Ancient DNA reveals male diffusion through the Neolithic Mediterranean route

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The Neolithic is a key period in the history of the European settlement. Although archaeological and present-day genetic data suggest several hypotheses regarding the human migration patterns at this period, validation of these hypotheses with the use of ancient genetic data has been limited. In this context, we studied DNA extracted from 53 individuals buried in a necropolis used by a French local community 5,000 y ago. The relatively good DNA preservation of the samples allowed us to obtain autosomal, Y-chromosomal, and/or mtDNA data for 29 of the 53 samples studied. From these datasets, we established close parental relationships within the necropolis and determined maternal and paternal lineages as well as the absence of an allele associated with lactase persistence, probably carried by Neolithic cultures of central Europe. Our study provides an integrative view of the genetic past in southern France at the end of the Neolithic period. Furthermore, the Y-haplotype lineages characterized and the study of their current repartition in European populations confirm a greater influence of the Mediterranean than the Central European route in the peopling of southern Europe during the Neolithic transition.

The Neolithic expansion was a major event in the European settlement and its impact on the European gene pool is still highly debated in terms of genetic flow and dispersal routes (i.e., Mediterranean vs. Central European) (1–4). In this context, molecular analyzes of ancient human populations of the end of the Neolithic are crucial to understand the origin and genetic structure of the European population. Because DNA is a very fragile molecule, rarely well preserved in ancient European specimens, only few molecular analyzes have been carried out on Neolithic remains, and they have often been limited to the study of mtDNA (4–9). The few published studies on nuclear DNA concern a small number of individuals (10–13). In the present work, the particularly good preservation of DNA in the samples excavated from a collective burial of the end of the Neolithic period (3000 B.C.) (14) allowed us to perform a study of short tandem repeats (STRs) and/or SNPs located on the nuclear DNA (Y-chromosome and autosomes) and mitochondrial DNA. Concretely, we analyzed DNA extracted from 53 individuals buried in Cave I of Treilles located in the Grands Causses region, at Saint-Jean-et-Saint-Paul, Aveyron, France (Fig. 1). The Treilles cultural group is a well identified archeological complex of the late Stone Age period, preserved of any major late Neolithic population movements as suggested by the absence of the Bell–Beaker culture influence in the second part of the third millennium B.C. The study of this cultural group should give a snapshot of the local genetic pool of the end of the Neolithic period in southern France before all recent migrations.

The two main objectives of this ancient DNA work were (*i*) to understand the structure of the Treilles community and its funeral practices by determining the sex of the individuals buried as well as putative close familial relationships; and (*ii*) to estimate the biogeographical origins of the specimens under study, and to infer the patterns of peopling of the region in this transitional period. To trace back the maternal and paternal lineages, we determined both mtDNA and Y-chromosomal haplogroups. We also typed a particular polymorphism associated with lactase persistence (i.e., ability to digest raw milk at adulthood) probably carried in western Europe with the Linearbandkeramic culture during the Neolithic (15).

Results

Necropolis Recruitment. Partial autosomal profiles were obtained for 24 of the 53 specimens under study (Table S1). The amelogenin locus indicates that 22 individuals were male and two were female (subjects 573 and 614). For five samples (samples 571, 581, 603, 609, and 637), the molecular sex could not be determined. Autosomal STR kinship analyzes highlighted at least three close familial relationships within the necropolis: individuals 604 and 636 have a 99,9979% probability to have a father/son relationship [likelihood ratio (LR), 48,400]. Individuals 612 and 583 could be siblings (LR, 66,400), with a probability of 99.9985%, and subject 612 could also be the father of 616, with a probability of 99.9995% (LR, 22,4000).

Mitochondrial Results. Reproducible HVI sequences were obtained for 29 of the 53 individuals tested. They were classified into 13 different haplotypes, which yielded a relatively high haplotype diversity (H) of 0.8966 ± 0.0354. All the haplogroups inferred by HVI sequencing were confirmed by typing of the mitochondrial coding region SNPs, for which the typing rate was as high as 98% (Table S2). Thanks to these coding region positions, the 13 haplotypes previously found could be classified in 11 different haplogroups or subhaplogroups: H1, H3, HV0, V, K1a, T2b, U, U5, U5b1c, X2, and J1.

Analysis of the F_{ST} genetic distances based on HVI variation showed that the Treilles specimens were genetically close to all current European populations. Indeed, F_{ST} values were between 0 and 0.06 for all populations included in the database (Table S3 and Figs. S1 and S2). The study of shared lineages showed furthermore that the Treilles maternal lineages are found in all present-day European populations with percentages as high as nearly 18% (Fig. 2 and Table S4).

Nonrecombining Region of Y-Chromosome Results. From the 22 ancient male specimens studied, three complete and 18 partial Y-STR-haplotypes were obtained (Table S5). Although all loci could not be clearly amplified for all specimens, most of the ancient individuals' Y-STR haplotypes seem closely linked. This explains the very low average gene diversity over all loci obtained (H, 0.361664 ± 0.196576). Only individuals 577 and 596 seemed different from the other ones. Among the six nonrecombining region of Y-chromosome (NRY) SNPs typed to confirm the af-

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Fig. 1. Location of the Grands Causses region (bounded by square) and of cave I of Treilles at Saint-Jean-et-Saint-Paul (France).

filiation to haplogroups previously deduced from STR haplotypes, only three gave workable results (P15, M438, and P37.2). Nevertheless, the 22 male individuals were confirmed to belong to the Y-haplogroup previously inferred. As expected from Y-STR data, all samples were found to belong to Y-haplogroup G2a except samples 577 and 596, which belong to haplogroup I2a. Cross-population comparison tests showed a great or very great genetic differentiation between Treilles male samples and current western Eurasian populations (F_{ST} values >0.15 and as high as >0.45) except for Basque and Spanish populations, with F_{ST} values of 0.0014 and 0.007, respectively (Table S6 and Figs. S3 and S4). The analysis of shared lineages showed that the Treilles haplotypes are rarely observed in current western European populations: among the 4,791 haplotypes carried by the 10,488 European individuals included in the databases, the Treilles haplotypes were observed only 11 times (Table S7). The highest percentage of shared lineages were found mainly in Mediterranean populations: 2.06% in Cypriot, 1.98% in Portuguese, 0.7% in Turkish, 0.38% in Italian, and 0.35% in Lebanese populations (Fig. 3).

To evaluate the molecular affinity between the G2a haplotypes from the Treilles samples and current G2a haplotypes found in European populations, we constructed a median-joining network of the G2a paternal haplogroup frequently observed in our ancient samples. The Treilles G2a haplotypes are located at the periphery of the network in a particular branch, suggesting that they are probably not the ancestral haplotypes (Fig. S5). Furthermore, they are located on a Mediterranean branch clearly differentiated from the Caucasian G2a, in which G2a is currently the most frequent in Europe, as high as approximately 30% (16).

Lactase Persistence Results. The LP-13910-C/T SNP associated with lactase persistence was successfully typed for 26 of the 29 ancient samples tested. All were homozygous C/C for this marker, which suggests that the ancient Treilles individuals were probably not able to digest fresh milk.

