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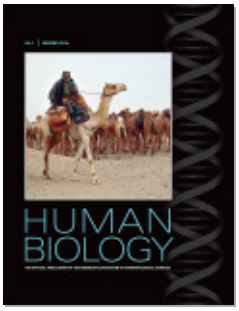

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Twenty Nuclear DNA Polymorphisms in a Moroccan Population: A Comparison with Seven Other Human Populations

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Abstract A south central Moroccan sample was analyzed for 20 nuclear DNA polymorphisms (restriction fragment length polymorphisms). The population was chosen on the basis of available information on its history, making it suitable for comparisons with data from other European populations. The markers analyzed have been studied previously in several human groups from different continents, and data on African and European samples have been compared to evaluate the genetic affinity of the studied sample with other populations, especially with two Spanish groups: Basques and Andalusians. Heterozygosity levels showed intermediate values between the African and European groups and higher than those found so far in an African group for the studied markers. Genetic distances closely matched geographical relationships through neighbor-joining tree and correspondence analysis, the Moroccans being closer to the European groups than the sub-Saharan Africans included in the analysis. Allele distributions revealed specific population associations with large weight of several alleles in the differentiation of some groups. Gene flow from sub-Saharan Africa appears to be relevant in understanding the differentiation of present Moroccan populations.

North Africa is a particularly interesting region because although it belongs to continental Africa, nineteenth-century anthropologists classified its light-skinned populations as Caucasoid. It is commonly accepted that two important but not impermeable geographical barriers, the Sahara Desert and the Mediterranean Sea, have conditioned the peopling of this region. However, several questions about the demographic history of northern Africa remain open, such as the level of gene flow associated with Arab expansions, the contribution of North African peoples to the gene pool of the northern Mediterranean shore, and the potential sub-Saha-

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ran genetic influence associated with the historically documented north-south trade routes.

The genetic structure of most Mediterranean populations can be considered to be the result of a complex pattern of isolation periods, mutual interactions, and recurrent colonizations. These populations constitute a useful model for detecting potential genetic traces of ancient and/or historical movements in the present populations. In historical times, an important population movement relating both sides of the Mediterranean was the conquest of the Iberian Peninsula and other northern Mediterranean regions by North Africans soon after the first Muslim invasion of North Africa in the 7th century. More than eight centuries of Muslim domination in the southern part of the Iberian Peninsula entailed an important cultural legate and, likely, population exchanges with North Africa were frequent. It is difficult to think of an effective expulsion of North African genes after such a long period of coexistence, especially in some regions like La Alpujarra, a rural mountainous area from the southeast of the Iberian Peninsula (Vincent 1987).

The position of the examined Moroccan population within the genetic landscape of Mediterranean populations has been previously focused through such classical markers as blood groups (Fernández-Santander et al. 1999) and enzyme polymorphisms (Kandil et al. 1999). These data are consistent with a certain degree of demographic impact of the long historic Arabic presence in the Iberian Peninsula. However, either the general picture or the antiquity of possible north-south demic contacts in the Western Mediterranean is far from clear. Other recent genetic studies seem to support different interpretations. Thus, surveys on HLA variation showed a close genetic similarity between some North African (Algerians) and Spanish populations that has been interpreted as the result of pre-Neolithic gene flow (Arnaiz-Villena et al. 1999). In contrast, other genetic data (classical polymorphisms, Simoni et al. 1999; autosomal STRs, Bosch et al. 2000; Y-chromosome haplotypes, Bosch et al. 1999; mtDNA, Rando et al. 1998; *Alu* insertion polymorphisms, Comas et al. 2000) emphasized a clear north-south differentiation in the Western Mediterranean that has been explained as the result of parallel, but independent, Neolithic human expansions on the north and south shores of the Mediterranean Sea (Comas et al. 2000)

Here we analyze the distribution of 20 nuclear restriction fragment length polymorphisms (RFLPs) in a Moroccan population sample providing new data on the genetic characterization of North African populations. These markers constitute a subset of the battery of 100 nuclear DNA RFLPs outlined some time ago for the systematic study of the diversity of the human genome (Bowcock et al. 1987). The information is applied to assess the relationship of the Moroccan population with other human groups from the European and African continents, paying special attention to Iberian Peninsula populations. This study of autosomal DNA markers with low mutation rates adds a new perspective to recent genetic information on north-south relationships in the Western Mediterranean (Gómez-Casado et al. 2000; Bosch et al. 2000).

