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GENETIC STRUCTURE OF TUNISIAN ANDALUSIAN COMMUNITIES REVEALED BY UNIPARENTAL MARKERS

ABSTRACT: Andalusian groups lived in typical villages in the north of Tunisia. The settlement of the Moor after the later wave of massive migration from Iberian Peninsula (1609–1614) resulted in the formation of these communities. The genetic relationship of these communities to North African and Iberian population was analysed in this paper through the typing of the HVSI region of mtDNA and 7 YSTR in four principal Andalusian villages (Testour, Slouguia, Qalaat El Andaleus and El Alia).

Statistical analysis (Fst distance, AMOVA and PCA) from the data of both markers (mtDNA and 7YSTR) revealed a North African structure concerning these populations. The analysis of mtDNA haplogroups in every population shows a remarkable contribution of sub-Saharan and North African lineages (U6a) as well as Eurasian ones. The distribution of "Iberian" haplogroups (H1, V...) does not reflect the cultural criteria. The typical Andalusian village (Testour) lacks these lineages. However, its neighbouring village (Slouguia) undergoes an Iberian print attested by a higher frequency of these haplogroups. Less frequency of these types was observed in El Alia and Qalaat el Andaleus. These results were globally in accordance with a little contribution of Iberian men and women in the present genetic structure of Andalusian communities. This conclusion was explained by historical data of Moor literature in Tunisia.

KEY WORDS: Tunisia – Andalusian communities – mtDNA – YSTRs

INTRODUCTION

Tunisia, like the other countries of North Africa, has been settled since the Palaeolithic by Iberomaurisian (Newman 1995), then came the Capsians. Most of this

archaeological trace of civilisation is found around Gafsa, a town located in southern Tunisia. In fact, Berber civilisation has been expanded uniformly in Tunisia since the Neolithic and continued until the beginning of historical time with the settlement of several civilisations:

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Phoenicians, Romans, Vandals and Byzantines. Other demographic events characterize Tunisian population history: Arabic expansion along with Islamic conquest (7th AC) and Bedouin invasion (11th AC) which deeply influenced Berber populations as far as language, customs and demography are concerned (especially with Bedouin invasion). In recent times, the most important population movement from Iberia to North Africa was manifested in the expulsion of the Moor following Grenada capitulation (1492 AC). Tunisia received a large number of immigrants estimated to be over than 80000 Andalusians (Abdulwahab 1917). They settled in north of Tunisia in three principal regions: the Cap Bon Peninsula, near Medjerda River and around Bizerta town (Figure 1).

Andalusian villages, situated in the north of Tunisia, were typical and similar to ancient Arabic towns in Spain (Latham 1957). Many archaeological and cultural prints were clearly exogenous like houses and mosques architecture (Marcais 1942, Saadaoui 1990),

arabo-andalusian music (Marzouki 1994), weddings clothes and culinary tradition (Skhiri 1968, 1969, De Epelza 1980). Andalusian villages witnessed many demographic changes such as population decrease caused by epidemics and immigrations (Hamrouni 2000) as well as the settlement of other groups. Andalusians, however, were not mixed to autochthonous until the nineteenth century. Nowadays, Andalusian families are identified in their villages by their original Andalusian names which have been deviated to Arabic ones like Alakanti, Arrouich, El Bantour, Cherkinou, Dana, Dermoul, Filiboû, Jbis, Jhin, Jourji, El Kandi, Kristo, landil, Lichou, Makarirou, Markikou, Markou, Menara, Merrichkou, Metchinech, Midîna, Ranyoûn, Sirisou, Tabarinou, Taloûcha, Tribilia, Zafrân (Marcais 1942, Limam 1981).

Meanwhile, many genetic studies have been carried out in the North as well as the South coast of the Mediterranean Sea (Bosch *et al.* 2000a, Comas *et al.*