Discussion

Authenticity of Results. The main issues in ancient DNA studies is to avoid contamination by modern DNA templates and to produce authentic data. During all the steps of this study, extensive precautions were taken to avoid the amplification of contaminating contemporary DNA molecules (*SI Materials and Methods*). Despite the fact that not all of the classical authenticity criteria (17) could be satisfied, the following data support the authenticity of the results: (*i*) extraction controls, PCR blanks, and amplified products from animal remains were always negative; (*ii*) autosomal profiles were different from each other and different from those of researchers recently in contact with the samples; (*iii*) there was an inverse relationship between the amplification efficiency and length of the amplification products, especially with STR markers, which is characteristic of ancient degraded DNA; (*iv*) results of amplifications performed several times on various extractions were always concordant between each others; (*v*) results of SNP genotyping were also 100% concordant with mitochondrial and Y-chromosome haplotypes previously deduced from HVI sequencing and Y-STRs analysis; and (*vi*) results obtained are consistent with what can be expected on European ancient remains, as all samples were unambiguously affiliated to European haplogroups.

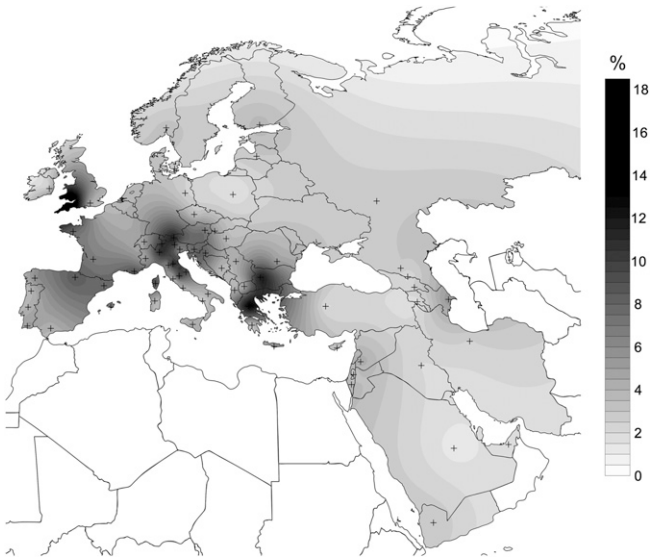

Fig. 2. Map showing mitochondrial lineages shared between Treilles individuals and current European populations. Crosses denote the location of modern-day populations used in the analysis. The gray gradient indicates the percentage of shared lineages between modern local populations and ancient samples: the highest percentages are in black and the weakest are in gray.

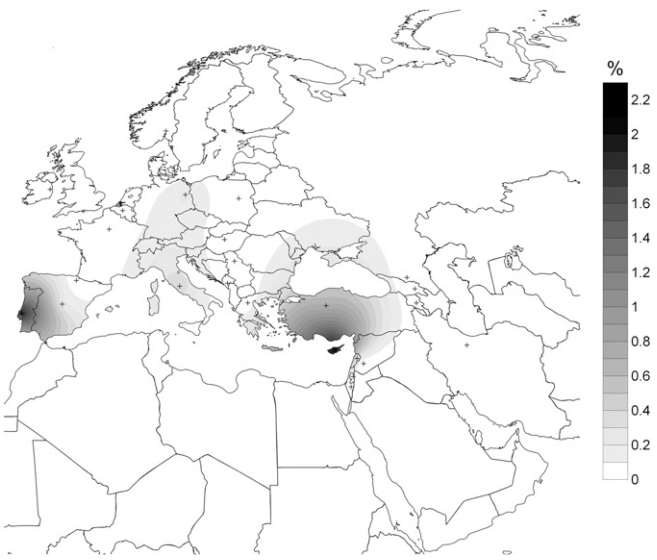

Fig. 3. Map showing the Y-lineages shared between Treilles individuals and current European populations. Crosses denote the location of modern-day populations used in the analysis. The gray gradient indicates the percentage of shared lineages between modern local populations and ancient samples: the highest percentages are in black and the weakest are in gray.

Social and Burial Implication. According to molecular data, 22 individuals were male and two were female. Morphometric analysis on 30 well preserved hipbones (not included in the analysis) also showed an imbalance of sex ratio: 20 male and 10 female (14). Furthermore, in the Treilles samples, a very low gene diversity was calculated from Y-haplotypes (H, 0.361664 ± 0.196576), combined with a high gene diversity from HVI haplotypes (H, 0.8966 ± 0.0354). We can thus hypothesize that the necropolis was only dedicated to male specimens of the same paternal lineage (18).

In present-day populations, this particular sex-specific genetic structure often involves a limited gene flow within the male component of the populations and suggests that the communities are patrilocal (19). In our ancient samples, this genetic structure suggests that the community that used this burial cave was patrilocal, or that it reflects a particular funeral rite.

Maternal and Paternal Origins. The results of the mitochondrial and Y-chromosome analyzes suggest that the maternal and paternal biogeographical origins of the Treilles samples might be substantially different.

Concerning the maternal origin, the gene pool of the Treilles samples seems to reflect a combination of the main events of the settlement of southwest Europe. Most of the mitochondrial haplogroups have an ancient ancestry consistent with the oldest episodes of settlement of western Europe from the Near East during the upper Paleolithic (52% of individuals are U, U5, HV0, X2, K1a, or T2b) (20–22) or from the Franco-Cantabrian region during the late glacial recolonization of the continent after the late glacial maximum (28% of individuals are H1, H3, V, or U5b1c) (23–25). A frequent haplogroup in Neolithic samples (4, 5, 8), haplogroup J1, found in six of 29 of the Treilles individuals, indicates also a Neolithic contribution of approximately 20% in the gene pool of our ancient samples (24, 26). The great haplotype diversity of the U5 cluster, one of the most ancient haplogroups found in Europe and very frequent in Neolithic and Mesolithic specimens (20), confirms moreover that part of the maternal lineage of Treilles samples is probably very ancient, originating from the upper Paleolithic. Similarly, the lack of haplotype diversity within haplogroup J1 confirms a probable recent origin of this haplogroup in the genetic pool of the Treilles samples.

On the contrary, similarly to southern Europe Neolithic specimens (4), there is no evidence in the Treilles samples of the N1a haplogroup, which was highly present in central Europe and Atlantic coast Neolithic cultures (6, 8). According to mitochondrial data, the Neolithic wave in the Treilles genetic pool is thus more likely to be Mediterranean than central European in origin.

The biogeographical origin of male samples appears less diverse. Treilles males belong to only two different haplogroups: I2a and G2a. In the Y phylogeny, haplogroup I is widespread over Europe but virtually absent elsewhere (27). Subclade I2a (formerly I1b1) probably originated in southern Europe during the Ice Age. Haplogroup G may represent a male contribution to a demic diffusion of farmers (1) from the Middle East to Europe (16, 28). G2a (formerly G2) is the major subclade of haplogroup G (29). Its origin in Europe is still unclear, but it could be a good marker for the Neolithic migrations of farmers into Europe (30).

