Variation of Rhesus Haplotype Frequencies in North Africans and in Worldwide Population Analyses

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ABSTRACT The Rhesus (Rh) blood group system is one of the most highly polymorphic genetic systems used in the investigation of human genetic relationships. In this paper the researchers aimed to expand the determination of the Rh haplotype frequencies in new samples from North African populations providing comparative analyses within and between these populations. A total of 771 blood samples were collected from three North African countries. Results reveal a general genetic homogeneity between North African populations when samples representative of wide areas were considered, regardless of their current linguistic status. However a significant micro-differentiation could be noted when small areas were considered. North African populations would possess a low ancient genetic sub-Saharan component. Analyses of the Rh haplotype frequency variation showed that worldwide populations represent a network of genetic relationships having adequate statistics and a general correspondence with geography coupled to historical patterns of gene flow and genetic drift influence.

INTRODUCTION

Until what extent the ethnic and genetic composition of present-day North African populations reflects the history and geography of North Africa is a matter of high interest for anthropologists and historians. North Africa has been a vital crossroads throughout history and historians name "Berbers" the people living in it since about 6000 years. This ancient Berber background would be only slightly influenced by different civilizations settled in this region such as Phoenicians (814 BC – 238 BC) and Romans (146 BC – 253 AD) because Berbers were opposed continually to these invaders, who were unable to dominate all North African regions and generally left the country after their defeat due to the arrival of a new power. On the other hand, the large expansion of Arabs, during the 7th century AD, may have added a substantial Arab component to North African populations. In fact, although at first the Arabs met remarkable resistance from Berbers, they persuaded them to adopt Islam, to learn the Arabic language and to accept inter-marriage. Thus, Arabs settled permanently in North Africa. However, in spite of the general adoption of the Muslim Arab culture some Berber groups have kept their Berber language and some of their traditions until now (El Moncer et al. 2010). Thus, the current North African populations (Moroccans, Algerians, Tunisians and Libyans) have similar structures: each one is composed of a general population (Arab-Berber) of Arabic speakers (a mixture mainly between Berbers and Arabs) and some Berber groups often speaking both Berber and Arab languages. Although these Berber groups are large in Morocco and Algeria and moderate in Libya, they are few and small in Tunisia (Ben Halima et al. 2014). Agreeing with the geographic continuity, Egypt with the exception of its eastern part "Sinai" represents a genetic continuity with North Africa (Salem et al. 2014). Several classic and molecular markers have been determined and analyzed in samples from North African populations (e.g., Lefranc et al. 1979; Chaabani et al. 1984a, 1984b; Chaabani and Cox 1988; Ghanem et al. 1988; Chaabani et al. 1989; Cherni et al. 2005, Coudray et al. 2006, El Moncer et al. 2010; Triki-Fendri et al. 2013, Ben Halima et al. 2014, Bahri et al. 2014).

Although the use of molecular markers is now considered as a preferable manner for studying human genetic variation, some researchers continue to determine classic markers such as

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the case of ABO and Rh D antigens (e.g., Benahadi et al. 2013; Weinstock et al. 2014) studied in a large number of world populations. However, Rh haplotypes (Rh D and RhCE antigens) have been less studied and obtained results are often presented as a simple phenotypic enumeration with little statistical analyses (e.g. Makroo et al. 2013; Benahadi et al. 2014).

The aim of this research is to provide a deepened study on the variation of Rh haplotype frequencies by applying all necessary and adequate statistical analyses. First, we Expand the determination of Rh haplotype frequencies in new samples from three North African countries (Morocco, Tunisia and Libya) performing analyses among these samples and others already studied (e.g., Fernandez-Santander et al. 1999; Chaabani et al. 2000; Harich et al. 2002; El Ossmani et al. 2008; Metri et al. 2012). Second we revise and analyze a large number of world populations to reveal the usefulness of the Rh system in the exploration of human evolutionary relationships.

INDIVIDUALS AND METHODS

A total of 771 blood samples of healthy unrelated North Africans of both sexes were collected in analyses laboratories after an informed consent following the Ethical Committees of the University of Monastir and the Tunisian Association of Anthropology that adhere to the principles of the Declaration of Helsinki. Two hundred and twenty nine are collected from different regions representative of the general Libyan population (Arab-Berber), 103 from several regions of the general Moroccan population, Arab-Berbers, (Mor 1) and 439 from the general Tunisian population (Arab-Berber): 241 from a small area in the North (Tun 1) and 198 from several regions of the Centre (Tun 2).