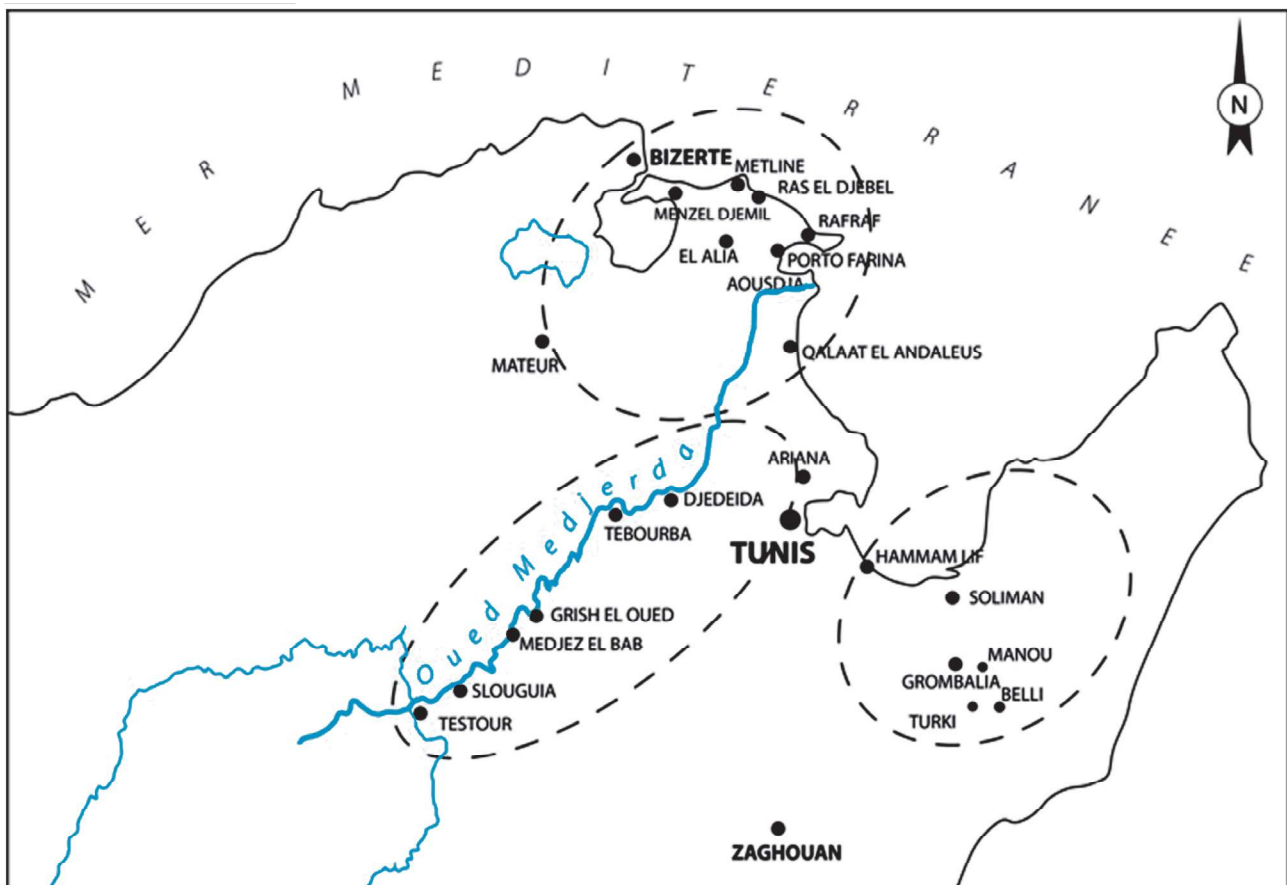


FIGURE 1. Localities of Andalusians insertions in North of Tunisia.

2000, Plaza *et al.* 2003), namely in the Iberian Peninsula and North western Africa. The result of these studies shows a different genetic structure between Iberian North African populations the present day genetic structure. This implies that Gibraltar strait has acted as a strong barrier to genetic flows in both directions. This conclusion was supported by different marker studies (Alu insertion Autosomal STRs, YSTRs, mtDNA).

Y chromosome and mitochondrial DNA (mtDNA) polymorphisms are particularly suitable for population genetic studies since they share the special state of haploidy, lack of recombination, uniparental inheritance and reduction in effective population size relative to autosomes. Important inferences concerning population origins and movements are made possible by simultaneous study of Y chromosome and mtDNA. Consequently, they give complementary information about male (Y chromosome) and female (mtDNA) lineages (Torroni *et al.* 1996, Macaulay *et al.* 1999, Kaysar *et al.* 2001, Salas *et al.* 2002, Jobling, Tyler-Smith 2003, van Oven M and Kayser M 2009, Karafet *et al.* 2008). MtDNA and Ychromosome haplogroups are potentially informative about the matrilineal and patrilineal origin respectively in a given population because of their ethno-geographical specificities. This idea can lead us to classify mtDNA lineages into three major groups: Eurasian H, V, R, U, J, T, I, X, W) sub-Saharan (L1, L2, L3, L4, L5, L6) and North African haplogroup (U6 and M1) (Torroni *et al.* 1996, Watson *et al.* 1997, Rando *et al.* 1998, Macaulay *et al.* 1999, Richards *et al.* 2000, Salas *et al.* 2002, Rosa *et al.* 2004, van Oven M and Kayser M, 2009).

Y chromosome lineages are defined by two types of markers: SNP and INS/DEL markers whose combination define haplogroups with ethno-geographical specificities on one hand and YSTR on the other hand with more rapid evolution and that are used to define haplotypes within a given haplogroup.

In order to evaluate the contribution of recent flows from the Iberian Peninsula to the genetic structure of Andalusian communities, we have analysed mtDNA HVSI sequences and 7Y-STR in four Andalusian communities situated in the North of Tunisia.

MATERIALS AND METHODS

Samples

Samples were obtained from unrelated Andalusian samples distributed in four principal Andalusian communities in the North of Tunisia (Testour: 40 samples, Slouguia 25, Qalaat El Andaleus 33, El Alia 45). This

sampling was based upon a patronymic criterion. This study was conducted after receiving ethics approval by the local health authorities. Informed consent was obtained from all participants before blood sampling. Principles of confidentiality were strictly applied during all process.

Total DNA was extracted from fresh blood using standard Phenol-Chloroform protocol.

mtDNA HVSI region sequencing

HVSI region of mtDNA was amplified through the use of specific primers: L15996 5'-CTCCACCATTAGCACCCAAAGC-3' and H16401 5'-TGATTTCACGGAGGAGGATGGTG- (Vigilant *et al.* 1989). The amplified product was purified with QIAGEN kit. The sequencing reaction was performed using the big day Terminator cycle sequencing kit with ampli taq DNA polymerase (Applied Biosystems). The sequencing products were run in an automatic Sequencer ABI377 (Applied Biosystems).

Phylogenetic analysis

Sequence alignment was performed using the CLUSTAL program. Each HVSI sequence was assigned to a given haplogroup in comparison to other published data related to other population studies (Torroni *et al.* 1996, Watson *et al.* 1997, Rando *et al.* 1998, Macaulay *et al.* 1999, Richards *et al.* 2000, Salas *et al.* 2002, Rosa *et al.* 2004).

Statistical analyses were carried out using the Arlequin 2000 software package. Population genetic structure and relative affinities between populations were assessed using the Analysis of MOlecular VARIances (AMOVA) (Excoffier *et al.* 1992). Genetic distances between populations using the HVSI region of mtDNA were calculated by the following equation:

$$D = d_{ij} - (d_{ii} + d_{jj}) / 2$$

- d_{ij} is the distance between population i and j.
- d_{ii} and d_{jj} are the intra-population distance.

In addition, we used the formula: $I = H_{xy} / \sqrt{H_x H_y}$ (Mayer *et al.* 2003) as an estimation of identity (I) between populations (where H_{xy} is the number of haplotypes shared between populations x and y, H_x is the number of different haplotypes present in population x, and H_y is the number of different haplotypes present in population y).

In this measure, it is supposed that selective forces are negligible, and that the relative similarity between populations is only due to the counteracting forces of gene flow and genetic drift due to founder effects and different demographic histories. We also used the Principle Coordinate Analysis (PCA) to visualise the statistical positions of populations compared to their geographical positions.

YSTR analysis

We reported the results published in Cherni *et al.* 2005a, in which 11YSTR were typed. In this paper, we only consider 7 YSTRs (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393), which are mostly investigated in Eurasian populations. For this analysis we used more statistical parameters like the number of shared haplotypes, Fst genetic distances, AMOVA and PCA.

RESULTS***MtDNA diversity***

As described in *table 1*, genetic diversity parameters in Andalusian populations are not significantly distinct from the other populations. Considering MPD value, three populations (Slouguia, Qalaat el Andaleus and Al

Alia) have European profile. For Testour population, however, this parameter seems to be an important indicator of North African profile.