The low percentage (<2%) of shared lineages between Treilles and current populations, and the fact that the ancestral and current G2a haplotypes do not seem related, imply that the G2a lineage of Treilles was probably lost between the end of the Neolithic and today. Few ancient data are currently available on Y-haplogroups to confirm this hypothesis, but G2a haplotypes have been found in other prehistoric remains; two ancient DNA studies revealed the presence of G2a in the Czech Republic during the seventh century (31) and in a German sample of a central European Neolithic culture (13), whereas this haplogroup is very rare in these places nowadays (32).

Anyway, even if the lineages shared between Treilles individuals and present-day populations are small, their location along the Mediterranean coast is consistent with an origin of part of the males' gene pool in the Mediterranean Neolithic expansion (33).

Recent studies on modern samples link the geographical distribution of the R1b-M269 haplogroup to its spread from the Near East during the Neolithic (34). More specifically, subclade R1b-S116 has been linked with the early north-central European plain colonization (35). This haplogroup was not found in the Treilles samples. The Treilles group is strongly structured by paternal lineage, implying a low diversity among paternal lineages. The absence of the R1b haplogroup in the ancient samples could be linked to this particular genetic structure but it could also be caused by the absence of a Danubian route influence in the southwestern Mediterranean male gene pool. The latter hypothesis is highly compatible with shared lineages distribution.

In summary, even if the maternal lineages seem to have more diversified origins in time and space, both mitochondrial and NRY studies reveal a contribution of the Neolithic wave in the gene pool of the Treilles specimens. Furthermore, our results also show that, at least for the gene pool of the male samples, the Neolithic dispersals had to take place along the Mediterranean route.

Lactase Persistence in the Treilles Individuals. The allele T located at position 13,910 bp upstream of the lactase gene is a polymorphism strongly associated with the ability to produce lactase, an intestinal enzyme that aids the digestion of untransformed milk. Largely widespread in northern and western current Europe, the 13910T allele is present in 43% of the present French population (36). This polymorphism is very rare or absent in Mesolithic Scandinavian samples and in early Neolithic Europeans (10, 12). According to a recent study, the T allele probably appeared in Europe in a region between the Balkans and central Europe and spread with the dissemination of the Linearbandkeramic culture over central Europe (15). This allele was not found in Treilles samples. This suggests that the Treilles individuals probably did not directly acquire the possibility to digest fresh milk from the farming communities of central Europe. This could also imply that the Treilles community was closer to the Mediterranean agropastoral cultures, which have an economy based on farming of sheep/goat and consumption of fermented milk (15) than to central European cultures, which practiced dairy farming. This finding also suggests that the peopling of southern France during the Neolithic expansion is more likely to have originated from the Mediterranean Sea than the central European plains.

Conclusion

All three systems used in this work to estimate the genetic origin of the Treilles samples (mtDNA, NRY, and lactase persistence SNP) are consistent with a substantial contribution of the Mediterranean Neolithic spread into the gene pool of ancient specimens. The absence of the mitochondrial haplogroup N1a and of the R1b Y-chromosomal haplogroup, both potentially associated with the spread of a Neolithic culture in Central Europe, confirms moreover the probable heterogeneity of Neolithic dispersals into Europe.

However, data obtained on the Y-chromosome suggest that the Treilles group was strongly structured by paternal lineage, and thus these data provide information on only a limited part of all of the existing lineages of southern European populations living nearby at the same period. New ancient Y-chromosomal studies from adjacent ancient populations will be needed in the future to give a complete overview on the Neolithic male diffusion through the Mediterranean route.

Materials and Methods

Samples. The cave of Treilles is a collective burial site containing a minimum number of 149 individuals buried over a period of one or two centuries (14). Babies and young children were less represented than would be expected from the natural mortality of a community (63 children and subadults and 86 adults), and the adults' bodies were partially disarticulated, a widespread ritual in the French Neolithic (37). Consequently, to sample each individual only once, we used mandibular teeth without carious lesions and still fixed to the mandible. All mandibles still bearing teeth were collected. Molecular analyzes were thus performed on teeth from 53 individuals. Sampling was

done by two laboratory members at the Natural History Museum of Toulouse (France), where the bone collection is preserved.

DNA Extraction. The teeth were first decontaminated with bleach, rinsed with ultrapure water, exposed to UV light (254 nm) on each side during 30 min, and powdered in a grinder mill under liquid nitrogen. Two hundred milligrams of the tooth powder were suspended in an extraction buffer and incubated overnight at 50 °C. Purification and concentration steps were then performed as previously described (38). Between three and six extractions were carried out for each individual, depending on the powder quantity retrieved from each tooth.

Nuclear Quantification. For one DNA extract per sample, a nuclear quantification was performed on an ABI Prism 7000 Sequence Detection System by using the Quantifiler Human DNA Quantification Kit (Applied Biosystems) according to the manufacturer's protocol.

Autosomal Analysis. Sixteen autosomal STR loci were analyzed using the AmpFiSTR Identifiler Plus and the MiniFiler PCR Amplification Kits (Applied Biosystems). Capillary electrophoreses were performed on a 3500 Genetic Analyzer and the STRs profiles were analyzed with GeneMapper 4.1 software. Two amplifications were performed on three or four different DNA extracts for each sample.

mtDNA Analysis. Mitochondrial haplogroups were determined for each ancient sample on the basis of the HVI haplotype and of SNPs chosen on the mtDNA coding region according to the latest mtDNA phylogeny (39). Three hundred eighty-one base pairs of the HVI region of the mtDNA were amplified and sequenced in two overlapping fragments (40). Twenty-one diagnostic SNPs of the mitochondrial coding region were typed to clarify the haplogroup status inferred from HVI sequences. Typing was performed using the iPLEX Gold technology (Sequenom) as described by Mendisco et al. (38). Two multiplexes containing a total of 28 SNPs located on mtDNA, the NRY, and the *MCM6* gene were designed with MassArray Assay design software (version 4.0). The typing reactions were performed twice on two different DNA extracts.

Y-Chromosomal Analysis. Y-chromosomal analyzes were made on the 22 ancient male samples. Haplotypes were obtained from the analysis of 17 Y-STRs loci using the AmpFiSTR Yfiler PCR Amplification Kit (Applied Biosystems). Haplogroups deduced with the haplogroup predictor software (41) were then tested by SNP typing by using iPLEX Gold technology (Sequenom). We chose the six Y-SNP markers characteristic of the haplogroups and subhaplogroups G (M201), G2 (M287) and G2a (P15) (42), and I (M170), I2 (M438), and I2a (P37.2) (43) to confirm the assignment to the haplogroups initially inferred.

Lactase Persistence Typing. One SNP located in the *MCM6* gene and found to be associate with hypolactasia, more commonly known as lactose intolerance in European Caucasian populations, was added into the multiplex 2 of the SNP typing (LP-C/T13910; Rs4988235).

Statistical Analysis. All statistical analyses performed on the Treilles data are detailed in *SI Materials and Methods*.

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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).

1. Keyser C, et al. (2009) Ancient DNA provides new insights into the history of south Siberian Kurgan people. *Hum Genet* 126:395–410.
2. Kalinowski S, Wagner A, Taper M (2006) ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Mol Ecol Notes* 6: 576–579.
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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.
6. Tishkoff SA, et al. (2007) History of click-speaking populations of Africa inferred from mtDNA and Y chromosome genetic variation. *Mol Biol Evol* 24:2180–2195.

