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Original scientific paper

Y-Chromosome-Specific Microsatellite Variation in a Population Sample from Sardinia (Italy)

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A B S T R A C T

*The genetic variability at seven Y-chromosomal microsatellite loci was studied among 113 Sardinian males from the regions of Campidano of Cagliari, Nuorese and Gallura. The allelic and haplotypes frequency distributions are compared between our sample and from the available literature data on Mediterranean and European populations. As a result, the Sardinian samples showed a very high allele frequency in the DYS19*17, a rarity in the rest of Europe, probably due to the founder effect. The analysis has shown an intra-population genetic heterogeneity and genetic differentiation from other Mediterranean and European population deal with. The results reported in this work showed that of the Euro-Mediterranean populations, the Corsican of the South seems to have the most genetic affinity with the Sardinians, thereby reaffirming the observations from previous works that had suggested a certain level of genetic similarity*

Introduction

The settling of Sardinia seems to have taken place more recently than in other European and Mediterranean regions. According to archaeological evidence, the arrival of man in Sardinia could be dated back to the lower Paleolithic period (500,000 to 150,000 years ago), when the sea retreated during the last glaciation, creating the formation of the Sardinia-Corsica landmass and reducing its distance from the mainland. Evidence of man's presence appears in the findings of Riu Altana which date back to the old Clactonian period and are greatly similar to the industries found in some areas on the Italian mainland (Abruzzo, Gargano, Emilia)[1].

During the Neolithic and Aneolithic periods an influx of people originating from different areas of the Mediterra-

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nean, south-west Europe and Asia Minor seems to have taken place. This was a consequence of the contact brought about by the trade in obsidian, lithic products and cooper and also by the development of agriculture and pottery[2].

From the 17th to the 3rd century BC, Sardinia saw the development of the Nuragic civilization. Infact, the protagonist of Sardinian prehistory is the Nuraghe, which is a truncated cone tower, built with huge square blocks of stone, usually located in panoramic positions.

The importance of the contribution from other ethnic groups to the genetic constitution of the Sardinian nuragical Bronze Age population has yet to be established. Certainly elements of numerous other ethnic groups: Corsican, Ligurian, Iberian, Balearic, Central European, Libyan, Anatolian, Etruscan were knit into the autochthonous populations[2]. Furthermore, in 800 BC there began a long series of invasions that continued until the end of the last century (Phoenician, Carthaginian, Roman, Vandal, Byzantine, Pisan, Genoese, Aragonese, Catalan, Piemontese). Since the influence of these invaders was mainly limited to the coastal areas, its effects are not thought to have much significance on the genetic structure of the Sardinian population. Such historical events have led to the distinctive anthropological, genetic and culture characteristics of the present day Sardinia. The study of numerous genetic markers in the numerous works produced on this subject has made evident clear-cut differences between Sardinians and other populations of the Mediterranean Basin[3–7]

Over the last decade there has been a number of different definitions given to the Y Chromosome, names such as, »functional wasteland«, »non-recombining desert« and »gene-poor chromosomes« are some of the examples[8]. Unique in many aspects, the Y chromosome has demonstrated to be extremely informative in disentangling the history of human variation. In this work we used the properties of the non-recombining portions on the Y-chromosome to investigate the genetic structure of the Sardinian population.

Our aim is to analyze, for the first time, the distribution of 7 highly polymorphic Y-chromosome specific microsatellites (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393) in Sardinia, in order to contribute to the study of Sardinian population peculiarities with respect to the other circum-Mediterranean populations and to analyze the island's internal micro-geographical variabilities.

Material and Methods

DNA was extracted from fresh blood of a total of 113 unrelated, native Sardinian males. The samples analyzed come from the regions of Campidano of Cagliari (33), Nuorese (46) and Gallura (34) (Figure1).

Amplification of STR loci was performed in three non-overlapping multiplex PCR systems: triplex (comprising loci DYS19, DYS390 and DYS393), duplex I (comprising DYS389I–II) and duplex II (comprising DYS 391 and DYS392). The primer sequences for DYS 19; DYS 389I–II; DYS 390, DYS 391, DYS 392, and DYS 393 were those described by Kayser et al.[9]. Electrophoresis was performed according to the method of Kaska et al.[10]. After electrophoresis the gel was silver stained, following the method of Budowle et al.[11]. The alleles were named according to the number of repeats at each locus as recommended by DNA Commission of the International Society for Forensic Hemogenetics[12]. For each group sample, and for each locus, allele frequencies were calculated by simple gene counting. An Y-STR haplotype comprising seven loci was constructed for each individual with the loci in the order DYS19; DYS389I; DYS389II; DYS390;