All blood samples were investigated for rhesus antigens by the classical haemagglutination method with five antisera: anti-D, anti-C, anti-c, anti-E and anti-e. Positive and negative controls for the polymorphisms examined were included in each test series. Rhesus frequencies were computed by means of a maximum-likelihood standard procedure based on the Hardy–Weinberg equilibrium hypothesis. The geographical origin of the samples studied in this paper and those used in comparisons are indicated in Figure 1.

Population comparisons (exact test of population differentiation) and hierarchical analyses of molecular variance (AMOVA) were estimated
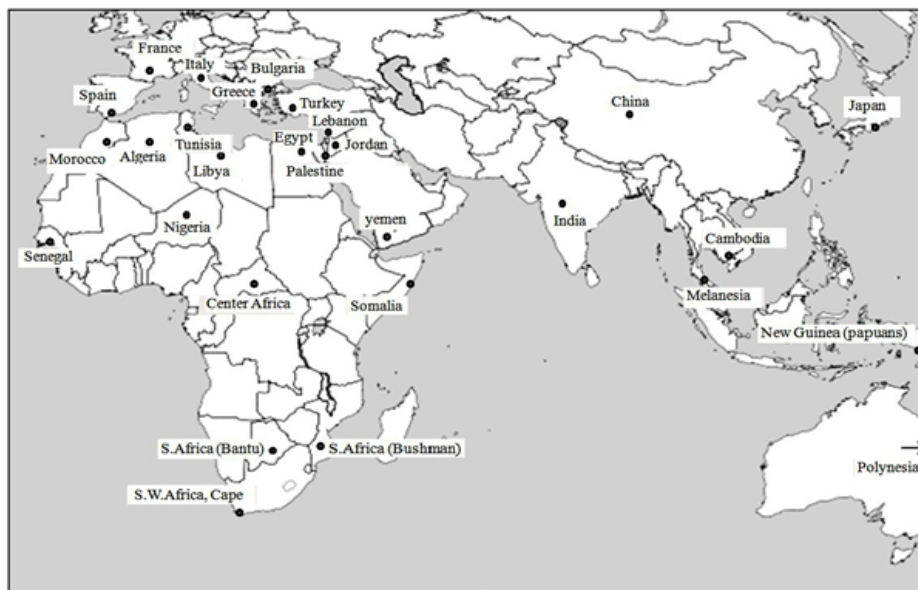

Fig. 1. Geographical location of population samples studied in the present paper and those used for comparison
Populations from four Islands located in South Europe were also used and not indicated in the figure (Corsica, Sardinia, Sicilia, and Crete)

using the Arlequin v 3.0 package (Schneider et al. 2000) AMOVA values of F_{SC} (genetic variance among population within groups), F_{CT} (genetic variance among groups) and F_{ST} (genetic variance among populations) were obtained for the different geographical partitions. F_{ST}-genetic distances between pairs of populations (Reynolds et al. 1983) were computed by means of the Phylip 3.5 package (Felsenstein 1989). Distance errors were estimated through bootstrapping methods. Genetic relationships among populations were depicted through two Multidimensional Scaling (MDS) plots from the distance matrix.

RESULTS

Rhesus phenotype distributions observed in the 4 North African samples from Tunisia, Morocco and Libya are presented in Table 1. All samples fitted the Hardy–Weinberg equilibrium. Corresponding Rh haplotype frequencies are recapitulated with those found in other samples from North Africa in Table 2, while those found in other world populations are recapitulated in Table 3.

Rh haplotype frequency comparisons, checked through the exact test of population differentiation, yielded no significant difference (p = 0.054) between the two samples Tun 2 and Tun 3 collected from wide areas in the Centre and in the South of Tunisia respectively, while the difference becomes significant (p < 0.001) if we incorporate into this comparison Tun 1 collected from a small area in the North. No significant difference (p = 0.335) was found between

the sample of Moroccan Berbers (Mor Ber) and the two samples of the general population (Arab Berber) (Mor 1 and Mor 2) collected from wide areas. But adding to this comparison the sample Mor 3 (Arab Berbers) collected from a small area (Beni-Mellal region) the difference becomes significant (p = 0.0114). A comparison among the general North African populations (all samples collected from wide areas considered together for each of the three countries, Libya, Morocco and Tunisia) yields, on the whole, no significant difference (0.063).

