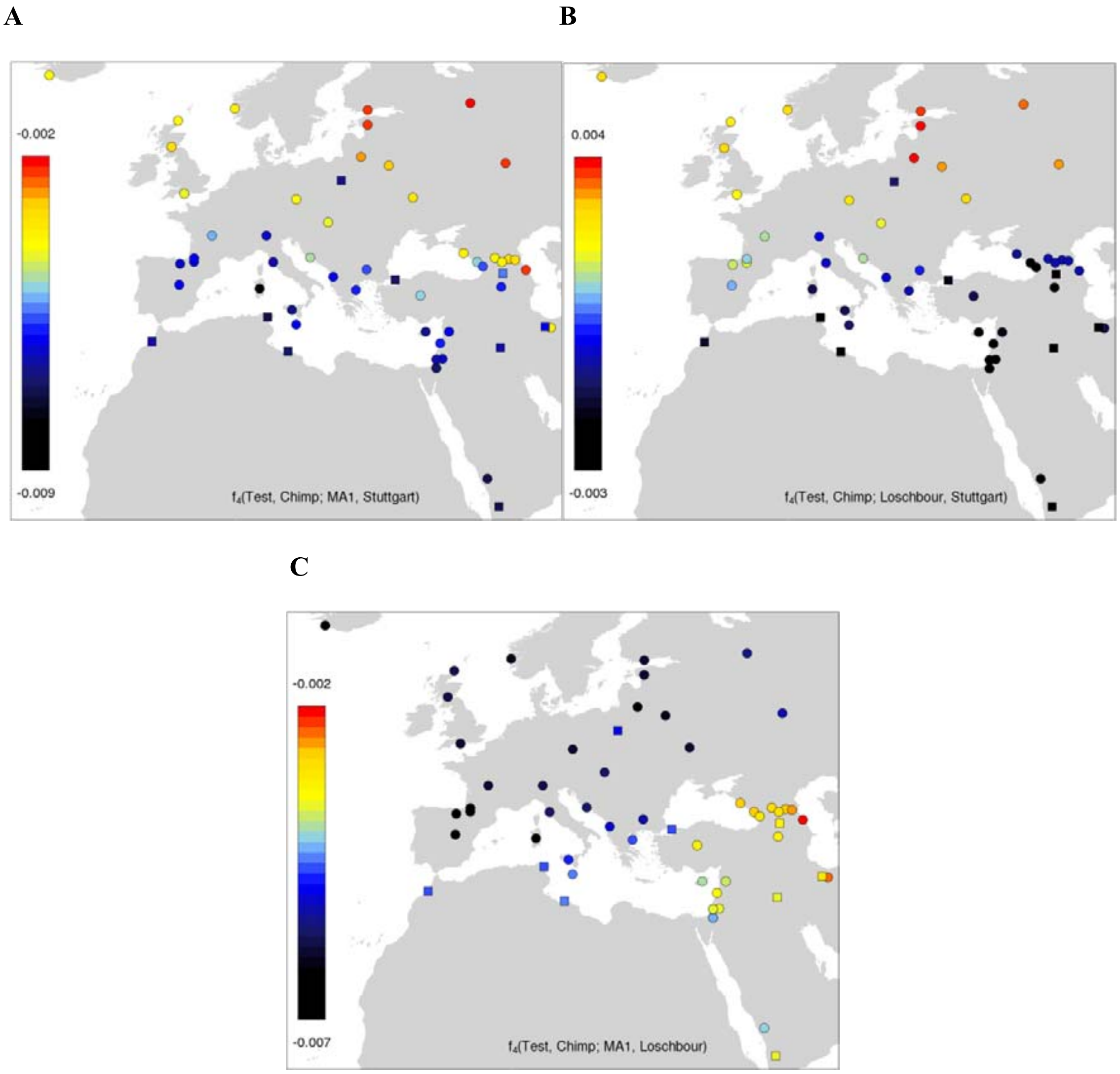
Extended Data Figure 4: ANE ancestry is present in both Europe and the Near East but WHG ancestry is restricted to Europe, which cannot be due to a single admixture event. (*x*-axis) We computed the statistic *f_4(Test, Stuttgart; MA1, Chimp)*, which measures where MA1 shares more alleles with a test 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) gene flow only in Europeans.