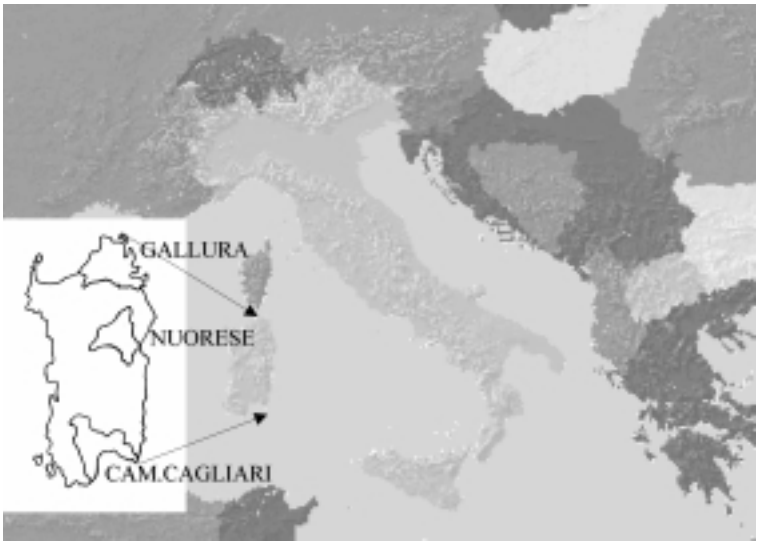

Fig. 1. Geographic location of the Sardinian samples.

DYS391; DYS392; DYS393. Gene diversity (D) at each given locus was computed as $D = 1- p_i^2$, where p_i is the allelic frequency. D is equivalent to the expected heterozygosity for diploid data. It is defined as the probability that two randomly chosen alleles are different in the sample[13]. Haplotype diversity (H) was computed with the same equation of gene diversity using haplotype frequencies instead of allele frequencies. In a pairwise analysis, allele frequencies at the 7 loci were compared between all pairs of the three Sardinian samples, by the Fisher exact test-based, genic comparison option included in GENEPOP[14]. We have been comparing allele frequency distribution between our sample and the available data from literature on Italian[15,16] European 15, 17–20 and Worldwide populations[21]. On the basis of the genetic distances according to Cavalli-Sforza[22], a neighbor-joining (NJ) tree was constructed by the PHYLIP version 3.2c program. Genetic relationship among the Sardinian and compared population were calculated using the Harpending and Jenkins[23] method. The eigenvalues and the eigenvectors of the R-matrix were extracted and the populations were located in a plot according to their scores for the first two eigenvectors. The genetic structure among our population samples and compared populations, on the seven-loci Y-STR haplotypes were analyzed with consideration for the molecular differences between individual haplotypes. In addition to differences in haplotype frequencies, resulting in estimates of st (Rst) values, a Fst analogue. Significance levels of the genetic–variance component, as well as st values were estimated by the use of 10,000 permutations, as implemented in Arlequin package[24].

Pairwise genetic distances between populations were computed as a linearization of the st values, as described in Kayser et al.[15]. On the basis of these adjusted st values, a neighbor-joining (NJ) tree was constructed by PHYLIP package and the resulting tree was visualized using TREEVIEW.

Results

The distribution of allele frequencies and gene diversity values for the 7 Y-chromosome specific microsatellites (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393), were estimated (Table 1). All loci tested were founded to be highly polymorphic and gene diversity values (D) computed for each Y chromosome microsatellite locus for all the three Sardinian samples showed different values from one sample to another (Table 1). It ranges from a minimum of 0.219 for DYS389I in Gallura whereas Nuorese's sample displayed the highest gene diversity value (0.793) for the DYS19 locus.

The Nuorese and Campidano of Cagliari samples showed a very high allele frequency in the DYS19*17, respectively 0.261 and 0.212 probably due to the founder effect, a rarity in the rest of Europe that shows a maximum value of 0.067 in Basques and a minimum value of 0 for the Swiss and 0.05 for the Italians[15].

With regards to the possible pairwise allele-frequency comparison between the Sardinian populations, there were no significant differences between the Nuorese and Campidano of Cagliari samples. In contrast allele frequencies for DYS19, DYS389I and II and DYS392 showed significant differences between Gallura and Nuorese, and for DYS19 and DYS389II between Gallura and Campidano of Cagliari (Table 2).

In order to investigate the genetic relationship between the three Sardinian samples and the compared population, genetic distance analysis was carried out according to Cavalli-Sforza[22] method. The values vary from a minimum of 0.008 (Hungarian-Germans to a maximum of 0.216 (Southern Corsica-Sahara). The genetic distances within Sardinia varied from a recorded maximum of 0.053 (Gallura-Nuorese) to the minimum value of 0.016 (Nuorese-Cam. Cagliari). Compared with the others populations, Sardinians showed the lowest genetic distance values (0.025) between Campidano of Cagliari and Bavarian, and the highest value (0.167) between Nuorese and Saharan people. A genetic tree (Figure 2) based on the matrix of the genetic distances in Table 3 was constructed with the neighbor-joining method after 100 repetitions. The tree shows a clear differentiation of the Sardinian samples from the other European population except for the South Corsican sample. The cluster that shows Sardinian populations is divided in two branches one with Nuorese and Campidano of Cagliari and the other with South Corsica and Gallura. The African North- West populations occupy an out-group position, and are located far away with respect to the European groups that cluster together. Inside the European cluster we can see a tight cluster containing the Italian, German, Bavarian, Swiss, Dutch, Hungarian and Tuscany population. The Spanish populations form a distinct cluster with the Corsican samples from Balagna and Corte respectively. Genetic variation within Sardinia and between compared populations, were also evaluated throughout the analysis of the R-matrix[23]. Analysis of the R-matrix reveals that the greatest contribution to variability seen from the population comparison is given by the Saharan populations, followed in the European populations by the Basques. In the Sardinian populations the greatest contribution to variability is given by the Nuorese sample (Table 3).