The genetic relationships among North African, European and Middle Eastern populations are represented in a multidimensional scaling plot based on the Reynolds distance matrix from Rh haplotype frequencies (Fig. 2). The plot revealed two major groups of samples. In the first, North African populations are assembled showing that (1) samples collected from small areas such as Tun 1 and Mor 3 are relatively distant from the other North African samples, (2) Arab-Berber and Berber samples are genetically close in each of Algeria and Morocco, and (3) the sample from general populalion of Western Egypt (Egy) comes within this North African group, while that from the East part of Egypt "Sinai" (Egy Sin), is moved away from this group. This genetic discontinuity between the two parts of Egypt already revealed by molecular marker analyses would have been due to historic events and maintained thanks to geo-climatic and social factors (Salem et al. 2014) (this peculiar genetic position of Sinai is also evident in Figure 3). In a second group the European populations

Table 1: Rhesus phenotype distributions in population samples from Libya, Tunisia and Morocco.

	Libya (Lyb)		Tunisia (North) (Tun 1)		Tunisia (Centre) (Tun 2)		Morocco (Mor 1)	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
DCec	72	81.15	55	52.86	64	54.24	46	37.53
Dce	35	34.77	25	22.23	23	22.24	11	10.96
DCe	31	30.96	23	24.94	25	23.19	16	15.97
ce	21	20.88	26	25.99	14	13.9	12	11.97
DEc	20	12.68	4	03.65	7	8.19	0	1.34
DEce	19	19.54	15	18.97	25	30.03	10	10.4
DCEec	18	21.73	34	30.49	24	31.18	5	11.58
DCEe	5	4.71	4	6.75	2	3.22	1	2.34
DCEc	4	1.71	5	4. 29	2	1.87	1	0.91
Cec	2	1.93	24	17.58	6	5.28	0	0.77
Ece	2	01.93	13	12.76	4	3.98	1	0.77
Ce	0	0.04	3	2.94	1	0.64	0	0.01
CcEe	0	0.09	2	3.26	1	0.99	0	0.23
Total	229	229	233	233	198	198	103	103

Table 2   Haplotype Rh frequencies in North African populations

Populations	Rh Haplotypes								Ref
	CDE	CDe	cDE	cDe	CdE	Cde	cdE	cde	
Libia (Lyb): GP	0.028	0.354	0.078	0.191	-	0.014	0.014	0.302	PS
Tunisia: GP									
North (Tun 1)	0.049	0.221	0.068	0.120	0.021	0.113	0.082	0.334	PS
Centre (Tun 2)	0.022	0.300	0.169	0.161	0.001	0.057	0.038	0.265	PS
South (Tun 3)	0.014	0.383	0.103	0.195	0.008	-	0.099	0.286	1
Algeria:									
Alger, GP (Alg 1)	-	0.441	0.098	0.198	-	0.012	-	0.251	2
Telmcen, GP (Alg 2)	0.019	0.364	0.122	0.247	-	0.002	0.005	0.250	3
Tizi Ouzou, Berbers (Alg 3)	0.002	0.434	0.083	0.182	-	0.018	0.004	0.277	2
Morocco									
GP (Mor 1)	0.012	0.383	0.104	0.131	0.003	0.011	0.014	0.341	PS
GP (S. Centre) (Mor 2)	0.007	0.339	0.124	0.156	-	0.069	0.013	0.292	5
GP (Beni - Mellal) (Mor 3)	-	0.382	0.076	0.225	-	0.065	0.073	0.179	6
Berbers (Mor 4)	0.052	0.307	0.079	0.233	-	0.029	0.017	0.283	4
Egypt									
West part (Egy)	0.005	0.326	0.133	0.186	-	0.011	0.005	0.337	7
East part "Sinai" (Egy Sin)	-	0.258	0.114	0.159	-	0.003	0.011	0.455	8*

