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HLA genes in Macedonians and the sub-Saharan origin of the Greeks

Key words:
Macedonians; Greeks; Ethiopians; Mediterraneans; Berbers; Sudan; Turks; Egyptians; Sahel; Africa

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Abstract: HLA alleles have been determined in individuals from the Republic of Macedonia by DNA typing and sequencing. HLA-A, -B, -DR, -DQ allele frequencies and extended haplotypes have been for the first time determined and the results compared to those of other Mediterraneans, particularly with their neighbouring Greeks. Genetic distances, neighbor-joining dendrograms and correspondence analysis have been performed. The following conclusions have been reached: 1) Macedonians belong to the "older" Mediterranean substratum, like Iberians (including Basques), North Africans, Italians, French, Cretans, Jews, Lebanese, Turks (Anatolians), Armenians and Iranians, 2) Macedonians are not related with geographically close Greeks, who do not belong to the "older" Mediterranenan substratum, 3) Greeks are found to have a substantial relatedness to sub-Saharan (Ethiopian) people, which separate them from other Mediterranean groups. Both Greeks and Ethiopians share quasi-specific DRB1 alleles, such as *0305, *0307, *0411, *0413, *0416, *0417, *0420, *1110, *1112, *1304 and *1310. Genetic distances are closer between Greeks and Ethiopian/sub-Saharan groups than to any other Mediterranean group and finally Greeks cluster with Ethiopians/sub-Saharans in both neighbour joining dendrograms and correspondence analyses. The time period when these relationships might have occurred was ancient but uncertain and might be related to the displacement of Egyptian-Ethiopian people living in pharaonic Egypt.

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The highly polymorphic HLA system has been validated as useful for distinguishing and/or relating populations (and individuals) in many research studies since the first International HLA Anthropology Workshop (Evian, 1973) and in all the subsequent seven International Workshops. HLA gene frequencies correlate with geographically related populations. The existence or absence of gene flow among neighbouring ethnic groups may be assessed with the study of HLA frequencies and the corresponding genetic distances (1, 2).

Ancient Macedonians were among the peoples that lived between northern Greece (Thessaly) and Thrace in the Balkans and were considered by the classical Greeks as "non-Greek barbarians" that could not participate in the Greek Olympic Games (3). Hero-

dotus wrote that "Macedonians" were "Dorians" and were never admitted to the Greek community (4). They did not speak Greek but another language presently unknown and of which only proper names remain; nowadays, they speak a Slavic language (5). Macedonians fought against the Greeks between 357–336 B.C. under King Philip II. They defeated the Greeks at the Battle of Chaironea (338 B.C.). The Macedonian empire extended from the Balkan Peninsula to the Himalayas and to North Africa during the reign of Philip's son, Alexander the Great (6). Thereafter, Macedonia was conquered by the Romans and has been disputed in more recent times by Serbs and/or Bulgars. Ottoman Turks controlled Macedonia between 1380–1912 A.D., and it was integrated into Yugoslavia in 1946. In 1991, after the partition of Yugoslavia, a referendum gave Macedonia its independence. The present ethnic groups within the country are: 1) Macedonians: 1,279,000; 2) Albanians: 377,000; 3) Turks: 87,000; 4) Serbs: 44,000; and 5) others: 40,000. The northernmost region of Greece is also known as Macedonia and this is why Greece has opposed the independence of the country while it bears the same name (7).

Furthermore, we have found that the Greeks did not cluster together with other Mediterranean populations, including both western (Iberians, Algerians, Berbers) and eastern (Cretans, Jews, Lebanese, Egyptian, Turks-Anatolians) Mediterraneans (8–10).

The aim of the present work is to determine the relative contributions of Macedonians and Greeks to the present-day genetic pool of Mediterranean peoples. For these purpose, both HLA class I and class II DNA typings have been studied in Macedonians for the first time. The genetic relationship of Macedonians and Greeks to other Mediterraneans, including North Africans (Berbers from Agadir and El Jadida areas and Algerians from Algiers), Iberians (Spaniards, Basques and Portuguese) and Greeks (from Attica, Aegean and Cyprus) were calculated. In addition, sub-Saharan and other Africans were compared with all available Mediterranean groups in order to solve the question of the unique Greek HLA profile.