The first two eigenvector together accounts for 62.53% of the total variation. Individually, the first eigenvector accounts for 40.76% and the second for 21.77% of the total variation. The plot of the two eigenvector (Figure 3) shows that the first eigenvector clearly differentiates the North West African populations from the European and Mediterranean populations.

TABLE 1
ALLELE FREQUENCIES OF 7 STRS POLYMORPHISM IN THE SARDINIAN POPULATIONS

Markers	Alleles	Gallura	Nuorese	Cam. Cagliari
DYS19	13	0.2647	0.1522	0.1212
	14	0.3824	0.2174	0.2121
	15	0.2353	0.1957	0.3636
	16	0.0882	0.1739	0.0909
	17	0.0294	0.2609	0.2121
	N	34	46	33
	D	0.720	0.793	0.755
DYS389I	11	0.0303	0.0000	0.0000
	12	0.0000	0.2000	0.0833
	13	0.8788	0.7500	0.8333
	14	0.0909	0.0500	0.0833
	N	33	40	24
	D	0.219	0.395	0.292
DYS389II	27		0.0256	0.0500
	28	0.0606	0.3846	0.3500
	29	0.4242	0.2564	0.2000
	30	0.4242	0.3077	0.2500
	31	0.0606	0.0256	0.1500
	32	0.0303	0.0000	0.0000
	N	33	39	20
	D	0.632	0.690	0.750
DYS390	22	0.1250	0.1463	0.1600
	23	0.4583	0.4878	0.4000
	24	0.3333	0.2195	0.3200
	25	0.0833	0.1463	0.1200
	N	24	41	25
	D	0.656	0.671	0.698
DYS391	9	0.0769	0.0263	0.0312
	10	0.6538	0.8158	0.6562
	11	0.2692	0.1579	0.2812
	12	0.0000	0.0000	0.0312
	N	26	38	32
	D	0.494	0.309	0.488
DYS392	11	0.3600	0.8684	0.7083
	12	0.1600	0.0789	0.0417
	13	0.3200	0.0526	0.2083
	14	0.1600	0.0000	0.0417
	N	25	38	24
	D	0.717	0.237	0.451
DYS393	9	0.0000	0.0488	0.0400
	12	0.1905	0.2439	0.1200
	13	0.6190	0.5122	0.6400
	14	0.1905	0.1951	0.2000
	N	21	41	25
	D	0.544	0.638	0.534

N = number of the individuals; D = gene diversity according to Nei (1973)

TABLE 2
ANALYSIS OF DIFFERENTIATION, BY FISHER'S EXACT TEST.
PAIRWISE ALLELE-FREQUENCY COMPARISON BETWEEN SARDINIAN POPULATIONS

	Nuorese-Gallura	Nuorese-Cam. Cagliari	Gallura-Cam. Cagliari
DYS19	0.019	0.529	0.053
DYS389I	0.013	0.499	0.293
DYS389II	0.011	0.489	0.013
DYS390	0.748	0.815	1
DYS391	0.320	0.351	0.895
DYS392	0.000	0.11	0.098
DYS393	0.879	0.727	0.949

Fig. 2. Neighbor-joining tree for the Sardinian and other European and Worldwide populations.

The Sardinian samples are found at one end of the European population distribution, furthermore samples from the south of Corsica are show between the samples from Central and South Sardinia (Nuorese and Cam. Cagliari). Spanish and Basques lie at the other edge of the European cluster.

The Y haplotype frequency distribution in Sardinian samples were constructed from the seven Y STR loci studied (DYS19; DYS389I–II; DYS390; DYS391; DYS392; DYS393) (Table 4). From a total of 69 chromosomes with complete typing, 55 distinct haplotypes were obtained. The two most frequent haplotypes 4 (13–13–30–25–10–11–13) and 44 (16–13–28–24–10–11–13) were found in 5% Cam. Cagliari and 9% Nuorese and in 11% Cam. Cagliari and 6% Nuorese, respectively.

Haplotype 4 was only founded in 0.4% of the other European samples, while haplotype 44 was not present in the European and North African populations[15,21].

Nuorese shared 4 haplotypes with Cam. Cagliari and 2 with Gallura, on the other hand Gallura and Cam. Cagliari did not share any haplotypes.