GP : General population Arab-Berber
References : Present study (PS) ; 1. Chaabani et al. 2000 ; 2. Aireche and Benabadji 1988 ; 3. Metri et al. 2012; 4. Harich et al. 2002; 5. Fernandez-Santander et al. 1999; 6. El Ossmani et al. 2008; 7. Mourant et al. 1976; 8. Bonné et al. 1971* (*quoted by Mourant et al.1976)
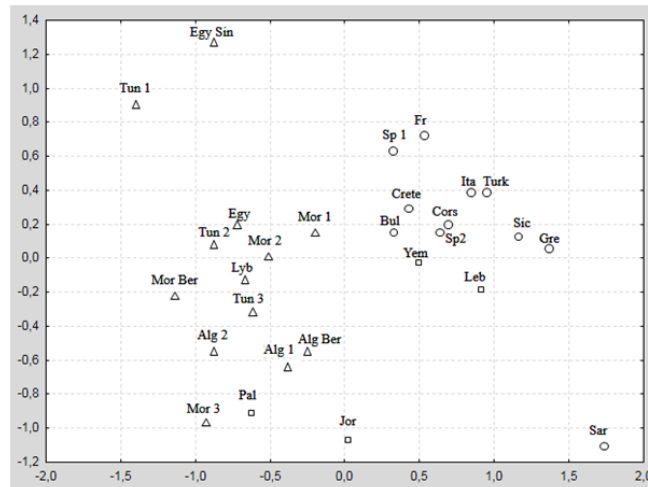

Fig. 2. Multidimensional scaling plot based on Raynolds distance matrix, data from Rh haplotype frequencies in 28 samples from 14 Mediterranean and Middle Eastern countries (Stress of 0.137)
Frequencies obtained in North Africans (Δ) are indicated in Table 2. Frequencies obtained in European (o) and Middle Eastern (□) populations are indicated in Table 3.
North Africa: Tunisia, North (Tun 1); Tunisia, Centre (Tun 2); Tunisia, South (Tun 3); Morocco, GP* from different regions (Mor 1); Morocco, GP* from S. Centre regions (Mor 2); Morocco, GP* from Beni Mellal region (Mor 3); Morocco, Berbers (Mor Ber); Algeria, GP* from different regions (Alg 1); Algeria, GP from the region of Telmcen (Alg 2); Algeria, Berbers from Tizi Ouzou (Alg Ber); Libia (Lyb); Egypt West part (Egy) ; Egypt East part, Sinai (Egy Sin).
Europe: France (Fr); France: Corsica (Cors); Spain (Sp 1); South Spain (Sp 2); Bulgaria (Bul); Greece (Gre); Greece: Crete (Crete); Italy, Lazio (Ita); Italy: Sardinia (Sar); Italy: Sicily (Sic); Turkey (Turk).
 Middle East: Lebanon (Leb); Palestine (Pal); Jordan (Jor); Yemen (Yem).
* GP: general population Arab-Berber

Table 3: Rh Haplotype frequencies considered in Europe, Middle East, Asia and sub-Saharan Africa

Populations	Rh Haplotypes								Ref
	CDE	CDe	cDE	cDe	CdE	Cde	cdE	cde	
Europe									
France (Fr)	0.004	0.425	0.130	0.028	-	0.013	0.005	0.390	1
France: Corsica (Cors)	0.004	0.469	0.164	0.046	-	0.013	-	0.304	2
Spain (Sp 1)	0.015	0.409	0.147	0.040	-	0.004	0.007	0.378	3
S. Spain (Sp 2)	0.006	0.172	0.138	0.062	0.006	-	0.009	0.313	4
Bulgaria (Bul)	-	0.424	0.140	0.074	-	0.014	0.001	0.309	5
Greece (Gre)	-	0.561	0.101	0.015	-	0.014	0.001	0.309	6
Greece: Crete (Crete)	-	0.430	0.148	0.050	-	0.057	-	0.315	7[*]
Italy, Lazio (Ita)	0.008	0.486	0.121	0.025	-	0.014	0.001	0.346	8
Italy: Sardinia (Sar)	0.020	0.643	0.089	0.036	-	0.025	-	0.187	9
Italy: Sicily (Sic)	0.010	0.539	0.109	0.015	-	0.016	-	0.311	10
Turkey (Turk)	-	0.482	0.171	0.013	-	0.014	-	0.320	11
Middle East									
Lebanon (Leb)	-	0.518	0.114	0.067	-	0.013	0.002	0.268	12
Palestine (Pal)	0.021	0.383	0.128	0.205	0.058	0.030	-	0.188	13
Jordan (Jor)	-	0.305	0.233	0.128	-	-	-	0.322	14
Yemen (Yem)	-	0.459	0.149	0.099	-	0.014	-	0.271	15
Asia									
India, North W (Ind N)	0.007	0.592	0.146	0.047	-	0.015	0.000	0.190	16
India, GP* (Ind GP)	0.012	0.632	0.097	0.056	0.001	0.021	0.004	0.177	18
China, Hong Kong (Chi)	0.004	0.729	0.187	0.033	0.003	0.018	0.000	0.023	19
Japan (Jap)	-	0.608	0.280	0.001	-	0.000	0.000	0.040	20
Polynesia (Pol)	-	0.680	0.240	0.064	-	0.000	0.000	0.000	21
New Guinea (N Gui)	-	0.880	0.096	0.008	-	0.000	0.000	0.000	11
Melanesia (Mel)	0.011	0.753	0.126	0.106	-	0.000	0.000	0.000	22
Cambodia (Cam)	0.014	0.734	0.173	0.059	-	0.014	0.003	0.001	23
Sub-Saharan Africa									
Nigeria (Nig)	-	0.060	0.115	0.590	-	0.031	0.000	0.202	11
Senegal (Sen)	0.002	0.016	0.079	0.632	-	0.000	0.003	0.268	24
Centre – Africa (C Afr)	-	0.019	0.104	0.623	-	0.000	0.000	0.153	25
Somalia (Som)	-	0.155	0.013	0.628	-	0.006	0.007	0.116	26
S Africa, Bantu (Ban)	-	0.036	0.086	0.721	-	0.013	0.000	0.144	22
S Africa, Bushman (Bus)	-	0.034	0.005	0.891	-	0.000	0.000	0.083	22
S Africa, Cape (SW Afr)	-	0.070	0.098	0.691	-	0.015	0.000	0.127	27