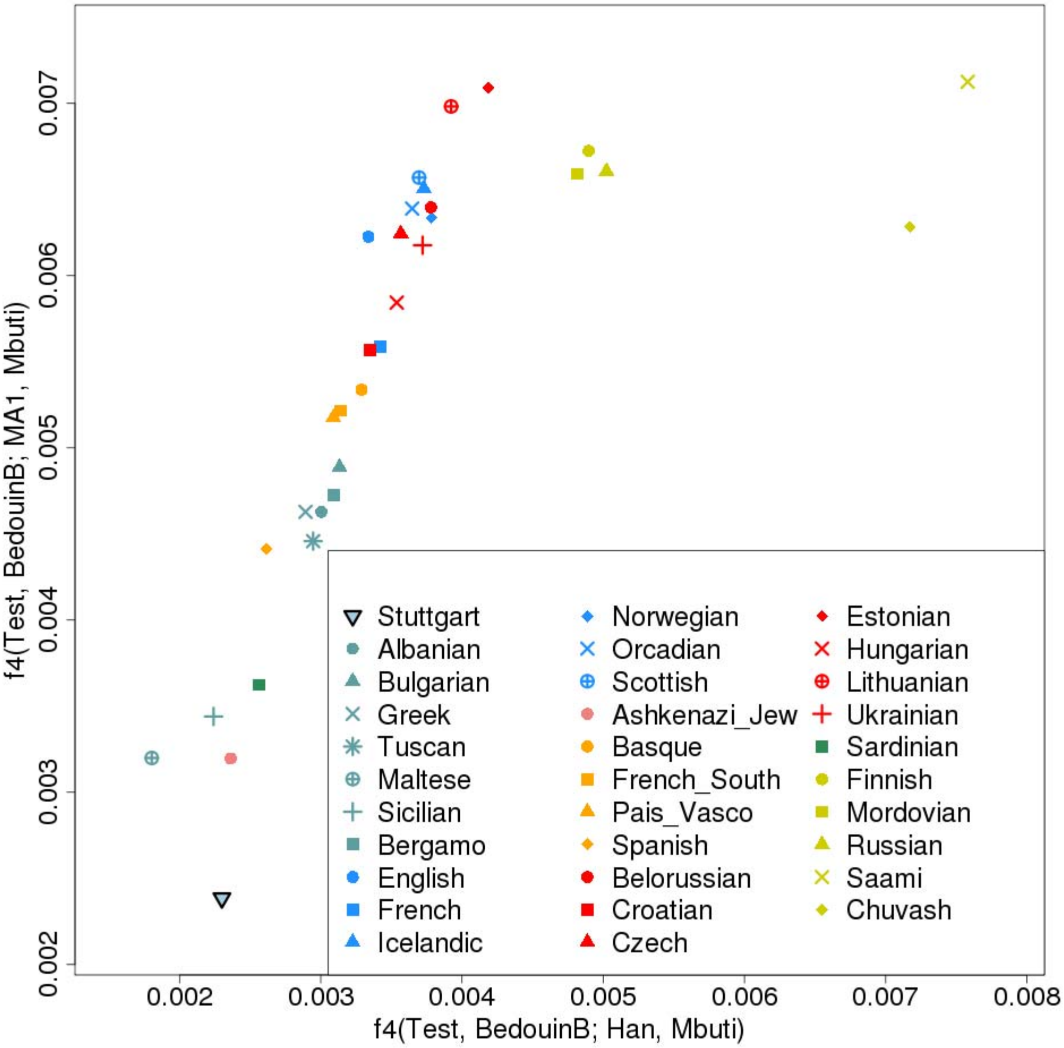
Extended Data Figure 5: MA1 is the best surrogate for ANE for which we have data. Europeans share more alleles with MA1 than with Karitiana, as we see from the fact that in a plot of *f_4(Test, BedouinB; MA1, Chimp)* and *f_4(Test, BedouinB; Karitiana, Chimp)*, the European cline deviates in the direction of MA1, rather than Karitiana (the slope is >1).