For the number of shared haplotypes and similarity indexes (I), reported figures are similar for both Tunisian Andalusians with Iberian Peninsula as well as with other populations from North Africa. However, some statistics make a link between Tunisian Andalusians and North West African populations, namely Berbers and Arabs from Morocco (*Table 2*).

mtDNA Haplogroups composition

Haplogroups frequency is presented in *table 3*. The four mentioned populations have a variable frequency of Eurasian haplogroups. Lowest and highest frequencies of Eurasians are found in Testour (50%) and Slouguia/ El Alia (68%) respectively. Qalaat El Andaleus has an intermediate frequency of these lineages (57%).

TABLE 1. Diversity parameter reported in Tunisian, North African and Iberian populations. SE; stands for standard error. ^a Mean Pairwise Difference.

Populations	Number of samples	Number of haplotypes (%)	Number of polymorphic sites (%)	Gene diversity (\pm SE)	M.P.D ^a	References
<i>Tunisian Andalusians</i>						
Testour	40	29 (72.5)	52 (14.4)	0.958 \pm 0.0272	6.266	Present study
Slouguia	24	19 (79.2)	33 (9.1)	0.978 \pm 0.0187	5.163	Present study
Qalaat El Andaleus	33	21 (63.6)	29 (8.0)	0.960 \pm 0.0184	5.326	Present study
El Alia	45	26 (57.8)	41 (11.8)	0.957 \pm 0.0180	5.428	Present study
<i>Iberian Peninsula</i>						
Portugal	241	144 (59.8)	97 (26.9)	0.959 \pm 0.010	4.617	Pereira <i>et al.</i> 2000
Galicia	92	53 (57.6)	56 (15.6)	0.930 \pm 0.023	3.112	Crespillo <i>et al.</i> 2000
Spain	118	81 (68.6)	57 (15.8)	0.969 \pm 0.011	4.130	Salas <i>et al.</i> 1998
Andalusia	50	40 (80)	54 (15)	0.979 \pm 0.0128	5.109	Mayer <i>et al.</i> 2003
Cantabria	88	51 (57.9)	52 (14.4)	0.964 \pm 0.0106	3.514	Mayer <i>et al.</i> 2003
Castile	38	29 (76.3)	35 (9.7)	0.964 \pm 0.0213	3.947	Mayer <i>et al.</i> 2003
Lebanigos	71	37 (51.3)	44 (12.2)	0.928 \pm 0.0233	3.784	Lagurra <i>et al.</i> 2001
Leonese	62	42 (68.8)	52 (14.4)	0.932 \pm 0.0279	3.824	Lagurra <i>et al.</i> 2001
Pasiegos	81	29 (35.3)	33 (9.1)	0.944 \pm 0.0114	3.800	Lagurra <i>et al.</i> 2001
<i>Northern Africa</i>						
Algeria	86	31 (36.0)	37 (10.3)	0.945 \pm 0.010	4.882	Corte Real <i>et al.</i> 1996
Bedouin	29	27 (93.1)	51 (14.2)	0.995 \pm 0.011	7.512	Di Rienzo, Wilson 1991
Egypt	68	59 (86.8)	71 (19.7)	0.993 \pm 0.005	7.075	Krings <i>et al.</i> 1999
Mauritania	30	23 (76.7)	30 (8.3)	0.975 \pm 0.017	6.025	Rando <i>et al.</i> 1998
Morocco Berber	60	38 (63.3)	48 (13.3)	0.963 \pm 0.015	4.594	Pinto <i>et al.</i> 1996
Morocco Non Berber	32	29 (90.6)	46 (12.8)	0.988 \pm 0.014	6.026	Rando <i>et al.</i> 1998
Souss	50	34 (68.0)	38 (10.6)	0.961 \pm 0.018	4.604	Brakez <i>et al.</i> 2001
Tuareg	23	21 (91.3)	40 (11.1)	0.992 \pm 0.015	6.838	Watson <i>et al.</i> 1997
Western Sahara	25	20 (80.0)	31 (8.6)	0.973 \pm 0.022	5.340	Rando <i>et al.</i> 1998, Plaza <i>et al.</i> 2003

TABLE 2. MtDNA shared haplotypes between Tunisian Andalusians, North African and Iberians populations. TR Testour, AL El Alia, QL Qalaat El Andaleus, SL Slouguia, CN Cantabria, CS Castille, LB Lebanigos, LO Leonese, PS Pesigos, GL Galicia, PT Portugal, AD Andalousia, BS Basque, CT Catalan, MZ Mzab, TG Tuareg, MA Morocco Arabs, MB Morocco Berbers, EG Egypt, SH West Sahara. Under the diagonal is the number of shared haplotypes between populations. Diagonal represent different haplotypes characterized in these populations. Above the diagonal indices of similarity calculated from the two last data.