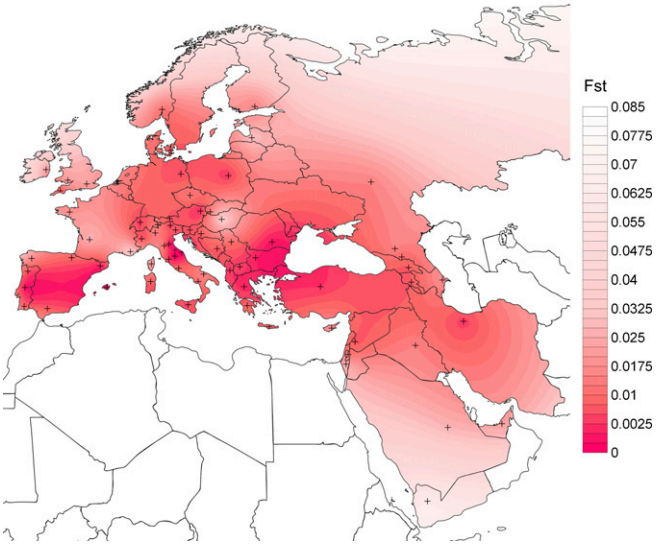

Fig. S1. Spatial distribution of the genetic matrilineal distances between Treilles samples and modern Western Eurasian populations.

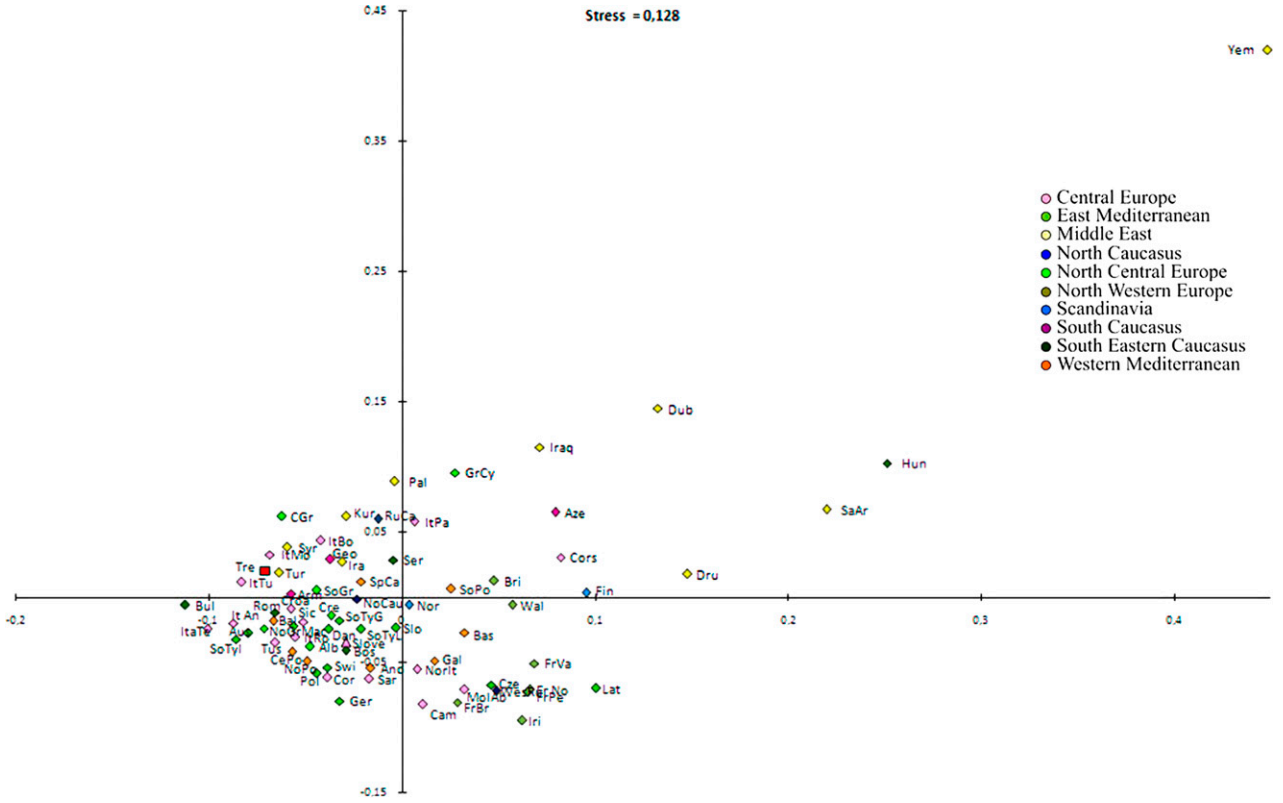

Fig. S2. Multidimensional scaling plot of genetic distances calculated for mtDNA data. The red square represents Treilles samples.

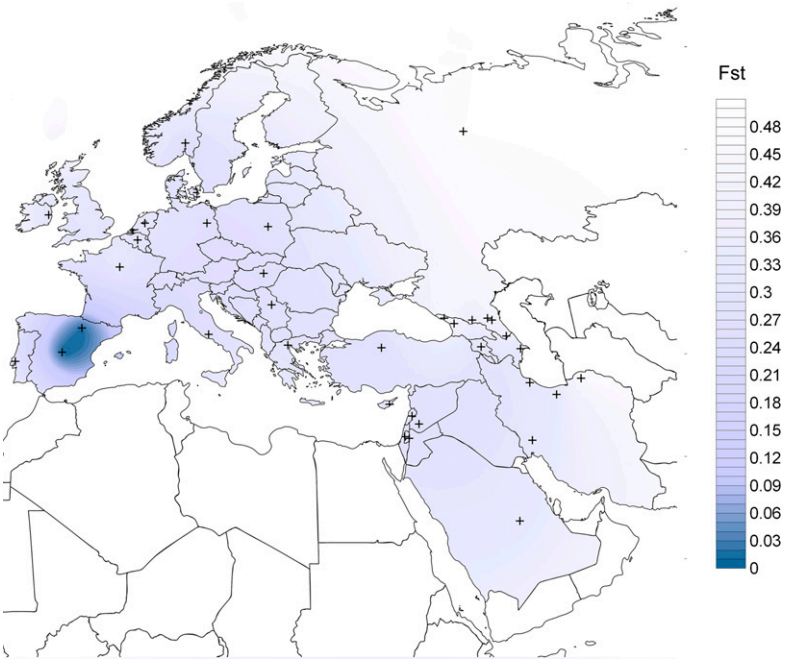

Fig. S3. Spatial distribution of the genetic patrilineal distances between Treilles male samples and modern Western Eurasian populations.