Materials and Methods

Population Sampling. Blood specimens were obtained from 101 (58 males and 43 females) Moroccan unrelated and apparently healthy adults whose four grandparents originated in the same region. All sampled subjects were ethnically Arabs from the Doukkala region in south-central Morocco, and were selected on the basis of linguistics, physical characteristics, and family history information, under the expertise of the staff of the Department of Biology at the El Jadida University. Thus, the collected sample may be considered as representative of the present Arab-speaking population from south-central Morocco as a result of the Arabization of local populations. Seventy healthy individuals of both sexes were tested for 20 nuclear RFLP markers.

Although the complete set of DNA polymorphisms typed here has not been extensively studied, comparable data from four European and three African populations were gathered for comparisons. European groups comprise a general mixed sample of people from North and Central Europe (Bowcock et al. 1991), a more homogenous group from North Italy (Matullo et al. 1997), and two Spanish samples from Basques of the Guipuzcoa province (Moral et al. unpublished data) and La Alpujarra in southeast Spain (Fernández-Santander et al. 2001). The three sub-Saharan African groups included in the comparisons were East Senegal (Poloni et al. 1995) and two Pygmy samples from the Central African Republic and The Congo (Bowcock et al. 1991).

DNA Analysis. Genomic DNA was extracted from peripheral blood by the phenol-chloroform standard procedure and digested with 11 different restriction enzymes. The 20 nuclear RFLPs correspond to digestion patterns of 15 genomic loci with one or more enzymes.

Electrophoresis and Southern blotting were carried out as described in Feder et al. (1985). DNA samples were size-fractionated by 16-hour electrophoresis in agarose gel (0.85% or 1.2%), capillary transferred in a 20 × SSPE buffer onto Zetabind filters, and then hybridized with specifically labeled probes. A complete description of the probes and their sources was given in Bowcock et al. (1987). DNA probes were labeled with P^{32} by the random oligonucleotide priming technique (Feinberg and Vogelstein 1983). Fragment sizes were measured as compared to a lambda bacteriophage (supplied by Boehringer Mannheim, and double-digested with *Eco*RI and *Hin*dIII). Autoradiographs were interpreted according to definitions commonly used (see Bowcock et al. 1987).

Statistical Analysis. Allele frequencies were estimated by direct counting, and Hardy-Weinberg equilibrium was checked by chi-square and Fisher's exact test. Genetic differentiation between populations was estimated by Wright's (1978) F_{ST} as a measure of the interpopulation variance of allele frequencies. Significance was determined with an adaptation of the chi-square test after Work-

mann and Niswander (1970). Pairwise chi-square comparisons were carried out for every marker between the studied sample and the remaining populations. The alpha level was adjusted by the *Bonferroni inequality* (adjusted alpha = overall alpha/number of test) both in chi-square and F_{ST} analyses. Genetic distances among populations, defined as coancestry coefficients (Reynolds et al. 1983), were computed with the program Phylip 3.5 C (Felsenstein 1989). A neighbor-joining tree (Saitou and Nei 1987) displaying population relationship was inferred from distances, and its typology was checked by bootstrapping using 1000 iterations. Genetic relationships among populations were also described by correspondence analysis from the frequencies of 57 independent alleles present in the whole of the eight compared populations. Matrices of sample kinship coefficients were calculated for each allele, and then averaged to yield one overall matrix. From this matrix a genetic map was obtained where alleles and populations were represented together giving information about relations among human groups and alleles mainly associated with the population representation (Harpending and Jenkins 1973). Sub-Saharan genetic influence in the Moroccan population was estimated through the m_y admixture coefficient (Bertorelle and Excoffier 1998).

Results

Although the analysis was performed in a sample of 70 individuals, differences in number of chromosomes analyzed were due to DNA availability and methods used for typing each RFLP. The average number of individuals by locus was 58 ± 1.9. Allele frequencies for the 20 markers are shown in Table 1. Most polymorphisms were biallelic and three were multiallelic (*RB1/Dra*I, *D17S71/Pvu*II, *D7S1/Hin*dIII), depending on the variable number of restriction sites in the studied fragment. The Moroccan population showed good agreement with Hardy-Weinberg equilibrium for most markers. In general, the allele frequencies in the Moroccan population were within the European variation range showing clear differences from sub-Saharan groups. For example, *D17S71/Pvu*II polymorphism showed the typical European biallelic pattern instead of the African multiallelic one (Bowcock et al. 1991).