Populations used for the present work

Identification numbers	Region and population	n^1	References
1	Macedonians	172	Present study
2	Moroccans (El Jadida)	98	22
3	Berbers (Souss)	98	29
4	Moroccan Jews	94	30
5	Spaniards	176	9
6	Basques	80	9
7	Portuguese	228	15
8	French	179	16
9	Algerians (Algier)	102	8
10	Sardinians	91	16
11	Italians	284	16
12	Jews (Ashkenazi)	80	31
13	Jews (non-Ashkenazi)	80	31
14	Cretans	135	10
15	Greeks (Aegean)	85	2
16	Greeks (Attica)	96	2
17	Greeks (Cyprus)	101	2
18	Lebanese (NS)[2]	59	2
19	Lebanese (KZ)[3]	93	2
20	Iranians	100	32
21	Turks	228	Arnaiz-Villena et al. (unpublished. results)
22	Armenians	105	16
23	Egyptians (Siwa)	101	2
24	Oromo	83	2
25	Amhara	98	2
26	Fulani	38	2
27	Rimaibe	39	2
28	Mossi	42	2
29	San (Bushmen)	77	16
30	Senegalese	192	16
31	South-African-Blacks	86	16

[1]*n*=number of individuals analysed for each population; [2]NS=Niha el Shouff (town); [3]KZ=Kafar Zubian (town)

Table 1

Material and methods

Population samples

Samples from one hundred and seventy-two unrelated Macedonians in Skopje (Institute of Blood Transfusion, Tissue Typing Laboratory), the Republic of Macedonia capital, were used for HLA genotyping and phylogenetic calculations. All were Macedonian language speakers and their ancestors did not belong to a country minority group (detailed above). The origin of all other populations used for comparisons is given in Table 1.

HLA genotyping, DNA sequencing and statistics

Generic HLA class I (A and B) and high-resolution HLA class II (DRB1 and DQB1) genotyping was performed using a reverse dot-blot technique with the Automated Innolipa system (Innogenetics N.V., Zwijndrecht, Belgium). HLA-A, -B, -DRB1, and -DQB1 allele DNA sequencing was only done when indirect DNA typing (reverse dot-blot) yielded ambiguous results (11). Statistical analysis was

HLA-A, -B, -DRB1, and -DQB1 allele frequencies in the Macedonian population *Table 2*

Alleles	Allele frequencies %	Alleles	Allele frequencies %	Alleles	Allele frequencies %
HLA-A		B*58	0.9	HLA-DQB1*	
A*01	13.7	B*40(B60)	2.0	02	14.5
A*02	25.6	B*40(B61)	1.5	0203	0.9
A*03	11.9	B*15(B62)	1.5	0301	25.0
A*11	7.6	B*14(B65)	0.6	0302	6.4
A*23	3.5	B*78	0.3	03032	2.9
A*24	16.3	HLA-DRB1*		0304	0.3
A*25	1.7	0101	5.2	0305	0.3
A*26	6.7	0301	9.0	0402	1.5
A*29	1.2	0401	1.2	0501	7.3
A*30	1.7	0402	1.7	0502	17.2
A*31	2.0	0403	3.2	05031	5.8
A*32	3.2	0404	0.6	0601	2.9
A*33	1.2	0405	0.3	0602	6.9
A*68	3.8	0415	0.3	0603	5.8
		0424	0.3	0604	1.7
HLA-B		0432	0.3	0606	0.3
B*07	6.4	0701	6.4	0609	0.3
B*08	6.7	0801	1.7		
B*13	1.5	0804	0.3		
B*14	0.3	0901	0.3		
B*15	0.3	1001	2.0		
B*18	16.6	1101	7.3		
B*27	4.1	1103	0.9		
B*35	14.8	1104	16.6		
B*37	2.0	1201	0.6		
B*38	5.2	1301	4.4		
B*39	1.7	1302	2.3		
B*41	1.2	1305	0.9		
B*44	7.8	1307	0.3		
B*45	0.3	1401	5.8		
B*49	1.5	1404	0.6		
B*50	0.6	1407	0.3		
B*51	14.8	1501	9.0		
B*52	2.3	1502	2.6		
B*53	0.3	1505	0.3		
B*56	0.3	1601	13.9		
B*55	2.6	1602	1.5		
B*57	2.0				

Alleles DQB1*0201 and 0202 were all assigned as DQB1*02. Number in brackets indicates the serologic antigen most probably corresponding to the genetic allele obtained

performed with Arlequin v1.1 software kindly provided by Excoffier and Slatkin (12). In summary, this program calculated HLA-A, -B, -DRB1 and -DQB1 allele frequencies, Hardy-Weinberg equilibrium and the linkage disequilibrium between two alleles at two different loci. Linkage disequilibrium (D′; also named LD, see ref. 13) and its level of significance (*P*) for 2×2 comparisons were determined using the formulae of Mattiuz and co-workers (14) and the 11th International Histocompatibility Workshop methodology (13).