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 than that 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 SI12. 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. We thank Pontus Skoglund for sharing plotting software that we modified to produce these visualizations.



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

Population	N	Latitude	Longitude	Ref$_1$	Ref$_2$	Lowest f_3(Population; Ref1, Ref2)	Z	f_4(Stuttgart, Population; Loschbour, Chimp)	Z	f_4(Stuttgart, Population; MA1, Chimp)	Z
Abkhasian	9	43.00	41.02	MA1	Stuttgart	-0.0053	-2.9	0.002	4.2	-0.0023	-4.7
Adygei	1	44.00	39.00	Piapoco	Stuttgart	-0.0073	-5.9	0.0013	2.6	-0.0029	-6
Albanian	6	41.33	19.83	MA1	Stuttgart	-0.0121	-7	-0.0009	-1.8	-0.0027	-5.4
Armenian	1	40.19	44.55	Gujarati3	Stuttgart	-0.007	-8.2	0.0022	4.5	-0.0016	-3.3
Ashkenazi_Jew	7	52.23	21.02	MA1	Stuttgart	-0.0057	-3.4	0.0008	1.7	-0.001	-2
Balkar	1	43.48	43.62	Piapoco	Stuttgart	-0.0113	-8.9	0.0014	2.9	-0.0027	-5.6
Basque	2	43.04	-0.65	Iraqi_Jew	Loschbour	-0.0083	-10.3	-0.0034	-7.2	-0.0032	-6.7
Bedouin1	2	31.00	35.00	Esan	Stuttgart	-0.0162	-18.2	0.0062	13	0.0026	5.4
Bedouin2	1	31.00	35.00	Esan	Stuttgart	0.0089	7.8	0.0046	9.3	0.0019	3.9
Belorussian	1	53.92	28.01	Georgian	Loschbour	-0.0133	-17.6	-0.0035	-6.9	-0.0042	-8.6
Bulgarian	1	42.16	24.74	MA1	Stuttgart	-0.013	-8.2	-0.0012	-2.5	-0.0028	-5.9
Chechen	9	43.33	45.65	MA1	Stuttgart	-0.0056	-3.2	0.0011	2.3	-0.0031	-6.2
Croatian	1	43.51	16.45	MA1	Stuttgart	-0.0114	-6.7	-0.0023	-4.7	-0.0035	-7.4
Cypriot	8	35.13	33.43	MA1	Stuttgart	-0.0057	-3.2	0.0019	3.9	-0.0012	-2.5
Czech	1	50.10	14.40	Georgian	Loschbour	-0.0137	-17.9	-0.0032	-6.6	-0.004	-8.2
Druze	3	32.00	35.00	MA1	Stuttgart	-0.0024	-1.5	0.0028	5.9	-0.0006	-1.3
English	1	50.75	-2.09	Iraqi_Jew	Loschbour	-0.0129	-14.8	-0.0032	-6.5	-0.0041	-8.5
Estonian	1	58.54	24.89	Abkhasian	Loschbour	-0.0124	-15.1	-0.0043	-8.5	-0.0051	-10.1
Finnish	7	60.20	24.90	Abkhasian	Loschbour	-0.0102	-11.3	-0.0035	-6.9	-0.0045	-9.1
French	2	46.00	2.00	MA1	Stuttgart	-0.0131	-8.4	-0.0027	-5.6	-0.0036	-7.7
French_South	7	43.44	-0.62	Iraqi_Jew	Loschbour	-0.0095	-9.5	-0.003	-6.2	-0.0031	-6.2
Georgian	1	42.50	41.85	Gujarati3	Stuttgart	-0.0036	-4	0.002	4.2	-0.0019	-3.9
Georgian_Jew	7	41.72	44.78	Gujarati3	Stuttgart	-0.0009	-0.9	0.0022	4.3	-0.0017	-3.4