The average heterozygosity in Morocco was 0.376 ± 0.03, and there were no significant differences between coding (0.398) and anonymous regions (0.336) (U-Mann Whitney = 36.0, $p = 0.485$). Highest heterozygosity values were present in *RB1/Dra*I, *D13S41/Taq*I, *D13S6/Xmn*I, and *APOA/Xmn*I loci (Table 1). Some Moroccan markers showed the highest heterozygosity ever described in any population, such as *RB1* polymorphisms. Average heterozygosity of the Moroccan population (0.376 ± 0.03) was slightly higher than some African groups values (Senegalese = 0.346 ± 0.04; Congo Pygmies = 0.342 ± 0.04) and lower than the European populations included in the same analysis (Alpujarra = 0.399 ± 0.02; North Europeans = 0.391 ± 0.03; Basques = 0.412 ± 0.02; Italians = 0.399 ± 0.03).

As for population diversity of the typed loci, the average heterozygosity across the eight populations included in the analysis was 0.381± 0.03 (ranging

Table 1. Gene Frequencies, Number of Chromosomes Analyzed (*n*), and Proportion of Heterozygotes in the Individuals Typed for the 20 Markers in the Moroccan Population[a]

Locus/Enzyme	*Allele Size (kb)*	*Gene Frequency (n)*	*Proportion of Heterozygotes*
*NGFB/Taq*I	6.0	0.164 ± 0.03	0.300
	4.3/1.7	0.836 ± 0.03	
		(140)	
*CD8A/Dra*I	3.0	0.858 ± 0.03	0.283
	2.6	0.142 ± 0.03	
		(120)	
*D7S1/Hin*dIII	1.6	0.862 ± 0.03	0.277
	7.4	0.106 ± 0.03	
	2.8	0.032 ± 0.02	
		(94)	
*METD/Taq*I	6.0	0.889 ± 0.03	0.111
	4.4	0.111 ± 0.03	
		(108)	
*PLAT/Eco*RI	2.9	0.581 ± 0.04	0.426
	2.5	0.419 ± 0.04	
		(136)	
*APOA1/Xmn*I	8.3	0.735 ± 0.04	0.490
	6.6	0.163 ± 0.04	
	8.0	0.102 ± 0.03	
		(98)	
*A2M/Pvu*II	8.5	0.186 ± 0.04	0.176
	8.0	0.814 ± 0.04	
		(102)	
*A2M/Eco*RV	9.0	0.094 ± 0.03	0.156
	7.8	0.906 ± 0.03	
		(128)	
*A2M/Hin*fI	0.9	0.500 ± 0.05	0.424
	0.6	0.500 ± 0.05	
		(118)	
*A2M/Bgl*II	6.5	0.633 ± 0.04	0.299
	3.6	0.367 ± 0.04	
		(128)	
*A2M/Hae*III	2.0	0.333 ± 0.04	0.349
	1.7	0.667 ± 0.04	
		(126)	
*D13S62/Xmn*I	7.0	0.918 ± 0.03	0.122
	6.1	0.082 ± 0.03	
		(98)	
*D13S32/Eco*RV	5.9	0.235 ± 0.04	0.382
	3.9	0.765 ± 0.04	
		(136)	
*D13S41/Taq*I	8.5	0.493 ± 0.04	0.515
	2.4	0.507 ± 0.04	
		(136)	
*RB1/Dra*I	1.30	0.025 ± 0.01	0.617
	1.25	0.117 ± 0.03	

Table 1. (Continued)

Locus/Enzyme	*Allele Size (kb)*	*Gene Frequency (n)*	*Proportion of Heterozygotes*
	1.20	0.225 ± 0.04	
	1.10	0.433 ± 0.04	
	1.05	0.042 ± 0.02	
	1.00	0.050 ± 0.02	
	0.90	0.108 ± 0.03	
		(120)	
*RB1/Bam*HI	4.5	0.524 ± 0.05	0.429
	2.3/2.2	0.476 ± 0.05	
		(84)	
*D13S1/Taq*I	6.95	0.333 ± 0.04	0.435
	5.95/1.4	0.667 ± 0.04	
		(138)	
*D13S6/Xmn*I	8.0	0.684 ± 0.05	0.510
	7.0	0.316 ± 0.05	
		(98)	
*HP/Bam*HI	7.5	0.415 ± 0.05	0.362
	9.2	0.585 ± 0.05	
		(94)	
*D17S71/Pvu*II	3.2	0.125 ± 0.03	0.183
	2.9	0.875 ± 0.03	
		(120)	

a. Loci with the d-designation are the anonymous loci whereas the remainder are seated on coding regions.