GP: General population (various Indian population groups)*
References (Ref.): 1. Goudemand and Salmon 1980; 2. Memmi 1999; 3. Moreno and Moral 1983; 4. Fermandez-Santander et al. 1999; 5. Baltova 2005; 6. Tsiakalos et al. 1980; 7. Barnicot et al. 1965 (* quoted by Mourant et al. 1976); 8. Piazza et al. 1989; 9. Vona et al. 1994; 10. Vona et al. 1998. 11. Mourant et al. 1976; 12. Ruffré and Taleb 1965; 13. Skaik 2011; 14. Nabulsi et al. 1997; 15. Chaabani et al. 2000. 16. Kumar and Bhasin 2011. 18. Bhasin and Walter 2007; 19. Mackey et al. 1969. 20. Missawa et al. 1974. 21. Leai and Bloom 1982. 22. Schanfield 1980. 23. Jaulmer et al. 1987. 24. Bouloux et al. 1972. 25. Langaney et al. 1978. 26. Sistonen et al. 1987. 27. May and du Toit 1990

are assembled except for that of Sardinia (Italian Island). In fact Sardinians appeared evidently distant from Europeans with a peculiar genetic profile that would be the result of several possible factors such as that Sardinia was isolated during long periods interrupted by the arrival of several groups of different origins. Similar peculiar genetic profile with more detailed explanations was already deduced from data analyses of several molecular and other classic markers (Calo et al. 2008). For the Middle East populations, Palestinians are nearer to North Africans,

while Lebanese, Yemenites and Jordanians show intermediate positions between South and North Mediterranean populations (this is also noted in Figure 3).