	TR	AL	QL	SL	CN	CS	LB	LO	PS	GL	PT	AD	BS	CT	AG	TG	MA	BR	AR	EG	SH
TR	29	0.131	0.121	0.085	0.104	0.068	0.061	0.057	0.068	0.076	0.077	0.029	0.071	0.071	0.067	0.122	0.116	0.12	0.069	0.048	0.077
AL	2	26	0.213	0.179	0.082	0.109	0.096	0.121	0.109	0.161	0.114	0.062	0.113	0.111	0.106	0.086	0.082	0.159	0.073	0.051	0.081
QL	3	5	21	0.15	0.061	0.04	0.036	0.101	0.081	0.03	0.018	0.035	0.042	0.082	0.039	0.143	0.091	0.071	0.041	0.028	0.03
SL	2	4	3	19	0.064	0.043	0.064	0.071	0.128	0.063	0.057	0.109	0.132	0.043	0.124	0.1	0.096	0.112	0.128	0.03	0.126
CN	4	3	2	2	51	0.03	0.16	0.19	0.25	0.25	0.14	0.36	0.22	0.11	0.126	0.031	0.088	0.182	0.052	0.091	0.096
CS	2	3	1	1	1	29	0.122	0.143	0.069	0.179	0.155	0.147	0.143	0.07	0.122	0.041	0.194	0.181	0.034	0.097	0.102
LB	2	3	1	2	7	4	37	0.152	0.183	0.158	0.123	0.104	0.158	0.062	0.059	0.036	0.069	0.24	0.061	0.043	0.045
LO	2	4	3	2	9	5	6	42	0.172	0.17	0.154	0.146	0.238	0.175	0.083	0.067	0.129	0.175	0.115	0.079	0.085
PS	2	3	2	3	9	2	6	6	29	0.128	0.108	0.147	0.107	0.105	0.067	0.041	0.116	0.151	0.069	0.024	0.026
GL	3	6	1	2	12	7	7	8	5	53	0.137	0.174	0.211	0.182	0.099	0.03	0.172	0.178	0.128	0.102	0.075
PT	5	7	1	3	12	10	9	12	7	12	144	0.092	0.112	0.173	0.105	0.037	0.087	0.122	0.124	0.108	0.08
AD	1	2	1	2	15	5	4	6	5	8	7	40	0.183	0.09	0.057	0.035	0.033	0.077	0.059	0.144	0.022
BS	2	3	1	3	8	4	5	8	3	8	7	6	27	0.218	0.104	0.042	0.04	0.094	0.071	0.025	0.079
CT	2	3	2	1	4	2	2	6	3	7	11	3	6	28	0.102	0.034	0.079	0.092	0.07	0.074	0.104
AG	2	3	1	3	5	4	2	3	2	4	7	2	3	3	31	0.039	0.15	0.204	0.1	0.094	0.222
TG	3	2	3	2	1	1	1	3	1	1	2	1	1	1	1	21	0.137	0.071	0.041	0.028	0.12
MA	3	2	2	2	3	5	2	4	3	6	5	1	1	2	4	3	23	0.271	0.116	0.109	0.115
BR	4	5	2	3	8	6	3	7	5	8	9	3	3	3	7	2	8	38	0.181	0.106	0.245
AR	2	2	1	3	2	1	2	2	2	5	8	2	2	2	3	1	3	6	29	0.121	0.102
EG	2	2	1	1	5	4	2	4	1	4	7	1	3	4	4	1	4	5	5	59	0.107
SH	3	3	1	4	7	6	3	4	4	7	11	3	3	3	9	4	11	11	4	6	53

Eurasian haplogroups in Testour population are essentially represented by the European haplogroup H (35.8 %). Remainder lineages are represented by other Near Eastern haplogroups (J, T, I and K) (14.4%). We also notice the lack of South-Western European lineages in this population (H1, V) (Torroni *et al.* 1998). However, its neighbouring village (Slouguia) has a higher frequency (32%) of South-Western European lineages (H1, V, R* and R1). El Alia population has, in addition to European haplogroups H (28.8) and U5b (2.2%) other South-Western European haplogroups (H1, V) but with less frequency (6.6%). The remainder are represented by Near Eastern originated lineages (J, T, W and R0a) (28.8%). In addition to H haplogroup (27.2%), Qalaat El Andaleus presents a weak contribution of "Iberian" haplogroups (V) (3%), remainder lineages are presented by Near Eastern lineages (J, T, I, U4, R0a and X) (27%).

For sub-Saharan haplogroups, variable frequency is observed in Andalusian populations. The highest

frequency of sub-Saharan lineages (40.6%) is found in Testour population. Whereas frequency of these haplogroups in the other populations is clearly low (the lowest frequency is in El Alia population (11%)).

North African lineages are mostly represented in Al Alia population (11%). In the other populations it does not exceed 5% (Qalaat El Andaleus 3%, Slouguia 4% and Testour 5%). Compared to several populations from the Iberian Peninsula like isolates (Maragatos, Pasiegos, Lebanigos, Leonese) and Cosmopolitan (Spain, Andalusia, Portugal), Tunisian Andalusians differ from these populations in terms of the over presence of North African and sub-Saharan lineages. The distribution of haplogroups among Tunisian Andalusians is quite similar to North African population (Figure 2).

Genetic distances

Fst distances between Tunisian Andalusians and other populations from North Africa and Western Europe are

TABLE 3. Distribution of Eurasian, sub-Saharan and North African mtDNA haplogroups among Tunisian Andalusians.

Haplogroups	Testour	Slouguia	Q. Andaleus	El Alia
	40	25	33	45
L3b	0.025	-	-	-
L3b1	0.025	-	-	-
L3b2	-	-	0.03	-
L3d	-	0.04	-	-
L3e1a	0.025	-	-	-
L3e2b	-	-	0.03	-
L3e5	-	-	-	0.022
L3f	0.05	0.04	-	-
L3f1	-	0.04	-	-
L3*	0.025	-	-	-
L2a	0.077	0.04	0.03	0.022
L2a1	0.077	-	0.03	-
L2c	-	0.04	-	-
L2c1	0.025	-	-	-
L2c2	-	-	-	-
L1b1	0.077	-	0.03	0.066
Sub-Saharan Haplogroups	0.406	0.2	0.15	0.11
H	0.358	0.12	0.272	0.288
H1	-	0.04	-	0.044
V	-	0.2	0.03	0.022
R0a	-	-	0.06	0.044
R*	-	0.04	-	-
R1	-	0.04	-	-
J	0.025	0.04	-	-
J2	0.025	-	-	0.044
T	0.025	0.12	0.09	0.09
T1	0.025	0.08	-	0.066
T2	-	-	-	-
K	0.025	-	-	-
U4	-	-	0.06	-
U5a	-	-	-	0.044
U5b	-	-	-	0.022
I	-	-	0.03	-
X	0.025	-	0.03	-
W	-	-	-	0.022
Eurasian Haplogroups	0.508	0.68	0.572	0.686
U6a	0.05	0.04	0.03	0.11
Nord African Haplogroups	0.05	0.04	0.03	0.11
Others	0.025	0.08	0.242	0.09

calculated using frequencies of mtDNA haplotypes. Significant genetic differences are observed among both Tunisian Andalusians and Iberian populations (the one exception is shown by Slouguia population which represents a non significant distance with Castile and Andalusia). However with North African populations, distances are generally not significant, especially with populations from Morocco (*Table 4*).

PC analysis is performed by SAS program using Fst distances between studied populations. First and second PC account respectively for 63.19% and 30.81% of the variation. The picture emerging from this analysis shows the clear segregation between two clusters represented by Iberian and North African populations. Tunisian Andalusians belong to the second cluster (*Figure 3*).