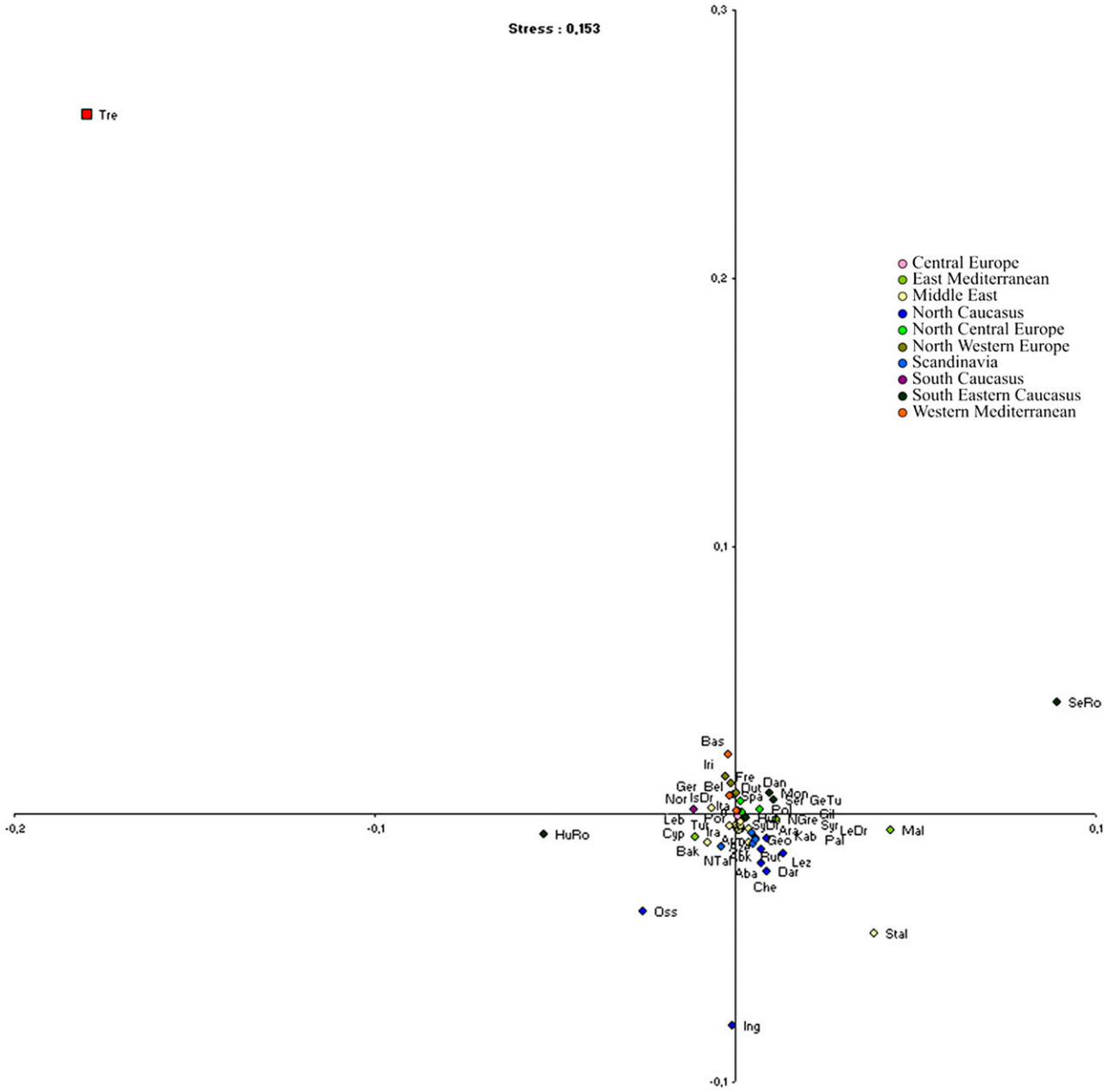

Fig. S4. Multidimensional scaling plot of genetic distances calculated for Y-chromosomal data. The red square represents Treilles samples.

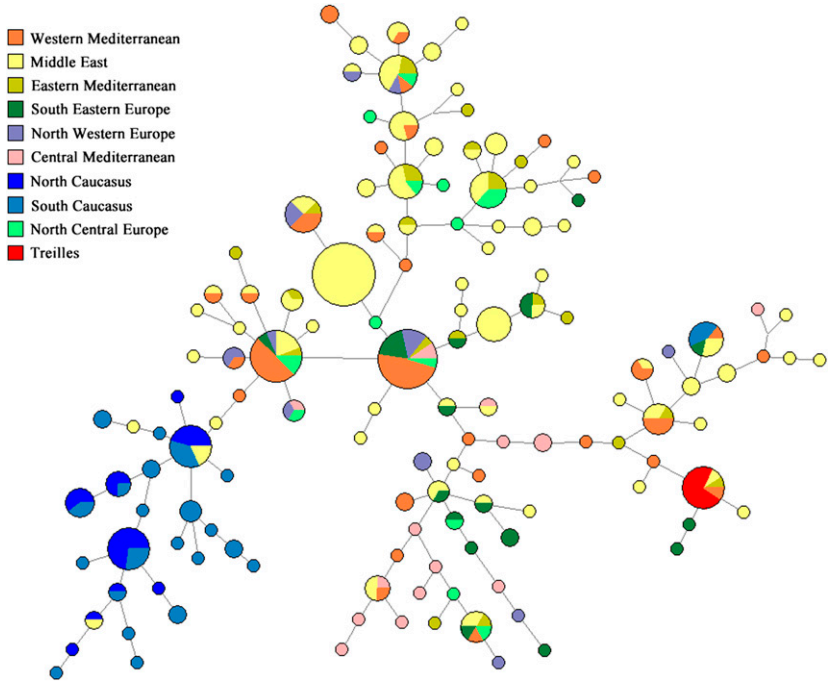

Fig. S5. Median joining network of Y-G2a haplotypes in current western European populations and in the Treilles male specimens (in red).