In addition, the most frequent complete haplotypes were deduced following a methodology used in the 11th International Histocompatibility Workshop: 1) the 2, 3, and 4 HLA loci haplotype frequencies (2, 15, 16); 2) the haplotypes previously described in other populations (2, 16); and 3) haplotypes which were assigned if they appeared in two or more individuals and the alternative haplotype was well defined. In order to compare allelic and haplotype HLA frequencies with other populations, the reference tables used were those of the 11th and 12th International HLA Workshops (2, 16; see also Table 1). Phylogenetic trees (dendrograms) were constructed with the allelic frequencies by applying the Neighbor-Joining (NJ) method (17) with the genetic distances between populations (DA, 18) and using DISPAN software containing the programs GNKDST and TREEVIEW (19, 20). A three-dimensional correspondence analysis and its bidimensional representation was carried out using the VISTA v5.02 computer program (21, http:/forrest.psych.unc.edu). Correspondence analysis comprises a geometric technique that may be used for displaying a global view of the relationships among populations according to HLA (or other) allele frequencies. This methodology is based on the allelic frequency variance among populations (similarly to the classical principal components methodology) and on the display of a statistical projection of the differences.

Genetic distances between populations (DA) between Macedonians and other populations ($\times 10^2$) obtained by using HLA-DRB1 allele frequencies (see Table 1 for populations identification)

HLA-DRB1 (DA)	
Cretans	8.38
Italians	10.45
French	14.41
Sardinians	17.66
Spaniards	17.76
Moroccan Jews	17.78
Non-Ashkenazi Jews	17.83
Lebanese (KZ)	20.98
Ashkenazi Jews	21.87
Algerians (Algiers)	22.37
Lebanese (NS)	23.29
Greeks (Attica)	23.69
Moroccans	25.47
Berbers (Souss)	28.50
Spanish-Basques	30.50
Greeks (Cyprus)	33.28
Greeks (Aegean)	37.52
South African Negroids	38.22
Senegalese	41.76
Oromo	43.26
Amhara	51.74
Mossi	53.46
Rimaibe	55.95
San (Bushmen)	57.78
Fulani	61.01

Table 3

Results

Characteristic HLA allele frequencies of the Macedonian population compared to other Mediterraneans

The expected and observed allele frequencies for HLA-A, -B, -DRB1 and -DQB1 loci do not significantly differ and the population sample is in Hardy-Weinberg equilibrium. Table 2 shows the HLA allele frequencies found in the Macedonian population. Fourteen different HLA-A and twenty-eight different HLA-B alleles were observed in the Macedonian population. Six HLA-A alleles and seven HLA-B alleles had frequencies higher than 5% (A*01, A*02, A*03, A*11, A*24, A*26, B*07, B*08, B*18, B*35, B*38, B*44 and B*51) and these are characteristic of Mediterranean populations (8–10, 22). With regard to the HLA class II alleles, thirty-one different DRB1 alleles were found and only six had frequencies higher than 5%; DQ allele frequencies reflect the DRB1 locus allele distribution due to the strong linkage disequilibrium between these two loci.

Two types of analyses were carried out to compare Macedonian HLA frequencies with other Mediterranean population frequencies: 1) with DRB1 data, which is probably a more informative and discriminating methodology; and 2) with generic (low-resolution) DR-DQ data. These two types of analysis were both performed because some of the populations used for comparison lacked HLA-A and -B data [Berbers (from Souss, Agadir area, Morocco), Jews (Ashkenazi), Jews (Morocco), Jews (non-Ashkenazi), Lebanese (NS and KZ), see Table 1], or high resolution HLA-DQ data [(Greeks (Attica), Greeks (Cyprus), Greeks (Attica-Aegean), see Table 1]], or only generic HLA-DR and