In European samples the most frequent haplotype was 14–13–29–24–11–

TABLE 3
CAVALLI-SFORZA'S GENETIC DISTANCES (ABOVE THE DIAGONAL) AND R-MATRIX VALUES
(RII ON THE DIAGONAL AND RIJ BELOW THE DIAGONAL) AMONG SARDINIAN AND COMPARED POPULATIONS

	*GA*L	NUO	CAG	COS	COC	CNW	TOS	ITA	BAS	SPA	SVI	OLA	BAV	UNG	GER	ARA	BER	SAH	ALG
GAL	0.042	**0.053**	0.036	0.052	0.049	0.066	0.042	0.029	0.103	0.054	0.035	0.043	0.030	0.039	0.035	0.092	0.089	0.150	0.110
NUO	-0.016	**0.222**	0.016	0.068	0.104	0.120	0.073	0.038	0.164	0.120	0.060	0.050	0.037	0.035	0.040	0.091	0.106	0.167	0.118
CAG	0.013	0.046	0.070	0.061	0.072	0.106	0.053	0.028	0.128	0.080	0.039	0.036	0.025	0.027	0.027	0.092	0.105	0.162	0.116
COS	0.029	0.013	0.032	0.126	0.079	0.115	0.102	0.052	0.154	0.103	0.061	0.075	0.062	0.053	0.062	0.123	0.113	**0.216**	0.143
COC	0.015	-0.052	-0.009	-0.001	0.067	0.053	0.052	0.050	0.057	0.043	0.034	0.038	0.038	0.055	0.046	0.140	0.138	0.204	0.159
CNW	0.014	-0.057	-0.023	-0.015	0.042	0.094	0.062	0.063	0.047	0.047	0.064	0.058	0.064	0.077	0.074	0.121	0.136	0.197	0.152
TOS	0.016	-0.022	-0.002	-0.018	0.025	0.030	0.108	0.025	0.093	0.055	0.028	0.031	0.027	0.042	0.039	0.113	0.126	0.168	0.147
ITA	0.008	0.016	0.009	0.016	0.001	0.006	0.014	0.023	0.093	0.048	0.016	0.019	0.017	0.016	0.021	0.069	0.083	0.140	0.100
BAS	-0.015	-0.078	-0.041	-0.051	0.056	0.066	0.014	-0.013	0.129	0.042	0.081	0.080	0.091	0.097	0.093	0.121	0.147	0.193	0.155
SPA	-0.002	-0.066	-0.025	-0.034	0.034	0.042	0.006	-0.008	0.072	0.073	0.041	0.050	0.053	0.054	0.053	0.086	0.095	0.137	0.109
SVI	0.004	0.002	0.002	0.004	0.017	0.007	0.012	0.006	0.010	0.007	0.023	0.023	0.018	0.024	0.020	0.087	0.099	0.160	0.105
OLA	0.002	0.008	0.008	-0.003	0.024	0.024	0.018	0.009	0.020	0.009	0.012	0.042	0.016	0.025	0.022	0.095	0.114	0.179	0.129
BAV	0.009	0.009	0.014	0.010	0.015	0.011	0.014	0.005	-0.005	-0.004	0.010	0.016	0.030	0.012	0.011	0.096	0.099	0.156	0.122
UNG	-0.005	0.023	0.013	0.019	-0.006	-0.009	-0.007	0.007	-0.019	-0.012	0.002	0.004	0.012	0.025	0.008	0.073	0.078	0.132	0.092
GER	0.001	0.010	0.013	0.012	0.005	-0.007	-0.003	0.000	-0.012	-0.009	0.005	0.008	0.014	0.017	0.030	0.078	0.087	0.139	0.095
ARA	-0.029	0.003	-0.026	-0.025	-0.056	-0.045	-0.050	-0.017	-0.025	-0.020	-0.026	-0.039	-0.040	-0.015	-0.022	0.114	0.025	0.061	0.032
BER	-0.023	-0.020	-0.027	-0.019	-0.053	-0.049	-0.051	-0.023	-0.043	-0.024	-0.033	-0.047	-0.032	-0.013	-0.022	0.096	0.145	0.051	0.041
SAH	-0.037	-0.030	-0.041	-0.067	-0.065	-0.070	-0.042	-0.037	-0.036	-0.016	-0.040	-0.064	-0.044	-0.021	-0.024	0.116	0.143	**0.261**	0.075
ALG	-0.028	-0.010	-0.026	-0.027	-0.060	-0.059	-0.062	-0.023	-0.030	-0.022	-0.023	-0.051	-0.043	-0.012	-0.017	0.106	0.094	0.113	0.181

Gallura = GAL; Nuorese = NUO; Cam. Cagliari = CAG; Corsica South = COS; Corsica centre = COC ; Corsica north west = CNW; Tuscany = TOS; Italian = ITA; Basques = BAS; Spanish = SPA; Swiss = SVI; Dutch = OLA; Bavarian = BAV; Hungarian = UNG; German = GER; Arabes = ARA; Berbers = BER; Saharian = SAH; Algerian = ALG