Analysis of Molecular Variance (AMOVA)

The analysis of molecular variance (AMOVA) was performed in order to detect any genetic structure within the present samples we have set. We have defined three groups: Tunisian Andalusians, North African and Iberian groups. When we consider the structures represented by the two groups (Tunisian Andalusians and Iberians), we find a significant intergroup figure ($p \leq 0, 05$). This result indicates the genetic difference between these two groups. However, when we analyse the structure defined by Tunisian Andalusians and North African populations, we notice that the inter-group value of variance is not significant (*Table 5*). These results are in accordance with the North African structure of the Tunisian Andalusian communities.

YSTR Analysis

64 Y-STR haplotypes were observed in the four Tunisian Andalusian populations. The number of haplotypes as well as the gene diversity (*Table 6*) show low values in Qalaat El Andalous, suggesting a genetic drift in this community.

We have compared Tunisian Andalusians to North African and Iberian populations only for 7Y-STRs described in materials and methods. Shared haplotypes were determined for all pairs of populations: Tunisian Andalusians, Iberian and North African populations (*Table 7*). The result shows that Tunisian Andalusian populations have more in common with North Africans than with Iberian population. Consequently, the genetic distances are more important and significant with Iberians (*Table 8*). The PC plot (*Figure 4*) shows more geographic structuring than with mtDNA. In this picture, Tunisian Andalusians are clustered together with North African populations. These clusters were clearly separated

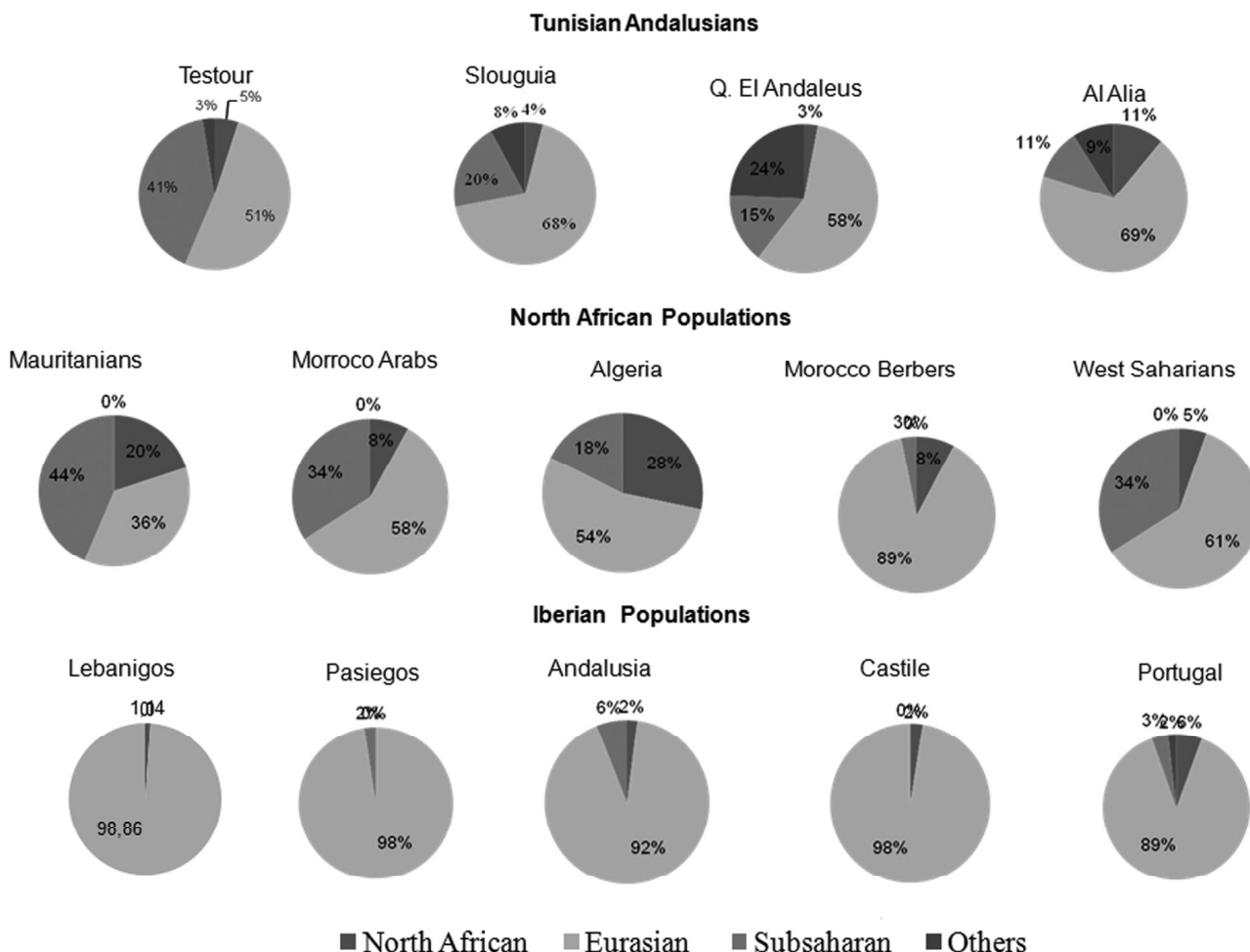


FIGURE 2. Global composition of haplogroups in Tunisian Andalusians, North African and Iberian populations.

from those of Iberian populations. In this picture too, Qalaat el Andalous is isolated by a drift effect. The AMOVA results (Table 5) indicate the same conclusion reported with mtDNA. Moreover, the intergroup statistics between Tunisian Andalusians and Iberians are more important and significant than those estimated with mtDNA. This observation is in relation with the geographic structuring of Y-SYR shown in other investigations (Kaysar *et al.* 2001).

