

# Supporting Information

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## SI Materials and Methods

**Ancient DNA Procedures.** Drastic precautions were taken to avoid contaminations by modern DNA templates (1): pre-PCR and post-PCR procedures were carried out in two separate laboratories located on two separate floors. Pre-PCR procedures were performed in a dedicated laboratory under laminar flux. Workbenches, surfaces, and all equipment were systematically wiped with bleach, rinsed with ultrapure water, and irradiated for at least 2 h with UV light before each manipulation. Laboratory access was limited to authorized personnel only who always wore gloves, overshoes, laboratory coats, and face masks. Pipettes, plastic ware, and aerosol-resistant tips were sterile and used exclusively for ancient DNA work. DNA from people handling the anthropological material (members of the museum and laboratory staff) was also analyzed to rule out recent contamination. DNA extracted from sheep or goat bone fragments also retrieved in the ossuary were used as a negative control to detect potential contamination that could have occurred during excavation.

**Statistical Analyses.** To study putative genetic relationships between individuals from the ossuary, kinship was determined from autosomal STR profiles with ML-Relate software (2) and confirmed with DNA•VIEW Software (3), with which the LR was calculated assuming a prior probability of 0.5.

Human specimens from necropoles cannot be of course considered as a population in a statistical sense. Furthermore ancient DNA data could not be obtained for all the specimens buried, and Y-haplotypes were not determined for all male individuals. However, to try to characterize affinities between the ancient Treilles specimens and current European populations, we performed cross-population comparisons from HVI sequences and partial Y-chromosomal haplotypes with the ARLEQUIN 3.1

software (4). Two databases were compiled for both uniparental markers. The mtDNA database comprises 14,699 HVI haplotypes associated with their corresponding haplogroup. The NRY database comprises 49 European populations representing 10,488 Y-STR profiles. References used to compile these databases are available in Table S8. For maternal lineages, comparisons were based on HVI haplotypes, and for paternal lineages, they were based on seven STR markers (DYS19, DYS389a, DYS389b, DYS390, DYS391, DYS393, and DYS439) and on the seven male individuals for whom complete datasets were obtained (195, 575, 584, 596, 615, 616, and 636). The pattern of genetic differentiation was visualized by multidimensional scaling plot (XLstat, version 7.5.2) and by plotting on a map all  $F_{ST}$  values obtained in the comparison between the Treilles population and each population in the database, using Surfer software (version 8.0; Golden Software).

The percentage of shared lineages between Treilles and each present-day population in the databases was graphically also plotted on a map by using Surfer software (version 8.0; Golden Software).

A haplotype network was generated for NRY haplogroup G2a\* from the Treilles data and all European data via the median-joining algorithm of Network, version 4.5.1.6. To obtain the most parsimonious networks the reticulation permissivity was set to zero. Datasets were preprocessed using the star contraction option in Network, version 4.5.1.6 (5). Because of the high level of reticulation in the G2a\* sample, Y-STR loci were subdivided into two mutation rate classes based on observed STR allelic variance and weighted as follows: 2 (low) for DYS391 and DYS392 and 1 (high) for DYS389I, DYS389II, DYS19, DYS393, and DYS390 (6).

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5. Forster P, Torroni A, Renfrew C, Röhl A (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Mol Biol Evol* 18:1864–1881.
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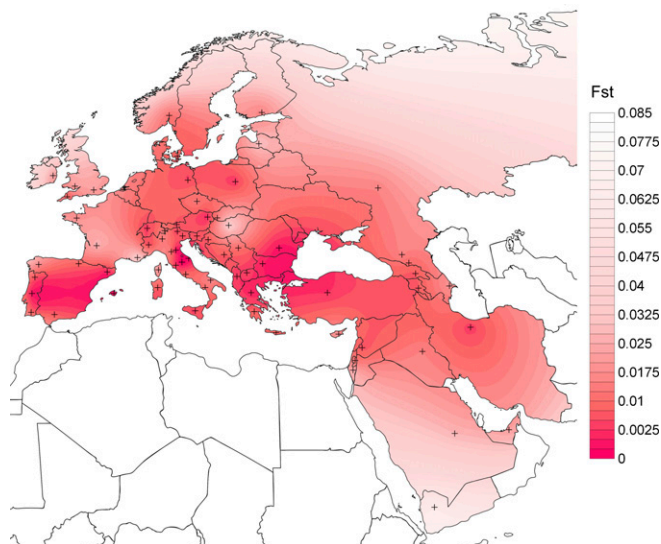


Fig. S1. Spatial distribution of the genetic matrilineal distances between Treilles samples and modern Western Eurasian populations.