from 0.623 for *RB1/Dra*I to 0.148 for *D13S62/Xmn*I marker). The mean value is higher than that reported in a previous paper for ten populations (Fernández-Santander et al. 2001), showing that the inclusion of the Moroccan population enlarges the variation range so far described for these markers.

Interpopulation differentiation was first approached by pairwise chi-square comparisons for every marker between the studied sample and the rest of the populations included in this analysis, indicating a considerable degree of genetic differentiation (Figure 1). Morocco exhibited significant differences from sub-Saharan Africa for at least half of the markers examined, whereas in the European group the percentage of significant differences was clearly lower. In the European group Basques showed the lowest number of significant differences from Morocco, followed by the two Mediterranean populations included in the analysis (southeast Spain and Italy). F_{ST} statistics revealed significant heterogeneity ($p < 0.01$) across populations for every polymorphism, except in the case of *A2M/Hin*fI. Average F_{ST} over the 20 loci was 0.098 ± 0.02 (ranging from 0.000 for *A2M/Hin*fI to 0.332 for *D7S1/Hin*dIII), similar to that reported for the same markers among ten populations (0.118 ± 0.01) in Fernández-Santander et al. (2001).

To assess the relationship between the Moroccan population and other Eu-

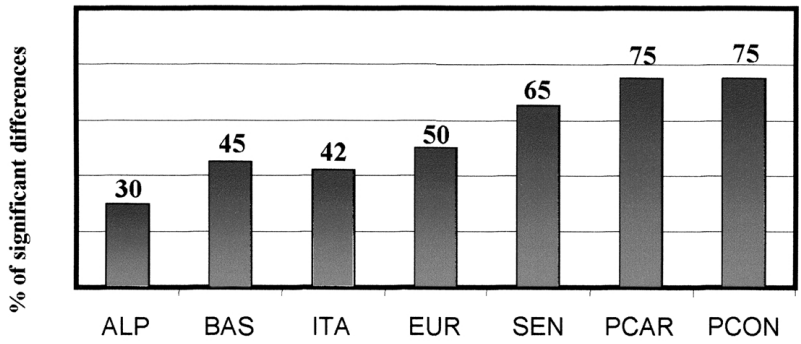

Figure 1. Percentage of significant differences tested by pairwise chi-square between Moroccan sample and the rest of populations included in the analysis. Abbreviations: MOR, Moroccans; ALP, Alpujarra; BAS, Basques; ITA, Italians; EUR, mixed Europeans; PCAR, Biaka Pygmies (Central African Republic); PCON, Mbuti Pygmies (The Congo); SEN, Senegalese.

ropean and sub-Saharan African groups, Reynolds' genetic distance coefficients were calculated (data not shown) and represented in a neighbor-joining tree (Figure 2). Distance estimates indicate that the closest populations to Morocco were La Alpujarra (0.041 ± 0.01) and Basques (0.043 ± 0.02), followed by Italians (0.045 ± 0.02). Average distance between sub-Saharan populations and Morocco was 0.155 ± 0.01, almost four times greater than the mean distance between the European groups and Morocco (0.046 ± 0.00). In general, lowest genetic distances corresponded to intra-European comparisons (average: 0.015); genetic distances among Africans were remarkably higher (average: 0.076), whereas the highest values corresponding to those between groups of the two continents (0.169). Consistently, the neighbor-joining tree in Figure 2 displayed two main population clusters: sub-Saharan Africa and the rest. The inclusion of Morocco within the European group reflects that genetic relationships closely matched geographical relationships. However, inside this group Morocco appears as the most differentiated population, a differentiation with a strong bootstrap support after 1000 iterations (85%). Among Europeans, the Mediterranean populations exhibited short branch-lengths, as is particularly evident for the two Spanish groups included in the comparison.