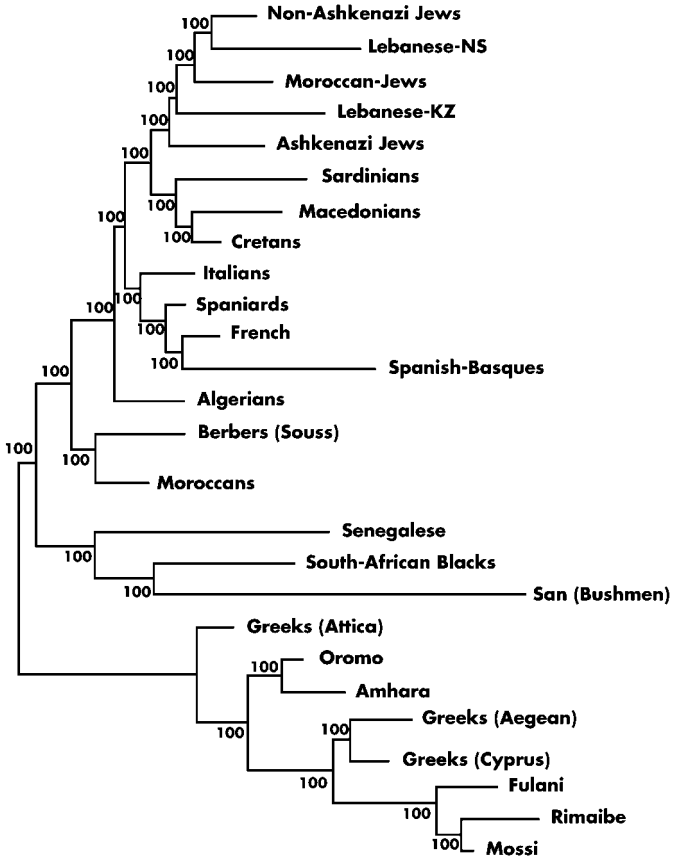

Fig. 1. **Neighbor-Joining dendrogram showing relatedness between Macedonians and other populations.** Genetic distances between populations (DA) were calculated by using HLA-DRB1 (high-resolution). Data from other populations were from references detailed in Table 1. Bootstrap values from 1000 replicates are shown.

-DQ data were available [Portuguese, Turks, Iranians, Armenians and Egyptians, see Table 1]. These partially HLA-typed populations should have been ignored, but they could be analyzed conjointly taking into account only either DRB1 or generic DR and DQ frequencies (Tables 3, 6, Figs 1–3). Analyses using DRB1and DQB1 conjointly were made but are not shown because only a few populations could be used and the results are concordant with the DRB1 analysis. Finally, it should be pointed out that class I generic typing tends to homogenize the comparisons based on DRB1 high-resolution typing (see ref. 22); one class I allele obtained by generic DNA typing may contain several class I alleles, while this is not the case for most DRB1 alleles.

Fig. 1 depicts an HLA class II (DRB1) neighbor-joining tree. Populations are grouped into three main branches with high bootstrap values: the first one groups both eastern (including Macedonians, Cretans, Jews, Lebanese) and western Mediterraneans (Europeans and North Africans; Sardinians are also included in the first group). The second branch is formed by African Negroid populations and the third one includes Greek and sub-Saharan populations. This distribution is also confirmed in the correspondence analysis (Fig. 2): the three groups are clearly delimited and a west to east Mediterranean gradient is shown. The Macedonian population shows the closest genetic distance with Cretans (Table 3) and no discontinuity is observed with eastern and western Mediterraneans reflecting the genetic similarity among these populations. It is evidenced that Cretans-Greeks distance is high. These results are confirmed using DR and DQ generic typings (see Fig. 3 and data not shown) which were used in order to include other Mediterranean populations (Iranians, Armenians, Egyptians and Turks, see Table 1). A DR-DQ neighbour-joining tree (data not shown) maintains the West to East Mediterranean gradient and also the group formed by Greeks and sub-Saharan populations. Turks (old Anatolians), Kurds, Iranians and Armenians have been shown specifically to cluster with the eastern Mediterranean groups (Arnaiz-Villena et al., submitted). On the other hand, genetic distances obtained by using DR-DQ generic typing allele frequencies (data not shown) show that Iranians (1.10×10^{-2}) and Cretans (1.54×10^{-2}) are the two populations closest to the Macedonians followed by the other Mediterranean populations. A discontinuity is found between Berbers (Souss) and Greeks (Attica) (9.59×10^{-2} vs. 12.42×10^{-2}) showing that the latter have a distant relationship with Mediterran-

Most frequent HLA-A, -B, -DRB1, and -DQB1 extended haplotypes in the Macedonian population and their possible origin

Haplotypes	HF (%)	Possible origin
A*01-B*08-DRB1*0301-DQB1*02[a]	4.9	Pan-European
A*02-B*18-DRB1*1104-DQB1*0301[b]	4.1	Mediterranean
A*02-B*51-DRB1*1601-DQB1*0502[c]	3.2	Macedonian
A*03-B*18-DRB1*1601-DQB1*0502[d]	2.6	Macedonian
A*01-B*52-DRB1*1502-DQB1*0601[e]	1.7	North African-Mediterranean
A*24-B*18-DRB1*1104-DQB1*0301[f]	1.5	Central-South-Eurasian
A*03-B*18-DRB1*1104-DQB1*0301[g]	1.5	Macedonian-Italian
A*25-B*18-DRB1*1501-DQB1*0602[h]	1.2	Iberian-Macedonian
A*26-B*38-DRB1*0402-DQB1*0302[i]	1.2	Macedonian-Turkish-Jewish