**Table S3.  $F_{ST}$  values calculated between Treilles and modern Western Eurasian population data**

Population	$F_{ST}$	$P$ value
<b>Middle East</b>		
Iranians	0.00338	0.25225 ± 0.0353
Saudi Arabians	0.02746	0.00000 ± 0.0000
Syrians	0.00588	0.14414 ± 0.0309
Iraqis	0.01515	0.07207 ± 0.0227
Druze	0.02639	0.00000 ± 0.0000
Yemenis	0.06229	0.00000 ± 0.0000
Kurds	0.01418	0.04505 ± 0.0203
Dubai	0.02235	0.00901 ± 0.0091
Palestinians	0.01156	0.02703 ± 0.0139
Turks	0.00216	0.27027 ± 0.0303
<b>North Caucasus</b>		
Russian Caucasians	0.0157	0.01802 ± 0.0121
Western Russians	0.01538	0.01802 ± 0.0121
Other North Caucasus populations	0.00965	0.05405 ± 0.0201
<b>South Caucasus</b>		
Georgians	0.00712	0.10811 ± 0.0264
Armenians	0.00719	0.05405 ± 0.0201
Azerbaijanis	0.01911	0.01802 ± 0.0121
<b>Northwestern Europe</b>		
British	0.02286	0.00000 ± 0.0000
Bretagne	0.01955	0.02703 ± 0.0139
Normandie French	0.02691	0.01802 ± 0.0121
Perigord-Limousin French	0.02691	0.00000 ± 0.0000
Var French	0.03602	0.00000 ± 0.0000
Welsh	0.02329	0.00901 ± 0.0091
Cornish	0.00762	0.17117 ± 0.0286
Irish	0.02224	0.00000 ± 0.0000
<b>North Central Europe</b>		
Germans	0.00461	0.13514 ± 0.0365
Danish	0.00769	0.11712 ± 0.0273
Czechs	0.01481	0.03604 ± 0.0148
Polish	0.00255	0.27027 ± 0.0470
Slovakians	0.01472	0.02703 ± 0.0194
Swiss	0.00295	0.27928 ± 0.0394
Austrians	-0.00027	0.43243 ± 0.0485
Latvians	0.03072	0.00000 ± 0.0000
South Tyrol Ladins	0.01427	0.03604 ± 0.0201
South Tyrol Germans	0.00664	0.20721 ± 0.0430
South Tyrol Italians	0.00259	0.23423 ± 0.0364
<b>Scandinavia</b>		
Norwegians	0.01138	0.06306 ± 0.0237
Finns	0.01576	0.25225 ± 0.0353
<b>Southeastern Europe</b>		
Bulgarians	0.00002	0.32432 ± 0.0473
Hungarians	0.03682	0.00000 ± 0.0000
Bosnians	0.00675	0.15315 ± 0.0305
Serbians	0.01092	0.06306 ± 0.0139
Romanian	-0.00144	0.54054 ± 0.0664
<b>Western Mediterranean</b>		
North Portuguese	0.00582	0.07207 ± 0.0227
Central Portuguese	-0.00126	0.53153 ± 0.0417
South Portuguese	0.00832	0.09009 ± 0.0271
Galicians	0.01786	0.02703 ± 0.0139
Spanish Catalans	-0.00049	0.43243 ± 0.0466
Andalusians	0.00766	0.11712 ± 0.0237
Balearic islanders	-0.00189	0.52252 ± 0.0297
Basques	0.00884	0.07207 ± 0.0297
<b>Central Mediterranean</b>		
Northeastern Italians	0.00767	0.12613 ± 0.0242
Tuscans	0.00231	0.25225 ± 0.0445
Acone Italians	-0.00272	0.57658 ± 0.0278
Bologna Italians	-0.00108	0.51351 ± 0.0526