In order to avoid the limitations of the bifurcating model imposed by the neighbor-joining tree, population genetic relationships were also assessed through correspondence analysis (Figure 3). The first two axes accounted for 80.2% of the total genetic variance. The first axis underlined again the separation between the sub-Saharan African and the European plus North Africa, while the second principal component distinguished between the European groups and the studied Moroccan sample. Although the population distribution in the figure is the result of small contributions of most alleles, a few alleles appear especially associated with the different population groups in the map. So, the 3.0 kb allele of

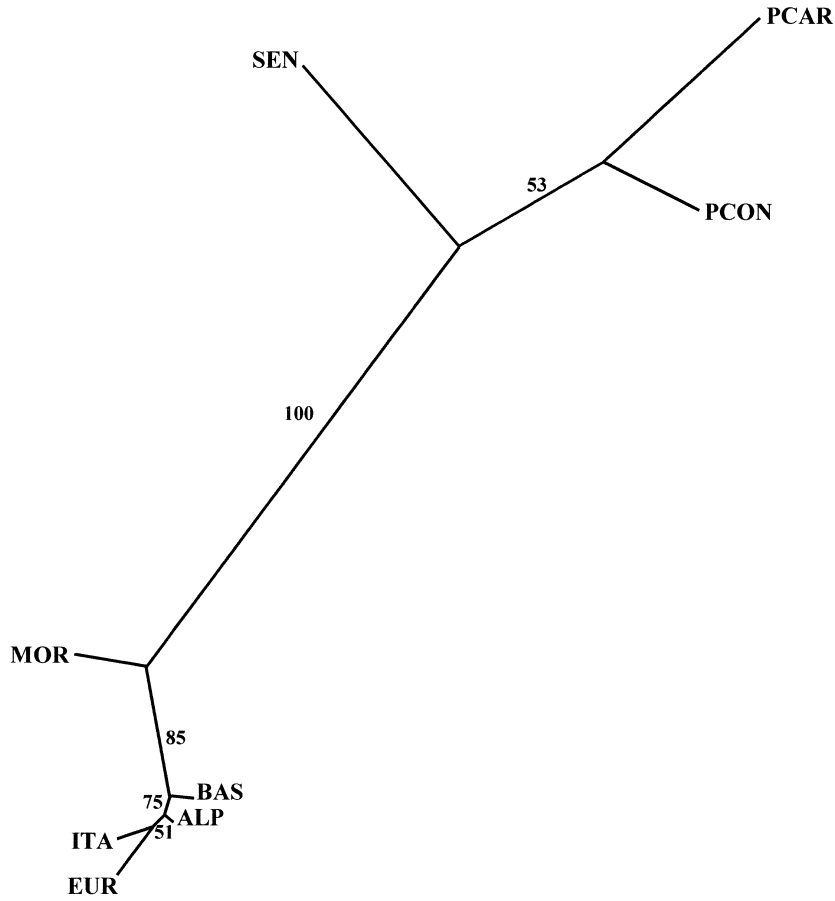

Figure 2. Neighbor-joining tree of eight populations tested on 19 markers. Robustness of the tree has been tested by the bootstrap method in more than 1000 resampled trees. Abbreviations of the populations are as in Figure 1.

*CD8A/Dra*I was mainly associated with the Moroccan position and may be seen as the one mainly responsible for the differentiation of this group in the context of the studied populations; 4.5 kb *RB1/Bam*HI, 1.2 kb *RB1/Dra*I, 7.0 kb *D13S6/Xmn*I, and 2.6 kb *CD8A/Dra*I were associated with the sub-Saharan African groups; and 2.3/2.2 kb *RB1/Bam*HI, 8.0 kb *D13S6/Xmn*I, and 1.1 kb *RB1/Dra*I were mainly responsible for the separation of the European populations.

Discussion

The present analysis on the distribution of 20 nuclear restriction fragment length DNA polymorphisms in an Arab-speaking population from south-central