HF: Haplotype frequency. [a]Also found in Basques (2.4%), Spaniards (3.4%), Britons (2.9%), Danes (3.4%), Cretans (1.1%), Germans (4.8%), Austrians (5.3%) and Yugoslavs (7.7%) (2, 9, 10, 15, 16). [b]This haplotype has been found in Albanians (3.9%), Italians (2.1%), Yugoslavs (3.5%), Turks (1.1%), Spaniards (1.1%) and Greeks (4.0%) (2, 16 and our own unpublished results). [c]and [d]Present only in Macedonians. [e]Partially (B52-DRB1*1502-DQB1*0601) found in Moroccans (1.5%), Cretans (2.5%), Spaniards (1.1%) and Italians (0.8%) (2, 16, 22). [f]Haplotype found in Armenians (2.1%) and Italians (0.7%) (2, 16). [g]Only found in Italians (0.8%) (2, 16). [h]Haplotype found only in Iberians, Portuguese (1.5%) and Spaniards (0.3%) (15). [i]Present in Turks (0.9%) and in Jews (our own unpublished results and 33). Other low frequency haplotypes present in Macedonians are also shared with central Europeans (A*03-B*07-DRB1*1501-DQB1*0602, HF: 0.8; A*02-B*13-DRB1*0701-DQB1*02, HF: 0.8; A*02-B*44-DRB1*0701-DQB1*02, HF: 0.6), western Europeans (A*02-B*07-DRB1*1501-DQB1*0602, HF: 0.6), north Africans (A*02-B*07-DRB1*1001-DQB1*0501, HF: 0.6) and Mediterranean-Europeans (A*23-B*44-DRB1*0701-DQB1*02, HF: 0.6) (2, 8–10, 16 and our own unpublished results)

Table 4

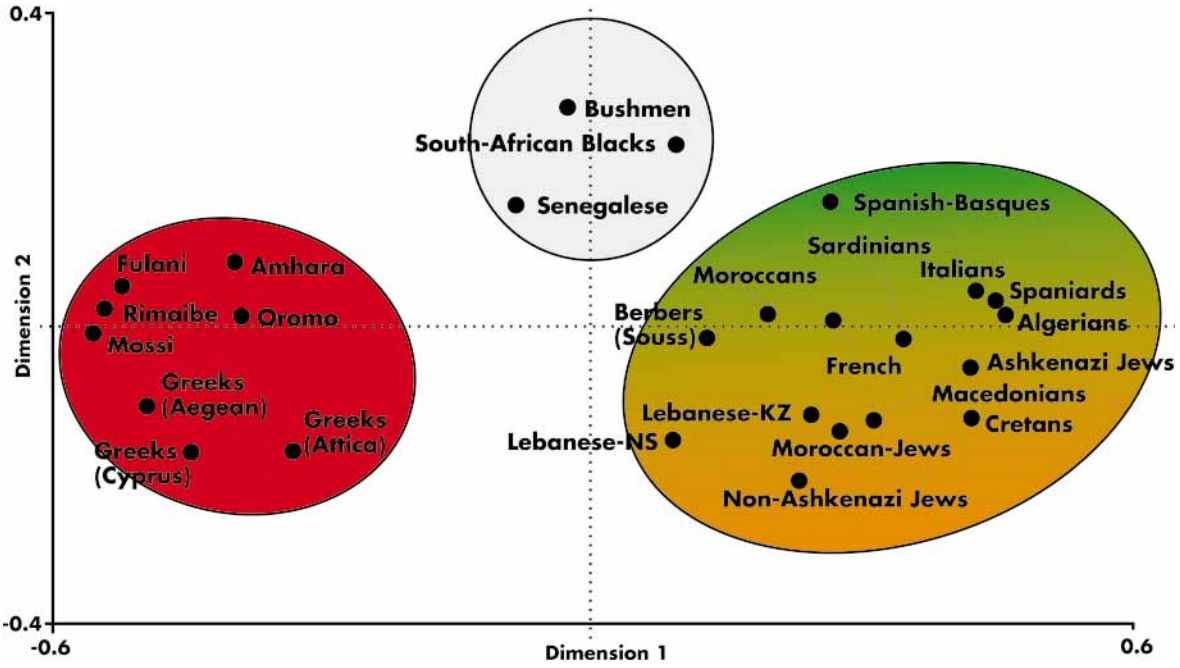

Fig. 2. **Correspondence analysis showing a global view of the relationship between Mediterraneans and sub-Saharan and Black African populations according to HLA allele frequencies in three dimensions (bidimensional representation).** HLA-DRB1 allele frequencies data.