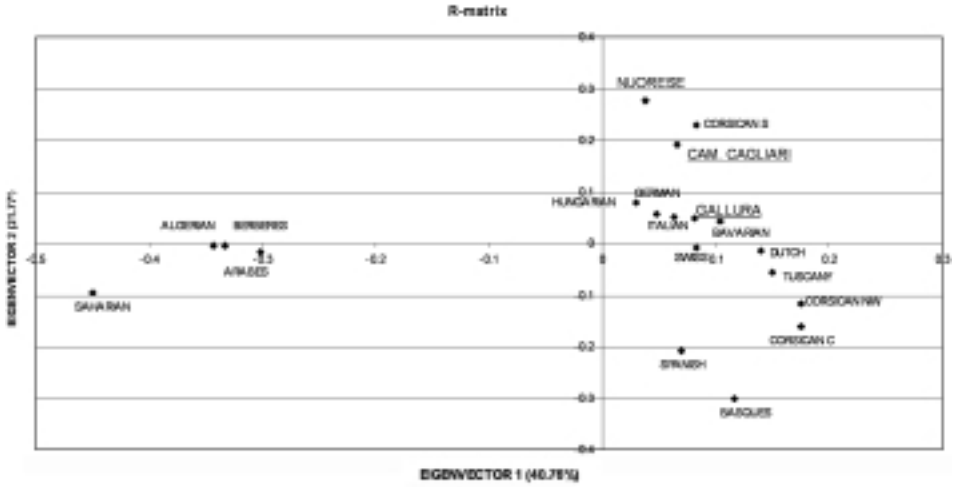

Figure 3. R-matrix analysis of the Sardinian and compared populations.

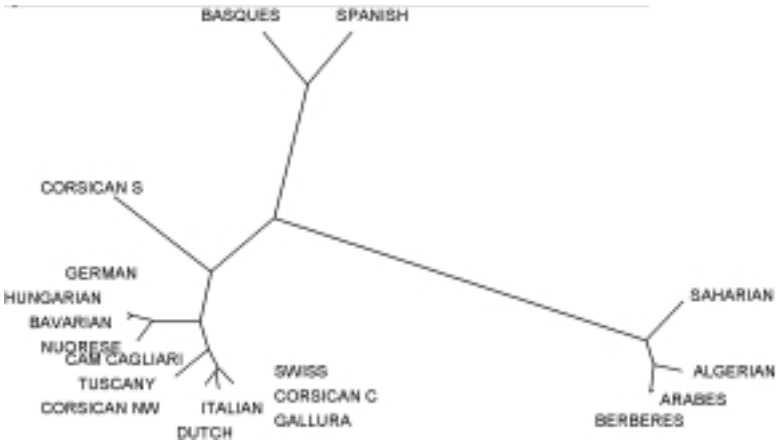

Fig. 4. Unrooted NJ tree, based on linearized Φst distances derived from AMOVA connecting Sardinian and comparison populations.

13–13 with 4.6% frequency, in Sardinia it was found that 5.5% of Gallura and 3% of Nuorese contained the same haplotype, however it was not present in Cam. Cagliari.

The st, molecular distances among microsatellite alleles are also taken in consideration, which are calculated as the square of the difference in repeat number. The matrix of pairwise st values is shown in Table 5. The highest Sardinian variance ($p<0.001$) was observed between the Gallura and Nuorese samples, instead Cam. Cagliari and Nuorese had a non-significant ($p>0.05$) st value. Nuorese showed with all compared populations significant values. The Gallura sample showed non-significant

TABLE 4
ABSOLUTE FREQUENCIES OF ALL SEVEN LOCUS Y-STR HAPLOTYPES AMONG SARDINIAN POPULATIONS

	Haplotype	Gallura	Nuorese	Cam. Cagliari	Total
1	13-13-29-22-10-14-13	1	...	...	1
2	13-13-29-24-10-12-13	1	...	...	1
3	13-13-30-24-10-12-13	...	...	1	1
4	13-13-30-25-10-11-13	...	3	1	4
5	13-13-30-25-10-11-14	...	2	...	2
6	13-13-32-25-10-12-13	1	...	...	1
7	13-14-31-24-10-11-13	...	1	1	2
8	14-12-28-24-10-11-14	...	1	...	1
9	14-12-29-23-11-11-13	...	1	...	1
10	14-13-28-23-10-11-13	...	2	...	2
11	14-13-29-23-10-11-12	...	1	...	1
12	14-13-29-23-11-13-12	1	...	...	1
13	14-13-29-23-11-13-13	1	...	...	1
14	14-13-29-24-10-13-14	1	...	...	1
15	14-13-29-24-11-13-13	1	1	...	2
16	14-13-29-24-11-14-12	1	...	...	1
17	14-13-29-24-11-14-13	1	...	...	1
18	14-13-29-24-12-13-13	...	...	1	1
19	14-13-30-23-10-11-12	...	3	...	3
20	14-13-30-24-10-13-13	1	...	...	1
21	14-13-31-22-10-11-13	...	...	1	1
22	14-14-30-23-11-13-13	...	...	1	1
23	14-14-30-24-10-13-13	1	...	...	1
24	15-12-27-23-10-11-13	...	1	...	1
25	15-12-29-23-10-11-14	...	...	1	1
26	15-12-30-22-9-12-13	...	1	...	1
27	15-13-28-22-11-11-14	...	1	...	1
28	15-13-28-23-10-11-12	...	1	...	1
29	15-13-28-23-10-11-13	...	1	...	1
30	15-13-28-24-10-13-13	...	...	1	1
31	15-13-29-23-10-11-13	...	1	...	1
32	15-13-29-23-10-12-13	1	...	...	1
33	15-13-29-23-10-13-13	...	...	1	1
34	15-13-29-23-11-13-12	1	...	...	1
35	15-13-29-24-10-11-13	...	1	...	1
36	15-13-30-22-9-11-14	...	...	1	1
37	15-13-30-22-11-11-14	...	...	1	1
38	15-13-31-23-10-12-14	1	...	...	1
39	15-13-31-25-11-11-13	...	...	1	1
40	15-14-30-22-10-11-14	1	...	...	1
41	15-14-30-22-11-11-14	1	...	...	1
42	16-12-29-23-10-12-14	...	1	...	1
43	16-13-28-23-10-11-13	1	1	...	2
44	16-13-28-24-10-11-13	...	2	2	4
45	16-13-29-22-10-11-12	...	1	...	1
46	17-12-27-23-10-11-13	...	...	1	1
47	17-12-29-22-10-11-14	...	1	...	1
48	17-13-28-23-10-11-9	...	...	1	1