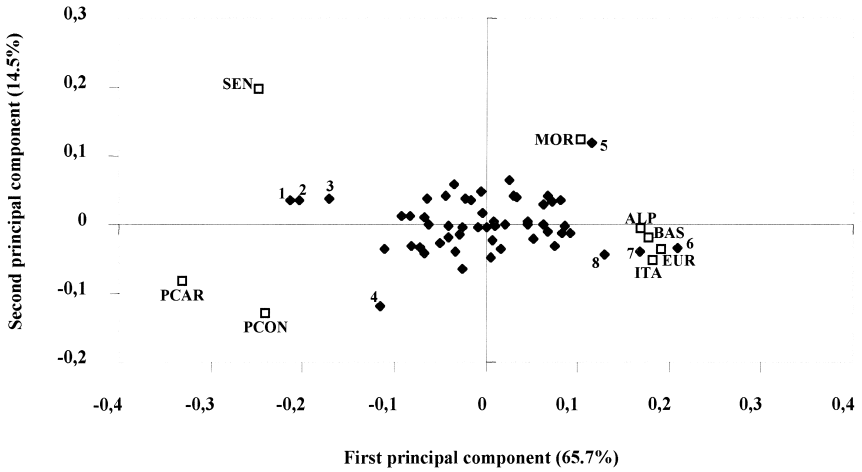

Figure 3. Principal components analysis of 57 independent alleles in the eight populations. Alleles are represented by black points and populations by empty squares. Abbreviations of populations are as in Figure 1, and numbers correspond to: 1: 4.5 kb allele of *RB1*/*Bam*HI; 2: 1.2 kb allele of *RB1*/*Dra*I; 3: 7.0 kb allele of *D13S6*/*Xmn*I; 4: 2.6 kb allele of *CD8A*/*Dra*I; 5: 3.0 kb allele of *CD8A*/*Dra*I; 6: 2.3/2.2 kb allele of *RB1*/*Bam*HI; 7: 8.0 kb allele of *D13S6*/*Xmn*I; 8: 1.1 kb allele of *RB1*/*Dra*I.

Morocco provides new molecular data to the knowledge of the genetic structure of present Moroccan populations, corroborating the genetic affinity of this North-African population within the general European and Mediterranean framework.

As for the markers examined, our results in Morocco confirm the usefulness of these polymorphisms to detect genetic variation between human populations according to previous surveys (see for instance, Bowcock et al. 1991; Linn et al. 1994; Poloni et al. 1995). In fact, the specific distribution found for some alleles associated with the population representation in the correspondence analysis reveals the discriminative power of some of them (for example, those from *RB1*/*Bam*HI, *RB1*/*Dra*I, and *D13S6*/*Xmn*I polymorphisms) for detecting genetic differentiation between human groups even at the microgeographical level.

The most conspicuous finding from our data is that the Moroccan population under study is closer to European populations than to sub-Saharan African populations. Morocco presents three-to-four times higher genetic distances to sub-Saharan Africa than to Europe. This sharp genetic differentiation might indicate that the Sahara Desert has acted as a stronger barrier to gene flow than the Gibraltar Straits. However, the relative proximity observed in the neighbor-joining tree and in the correspondence analysis between Morocco and sub-Saharan Africa as compared with European populations is also compatible with a certain sub-Saharan genetic influx into present Moroccan populations. A raw estimate of the sub-Saharan admixture in Morocco based on the allele frequencies of the 20 loci analyzed and assuming Europeans and Senegal samples as parental popula-

tions gave a bootstrap (10,000 iterations) average relative contribution of m = 0.2525 (bootstrap S.D. 0.050). These results agree with previous genetic studies (Lefranc et al. 1979; Bosch et al. 2000; Dios et al. 2001), even though our estimate is clearly lower than that from blood groups in Algeria (Aireche and Benabadji 1988). The remarkable sub-Saharan contribution (25%) found in this study points to remarkable gene flow that might have been particularly important before the formation of the Sahara Desert (5000 years BP, Said and Faure 1990), although the historical role of north-south trade routes cannot be discarded.

On the relationship with other European populations and, in particular, with the Iberian Peninsula, the present data also indicate a substantial differentiation. The distance between the Moroccan population and any European population (average 0.045) is much higher than the distance among European populations (average 0.015). Concerning this question, previous genetic data and interpretations are controversial (see, for instance, Arnaiz-Villena et al. 1999, and Comas et al. 2000). Our genetic distance analysis is concordant with the north-south differentiation evidenced by different kinds of genetic markers (classical, mtDNA, Y-chromosome, and *Alu* insertions). This differentiation has been interpreted as generated by parallel Neolithic waves along the two Mediterranean shores followed by a long period of isolation due to geographical and linguistic factors (Simoni et al. 1999; Comas et al. 2000). In such a scenario, a certain degree of particular differentiation should also be expected between North Africa and some European populations that have been traditionally interpreted as resulting from strong isolation, such as the Basques (Cavalli-Sforza et al. 1994). However, the 20 markers analyzed fail to reveal any singularity of Basques either in relation to other European populations (according to other data such as mtDNA [Bertranpetit et al. 1995] and Y-chromosome markers [Bosch et al. 1999]) or in relative comparisons with North Africa.