Table S1. Consensus STR autosomal profiles of the 24 Treilles human specimens

Sample name	Quantity, ng/μL	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	AMEL	D5S818	FGA
137	6.20×10^{-3}	(12)/13	(29)/30	(8/12)	12/12	16/17	6/6	12/12	11/12	17/(19)	12/15.2	17/17	(8/8)	12/16	X/Y	12/13	22/23
139	5.07×10^{-3}	(11/13)	31.2/33.2	9/9	11/11	—	—	9/10	11/12	24/25	—	(17/17)	—	14/15	X/Y	12/12	22/24
195	2.59×10^{-4}	10/14	30.2/31.2	9/12	11/11	15/17	6/9,3	8/11	9/(13)	17/19	12/14	16/17	9/(11)	(12)/14	X/Y	11/13	20/24
209	NA	12/14	30/30	(7)/10	(9/12)	18/18	6/(8)	9/(13)	9/9	(20/20)	13/14	(15)/17	(11/11)	13/19	X/Y	11/11	(19)/20
570	1.78×10^{-2}	10/14	28/31	11/12	11/(12)	18/18	—	8/8	11/11	17/21	12/13	17/20	(8/11)	(14)/17	X/Y	11/13	21/22
573	1.91×10^{-2}	11/14	31.2/31.2	(10/12)	9/(11)	16/17	7/7	9/(10)	(9/12)	(17/17)	14/15	14/16	(11/11)	(16/19)	X/X	12/12	21/23
575	1.23×10^{-2}	11/16	27/28	10/12	12/12	15/18	6/7	8/11	9/12	17/21	12/13	17/17	8/11	14/16	X/Y	10/11	23/24
577	5.19×10^{-3}	(10/10)	(29/32.2)	10/12	10/12	(16/16)	—	8/(12)	(9)/11	(21/21)	(14/14)	—	—	(13)/14	(X/Y)	(13/13)	24/25
579	1.97×10^{-2}	14/15	29/32.2	12/(13)	10/(12)	18/18	6/9.3	8/12	8/12	20/21	14/14	(15)/16	8/11	12/15	X/Y	12/13	22/23
583	7.51×10^{-3}	14/16	28/(30)	8/(12)	10/10	(15)/18	(9/9,3)	8/11	(14/14)	17/(20)	13/15	(15)/17	—	(14)/19	X/Y	(10)/12	24/24
584	1.21×10^{-2}	10/16	31.2/32.2	10/12	10/12	(15)/18	9/9	9/13	9/13	16/21	12/14	17/17	8/8	11/20	X/Y	11/(15)	20/24
587	6.75×10^{-3}	10/(13)	(24.2/24.2)	—	(13/13)	16/16	—	8/8	—	—	13/14.2	(15/17)	(11/11)	—	X/Y	12/12	—
588	3.38×10^{-3}	11/14	24.2/30	11/(12)	10/10	(16)/18	9,3/9,3	10/12	(12)/13	23/23	14/16	14/18	—	13/17	X/Y	11/12	21/24
592	1.52×10^{-3}	11/15	31/31.2	(10)/12	12/12	(17)/18	7/9.3	11/11	(12)/13	17/(23)	13/14	15/16	8/8	14/20	X/Y	11/12	24/24
593	1.63×10^{-2}	—	—	12/12	(10/10)	—	—	10/11	9/(11)	—	—	(17/17)	—	(12/17)	(X/Y)	—	25/26
596	1.18×10^{-2}	13/13	29/33.2	9/10	10/11	16/17	6/9.3	8/11	9/12	16/24	13/13	14/16	8/8	15/16	X/Y	11/12	19/25
600	4.43×10^{-3}	13/13	28/28	12/12	10/(11)	16/17	9/9.3	8/11	12/12	(17)/23	13/13	15/15	(8/8)	14/14	X/Y	12/12	23/25
604	NA	10/15	29.2/31.2	8/9	10/12	18/19	(6)/8	9/11	12/13	17/(25)	15/(16)	18/19	—	18/20	X/Y	11/12	(21)/26
611	Undet	14/14	(28/30)	—	(9/9)	14/15	9.3/9.3	(8/11)	—	—	(13)/14	16/17	(8/8)	(12/17)	X/Y	(11/11)	(19/19)
612	3.42×10^{-3}	10/15	(28/28)	—	10/10	(16)/18	(9/9.3)	8/8	11/11	17/20	13/15	15/15	(8/8)	12/19	X/Y	10/12	24/24
614	5.90×10^{-3}	10/13	28/29	(8/10)	11/11	17/18	(7/7)	(8/8)	11/12	(23/23)	12/12	14/14	—	18/19	X/X	10/13	21/21
615	1.85×10^{-2}	11/12	28/28	(9/11)	12/12	15/15	9/(9.3)	8/11	8/13	20/20	15.2/15.2	14/14	11/11	12/18	X/Y	11/11	20/20
616	3.34×10^{-2}	10/13	28/33.2	10/11	10/12	18/18	6/9.3	8/11	11/13	17/20	13/16	15/16	8/8	19/20	X/Y	11/12	22/24
636	2.01×10^{-2}	10/10	(31.2)/33.2	8/9	12/13	15/18	(6)/8	9/11	12/14	17/24	15/16	17/19	8/8	15/18	X/Y	12/12	21/21
Research team																	
1		13/13	28/31	10/11	10/12	15/17	8/9	12/13	12/12	18/24	13/15	17/17	8/11	11/16	X/Y	11/12	21/23,2
2		13/14	28/29	8/10	11/11	15/18	7/9.3	11/11	11/12	24/25	13/15.2	14/17	8/10	12/15	X/Y	11/13	21/22
3		12/14	29/29	9/11	12/12	14/18	9/9.3	12/12	11/11	17/19	13/13	15/15	11/11	14/17	X/X	11/11	21/23
4		10/11	30/32.2	10/10	11/12	16/17	9/9.3	8/11	11/12	20/23	14/14	16/18	11/12	12/13	X/X	11/13	19/25
5		10/13	29/30	9/11	10/11	14/18	9/9.3	11/11	11/14	17/22	14/15.2	14/18	8/12	15/17	X/Y	12/12	20/23

Dashes denote that alleles could not be clearly amplified for the locus in question. Consensus allelic profiles were built after two amplifications performed on at least three different DNA extracts for each sample. Alleles in brackets were observed just once. The five last profiles are those of the researchers of the Natural History Museum of Toulouse (France) and of the laboratory members who have recently been in contact with the samples.

The DNA quantity mentioned was obtained from one DNA extract per sample with the Quantifiler Human DNA Quantification Kit (Applied Biosystems). Undet, undetermined; NA, data not acquired.