**Table S3 Cont.**

Population	$F_{ST}$	$P$ value
Modena Italians	0.0145	0.05405 ± 0.0201
Pavia Italians	0.01635	0.09009 ± 0.0303
Roma Italians	0.01064	0.08108 ± 0.0286
Turino Italians	0.00218	0.32432 ± 0.0546
Terni Italians	-0.00498	0.58559 ± 0.0530
Molise-Abruzzo-puglia Italians	0.01832	0.02703 ± 0.0139
Campania Italians	0.01079	0.13514 ± 0.0311
Sicilians	0.00451	0.17117 ± 0.0212
Corsicans	0.02365	0.00000 ± 0.0000
Sardinians	0.00736	0.15315 ± 0.0273
Slovenians	0.00745	0.16216 ± 0.0353
Croatians	0.00696	0.18919 ± 0.0212
Eastern Mediterranean		
Macedonians	0.00487	0.23423 ± 0.0411
Albanians	0.0018	0.35135 ± 0.0515
Cretans	0.00892	0.13514 ± 0.0203
Cypriots	0.01888	0.02703 ± 0.0139
Northern Greek	-0.00061	0.45946 ± 0.0286
Central Greeks	0.00043	0.36036 ± 0.0664
Southern Greeks	0.00867	0.07207 ± 0.0182

$F_{ST}$  values calculated between mtDNA for Treilles (29 samples, 13 haplotypes) and modern Western Eurasian populations data (14,699 HVI haplotypes).



**Table S4. Shared mitochondrial lineages between Treilles and modern Western Eurasian populations**

Population	Shared lineages, %	
	No mismatches allowed	One mismatch allowed
<b>Middle East</b>		
Iranians	2,448	4,196
Saudi Arabians	1,198	2,994
Syrians	4,444	10,000
Iraqis	1,961	9,804
Druze	3,810	7,619
Yemenis	2,985	10,448
Kurds	3,448	8,621
Dubai	1,829	4,878
Palestinians	3,030	7,071
Turks	1,961	3,922
<b>North Caucasus</b>		
Caucasian Russians	2,970	8,911
Western Russians	2,778	6,481
Other North Caucasus populations	1,765	4,706
<b>South Caucasus</b>		
Georgians	2,732	5,464
Armenians	1,613	5,914
Azerbaijanis	5,556	13,889
<b>Northwestern Europe</b>		
British	3,896	11,688
Bretagne French	7.5	12.5
Normandie French	6.667	11,111
Perigord-Limousin French	6.667	11,111
Var French	9.091	22,727
Welsh	17,391	30,435
Cornish	16,667	29,167
Irish	2,564	6,410
<b>North-central Europe</b>		
Germans	2,564	4,029
Danish	2,857	5,714
Czechs	3,125	5,208
Polish	1,527	3,308
Slovakians	5,185	8,148
Swiss	4,651	8,527
Austrians	7,463	11,940
Latvians	2,941	5,882
South Tyrol Ladins	10,204	16,327
South Tyrol Germans	12,000	16,000
South Tyrol Italians	9,756	19,512
<b>Scandinavia</b>		
Norwegians	3,306	8,264
Finns	3,822	7,006
<b>South Eastern Europe</b>		
Bulgarians	12,500	29,167
Hungarians	3,623	7,246
Bosnians	3,497	6,993
Serbians	4,348	10,870
Romanian	5,000	12,500
<b>Western Mediterranean</b>		
Northern Portuguese	3.681	5.521
Central Portuguese	4.070	6.395
Southern Portuguese	5.298	7.285
Galicians	5.882	12.941
Spanish Catalans	7.527	10,753
Andalusians	4,000	10,000
Balearic islanders	7,317	24,390
Basques	8,602	12,903
<b>Central Mediterranean</b>		
Northeastern Italians	5,357	9,821

**Table S4 Cont.**

Population	Shared lineages, %	
	No mismatches allowed	One mismatch allowed
Tuscans	3,139	5,381
Acone Italians	9,091	18,182
Bologna Italians	11,111	25,000
Modena Italians	6,061	24,242
Pavia Italians	11,429	20,000
Roma Italians	3,797	10,127
Turino Italians	4,444	17,778
Terni Italians	10,000	30,000
Molisio-Abruzzo-puglia Italians	4,348	8,670
Campania Italians	2,564	12,821
Sicilians	4,587	7,339
Corsicans	9,677	19,355
Sardinians	3,822	7,006
Slovenians	7,813	14,063
Croatians	8,333	16,667
Eastern Mediterranean		
Macedonians	4,242	5,455
Albanians	4,225	11,268
Cretans	5,769	10,577
Cypriots	3,333	13,333
Northern Greek	2,885	4,327
Central Greeks	14,286	28,571
Southern Greeks	2,830	5,660

Mitochondrial shared lineages between Treilles (29 samples, 13 haplotypes) and modern Western Eurasian populations (14,699 HVI haplotypes). Analyses were performed for 0 or 1 mismatch.