An important historical event was the conquest of the Iberian Peninsula by North Africans under Arab leadership in the 8th century A.D. The conquerors left an important cultural legacy mainly in the southern part of the Peninsula (Andalusia), where the Muslim domination lasted for more than eight centuries. Although the demographic impact of this conquest is a controversial issue, it is not unlikely that a certain degree of population admixture might have happened. In this way, previous studies from classical polymorphisms on the same Moroccan population under study (Fernández-Santander et al. 1999; Kandil et al. 1999) and from short tandem repeat markers in other population samples (Bosch et al. 1999) support differential gene flow into South Iberia.

Data from this study indicate a comparable distance between the Moroccan population and populations from the Basque Country, southeast Spain, and other European countries. Close genetic relationships between North Africans and Basques have been suggested from the HLA haplotype variation (Arnaiz-Villena et al. 1997; Gómez-Casado et al. 2000) and other anthropological studies on dermatoglyphic finger patterns (Moral et al. 2000). Also, data on the Y-chromosome haplogroups (Scozzari et al. 2001) are consistent with North African gene flow to Iberia and Southern Europe. It is clear that data from this paper alone do not af-

ford a decision on the possible antiquity of the relations between North Africa and the Iberian Peninsula. However, the fact that the degree of genetic affinity between Morocco and the Basque Country is equivalent to that from southern Spain suggests that the relative genetic affinity observed could not be attributed to the historical invasion of the Iberian Peninsula, since the Basque Country was free of Muslim invaders, according to historical information. In summary, this study on the distribution of 20 autosomal RFLPs in the Arab-speaking population from south-central Morocco shows the strong genetic distance between North and sub-Saharan Africa, but with a remarkable contribution of sub-Saharan genes into the gene pool of the Moroccan sample, which may help to understand the relative present differentiation between the two shores of the Mediterranean Sea.

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Literature Cited

Aireche, H. and M. Benabadji. 1988. Rh and Duffy gene frequencies in Algeria. *Gene Geography* 2:1–8.

Arnaiz-Villena, A., J. Martínez-Laso, E. Gómez-Casado et al. 1997. Relatedness among Basques, Portuguese, Spaniards, and Algerians studied by HLA allelic frequencies and haplotypes. *Immunogenetics* 47:37–43.

Arnaiz-Villena, A., J. Martínez-Laso et al. 1999. Iberia: Population genetics, anthropology, and linguistics. *Hum. Biol.* 71 (5):725–743.

Bertorele, G., and L. Excoffier. 1998. Inferring admixture proportions from molecular data. *Mol. Biol. Evol.* 15:1298–1311.

Bertranpetit, J., J. Sala, F. Calafell et al. 1995. Human mitochondrial DNA variation and the origin of Basques. *Ann. Hum. Genet.* 59:63–81.

Bosch, E., F. Calafell, F.R. Santos et al. 1999. STR variation is deeply structured by genetic background on the human Y chromosome. *Am. J. Hum. Genet.* 65:1623–1638.

Bosch, E., F. Calafell, A. Perez et al. 2000. Genetic structure of north-west Africa revealed by STR analysis. *Eur. J Hum. Genet.* 8:360–366.

Bowcock, A.M., C. Bucci, J.M. Hebert et al. 1987. Study of 47 DNA markers in five populations from four continents. *Gene Geogr*. 1:47–64.

Bowcock, A.M., J.M. Hebert, J.L. Mountain et al. 1991. Study of an additional 58 DNA markers in five human populations from four continents. *Gene Geogr*. 5:151–173.

Cavalli-Sforza, L.L., P. Menozzi, and A. Piazza. 1994. *History and Geography of Human Genes*. Princeton, NJ: Princeton University Press.

Comas, D., F. Calafell, N. Benchemsi et al. 2000. Alu insertion polymorphisms in NW Africa and the Iberian Peninsula: Evidence for a strong genetic boundary through the Gibraltar Straits. *Hum. Genet*. 107:312–319.

Dios, S., J.R. Luis, J.C. Carril et al. 2001. Sub-Saharan genetic contribution in Morocco: Microsatellite DNA analysis. *Hum. Biol.*73:675–688.