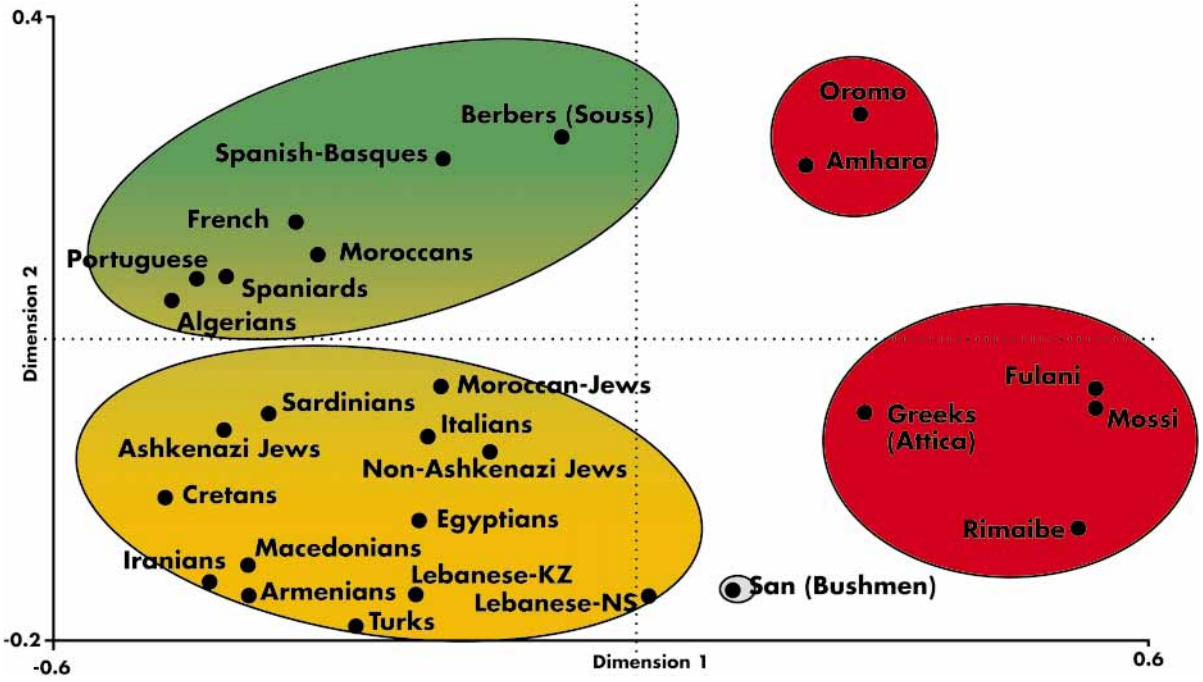

Fig. 3. **Correspondence analysis showing a global view of the relationship among West Mediterraneans (green), East Mediterraneans (orange), Greeks and sub-Saharan populations (red) and Blacks (grey) according to HLA allele frequencies in three dimensions (bidimensional representation).** HLA-DR and DQ (low-resolution) allele frequencies data.

Common HLA-DRB1 alleles between Greeks and sub-Saharan Africans

Allele	Greeks AF (%)			Ethiopia AF (%)		Sudan AF (%)	West Africans AF (%)		
DRB1	Attica	Attica/Aegean	Cyprus	Amhara	Oromo	Nuba	Rimaibe	Fulani	Mossi
0305[a]		2.5					0.8	0.2	0.8
0307[b]		2.2	3.2	2.1		1.3	0.8	0.2	0.8
0411[c]			0.4					0.6	
0413[d]	0.5	0.6	0.9	0.6	0.6				
0416[e]		0.6	0.9	0.6	0.6				
0417[f]			0.4			1.5		0.6	
0420[g]	0.1		0.4	0.6	0.6			0.6	
1110[h]		2.9	1.9		0.2		0.3		
1112[i]		2.9	1.9	0.4	0.2	2.7	0.3		
1304[j]			0.9				1.1	1.4	0.7
1310[k]			0.2			1.3	0.8	0.2	0.8

AF: Allele frequency. Some of these HLA-DRB1 alleles are also present in other populations: [a]Not found in other populations. [b]Present in Hungarians (0.4%). [c]Found in Amerindians and some Pacific peoples. [d]Not found in other populations. [e]Found in Hungarians (1.2%). [f]Present in Hva Island (Croatia, 0.3%) and Amerindian Yukpa (2.3%). [g]Found in Lebanese 0.1%. [h]Found in Hva Island (Croatia, 0.9%) and Hungarians (2.6%). [i]Also found in Lebanese (2.3%) and Hungarians (2.6%). [j]Not found in other populations. [k]Also present in Hva Island (Croatia, 1.0%) (2, 23)

Table 5

ean populations as previously described (10, 22) and cluster together with the sub-Saharan populations.

HLA-A, -B, -DRB1, and -DQB1 linkage disequilibria in Macedonians

Extended HLA haplotypes were determined in Macedonians and compared with those previously reported in other populations (Table 4). HLA-A-B and DRB1*-DQB1* two-loci linkage disequilibrium data (not shown) show that the most frequent combinations are characteristic of European and Mediterranean (western and eastern) populations (B*18-DRB1*1104, Haplotype Frequency (HF): 9.0; A*02-B*18, HF: 8.1; A*01-B*08, HF: 5.5; B*08-DRB1*0301, HF: 5.2; A*24-B*35, HF: 4.9 and B*07-DRB1*1501, HF: 4.1). The HLA-A-B-DR-DQ extended haplotypes found in the Macedonian population (Table 4) reflect common characteristics with the other "older" Mediterranean background (see footnote to Table 4). These haplotype results are concordant with those obtained by the allele frequency analyses (genetic distances, neighbor-joining trees and correspondence, see above).