Table S2. MtDNA haplotypes and haplogroups inferred for each Treilles specimen

			SNP typing results																							
Sample Name	HVI polymorphisms	Haplogroup inferred from HVI sequences	H- T7028C	J2- G15257A	T1- C12633T	T2- A14233G	T2B- G930A	U5- T3197C	U5B1C- T15191C	V- G4580A	X- T14470C	X1- T146C	H1-J1- G3010A	H3- T6776C	HV- T14766C	N/J- G13708A	K- A10550G	J/K1- A10398G	K1A- C497T	K2- T9716C	K2B- C2217T	T- A4917G	U- A12308G	X2- G1719A	Haplogroup inferred by SNP genotyping	Haplogroup inferred from the two techniques
137	16224C 16270T 16311C	U5 or K2b1	T	G	C	A	G	**C**	T	G	T	T	G	T	T	G	A	A	C	T	C	A	**G**	G	U5	U5
139	16069T 16126C	J	T	G	C	A	G	T	T	G	T	T	**A**	T	T	**A**	A	**G**	C	T	C	A	A	G	J1	J1
195	16192T 16270T	U5	T	G	C	A	G	**C**	T	G	T	T	G	T	T	G	A	A	C	T	C	A	**G**	G	U5	U5
209	16069T 16126C	J	T	G	C	A	G	T	T	G	T	T	**A**	T	T	**A**	A	**G**	—	T	C	A	A	G	J1	J1
570	16189C 16223T 16278T	X2	T	G	C	A	G	T	T	G	**C**	T	G	T	T	**A**	A	A	C	T	C	A	A	**A**	X2	X2
571	CRS	H*	T	G	C	A	G	T	T	G	T	T	G	T	—	G	—	A	C	—	C	A	**G**	G	U	U
573	16298C	HV0	T	G	C	A	G	T	T	G	T	T	G	T	**C**	G	A	A	C	T	C	A	A	G	HV	HV0
575	16227G 16256T 16270T 16362TC	U5	T	G	C	A	G	**C**	T	G	T	T	G	T	T	G	A	A	C	T	C	A	**G**	G	U5	U5
577	CRS	H ?	**C**	G	C	A	G	T	T	G	T	T	G	**C**	**C**	G	A	A	C	T	C	A	A	G	H3	H3
579	16224C 16270T	U5 or K2b1	T	G	C	A	G	**C**	T	G	T	T	G	T	T	G	A	A	C	T	C	A	**G**	G	U5	U5
581	CRS	H*	**C**	G	C	A	G	T	T	G	T	T	G	**C**	**C**	G	A	A	C	T	C	A	A	G	H3	H3
583	16069T 16126C	J	T	G	C	A	G	T	T	G	T	T	**A**	T	T	**A**	A	**G**	C	T	C	A	A	G	J1	J1
584	16126C 16294T 16296T 16304C	T2b	T	G	C	**G**	**A**	T	T	G	T	T	G	T	T	G	A	A	C	T	C	—	A	G	T2b	T2b
587	16069T 16126C	J	T	G	C	A	G	T	T	G	T	T	**A**	T	T	**A**	A	**G**	C	T	C	A	A	G	J1	J1
588	16126C 16294T 16296T 16304C	T2b	—	G	C	**G**	**A**	T	T	G	T	T	G	T	T	G	A	A	C	T	C	—	A	G	T2b	T2b
592	16183C 16189C 16223T 16278T	X	T	G	C	A	G	T	T	G	**C**	T	G	T	T	**A**	A	A	C	T	C	A	A	**A**	X2	X2
596	16269G	H	**C**	G	C	A	G	T	T	G	T	T	**A**	T	**C**	G	A	A	C	T	C	A	A	G	H1	H1
593	CRS	H*	**C**	G	C	A	G	T	T	G	T	T	**A**	T	**C**	G	A	A	C	T	C	A	A	G	H1	H1
600	CRS	H*	**C**	G	C	A	G	T	T	G	T	T	G	**C**	**C**	G	A	A	C	T	C	A	A	G	H3	H3
603	CRS	H*	**C**	G	C	A	G	T	T	G	T	T	**A**	T	**C**	G	A	A	C	T	C	A	A	G	H1	H1
604	16224C 16311C	K	T	G	C	A	G	T	T	G	T	T	G	T	T	G	**G**	**G**	**T**	T	C	A	**G**	G	K1a	K1a
609	16298C	HV0	T	G	C	—	G	T	T	—	T	T	G	T	**C**	G	A	A	C	T	C	A	A	G	HV	HV0
611	16189C 16192T 16270T 16311C	U5b1c	T	G	C	A	G	**C**	**C**	G	T	T	G	T	T	G	—	A	C	T	C	A	**G**	G	U5b1c	U5b1c
612	16069T 16126C	J	T	G	C	A	G	T	T	G	T	T	**A**	T	T	**A**	A	**G**	C	T	C	A	A	G	J1	J1
614	16224C 16311C	K	T	G	C	A	G	T	T	G	T	T	G	T	T	G	**G**	**G**	**T**	T	C	A	**G**	G	K1a	K1a
615	16183C 16189C 16223T 16278T	X	T	G	C	A	G	T	T	G	**C**	T	G	T	T	**A**	A	A	C	T	C	A	A	**A**	X2	X2
616	16069T 16126C	J	T	G	C	A	G	T	T	G	T	T	**A**	T	T	**A**	A	**G**	C	T	C	A	A	G	J1	J1
636	16183C 16189C 16223T 16278T	X	T	G	C	A	G	T	T	G	**C**	T	G	T	T	**A**	A	A	C	T	C	A	A	**A**	X2	X2
637	16298C	HV0	T	G	C	A	G	T	T	**A**	T	T	G	T	**C**	G	A	A	C	T	C	A	A	G	V	V
Research team																										
3	16270T	U5	T	G	C	A	G	**C**	T	G	T	T	G	T	T	G	A	A	C	T	C	A	**G**	G	U5	U5
5	CRS	H*	**C**	G	C	A	G	T	T	G	T	T	G	T	C	G	A	A	C	T	C	A	A	G	H	H
4	16093C 16189C 16270T 16274A	U5	T	G	C	A	G	**C**	T	G	T	T	G	T	T	G	A	A	C	T	C	A	**G**	G	U5	U5
1	CRS	H*	**C**	G	C	A	G	T	T	G	T	T	G	**C**	**C**	G	A	A	C	T	C	A	A	G	H3	H3
2	16129A 16223T	I	T	G	C	A	G	T	T	G	T	T	G	T	T	G	A	**G**	C	T	C	A	A	**A**	N1′5	I

Mitochondrial haplogroups were established by HVI sequencing as well as by SNP typing of coding positions of the mtDNA. SNPs in bold are variants at concerned positions.

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

Population	F_{ST}	*P* value
Middle East		
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
North Caucasus		
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
South Caucasus		
Georgians	0.00712	0.10811 ± 0.0264
Armenians	0.00719	0.05405 ± 0.0201
Azerbaijanis	0.01911	0.01802 ± 0.0121
Northwestern Europe		
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
North Central Europe		
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
Scandinavia		
Norwegians	0.01138	0.06306 ± 0.0237
Finns	0.01576	0.25225 ± 0.0353
Southeastern Europe		
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
Western Mediterranean		
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
Central Mediterranean		
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
Molisio-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
Middle East		
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
North Caucasus		
Caucasian Russians	2,970	8,911
Western Russians	2.778	6.481
Other North Caucasus populations	1,765	4,706
South Caucasus		
Georgians	2,732	5,464
Armenians	1,613	5,914
Azerbaijanis	5,556	13,889
Northwestern Europe		
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
North-central Europe		
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
Scandinavia		
Norwegians	3,306	8,264
Finns	3,822	7,006
South Eastern Europe		
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
Western Mediterranean		
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
Central Mediterranean		
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 S5. Y-haplogroups inferred from Y- STR haplotypes and NRY-SNPs typing results for the male specimens