Feder, J., H.M.D. Gurling, J. Darby et al. 1985. DNA restriction fragment analysis of the proopiomelanocortin gene in schizophrenia and bipolar disorders. *Am. J. Hum. Genet.* 37:286–294.

Feinberg, A.P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analyt. Biochem*. 132:6–13.

Felsenstein, J. 1989. PHYLIP. Phylogeny Inference Package (version 3.2). *Cladistics* 5:164–166.

Fernández-Santander, A., M. Kandil, F. Luna et al. 1999. Genetic relationships between South-Eastern Spain and Morocco: New data on ABO, Rh, MNSs and Duffy polymorphisms. *Am. J. Hum. Biol.* 11:745–752.

Fernández-Santander, A., F. Luna, G. Villegas et al. 2001. Seventy-three nuclear DNA polymorphisms in a Spanish population. A worldwide comparison with ten other populations. *Am. J. of Hum. Biol.* 13:212–219.

Gómez-Casado, E., P. Moral, J. Martínez-Laso et al. 2000. HLA genes in Arabic-speaking Moroccans: Close relatedness to Berbers and Iberians. *Tissue Antigens* 55:239–249.

Harpending, H., and T. Jenkins. 1973. Genetic distance among Southern African populations. In *Methods and Theories of Anthropological Genetics,* M.H. Crawford and P.L. Workman, eds. Albuquerque, NM: University of New Mexico Press, 177–199.

Kandil, M., P. Moral, E. Esteban et al. 1999. Red cell enzyme polymorphisms in Moroccans and southern Spaniards: New data for the genetic history of the western Mediterranean. *Hum. Biol.* 71:791–802.

Lefranc, G., L. Lange G. de Rivat, A. Langaney et al. 1979. Gm, Am and Km immunoglubulin allotypes of two populations in Tunisia. *Hum. Genet.* 50:199–211.

Linn, A.A., J.M. Hebert, J.L. Mountain et al. 1994. Comparison of 79 polymorphisms tested in Australians, Japanese and Papua New Guineans with those of five other human populations. *Gene Geogr*. 8:191–214.

Matullo, G., R.M. Griffo, J.L. Mountain et al. 1997. Seventy-five nuclear DNA polymorphisms in an Italian sample: A comparative worldwide study. *Gene Geogr*. 11:15–35.

Moral, P., M. Kandil, D. Zaoui et al. 2000. Relaciones genéticas entre las poblaciones de Marruecos y España: Datos de polimorfismos clásicos y caracteres dermatoglíficos. In *Tendencias actuales de investigación en la antropología física española*. Ed. Universidad de León, Spain.

Poloni, E.S., L. Excoffier, J.L. Mountain et al. 1995. Nuclear DNA polymorphism in a Mandeka population from Senegal: Comparison with eight other human populations. *Ann. Hum. Genet.* 59:43–61.

Rando, J.C., F. Pinto, A.M. Gonzalez et al. 1998. Mitochondrial DNA analysis of Northwest African populations reveals genetic exchanges with European, Near-Eastern, and sub-Saharan populations. *Ann. Hum. Genet.* 62:531–550.

Reynolds, J., B.S. Weir, and C.C. Cockerham. 1983. Estimation of the coancestry coefficient: Basis for a short-term genetic distance. *Genetics* 105:767–779.

Said, R., and H. Faure. 1990. Chronological framework: African pluvial and glacial epochs. In *General History of Africa*, J. Ki-Zerbo, ed. Paris, France: UNESCO, 146–166.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molec. Biol. Evol.* 4:406–425.

Scozzari, R., F. Cruciani, A. Pangrazio et al. 2001. Human Y-chromosome variation in the western Mediterranean area: Implications for the peopling of the region. *Hum. Immunol.* 62:871–884.

Simoni, L., P. Gueresi, D. Petterner et al. 1999. Patterns of gene flow inferred from genetic distances in the Mediterranean region. *Hum. Biol.* 71:399–415.

Vincent, B. 1987. *Minorías y marginados en la España del siglo XVI*. Granada, Spain: Ed. Excma. Diputación Provincial de Granada.

Workmann, P.L., and J.D. Niswander. 1970. Population studies in South Western Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Hum. Genet.* 22:24–49.

Wright, S. 1978. *Evolution and Genetics of Populations*. Vol. 4. Variability within and among Natural Populations. Chicago, IL: University Chicago Press.