49	17-13-28-23-10-11-12	…	1	1	2
50	17-13-28-24-10-11-12	…	1	…	1
51	17-13-28-24-10-11-13	…	…	1	1
52	17-13-29-23-10-11-13	1	…	…	1
53	17-13-29-23-11-11-12	…	1	…	1
54	17-13-30-22-11-11-12	…	1	…	1
55	17-13-30-23-11-13-14	…	1	…	1
Total					69
Haplotypes		18	26	17	
Individuals		18	33	18	

values with Central and North West Corsica, the Swiss, Dutch, and Bavarian samples. Cam. Cagliari showed non-significant values with the Italian, German, Bavarian, Hungarian samples. For the most of the pairwise population comparison the interpopulation differences (Table 5) were significant with 149 (87%) st values ($p<0.05$), 108 have high significant values ($p<0.001$), and 22 (13%) had non-significant st values. On the basis of the linearized st distances, an NJ tree was drawn (Figure 4). When the tree topology and the st significance (Table 5) are compared, the NJ tree provides a reasonable »fit« with most of the non-significant population, differences corresponding to the tight clusters, as Cam. Cagliari has done with Nuorese, Bavarians, Hungarians and Germans; Gallura with Central Corsica, Swiss, Italian and Dutch.

The dendrogramm constructed by linearized Rst value shows the differentiation of different branches for the North African, Basques, Spanish and Southern Corsica populations. The tree constructed from haplotype frequencies showed that besides the Sardinian samples, it confirms a differentiation between the Center-South to Gallura (Northern Sardinia).

Discussion

The population of Sardinia is known for its particular anthropological and genetic characteristics which tend to differentiate it, not only from the population of mainland Italy, but also from the other European populations. It is also possible to recognize regional differences within the Sardinian population itself in regards to the genetic structure, or in particular local characteristics[25,26]. This interesting situation is firstly due to the probable heterogeneity of the original Sardinian population, documented by studies on the ancient population of Sardinia, determined by complex historical events and cultural influences which have prevailed on the island in the course of time. This is accompanied by the isolation of the whole region from mainland, as well as areas and villages in inland Sardinia, due to the geographical terrain boundaries of the territory and cultural and linguistic barriers[27].

In the present study 7 Y-chromosome STR loci were typed in three Sardinian regions.

The Y is unique under many aspects. It is always in the haploid state, is full of repeated sequences but it is responsible for important biological roles such as sex determination and male fertility. Moreover, the Y chromosome is a powerful tool to study human populations and evolutionary pathways. The non-recombining portion of the Y retains a record of the mutational events that have occurred along male lineages throughout evolution. This is due to holoandrically trans-

TABLE 5
LINEARIZED $_{ST}$ DISTANCES (ABOVE THE DIAGONAL) AND ST VALUES
(BELOW THE DIAGONAL) AMONG SARDINIAN AND COMPARED POPULATIONS