**Table S6.  $F_{ST}$  values calculated between Y-chromosomal data of Treilles' samples and modern Western Eurasian population data (49 populations representing 10,488 Y-STR profiles)**

Population	$F_{ST}$	$P$ value
<b>Middle East</b>		
Iranians	0.29758	0.00000 ± 0.0000
Bakhtiari	0.32066	0.00000 ± 0.0000
Gilaki	0.32231	0.00000 ± 0.0000
Mazandarani	0.32759	0.00000 ± 0.0000
Syrians	0.28712	0.00000 ± 0.0000
Druze	0.28894	0.00000 ± 0.0000
Palestinians	0.27848	0.00000 ± 0.0000
Lebanese	0.27520	0.00000 ± 0.0000
Turks	0.26764	0.00000 ± 0.0000
<b>North Caucasus</b>		
Abazians	0.42472	0.00000 ± 0.0000
Abkhazians	0.44302	0.00000 ± 0.0000
Chechenians	0.42307	0.00000 ± 0.0000
Darginians	0.39692	0.00000 ± 0.0000
Ingushians	0.45255	0.00000 ± 0.0000
Kabardinians	0.31682	0.00000 ± 0.0000
<b>South Caucasus</b>		
Georgians	0.30749	0.00000 ± 0.0000
Armenians	0.29941	0.00000 ± 0.0000
Azerbaijanis	0.31764	0.00000 ± 0.0000
Lezginians	0.40088	0.00000 ± 0.0000
Ossetians	0.35485	0.00000 ± 0.0000
<b>Northwestern Europe</b>		
French	0.32143	0.00000 ± 0.0000
Irish	0.28895	0.00000 ± 0.0000
Belgians	0.28996	0.00000 ± 0.0000
Dutch	0.30891	0.00000 ± 0.0000
<b>North central Europe</b>		
Germans	0.26655	0.00000 ± 0.0000
Danish	0.27898	0.00000 ± 0.0000
Polish	0.27598	0.00000 ± 0.0000
<b>Scandinavia</b>		
Norwegians	0.26608	0.00000 ± 0.0000
<b>Southeastern Europe</b>		
Hungarian	0.26761	0.00000 ± 0.0000
Serbian	0.28178	0.00000 ± 0.0000
Serbian Romanian		
Montenegrin	0.27567	0.00000 ± 0.0000
<b>Western Mediterranean</b>		
Portuguese	0.27854	0.00000 ± 0.0000
Spanish	0.00724	0.00000 ± 0.0000
Basque	0.01392	0.00000 ± 0.0000
<b>Central Mediterranean</b>		
Italians	0.26635	0.00000 ± 0.0000
<b>Eastern Mediterranean</b>		
Maltese	0.37106	0.00000 ± 0.0000
Cypriots	0.29806	0.00000 ± 0.0000
Northern Greeks	0.28846	0.00000 ± 0.0000

**Table S7. Shared Y-lineages between Treilles and modern Western Eurasian populations (49 populations representing 10,488 Y-STR profiles)**

Population	Shared lineages, %
<b>Middle East</b>	
Iranians	0
Syrians	0
Druze	0
Palestinians	0
Lebanese	0.355
Turks	0.699
<b>North Caucasus</b>	
Other North Caucasus populations	0
<b>South Caucasus</b>	
Georgians	0
Armenians	0
Azerbaijanis	0
Other South Caucasus populations	0
<b>Northwestern Europe</b>	
French	0
Irish	0
Belgians	0
Dutch	0
<b>North Central Europe</b>	
Germans	0.226
Danish	0
Polish	0
<b>Scandinavia</b>	
Norwegians	0
<b>Southeastern Europe</b>	
Hungarians	0
Serbians	0
Serbian Romanians	0
Montenegrins	0
<b>Western Mediterranean</b>	
Portuguese	1.980
Galician	0
Catalan	0
Other Spanish	0.248
Basque	0
<b>Central Mediterranean</b>	
Italians	0.385
Sicilians	0
Sardinians	0
<b>Eastern Mediterranean</b>	
Maltese	0
Cypriots	2.062
North Greeks	0