Greek	2	39.84	23.17	MA1	Stuttgart	-0.0118	-7.4	-0.0004	-0.9	-0.0026	-5.6
Hungarian	2	47.49	19.08	MA1	Stuttgart	-0.0133	-8.4	-0.0025	-5.3	-0.0037	-7.8
Icelandic	1	64.13	-21.93	Abkhasian	Loschbour	-0.0121	-15.6	-0.0038	-7.7	-0.0043	-8.9
Iranian	8	35.59	51.46	Piapoco	Stuttgart	-0.0094	-7.2	0.0031	6.3	-0.0016	-3.2
Iranian_Jew	9	35.70	51.42	Gujarati3	Stuttgart	-0.0018	-2	0.0028	5.7	-0.0011	-2.2
Iraqi_Jew	6	33.33	44.42	Vishwabrah	Stuttgart	-0.0026	-2.6	0.003	6.1	-0.0005	-1
Italian	1	46.00	10.00	MA1	Stuttgart	-0.0106	-6.2	-0.0018	-3.9	-0.0028	-5.8
Jordanian	9	32.05	35.91	Esan	Stuttgart	-0.0145	-14.3	0.0048	9.6	0.0014	2.8
Kumyk	8	43.25	46.58	Piapoco	Stuttgart	-0.0111	-8.2	0.0015	3.1	-0.0028	-5.7
Lebanese	8	33.82	35.57	Esan	Stuttgart	-0.0105	-9.4	0.0038	7.7	0.0002	0.4
Lezgin	9	42.12	48.18	MA1	Stuttgart	-0.01	-6	0.0013	2.7	-0.0037	-7.5
Libyan_Jew	9	32.92	13.18	Esan	Stuttgart	-0.0051	-4.4	0.003	6.2	0.0004	0.9
Lithuanian	1	54.90	23.92	Abkhasian	Loschbour	-0.0119	-14.9	-0.0045	-9	-0.0048	-9.9
Maltese	8	35.94	14.38	MA1	Stuttgart	-0.0086	-4.9	0.0013	2.7	-0.0011	-2.3
Mordovian	1	54.18	45.18	Abkhasian	Loschbour	-0.0115	-14.4	-0.0028	-5.5	-0.0044	-9
Moroccan_Jew	6	34.02	-6.84	Esan	Stuttgart	-0.0062	-5.2	0.0021	4.3	-0.0001	-0.1
North_Ossetian	1	43.02	44.65	Piapoco	Stuttgart	-0.0093	-7.2	0.0014	2.9	-0.0028	-5.6
Norwegian	1	60.36	5.36	Georgian	Loschbour	-0.012	-14.8	-0.0035	-7.3	-0.0042	-8.7
Orcadian	1	59.00	-3.00	Armenian	Loschbour	-0.0102	-13.4	-0.0032	-6.7	-0.0042	-8.6
Pais_Vasco	5	42.80	-2.70	Iraqi_Jew	Loschbour	-0.0112	-9.9	-0.0035	-6.9	-0.0032	-6.4
Palestinian	3	32.00	35.00	Esan	Stuttgart	-0.012	-13.2	0.0047	10.2	0.0014	3.1
Russian	2	61.00	40.00	Abkhasian	Loschbour	-0.0119	-17.1	-0.003	-6.2	-0.0046	-9.4
Sardinian	2	40.00	9.00	Loschbour	Stuttgart	-0.0043	-2.6	-0.0016	-3.4	-0.0015	-3.3
Saudi	8	18.49	42.52	Kgalagadi	Stuttgart	-0.0042	-3.6	0.0042	8.6	0.0015	3.1
Scottish	4	56.04	-3.94	Iraqi_Jew	Loschbour	-0.0103	-8.3	-0.0034	-6.4	-0.0045	-8.7
Sicilian	1	37.59	13.77	MA1	Stuttgart	-0.0108	-6.5	0.0006	1.3	-0.0015	-3.2
Spanish	5	40.43	-2.83	Iraqi_Jew	Loschbour	-0.0126	-17.8	-0.0019	-4.2	-0.0024	-5.2
Syrian	8	35.13	36.87	Esan	Stuttgart	-0.0101	-8.7	0.0044	8.6	0.0012	2.4