Common alleles of Greeks with sub-Saharan Africans

In order to study the possible origin of the Greeks who remain outliers among Mediterraneans (10, 22), specific DRB1 alleles present in Greeks and not present in the other Mediterranean populations were searched in other geographically not very distant populations. Our own data, the 11th and 12th International Histocompatibility Workshops reference panels (2, 16, 23) and other previously described data were used (see Table 1). Table 5 shows the presence of these Greek alleles mainly in sub-Saharan populations from Ethiopia (Amhara, Oromo), Sudan (Nuba) and West Africa (Rimaibe, Fulani, Mossi). Some of these alleles are sporadically present in other populations without any relationships among them (see footnote to Table 5). It may be deduced from these data that sub-Saharans and Greeks share quasi-specific HLA-DRB1 alleles. The neighbor-joining tree (Fig. 1) and the correspondence analyses (Figs 2 and 3) confirm this Greek/sub-Saharan relatedness. The HLA-DRB1 genetic distances between Greeks and other Mediterraneans are shown in Table 6 and also support a sub-Saharan/Greek relatedness; genetic distances with HLA-DR and -DQ generic typings (not shown) give essentially the same results. No relationship of Greeks is seen with the Senegalese and South African Blacks (Bantu and people coming from the Guinea Gulf after the Bantu expansion, respectively (24)), nor with the present day Bushmen (24).

Two different types of problem regarding the obtained data are discarded: 1) mistakes in the HLA typings and 2) mistakes in the assignation of these specific alleles (DRB1*0417, *1112, etc, see Table 5). These problems are not likely to exist in the present work because; 1) HLA typings have been made by genetic technologies in three different Greek populations (2, 23) and 2) similar results are obtained when generic typing is used (DR-DQ analysis in Fig. 3; see also ref. 22).

Table 6

Genetic distances (DA) between the different groups of Greeks and other populations ($\times 10^2$) obtained by using HLA-DRB1 allele frequencies (see Table 1 for identification of populations)

HLA-DRB1 (DA)					
From Greeks (Attica) to		From Greeks (Cyprus) to		From Greeks (Aegean) to	
Greeks (Aegean)	7.35	Greeks (Aegean)	5.62	Greeks (Cyprus)	5.62
Greeks (Cyprus)	10.01	Greeks (Attica)	10.01	Greeks (Attica)	7.35
Mossi	16.00	Mossi	13.68	Mossi	10.92
Oromo	17.11	Rimaibe	17.48	Rimaibe	12.61
Rimaibe	20.69	Oromo	18.77	Oromo	19.85
French	20.87	Fulani	20.68	Fulani	20.43
Amhara	21.43	Amhara	24.10	Amhara	21.08
Moroccans	21.86	Non-Ashkenazi Jews	28.78	French	31.76
Non-Ashkenazi Jews	23.64	French	31.74	Libanese-NS	33.49
Macedonians	23.69	Libanese-NS	32.08	Non-Ashkenazi Jews	34.07
Fulani	23.85	Macedonians	33.82	Moroccans	35.10
Italians	26.28	Berbers (Souss)	34.43	Berbers (Souss)	37.02
Berbers (Souss)	26.53	Libanese-KZ	35.97	Macedonians	37.52
Cretans	27.08	Moroccan Jews	36.46	Italians	41.49
Moroccan Jews	27.56	Moroccans	36.58	Cretans	41.59
Libanese-NS	30.96	Cretans	37.45	Moroccan Jews	42.27
Libanese-KZ	31.13	Italians	41.26	Senegalese	44.34
Spaniards	33.61	Senegalese	43.23	Libanese-KZ	45.12
Algerians	33.68	Spaniards	45.25	Spaniards	51.17
Spanish-Basques	37.10	Sardinians	47.75	Algerians	51.71
Sardinians	37.87	Algerians	49.44	South African Negroids	53.48
Senegalese	38.94	Ashkenazi Jews	50.93	Sardinians	53.68
Ashkenazi Jews	40.10	South African Negroids	58.21	Ashkenazi Jews	55.33
South African Negroids	45.37	Spanish-Basques	59.57	Spanish-Basques	56.95
San (Bushmen)	62.90	San (Bushmen)	70.04	San (Bushmen)	66.68

Discussion

Macedonians

Our results show that Macedonians are related to other Mediterraneans and do not show a close relationship with Greeks; however they do with Cretans (Tables 3, 4, Figs 1–3). This supports the theory that Macedonians are one of the most ancient peoples existing in the Balkan peninsula, probably long before arrival of the Mycaenian Greeks (10) about 2000 B.C. Other possible explanation is that they might have shared a genetic background with the Greeks before an hypothetical admixture between Greeks and sub-Saharans might have occurred. The cultural, historical and genetic identity of Macedonians is established according to our results. However, 19th century historians focused all the culture in Greece ignoring all the other Mediterranean cultures present in the area long before the classical Greek one (25).