In order to estimate the genetic position of North African populations among worldwide populations, a second multidimensional scaling plot (Fig. 3) was carried out. This plot including 31 worldwide populations allowed distinguishing three population groups. The first is composed by populations from the Mediterranean and the Middle East. Asian populations are as-
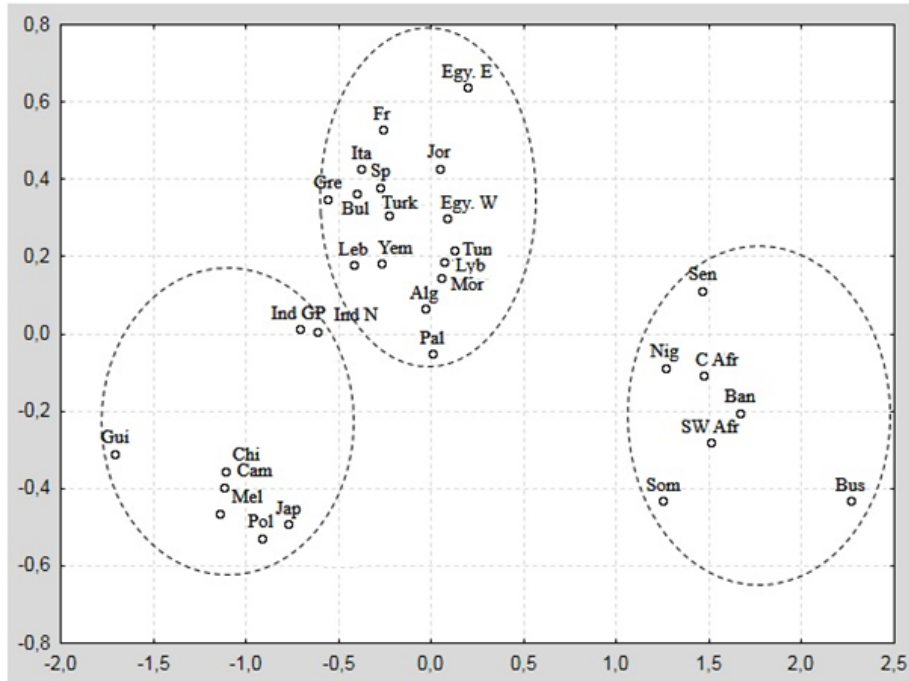
Fig. 3. Multidimensional scaling plot based on Raynolds distance matrix, data from Rh haplotype frequencies in 31 populations (Stress of 0.0324).
All corresponding frequencies are indicated in Table 2 and 3. For each of Tunisia, Algeria, Morroco and Spain populations the averages of frequencies of corresponding samples were considered.
North Africa: Tunisia (Tun); Morocco (Mor); Algeria (Alg); Libya (Lyb); Egypt West part (Egy W); Egypt East part, Sinai (Egy E).
Europe: France (Fr); Spain (Sp); Bulgaria (Bul); Greece (Gre); Italy, Lazio (Ita); Turkey (Turk).
Middle East: Lebanon (Leb); Palestine (Pal); Jordan (Jor); Yemen (Yem).
Asia: India, North W (Ind N); India, GP* (Ind GP); China, Hong Kong (Chi); Japan (Jap) ; Polynesia (Pol); New Guinea (N Gui); Melanesia (Mel); Cambodia (Cam).
Sub-Saharan Africa: Nigeria (Nig); Senegal (Sen); Senegal (Sen); Centre-Africa (C Afr); Somalia (Som); S Africa, Bantu (Ban); S Africa, Bushman (Bus); S Africa, Cape (SW Afr).
GP: General population (various Indian population groups)

sembled in the second where we note that Indian population is evidently nearer to those of the first group. This reflects the geographic position of India and possible ancient migrations such as those proposed for the Indo-Europeans (Mallory 1991). The third group, gathering sub-Saharan African populations, is clearly differentiated from the two other ones (average genetic distance, 0.303). On the other hand the average genetic distance between the Asian group and that composed by Mediterranean and Middle East samples showed a lower value (0.166).

To evaluate the genetic differentiation attributable to these 3 major worldwide population groups (group of Mediterranean and Middle Eastern populations, group of sub-Saharan Af-

ricans and group of Asians) a set of hierarchical analyses of molecular variance was conducted (Table 4. The overall genetic heterogeneity is significant between the 3 groups and also in the comparisons of these groups two by two. The genetic variance among these three groups (0.254, $p < 0.001$) and that between Sub-Saharan Africans and Asians (0.417, $p < 0.001$) was thirteen to eighteen times higher than those observed among populations within groups (0.0198, $p < 0.001$ and 0.022, $p = 0.001$ respectively). The genetic variance among groups when Mediterraneans and Middle Easterns on the one hand and Asians on the other hand were compared (0.092) was four times higher than that observed among populations within groups (0.021). The

Table 4: Hierarchical AMOVA analyses in 16 populations, data from Rhesus haplotype frequencies

	Hierarchical AMOVA		
	FSC among populations within groups	FST among populations	FCT among groups
Med. & M.E. / s.S.A. / As.	0.0198, p <0.001	0.269, p <0.001	0.254, p <0.001
Med. & M.E. / s.S.A.	0.0165, p <0.001	0.267, p <0.001	0.255, p <0.001
Med. & M.E. / As.	0.021, p =0.001	0.111, p <0.001	0.092, p <0.001
s.S.A. / As.	0.022, p =0.001	0.430, p <0.001	0.417, p <0.001