Tunisian_Jew	7	36.80	10.18	Gambian	Stuttgart	-0.0026	-2	0.0026	5.2	0.0002	0.5
Turkish	5	39.22	32.66	Piapoco	Stuttgart	-0.0129	-11.3	0.0018	3.8	-0.0019	-4
Turkish_Jew	8	41.02	28.95	MA1	Stuttgart	-0.0075	-4.3	0.0017	3.6	-0.0006	-1.3
Tuscan	8	43.00	11.00	MA1	Stuttgart	-0.0109	-6.4	-0.0011	-2.2	-0.0024	-5
Ukrainian	9	50.29	31.56	Georgian	Loschbour	-0.0134	-16.7	-0.0032	-6.4	-0.0041	-8.5
Yemenite_Jew	8	15.35	44.20	Esan	Stuttgart	-0.0027	-2.4	0.0046	9.1	0.0013	2.6

Extended Data Table 2: Confirmation of key findings on Human Origins San and Yoruba panels. We confirm key findings by showing selected *f*-statistics on a panel of 151,316 SNPs ascertained in a San individual and 115,577 SNPs ascertained in a Yoruba individual.

Statistic	**Interpretation**	**San panel (n=151,316)**		**Yoruba panel (n=115,577)**		**All SNPs (n=594,924)**	
		Value	**Z**	**Value**	**Z**	**Value**	**Z**
f_4*(Stuttgart, Armenian; Loschbour, Chimp)*	Stuttgart has Mesolithic European admixture	0.0012	2.2	0.0024	3.4	0.0022	4.5
f_4*(Lithuanian, Stuttgart; Loschbour, Chimp)*	Northern European populations have more WHG-related ancestry than Stuttgart	0.0035	6.1	0.0037	5.2	0.0045	9.0
f_4*(French, Stuttgart; MA1, Chimp)*	European populations have more ANE-related ancestry than Stuttgart	0.0026	4.1	0.0028	3.8	0.0036	7.7
f_4*(Lezgin, Stuttgart; MA1, Chimp)*	North Caucasian populations have more ANE-related ancestry than Stuttgart	0.0026	4.1	0.0028	3.5	0.0037	7.5
f_4*(French, Chimp; MA1, Karitiana)*	ANE-related ancestry in Europeans is better represented by MA1 than by Karitiana	0.0021	3.1	0.0019	2.5	0.0021	4.6
f_4*(Loschbour, Stuttgart; Onge, Chimp)*	Eastern non-Africans share more alleles with hunter-gatherers than with Stuttgart	0.0014	2.0	0.0028	3.3	0.0019	3.5
f_4*(Karitiana, Chimp; MA1, Loschbour)*	Karitiana share more alleles with MA1 than with Loschbour	0.0038	4.0	0.0041	3.7	0.0047	7.1
f_4*(Stuttgart, Chimp; Karitiana, Onge)*	West Eurasians share more alleles with Karitiana than other eastern non-Africans	0.0015	2.7	0.0016	2.2	0.0024	5.1

Extended Data Table 3: Admixture proportions for European populations. The estimates from the model with minimal assumptions are from SI13. The estimates from the full modeling are from SI12 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.