DISCUSSION

Confrontation of genetic result with historical data

Both markers (YSTRs and mtDNA) analysed in this paper indicate a genetic structure of Andalusian villages which are more similar to North African populations than to Iberians. Taking into account historical data, two

scenarios can explain this result: first, we can say that these Andalusians were expelled from Iberian Peninsula with a North African genetic structure. When we focused on the Moor literature, we found that Andalusians were considered as the descent of Arabs and Berbers who participated to the Islamic conquest of Iberia in the 7th century A.C (Abdulwaheb 1917). This analysis can lead us to say that these North African and Arab settlers have had economic and cultural impact in Iberian Peninsula during 10 centuries of settlement. However, no mixture with autochthonous people seems to have occurred. In the second scenario, we can say that Tunisian Andalusians arrived with intermediate genetic structures and many demographic events might have conveyed this structure to North Africa. As a matter of fact, it is proved that after their settlement in Tunisia, Andalusians have known a reduction in their number since 18th century due to epidemics and immigration. Moreover, many non-

	TR	AL	QL	SL	AN	CN	CS	LB	LE	PS	GL	SP	PT	BS	CT	AG	TG	SS	MB	MA	SH	EG
TR	0.0000	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-
AL	0.02681	0.0000	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-
QL	0.00665	0.02766	0.00000	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-
SL	0.01589	0.00412	0.01530	0.00000	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
AN	0.03986	0.01160	0.04018	0.01161	0.00000	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CN	0.07136	0.02902	0.07065	0.02394	0.01426	0.00000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CS	0.04930	0.01736	0.04741	0.01741	-0.00125	0.01287	0.00000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LB	0.07425	0.02707	0.07374	0.03139	0.01454	0.01688	0.01405	0.00000	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LE	0.05955	0.03372	0.06651	0.04033	0.01133	0.00844	0.01110	0.01317	0.00000	+	+	+	+	+	+	+	+	+	+	+	+	+
PS	0.07278	0.04476	0.06503	0.02796	0.02621	0.01290	0.02702	0.02501	0.02476	0.00000	+	+	+	+	+	+	+	+	+	+	+	+
GL	0.08505	0.03780	0.08354	0.04583	0.01542	0.01403	0.00427	0.00667	0.00949	0.03298	0.00000	+	+	+	+	+	+	+	+	+	+	+
SP	0.03818	0.02492	0.04092	0.02483	0.00315	0.01082	0.00652	0.01703	0.00511	0.02255	0.01282	0.00000	-	-	-	+	+	+	+	+	+	+
PT	0.03710	0.01369	0.03691	0.01299	0.00146	0.00731	0.00103	0.01024	0.00567	0.01865	0.01015	0.00187	0.00000	-	-	+	+	+	+	+	+	+
BS	0.05889	0.03061	0.07314	0.03526	0.00983	0.00686	0.01399	0.01420	0.00598	0.02375	0.01093	0.00882	0.00616	0.00000	-	+	+	+	+	+	+	+
CT	0.04560	0.02375	0.04674	0.02438	0.00480	0.01475	0.00091	0.01892	0.00045	0.03019	0.01317	0.00685	0.00006	0.00962	0.00000	+	+	+	+	+	+	+
AG	0.05398	0.05552	0.06256	0.08318	0.07937	0.09844	0.09682	0.11017	0.09225	0.12078	0.11951	0.07582	0.07558	0.10425	0.09141	0.00000	+	+	+	+	+	+
TG	0.03840	0.12923	0.06675	0.10674	0.16405	0.22317	0.19024	0.22689	0.20646	0.20584	0.25505	0.17554	0.17201	0.20714	0.18302	0.12928	0.00000	+	+	+	+	+
SS	0.01254	0.00853	0.02526	0.00762	0.01686	0.02475	0.01960	0.03487	0.03234	0.03734	0.03157	0.01064	0.01313	0.02122	0.02777	0.06574	0.10353	0.00000	+	+	+	+
MB	0.03695	0.0287	0.03927	0.00933	0.00199	0.01940	-0.00244	0.01222	0.01929	0.02767	0.01831	0.01091	0.00223	0.01999	0.01341	0.07332	0.16889	0.01374	0.00000	+	+	+
MA	0.00837	0.02105	0.00689	0.02465	0.02551	0.04000	0.03036	0.05678	0.03438	0.05358	0.05578	0.01839	0.02266	0.04348	0.02983	0.03413	0.07317	0.01017	0.02913	0.00000	-	+
SH	0.00644	0.03392	0.01323	0.02605	0.04492	0.05128	0.04889	0.06869	0.05091	0.05683	0.06973	0.03166	0.03739	0.04604	0.04522	0.03784	0.05760	0.00819	0.04331	-0.00010	0.00000	+
EG	0.01134	0.02778	0.01536	0.01354	0.03021	0.06501	0.04133	0.06350	0.05517	0.06067	0.07912	0.03967	0.03848	0.06321	0.03736	0.06456	0.06373	0.02819	0.03285	0.01492	0.02890	0.00000

TABLE 4. Fst genetic distances (and significations) based on HVSI of mtDNA between Tunisian Andalusians, North African and Iberian populations. TR Testour, AL El Alia, QL Qalaat, El Andaleus, SL Slouguia, AN Andalusia, CN Cantabria, CS Castille, LB Lebanigos, LE Leonese, PS Pesigos, GL Galicia, SP Spain, PT Portugal, BS Basque, CT Catalan, MZ Mzab, TGTuareg, SS Souss, MA Morocco Arabs, Morocco Berbers, SH West Saharan, EG Egypt. Under the diagonal Fst distances value, above the diagonal significance of distances; + significant distances, - not significant distances.