Sub-Saharan Africans (s.S.A.): - Somalians (n=1026) - Centre Africans (302) - W.S. Africans from Cape (n=1181) Asians (As.): - Indians (n=417) - Japanese (n=869) - Cambodians, Khmer (n=110). Mediterranean and Middle East (Med.&M.E.) populations: - Tunisians (n=605) - Moroccans (n=344) - Libyans (n= 229) - French (n=500) - S. Spanish (n=163) -Bulgarians (n=504) - Italy (n= 275) - Yemenites (n=210) - Palestinians from Ghaza (n=110) – Jordan (n = 180). (n: total number of individuals considered).

genetic variance among groups attributed to the comparison of Mediterraneans and Middle Easterns *versus* sub-Saharan Africans (0.255) was fifteen times higher than that among populations within groups (0.0165). With regard to haplotype frequencies, it is interesting to underline that R^1, R^0 and r were the haplotypes that mostly contributed to the high genetic differentiation among groups. For example, sub-Saharan Africans showed the lowest and highest haplotype frequencies of R^1 and R^0 respectively, whereas Asians showed the highest and lowest haplotype frequencies of R^1 and r respectively.

DISCUSSION

Three highly polymorphic immunologic systems GM, HLA and Rh are known by their usefulness in the study of the genetic history of human populations (Sanchez-Mazas and Pellegrini 1990; Chaabani 2014a). In this paper we provide for the first time, a detailed genetic analysis of Rh haplotype frequencies in several North African populations and a well-founded global analysis of Rh polymorphisms at a worldwide scale. Our results show that the most common Rh haplotypes found in the North African gene pool, R^1 and r (R^1: $0.227 – 0.383$; r: $0.265 – 0.341$), followed by R^0 and R^2 (R^0 : $0.120 – 0.233$; R^2: $0.068 – 0.169$), are generally the most widespread among world populations.

When wide areas are considered, no significant differences were obtained among North African populations (all samples considered together Morocco, Tunisia and Libya) and among Arab-Berber and Berber samples. This genetic homogeneity among North African populations was also provided among Moroccan, Algerian and Tunisian populations using other powerful classic or molecular markers (Coudray et al. 2006; Bahri et al. 2008; El Moncer et al. 2010) and agrees with historic data considering North African populations as having similar origins. However, a recent molecular study show that Libyan population appeared with higher genetic diversity compared with other North African populations (Ben Halima et al. 2014).

Rh haplotype polymorphism variation could show significant micro-differentiations among North Africans when samples from small areas were considered such as the case of Tun 1 towards Tun 2 and 3 samples or the case of Mor 3 towards the other Moroccan samples. As comparison with molecular marker analyses, we can quote a previous study (Bahri et al. 2008) in which using 8 *Alu* insertions authors have not found significant genetic differences between Tunisians of North, Center and South. However, a moderate significant difference appears when a higher number of *Alu* insertions (16 loci) were used (El Moncer et al. 2010). A micro-differentiation among samples from other small areas in different regions of Tunisia was also noted using other classic or molecular markers (e.g., Chaabani et al. 1984a; Chaabani and Cox 1988; Cherni et al. 2005, El Moncer et al. 2011).

If Rh haplotype frequencies in worldwide populations are examined (Table 3), it can noted that R^0 haplotype could be considered as specific to sub-Saharan African populations. In fact its frequency is high only in sub-Saharan groups (it ranges from 0.623 to 0.891); while it is very low in Europeans (0.011 – 0.074) and Asians (0.001 – 0.106) but not negligible in Middle Eastern Arab populations (0.099 – 0.205) and North African populations (0.150 to 0.247). This sug-

gests the presence of a slight sub-Saharan component in the gene pool of Middle Eastern Arabs and North African populations. Concerning the latter similar suggestions are already deduced from analyses of other protein and molecular markers such as those of IgG (GM haplotypes) and α_1-antitrypsin (Pi system) (Chaabani et al. 1984b; Chaabani and Cox 1988), those of immunoglobulin $C\gamma$ genes (Chaabani et al. 1989) and *Alu*/STR compound systems (Gonzalez-Perez et al. 2010; El Moncer et al. 2010). Historic data are in favor of the oldness of this sub-Saharan component in North Africa. In fact since about 5000 years before present (BP) the immense Sahara desert already had the current severe climate that represents a considerable barrier to human migration, but before it had better climate more accessible to people movements (Pons et al. 1991). Besides the first stage of Neolithic Age in North Africa designated "Neolithic with Sudan origins" is the only period characterized by some sub-Saharan ethnic contribution from Sudan. This cultural period was started about 9000 years BP in the extreme south of North Africa, where the current Saharan regions of Algeria are (Gragueb and Mtimet 1989). Hence the possible presence of slight sub-Saharan component in current North African populations could be traced back to the first stage of Neolithic Age. Namely it would be an original component already present in the gene pool of Berber ancestors' natives of North Africa.