	Full modeling of population relationships (individual fits)			**Full modeling of population relationships (averaged fits)**						**Modeling of population population relationships with minimal 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	0.640 ± 0.122	0.306 ± 0.169	0.054 ± 0.061	1.153	-1.325	1.366
Ashkenazi_Jew	0.931	0	0.069							0.977 ± 0.155	-0.057 ± 0.202	0.080 ± 0.058			
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	0.600 ± 0.099	0.283 ± 0.135	0.117 ± 0.048	-0.314	0.388	-0.445
Belorussian	0.418	0.431	0.151	0.426	0.397-0.464	0.408	0.338-0.443	0.167	0.150-0.199	0.303 ± 0.099	0.536 ± 0.140	0.161 ± 0.052	1.241	-0.917	0.107
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	0.677 ± 0.142	0.214 ± 0.192	0.109 ± 0.061	0.309	-0.268	0.125
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	0.579 ± 0.115	0.319 ± 0.151	0.103 ± 0.049	1.206	-1.24	0.97
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	0.499 ± 0.130	0.364 ± 0.172	0.138 ± 0.054	0.502	-0.46	0.24
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	0.438 ± 0.127	0.381 ± 0.178	0.181 ± 0.058	0.402	-0.186	-0.309
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	0.504 ± 0.093	0.341 ± 0.130	0.155 ± 0.047	-0.014	0.096	-0.237
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	0.086 ± 0.126	0.799 ± 0.190	0.115 ± 0.076	1.877	-1.626	0.952
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	0.537 ± 0.106	0.321 ± 0.141	0.142 ± 0.046	0.247	-0.169	-0.051
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	0.673 ± 0.126	0.200 ± 0.176	0.127 ± 0.059	-0.297	0.319	-0.319
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	0.694 ± 0.105	0.217 ± 0.137	0.089 ± 0.044	0.923	-1.231	1.629
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	0.428 ± 0.117	0.422 ± 0.166	0.150 ± 0.058	1.023	-0.863	0.406
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	0.390 ± 0.109	0.428 ± 0.148	0.182 ± 0.050	0.172	0.135	-0.775
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	0.279 ± 0.121	0.541 ± 0.169	0.180 ± 0.058	0.601	-0.312	-0.343
Maltese	0.932	0	0.068							1.356 ± 0.203	-0.587 ± 0.275	0.231 ± 0.095			
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	0.322 ± 0.123	0.510 ± 0.171	0.168 ± 0.061	0.772	-0.507	-0.134
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	0.426 ± 0.095	0.408 ± 0.132	0.166 ± 0.046	0.413	-0.23	-0.192
Pais_Vasco	0.713	0.125	0.163	0.612	0.561-0.660	0.292	0.214-0.365	0.096	0.072-0.126	0.620 ± 0.131	0.244 ± 0.174	0.136 ± 0.053	-0.065	0.276	-0.746
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	0.933 ± 0.142	0.008 ± 0.186	0.059 ± 0.056	-0.812	0.717	-0.324
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	0.313 ± 0.127	0.514 ± 0.179	0.172 ± 0.064	0.75	-0.52	-0.017
Sicilian	0.903	0	0.097							1.075 ± 0.163	-0.208 ± 0.221	0.133 ± 0.071			
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	0.905 ± 0.141	-0.069 ± 0.187	0.165 ± 0.057	-1.035	1.043	-0.88
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	0.765 ± 0.139	0.112 ± 0.190	0.123 ± 0.063	-0.104	0.058	0.055
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	0.283 ± 0.133	0.585 ± 0.196	0.133 ± 0.073	1.356	-1.064	0.374
Finnish										-0.257 ± 0.195	1.199 ± 0.292	0.058 ± 0.119			
Mordovian										-0.215 ± 0.163	1.162 ± 0.241	0.053 ± 0.108			
Russian										-0.256 ± 0.209	1.236 ± 0.308	0.021 ± 0.125			