**Table S8. References of the populations included in the databases**

Population (size)	References HVS-I	Population (size)	References Y-STR
<b>Middle East (<i>n</i> = 2,689)</b>		<b>Middle East (<i>n</i> = 2,482)</b>	
Iranians	1, 2	Iranians	3
Saudi Arabians	4–6		
Syrians	2, 7	Syrians	8
Iraqis	9		
Druze	10, 11	Druze	11
Yemenis	12		
Kurds	2, 13		
Dubai	14		
Palestinians	2	Palestinians	8
		Lebanese	15
Turks	2, 16–20	Turks	21, 22
<b>North Caucasus (<i>n</i> = 594)</b>		<b>North Caucasus (<i>n</i> = 78)</b>	
Caucasians Russians	2		
Western Russians	23		
Other North Caucasus populations	10, 19, 24, 25	Other North Caucasus populations	26
<b>South Caucasus (<i>n</i> = 652)</b>		<b>South Caucasus (<i>n</i> = 424)</b>	
Georgians	13, 19, 27, 28	Georgians	26
Armenians	2, 27, 29	Armenians	26
Azerbaijanis	27	Azerbaijanis	3, 26
		Other South Caucasus populations	26
<b>Northwestern Europe (<i>n</i> = 783)</b>		<b>Northwestern Europe (<i>n</i> = 408)</b>	
British	30		
French	31	French	32
Welsh	20		
Cornish	20		
Irish	20, 33	Irish	34
		Belgians	35
		Dutch	36
<b>North-Central Europe (<i>n</i> = 3,239)</b>		<b>North-Central Europe (<i>n</i> = 1,695)</b>	
Germans	20, 23, 37–39	Germans	36, 40
Danish	2, 20	Danish	41
Czechs	42		
Polish	23, 43, 44	Polish	45
Slovakians	29, 46		
Swiss	20, 47, 48		
Latvians	49		
Austrians	50		
South Tyrol Ladins	51, 52		
South Tyrol Germans	51		
South Tyrol Italians	51		
<b>Scandinavia (<i>n</i> = 712)</b>		<b>Scandinavia (<i>n</i> = 1,967)</b>	
Norwegians	53	Norwegians	54
Finns	55–57		
<b>Southeastern Europe (<i>n</i> = 909)</b>		<b>Southeastern Europe (<i>n</i> = 1,078)</b>	
Bulgarians	16		
Hungarians	58–60	Hungarians	61
Bosnians	62, 63		
Serbians	62	Serbians	64
Romanian	65	Serbian Romanians	66
		Montenegrins	64
<b>Western Mediterranean (<i>n</i> = 1,625)</b>		<b>Western Mediterranean (<i>n</i> = 1,442)</b>	
Portuguese	67, 68	Portuguese	69
Galicians	68, 70	Galicians	69, 71
Spanish Catalans	72, 73	Spanish Catalans	69
Andalusians	72, 74, 75		
Balearic islanders	75		
		Other Spanish	69, 71, 76, 77
Basques	2, 72, 78–80	Basques	69
<b>Central Mediterranean (<i>n</i> = 2,040)</b>		<b>Central Mediterranean (<i>n</i> = 562)</b>	
Northeastern Italians	52, 81–84	Northern Italians	85

Table S8 Cont.

Population (size)	References HVS-I	Population (size)	References Y-STR
Tuscans	75, 86, 87		
Other Italians: Accone, Bologna, Firenze, Modena, Pavia, Roma, Torino, Terni, Molisio- Abruzzo-puglia, Campania	84, 88, 89		
		Southern Italians	71
Sicilians	88, 90	Sicilians	71, 91
Corsicans	92		
Sardinians	20, 75, 93, 94	Sardinians	95
Slovenians	63		
Croatians	62		
Eastern Mediterranean (n = 1,298)		Eastern Mediterranean (n = 404)	
Macedonians	65, 88, 96, 97		
Albanians	65, 98		
Cretans	7, 88, 99	Maltese	8
Cypriots	100	Cypriots	8
Northern Greek	97, 100	Northern Greeks	101
Central Greeks	88, 97		
Southern Greeks	83, 88, 97		
Other Greeks	65		

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