Greeks are genetically related to sub-Saharans

Much to our surprise, the reason why Greeks did not show a close relatedness with all the other Mediterraneans analyzed (Tables 5, 6 and Figs 1–3) was their genetic relationship with sub-Saharan ethnic groups now residing in Ethiopia, Sudan and West Africa (Burkina-Fasso). Although some Greek DRB1 alleles are not completely specific of the Greek/sub-Saharan sharing, the list of alleles (Table 5) is self-explanatory. The conclusion is that part of the Greek genetic pool may be sub-Saharan and that the admixture has occurred at an uncertain but ancient time.

The origin of the West African Black ethnic groups (Fulani, Mos-

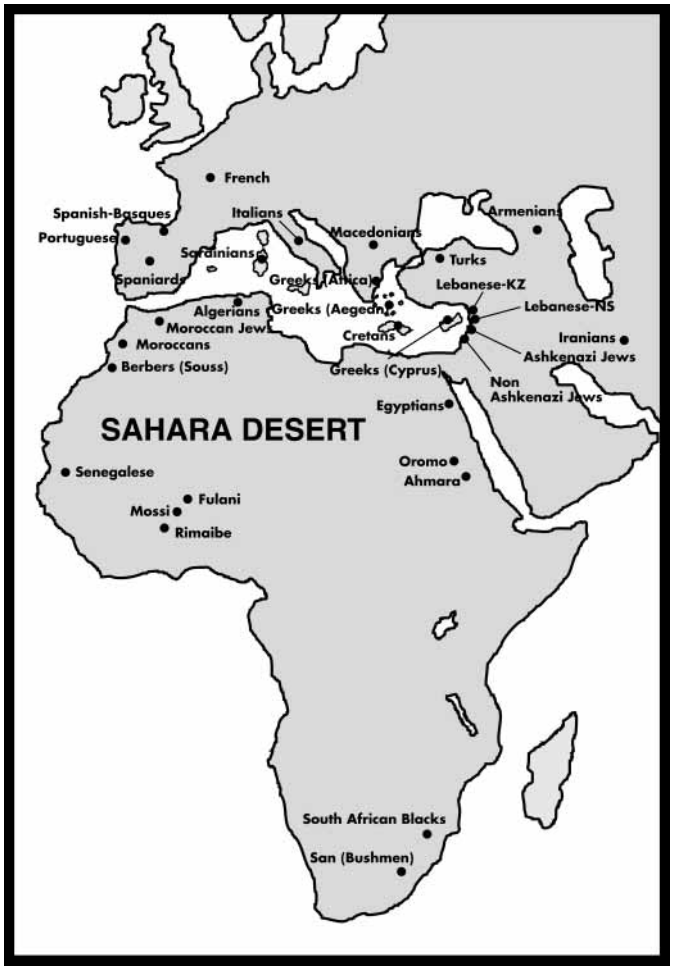

Fig. 4. **Map showing the location of the populations tested in the present work.**

si and Rimaibe sampled in Burkina-Fasso) is probably Ethiopian (26, 27) (Fig. 4). The Fulani are semi-nomadic hunters and gatherers and one of the few people in the area to use cows' milk and its by-products to feed themselves and to trade; their facial parameters show a Caucasian admixture. The Rimaibe Blacks have been slaves belonging to the Fulani and have frequently mixed with them (27). The Nuba people are now widespread all over Sudan, but are descendants of the ancient Nubians that ruled Egypt between 8th–7th centuries B.C. (28) and later established their kingdom at Meroe, North Khartoum. Two kinds of Nubians were described in ancient times: Reds and Blacks, probably reflecting the degree of Caucasian admixture. Both the Oromo and Amharic peoples live in the Ethiopian mountains (27). They obviously have in common a genetic background with the west-African groups mentioned above. Linguistic, social, traditional and historical evidence supports an east-to-west migration of peoples through the Sahel (southern Sahara strip), although this is still debated (26, 27).

Thus, it is hypothesized that there could have been a migration from southern Sahara which mixed with ancient Greeks to give rise to a part of the present day Greek genetic background. The admixture must have occurred in the Aegean Islands and Athens area at least (Figs 1 and 2). The reason why this admixture is not seen in Crete is unclear but may be related to the influential and strong Minoan empire which hindered foreigners establishment (10). Also, the time when admixture occurred could be after the overthrown of some of the Negroid Egyptian dynasties (Nubian or from other periods) or after undetermined natural catastrophes (i.e.: dryness). Indeed, ancient Greeks believed that their religion and culture came from Egypt (4, 25).

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