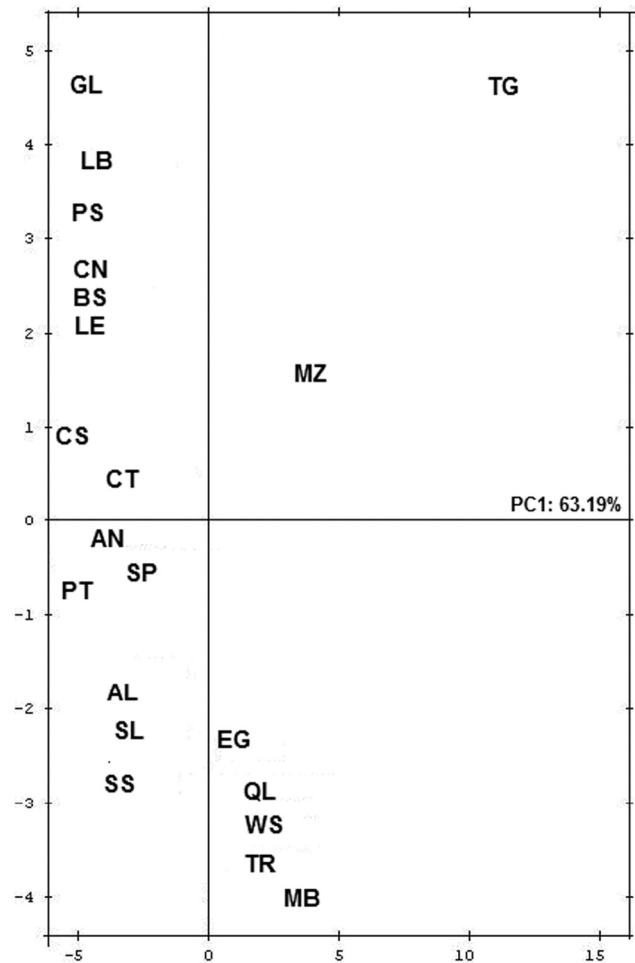


FIGURE 3. PC analysis of mtDNA performed with Tunisian Andalusians, North African and Iberian populations. GL Galicia, LB Lebanigos, PS Pasiegos, CN Cantabria, BS Basque, LE Leonese, CS Castille, CT Catalan, AN Andalusia, PT Portugal, SP Spain, MZ Mzab, TG Tuareg, SS Souss, WS West Saharan, MB Morocco Berbers, MA Morocco Arabs, MB Morocco Berbers, EG Egypt, AL El Alia, SL Slouguia, TR Testour, QL Qalaat EL Andaleus.

TABLE 5. Analyses of Molecular Variance (AMOVA) in Tunisian Andalusians, North African and Iberian populations; ns: non significant.

Markers	Groups	Within populations	Among populations within groups	Among groups
mtDNA	Tunisians Andalusians vs North Africans	96.25	3.91	-0.16 ns
	Tunisians Andalusians vs Iberians	95.41	1.68	2.91
YSTR	Tunisians andalusians vs North Africans	90.44	10.21	-0.65 ns
	Tunisians Andalusians vs Iberians	82.57	10.2	16.3

Andalusian families have borne Andalusian names in Andalusian villages and vice versa (De Epelza 1969). Finally, interbreeding with autochthonous people was observed in these small communities after the 19th.

Considering these four populations separately on the basis of mtDNA, Qalaat El Andaleus is firmly related to the others Tunisian Andalusian (number of shared haplotypes and similarity indexes...). In fact, this population represents the first region in which the Moors were assembled in the period between 1609–1614 before their final repatriation in the North of Tunisia (Abdulwaheb 1917). This population hosts R0a, haplogroup which is omnipresent in the Arabian Peninsula (Cerný *et al.* 2011), next to the North African haplogroup U6 (3%). This shows the Arab-Berber background in this population which was further enriched by mixing with non Andalusian in this region like: Louata, Tripolitains, Arabs of Kairouan and Khazmir from Morocco (Gafsi 1983).

The population of El Alia presents the highest frequency of U6 North African haplogroup (11%). Arabic imprint is also evident by the presence of R0a haplogroup (4.4%). This population was formed by a small community of Andalusian farmers (250 families; De Epelza 1980). In the 19th century, this region witnessed the settlement of migrating populations coming from Algeria.

On the basis of mtDNA, Testour town, which is considered as having the print of the Andalusian culture, has the highest level of sub-Saharan haplogroups compared to other Andalusian communities. Whereas, its genetic relation to Iberians was not evident due to the absence of South Western European haplogroups (H1, V, R...) (Torroni *et al.* 1998). However, Arab-Berber footprint is evident by the presence of middle eastern haplogroups (J and T) (10%) and U6 North African haplogroup (5%).

Particularly, Slouguia population seems to be as the nearest population to Iberians (Castile and Andalusia). This idea is supported by the high frequency of haplogroup V (20%) and haplogroup H1 (4%) which both have flourished in Iberia after the last glacial maximum

TABLE 6. Y-STRs diversity parameter in Tunisian Andalusians populations.

Population	Sample size	Number of haplotype	Gene diversity
Testour	48	31	0.9565 +/- 0.0208
Al Alia	43	26	0.9303 +/- 0.0211
Slouguia	22	16	0.9654 +/- 0.0241
Q.El Andaleus	19	3	0.2924 +/- 0.1274

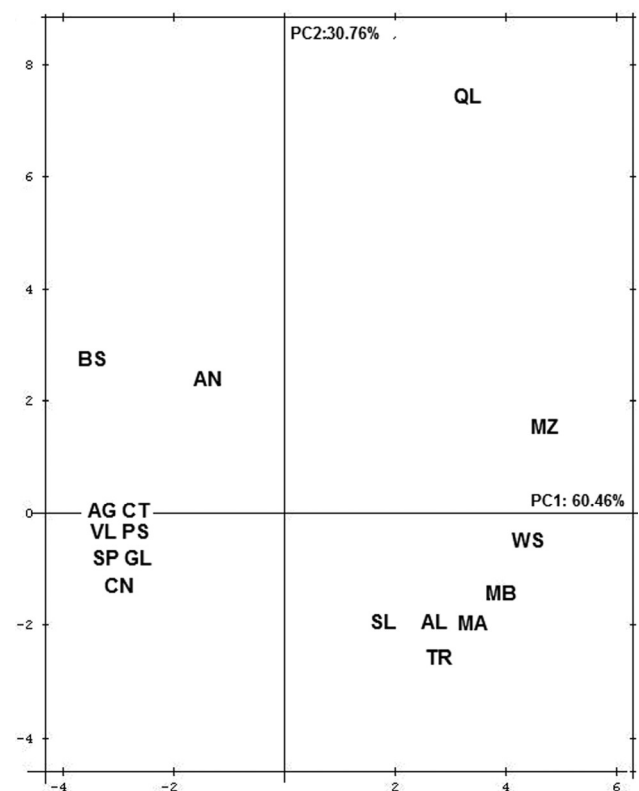


FIGURE 4. PC analysis of 7 YSTR performed with Tunisian Andalusians, North African and Iberian populations. BS Basque, AN Andalusia, AG Aragon, CT Catalan, VL Valencia, PS South Portugal, SP Spain, CN Cantabria, GL Galicia, MZ Mzab, WS West Saharan, MA Morocco Arabs, MB Morocco Berbers, SL Slouguia, AL El Alia, TR Testour, QL Qalaat El Andaleus.