	Y-STRs																NRY SNPs typing results							
Sample name	DYS456	DYS389I	DYS390	DYS389II	DYS458	DYS19	DYS385	DYS393	DYS391	DYS439	DYS635	DYS392	Y-GATA-H4	DYS437	DYS438	DYS448	Y–M287	Y-P15	Y-I-M170	Y-I2-M438	Y-I2A-P37.2	Y-G-M201	Positive Y-markers	Haplogroup Y
137	14	12	23	—	18	15	—	14	10	11	—	—	11	16	—	20	—	**T**	—	A	T	—	P15+	G2a
139	—	12	—	—	18	15	—	—	10	11	—	—	11	—	10	—	—	**T**	—	A	T	—	P15+	G2a
195	14	12	23	30	18	15	(13/15)	14	10	11	21	—	11	16	—	—	—	**T**	—	A	T	—	P15+	G2a
209	14	12	23	—	18	15	(13/15)	14	10	—	—	—	11	—	—	—	—	—	—	A	T	—		G2a (99.9%)
570	14	12	23	—	18	—	13/(15)	14	10	11	21	11	11	16	—	—	—	**T**	—	A	T	—	P15+	G2a
575	14	12	23	30	18	15	13/(15)	14	10	11	21	—	11	16	10	20	—	**T**	—	A	T	—	P15+	G2a
577	14	—	—	—	16	—	—	13	—	—	—	—	12	—	—	—	—	C	—	**G**	**C**	—	M438+ P37.2+	I2a
579	14	12	23	—	18	—	13/(15)	14	10	11	21	—	11	16	10	—	—	**T**	—	A	—	—	P15+	G2a
583	14	—	—	—	18	—	13/(15)	14	10	11	—	—	11	16	—	—	—	—	—	A	—	—		G2a (99.8%)
584	15	12	23	30	18	15	13/15	14	10	12	21	—	11	16	—	—	—	**T**	—	A	T	—	P15+	G2a
587	14	12	23	—	—	—	15	14	—	—	21	—	—	—	—	—	—	**T**	—	A	—	—	P15+	G2a
588	14	12	23	—	18	—	13/(15)	14	10	11	—	—	11	16	—	—	—	**T**	—	A	—	—	P15+	G2a
592	14	12	23	—	18	—	13/15	14	10	11	—	—	11	16	—	—	—	**T**	—	A	T	—	P15+	G2a
593	14	—	—	—	18	—	—	—	—	—	—	—	—	—	—	—	—	**T**	—	A	T	—	P15+	G2a
596	14	13	23	28	16	16	12	13	10	12	22	11	12	15	10	22	—	C	—	**G**	**C**	—	M438+ P37.2+	I2a
600	14	12	—	30	18	—	13/15	14	10	11	21	—	11	16	10	—	—	**T**	—	A	—	—	P15+	G2a
604	14	—	—	—	18	15	—	14	10	—	21	—	11	—	—	—	—	**T**	—	A	T	—	P15+	G2a
611	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**T**	—	A	T	—	P15+	G2a
612	14	12	—	—	18	—	(13/15)	14	10	11	21	—	11	—	—	—	—	**T**	—	—	—	—		G2a
615	14	12	23	30	18	15	13/15	14	10	11	21	11	11	16	10	20	—	—	—	A	T	—		G2a (100%)
616	14	12	23	30	18	15	13/15	14	10	11	21	11	11	16	10	20	—	**T**	—	A	—	—	P15+	G2a
636	14	12	23	30	18	15	13/15	14	10	11	21	11	11	16	9	—	—	**T**	—	A	—	—	P15+	G2a
Research team																								
1	15	14	24	30	18	13	13/14	13	9	10	21	11	11	14	10	20	A	C	A	A	T	—		E1b1b
2	16	13	24	28	17	14	11/12	13	11	13	24	13	11	15	12	20	A	C	A	A	T	—		R1b
5	15	14	24	30	18	14	12/14	13	11	11	24	13	12	15	12	19	A	C	A	A	T	G		R1b

Dashes denote that alleles could not be clearly amplified for the locus in question. Consensus Y-STR profiles were built after two amplifications from at least three different DNA extracts for each sample. Alleles in brackets were observed just once. The three last profiles are those of the male researchers of the Natural History Museum of Toulouse (France) and of the laboratory members who have recently been in contact with the samples. For samples 209, 583, 615, for which the Y haplogroup could not be confirmed by the typing of SNP, the determination of the haplogroup was conducted solely from the haplotype. The percentage of probability is shown in the last column. SNPs in bold are variants at concerned positions.

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
Middle East		
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
North Caucasus		
Abazinians	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
South Caucasus		
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
Northwestern Europe		
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
North central Europe		
Germans	0.26655	0.00000 ± 0.0000
Danish	0.27898	0.00000 ± 0.0000
Polish	0.27598	0.00000 ± 0.0000
Scandinavia		
Norwegians	0.26608	0.00000 ± 0.0000
Southeastern Europe		
Hungarian	0.26761	0.00000 ± 0.0000
Serbian	0.28178	0.00000 ± 0.0000
Serbian Romanian		
Montenegrin	0.27567	0.00000 ± 0.0000
Western Mediterranean		
Portuguese	0.27854	0.00000 ± 0.0000
Spanish	0.00724	0.00000 ± 0.0000
Basque	0.01392	0.00000 ± 0.0000
Central Mediterranean		
Italians	0.26635	0.00000 ± 0.0000
Eastern Mediterranean		
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, %
Middle East	
Iranians	0
Syrians	0
Druze	0
Palestinians	0
Lebanese	0.355
Turks	0.699
North Caucasus	
Other North Caucasus populations	0
South Caucasus	
Georgians	0
Armenians	0
Azerbaijanis	0
Other South Caucasus populations	0
Northwestern Europe	
French	0
Irish	0
Belgians	0
Dutch	0
North Central Europe	
Germans	0.226
Danish	0
Polish	0
Scandinavia	
Norwegians	0
Southeastern Europe	
Hungarians	0
Serbians	0
Serbian Romanians	0
Montenegrins	0
Western Mediterranean	
Portuguese	1.980
Galician	0
Catalan	0
Other Spanish	0.248
Basque	0
Central Mediterranean	
Italians	0.385
Sicilians	0
Sardinians	0
Eastern Mediterranean	
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
Middle East (*n* = 2,689)		Middle East (*n* = 2,482)	
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
North Caucasus (*n* = 594)		North Caucasus (*n* = 78)	
Caucasians Russians	2		
Western Russians	23		
Other North Caucasus populations	10, 19, 24, 25	Other North Caucasus populations	26
South Caucasus (*n* = 652)		South Caucasus (*n* = 424)	
Georgians	13, 19, 27, 28	Georgians	26
Armenians	2, 27, 29	Armenians	26
Azerbaijanis	27	Azerbaijanis	3, 26
		Other South Caucasus populations	26
Northwestern Europe (*n* = 783)		Northwestern Europe (*n* = 408)	
British	30		
French	31	French	32
Welsh	20		
Cornish	20		
Irish	20, 33	Irish	34
		Belgians	35
		Dutch	36
North-Central Europe (*n* = 3,239)		North-Central Europe (*n* = 1,695)	
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		
Scandinavia (*n* = 712)		Scandinavia (*n* = 1,967)	
Norwegians	53	Norwegians	54
Finns	55–57		
Southeastern Europe (*n* = 909)		Southeastern Europe (*n* = 1,078)	
Bulgarians	16		
Hungarians	58–60	Hungarians	61
Bosnians	62, 63		
Serbians	62	Serbians	64
Romanian	65	Serbian Romanians	66
		Montenegrins	64
Western Mediterranean (*n* = 1,625)		Western Mediterranean (*n* = 1,442)	
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
Central Mediterranean (*n* = 2,040)		Central Mediterranean (*n* = 562)	
Northeastern Italians	52, 81-84	Northern Italians	85

Table S8 Cont.

Population (size)	References HVS-I	Population (size)	References Y-STR
Tuscanians	75, 86, 87		
Other Italians: Acone, Bologna, Firenze, Modena, Pavia, Roma, Turino, 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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