	GAL	NUO	CAG	COS	COC	CNW	TOS	ITA	BAS	SPA	SVI	OLA	BAV	UNG	GER	ARA	BER	SAH	ALG
GAL	*	0.203	0.086	0.161	0.000	0.053	0.046	0.021	0.067	0.181	0.000	0.012	0.027	0.052	0.063	0.308	0.521	0.619	0.508
NUO	**0.1690**	*	0.000	0.114	0.289	0.278	0.257	0.072	0.570	0.754	0.127	0.138	0.064	0.045	0.068	0.265	0.415	0.655	0.507
CAG	0.0792	-0.0262	*	0.080	0.154	0.171	0.178	0.037	0.362	0.570	0.067	0.089	0.013	0.001	0.012	0.250	0.405	0.560	0.484
COS	0.1387	0.1026	0.0740	*	0.254	0.311	0.334	0.069	0.497	0.449	0.108	0.191	0.117	0.072	0.120	0.179	0.307	0.482	0.383
COC	-0.0171	**0.2244**	0.1335	**0.2025**	*	0.089	0.080	0.078	0.054	0.170	0.004	0.053	0.047	0.078	0.062	0.403	0.629	0.724	0.562
CNW	0.0503	**0.2177**	0.1457	**0.2374**	0.0820	*	0.000	0.033	0.099	0.185	0.019	0.069	0.046	0.055	0.077	0.243	0.456	0.544	0.387
TOS	0.0443	**0.2041**	**0.1514**	**0.2502**	**0.0737**	-0.0026	*	0.071	0.102	0.281	0.052	0.051	0.075	0.114	0.134	0.334	0.516	0.600	0.473
ITA	0.0210	**0.0670**	0.0353	0.0644	0.0720	0.0318	**0.0665**	*	0.180	0.268	0.008	0.024	0.018	0.020	0.051	0.135	0.255	0.374	0.274
BAS	0.0631	**0.3629**	**0.2660**	**0.3319**	0.0510	0.0898	**0.0922**	**0.1522**	*	0.090	0.100	0.204	0.164	0.181	0.177	0.503	0.754	0.739	0.607
SPA	**0.1532**	**0.4299**	**0.3632**	**0.3096**	**0.1450**	**0.1559**	**0.2194**	**0.2115**	**0.0824**	*	0.194	0.368	0.275	0.265	0.291	0.478	0.633	0.555	0.541
SVI	-0.0154	**0.1129**	0.0627	**0.0971**	0.0041	0.0185	**0.0494**	0.0082	**0.0905**	**0.1623**	*	0.007	0.013	0.032	0.040	0.190	0.320	0.412	0.318
OLA	0.0123	**0.1216**	0.0819	**0.1607**	0.0501	0.0646	**0.0486**	0.0234	**0.1692**	**0.2689**	0.0070	*	0.029	0.072	0.086	0.311	0.497	0.680	0.493
BAV	0.0264	0.0597	0.0131	**0.1050**	0.0447	0.0436	**0.0695**	0.0175	**0.1406**	**0.2154**	0.0127	0.0280	*	0.005	0.009	0.229	0.356	0.475	0.362
UNG	0.0498	0.0429	0.0006	0.0675	0.0727	0.0519	**0.1026**	0.0198	**0.1530**	**0.2092**	0.0308	**0.0670**	0.0045	*	0.000	0.158	0.257	0.347	0.276
GER	0.0589	0.0637	0.0115	**0.1073**	0.0585	0.0714	**0.1179**	**0.0487**	**0.1504**	**0.2254**	0.0386	**0.0795**	0.0086	0.0003	*	0.215	0.323	0.414	0.321
ARA	**0.2355**	**0.2095**	**0.2002**	**0.1516**	**0.2870**	**0.1958**	**0.2506**	**0.1188**	**0.3347**	**0.3236**	**0.1597**	**0.2373**	**0.1866**	**0.1362**	**0.1771**	*	0.005	0.089	0.029
BER	**0.3424**	**0.2934**	**0.2881**	**0.2348**	**0.3861**	**0.3130**	**0.3405**	**0.2032**	**0.4299**	**0.3876**	**0.2425**	**0.3322**	**0.2624**	**0.2042**	**0.2441**	0.0047	*	0.040	0.046
SAH	**0.3824**	**0.3959**	**0.3589**	**0.3250**	**0.4200**	**0.3522**	**0.3751**	**0.2723**	**0.4250**	**0.3567**	**0.2918**	**0.4048**	**0.3220**	**0.2574**	**0.2926**	0.0816	0.0383	*	0.083
ALG	**0.3368**	**0.3364**	**0.3259**	**0.2767**	**0.3596**	**0.2790**	**0.3209**	**0.2147**	**0.3779**	**0.3509**	**0.2410**	**0.3303**	**0.2658**	**0.2163**	**0.2431**	0.0285	0.0441	0.0768	*
H	1.0000	0.9830	0.9935	0.9852	0.9710	0.9762	0.9923	0.9941	0.9379	0.9671	0.9921	0.9830	0.9913	0.9952	0.9963	0.9207	0.9657	0.8670	0.8404

H = haplotype diversity. Underlined values are significant at the p<0.05 level; Boldface values are significant at the p<0.001 level

mission from father to son, without recombination at meiosis. Thus, the study of the different mutations this molecule has accumulated along its evolution may be highly informative in deducing the histories of human populations[8]. Due to their relatively high mutation rate, Y STRs are potentially polymorphic in all human populations and allow the human migration process to be traced on a historical timescale. On the other hand, because Y SNPs have a 100,000 times lower mutation rate, they are ideal for the study of human migration at an evolutionary, rather than a historical timescale. As has been suggested elsewhere[28] it will consist of a dual approach, using Y STRs as well as Y SNPs, to render the maximum amount of information.

The analysis of the frequencies was primarily aimed at evaluating the island's internal variability and secondly to study the genetic relationship between Sardinia population and the other European populations.

Comparison of allelic frequencies showed significant differences between North of Sardinia (Gallura) and the other two groups. As well as Scozzari et al.[29], it has been observed that Gallura occupies an intermediate position between Corsica and Sardinia, much like a reflection of the geographical location or the language spoken by its inhabitants. A similar result was also obtained by mtDNA analysis and interpreted as being due to a recent settling event from continental Italy, involving both Corsica and Gallura[30].