TABLE 7. 7YSTR shared haplotypes between Tunisian Andalusians, North African and Iberian populations. MZ Mzab¹, MB Morocco Berber², MA Morocco Arabs², SH Sahraouis¹, BS Basque³, CT Catalan³, AG Aragon³, CN Cantabria³, SP Spain³, VL Valencia³, GL Galicia³, PN Northern Portugal³, PS, Southern Portugal³, AL El Alia⁴, QL Qalaat El Andaleus⁴, SL Slouguia⁴, TR Testour⁴, AN Andalousia⁵. Below the diagonals number of shared haplotypes between populations. Diagonal represent the number of different haplotypes. 1, Bosch *et al.* 2001; 2, Bosch *et al.* 2001, Quintana-Murci *et al.* 2005; 3, Roewer *et al.* 2005; 4, Cherni *et al.* 2005b; 5, Prieto *et al.* 2003.

	MZ	AM	BM	SH	BS	CT	AG	CN	ESP	IM	VL	GL	PN	PS	QL	SL	TR	AN
MZ	20																	
AM	3	50																
BM	4	14	49															
SH	3	5	5	10														
BS	1	7	2	1	55													
CT	2	11	5	2	21	127												
AG	2	9	3	2	14	28	84											
CN	2	9	3	2	15	23	16	66										
SP	1	11	6	2	17	28	19	18	98									
IM	3	8	7	3	11	18	15	8	15	61								
VL	1	9	4	2	16	26	17	17	22	14	90							
GL	1	7	4	2	9	25	13	12	16	9	13	70						
PN	2	9	7	2	17	29	17	17	26	20	17	18	101					
AL	3	8	7	3	3	3	2	3	7	2	4	3	5	26				
QL	1	3	1	1	1	1	1	1	1	1	1	1	1	1	3			
SL	1	7	5	2	1	1	1	1	3	3	3	1	2	1	1	16		
TR	3	9	10	4	1	5	4	5	6	4	4	7	6	6	1	5	31	
AN	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	72

(Achilli *et al.* 2004). Historical data describe that the origin of Moor of Testour and Slouguia is respectively from Castile, Valencia and Aragon (Hopknes 1977). After the settlement of the Wselitia Berbers in Testour around 1762 A.C, many Andalusians were moved from Testour to Slouguia (Despois 1959, Valensi 1964).

Considering all analysed genetic data confronted with the historical information, we can conclude that the Andalusians have left the Iberian Peninsula with North African genetic structure. Were only the descendants of Arab-Berber conquerors who were repressed of Iberia in 1609 A.C. But this fact does not exclude Admixture with the indigenous already present in insertions regions and especially after the 19th century. The North African genetic structure is still proven by PC analysis and AMOVA. In fact, PC analysis based on data of the Y STR (Figure 4) shows that three Andalusians populations (Slouguia, Testour and Al Alia) were closely clustered with North African. Moreover, the influence of demographic factors is clearly shown by the isolation of Qalaat Al Andaleus in the PC plot.

Finally, to further draw solid conclusions about the origin of Andalusian in Tunisia, we plan to increase the

analyzed sample size by the recruitment of new Andalusian populations and the fine charecterization of maternal (mtNA) and paternal (YDNA) lineages having evolved in North Africa, the Arabian Peninsula and Iberia.

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TABLE 8. Fst genetic distances (and significations) based on 7YSTRs between Tunisian Andalusians, North African and Iberian populations. BS Basque, AN Andalusia, AG Aragon, CT Catalan, VL Valencia, PS South Portugal, SP Spain, CN Cantabria, GL Galicia, MZ Mzab, WS West Sahara, MA, Morocco Arabs, Morocco Berbers, SL Slouguia, AL El Alia, TR Testour, QL Qalaat El Andaleus. Under the diagonal Fst distances value, above the diagonal significance of distances; + significant distances, - not significant distances.

MZ	AM	BM	SH	BS	CT	AG	CN	SP	VL	GL	PN	PS	AL	QL	SL	TR	AN
MZ	0.0000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
AM	0.09348	0.0000	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
BM	0.12705	0.01595	0.0000	+	+	+	+	+	+	+	+	+	+	+	+	-	+
SH	0.20732	0.09105	0.05373	0.0000	+	+	+	+	+	+	+	+	+	+	+	+	+
BS	0.42147	0.28913	0.33481	0.38845	0.0000	+	+	+	+	+	+	+	+	+	+	+	+
CT	0.29280	0.18395	0.22726	0.28052	0.03200	0.0000	-	-	-	+	+	-	+	+	+	+	+
AG	0.31682	0.18427	0.23280	0.28143	0.02806	0.00111	0.0000	-	-	+	+	-	+	+	+	+	+
CN	0.27405	0.15571	0.19817	0.24623	0.04704	0.00288	0.00498	0.0000	+	-	-	-	+	+	+	+	+
SP	0.27878	0.16273	0.21439	0.25963	0.04277	0.00467	-0.00020	0.00725	0.0000	+	+	-	+	+	+	+	+
VL	0.29074	0.17391	0.22315	0.27239	0.04993	-0.00129	0.00156	0.00426	0.00284	0.0000	-	-	+	+	+	+	+
GL	0.27393	0.14981	0.20149	0.25436	0.07826	0.00840	0.01459	0.00492	0.01310	-0.00084	0.0000	-	+	+	+	+	+
PN	0.28664	0.16801	0.21833	0.27658	0.05808	0.00614	0.00708	0.00380	0.00660	-0.00021	-0.00126	0.0000	+	+	+	+	+
PS	0.28939	0.16233	0.21219	0.26880	0.05714	0.00178	0.00521	0.00349	0.00534	-0.00485	-0.00265	-0.00304	0.0000	+	+	+	+
AL	0.14655	0.04166	0.05682	0.05231	0.29852	0.18735	0.18457	0.15743	0.16271	0.17176	0.14984	0.17636	0.16971	0.0000	-	-	+
QL	0.38770	0.26751	0.34558	0.46328	0.49485	0.35709	0.38824	0.35322	0.35649	0.34052	0.30606	0.33120	0.32367	0.33979	0.0000	+	+
SL	0.22095	0.05647	0.06032	0.07100	0.28444	0.17778	0.17420	0.14455	0.15668	0.16544	0.14308	0.16000	0.15651	0.01338	0.38746	0.0000	+
TR	0.11246	0.00270	0.01401	0.05939	0.30340	0.18619	0.18632	0.14944	0.16371	0.17309	0.14468	0.16517	0.16256	0.00965	0.30937	0.00492	0.0000
AN	0.34864	0.24547	0.29048	0.31154	0.20892	0.15822	0.15299	0.13945	0.14718	0.14810	0.14256	0.14444	0.14573	0.23135	0.38166	0.20758	0.23237

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