The genetic variation of Y STR haplotypes in the Sardinian populations are reflected in the haplotype diversity values; 0.983 in Nuorese, 0.9935 in Cam. Cagliari and 1.000 in Gallura. The lower value of Nuorese may be explained by their isolation and inbreeding. Lower haplotype diversity values are often observed in samples from small and isolated populations (Kayser et al., 2001). On the other hand the high value founded in Gallura can be simply due to a small sample size (n=18) rather than a high genetic variation within the sample. In the comparison, populations haplotype diversity varies from 0.8404 in the Algerian to 0.9963 in the German samples.

The genetic trees drawn from the 7 STRs loci allele frequencies and linearized values and the analysis of the R-matrix agree in highlighting the extent to which the Sardinians are differentiated in the comparison with other Euro-Mediterranean populations. Many studies have established numerous genetic, demographic and linguistic peculiarities of Sardinians[3]. The long period of prehistoric and historic isolation of Sardinia caused significant genetic differentiation between Sardinians and other European and Mediterranean populations, as well as between geographical and cultural areas of the island itself. Genetic drift and isolation from outside countries, together with the relatively small amount of internal migration led to the differentiation and the independent evolution of the Sardinian cantonal areas, which differ from a historical, cultural and linguistic point of view[27].

In a previous work Caglià et al.[34] concluded that a remarkable genetic diversity exists between continental Italy and Sardinia, as a consequence of the long lasting genetic isolation of the island. Distinctive patterns of allelic association found in the Sardinian and Italians unambiguously indicates that the Sardinian Y haplotype combination had to have undergone large and rapid variation in frequency. Scozzari et al.[29] found in Sardinian a haplogroup (HG2.2) that is common only to this island and they estimated it arrived 9,000 years ago from Western Europe at the time of the first human settlement on the island. Alternatively it could have originated in situ after the settling of the island. In any case, its rarity out-

side the island confirms the genetic peculiarity and the isolation of the Sardinians in the framework European variation[29]

Besides Nuorese and Cam. Cagliari samples showed a very high DYS19*17 allele frequency, a rarity in the rest of Europe. When we compared the Sardinian haplotypes, that carry the DYS19*17, to the populations of this work and with the forensic Y-STR database (http://ystr.charite.de/index_gr.html), no haplotype observed was shared except for 17–13–28–24–10–11–13, which was found in 3 individuals in Europe. We can therefore conclude that the distribution of the DYS19*17 in Sardinia can be due to the founder effect.

The results reported in this work showed that of the Euro-Mediterranean populations, the Corsican of the South seems to have the most genetic affinity with the Sardinians, thereby reaffirming the observations from previous works[31–33] which had suggested a certain level of genetic similarity between the populations of the two islands; a similarity resulting from a common initial peopling and the constant exchange of culture and individuals which accomplished far more than a geological phases which lead to the dividing of the Corsican-Sardinian mass.

From the tree constructed with the linearized st values, it is possible to see an affinity between Center-South Sardinia (Nuorese and Cam. Cagliari), Germans, Bavarians and the Hungarian populations. Due to the relatively high mutation frequency of STRs, it is difficult to say what proportion of STRs haplotype sharing is due to recurrent mutation and what proportion is due to a genuine shared common ancestry.

The results of the linearized st value tree were not perfectly equivalent to that of the tree constructed from the allele frequencies. Moreover culture, history and geography provide an explanation to the tree constructed from the allelic frequencies.

In conclusion, this study has confirmed the usefulness of Y-chromosome microsatellite polymorphisms to analyze the genetic structure of geographically closed populations and their use in micro-evolutionary studies, by highlighting internal variability within Sardinia and Corsica. In order to better clarify such relationship, Y chromosome microsatellite haplotypes combined with unique event polymorphism analysis may certainly be of great interest.

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VARIJABILNOST MIKROSATELITA KROMOSOMA Y U UZORKU STANOVNIŠTVA SARDINIJE (ITALIJA)

S A Ž E T A K

Ispitana je genetička varijabilnost sedam mikrosatelitskih lokusa na kromosomu Y kod 113 sardinijskih muškaraca iz regija: Campidano iz Cagliarija, Nuorese i Gallura. Uočene raspodjele učestalosti alela i haplotipova uspoređene su s onima dostupnim iz literature drugih mediteranskih i europskih populacija. Usporedba je pokazala u uzorku iz Sardinije vrlo visoku učestalost alela DYS19*17, čija je učestalost u ostalim europskim populacijama niska, a njegovo prisustvo u populaciji Sardinije vjerojatno treba pripisati učinku utemeljitelja. Analiza je pokazala unutar populacijsku genetičku heterogenost i genetičku različitost od drugih uspoređivanih mediteranskih i europskih populacija. U ovom radu prikazani rezultati su pokazali da od euro-mediteranskih populacija najveće genetičke sličnosti s populacijom Sardinije ima stanovništvo južnog dijela Korzike, što potvrđuje rezultate prethodnih istraživanja koja su ukazala na određeni stupanj genetičke sličnosti.