At a macro-geographic scale, the multidimensional scaling plot (Fig. 3) demonstrated the ability of Rh haplotypes to differentiate the major world populations by giving a clear representation of a complex network of genetic relationships that appears to be in correspondence with the geography coupled to historical patterns of gene flow and genetic drift influence. In fact, sub-Saharan African populations formed a well-differentiated cluster from the remaining world populations grouped in two close clusters: that of Asian populations on the one hand, and that of North African, southern European and Middle East populations on the other hand. The genetic affinity among populations of the last cluster could be attributed to the homogenizing effect of gene flow between Mediterranean and Middle East regions, connected by major ancient people movements such as that from Yemen to North Africa and to ancient Mesopotamia (Chaabani 2002; Cerny et al. 2011;

Bahri et al. 2012; Badro et al., 2013; Chaabani 2014b). However, in this last cluster we can note three sub-clusters corresponding to South Europeans, North Africans and Middle East populations. Similar networks of genetic relationships were already obtained when powerful known markers were used such as the case of GM markers (e.g., Chaabani et al. 2000) or the case of *Alu* insertion molecular markers (e.g., Bahri et al. 2012; Terreros et al. 2009). This proves the accuracy of the Rh polymorphisms in analyses of worldwide population relationships.

Our present data show that *D*-frequency is around 0.30 to 0.36 in North Africans. Compared with data shown by the other world populations quoted above, we note that *D*- frequency is evidently lower in sub-Saharan Africans (around 0.10 to 0.27) and in Asians (around 0.00 to 0.23), while it can reach 0.45 in Europeans. This appreciable *D*- frequency generally linked to no departures from Hardy-Weinberg equilibrium seems in disagreement with the genetic selection models dictating that there must have been some advantage to individuals who were heterozygous (balancing selection). What that advantage might have been is obscure, as the only obvious selection pressure in this case is against the heterozygote, reflected in morbidity and mortality of *D/d* infants born to *D–* alloimmunized women. Besides, this is supported by results of a recent study (Perry et al. 2012) in which authors tested the idea that positive selection for an as-of-yet unknown fitness benefit of the *RHD* deletion may have offset the otherwise negative fitness effects of hemolytic disease of the newborn. They found no evidence that positive natural selection affected the frequency of the *RHD* deletion and, therefore, they consider that the *D–* negative has arisen numerous times on different genetic backgrounds and its frequency variation may simply be explained by genetic drift / founder effect.

Concerning the global Rh system (*RHD* and *RHCE* genes), although it is not yet clear how natural selection has shaped population differentiation, some studies suggested that negative selection has globally reduced population differentiation giving exceptionally low F_{ST} values; while positive selection has ensured the regional adaptation of human populations by increasing population differentiation giving unusually high F_{ST} values (Cavalli-Sforsa 1966; Akey et al. 2002). Besides, Barreiro et al. (2008)

interpreted F_{ST} values below 0.05 as indicating low differentiation, so their corresponding loci have therefore been under evident negative selection pressures, while values above 0.65 indicated extreme differentiation and their loci have therefore been under evident positive selection pressures. Our results on Rh haplotypes (Table 4) show that the genetic variance among the three major world groups (F_{ST}) is 0.254, ranging from 0.092 to 0.417 when the three groups were compared in pairs. Thus, on the basis of the quoted considerations our F_{ST} values could not be in favor that Rh genes have been under important negative or positive selection. But in any case it would be impossible to demonstrate for any individual system that it was not been under slight or even moderate action of selection.

CONCLUSION

This paper expanded the determination of the Rhesus (Rh) haplotype frequencies by exploring new samples of North African populations and provided deepened analyses of the worldwide variation of Rh haplotype frequencies. The results assert a general genetic homogeneity among North African populations when samples representative of wide areas were considered, regardless of their current linguistic status. However a significant micro-differentiation could be noted when small areas were considered. Although North African populations are distinct from sub-Saharan Africans, they would possess a low ancient genetic sub-Saharan component. Rh haplotype frequency analyses of worldwide populations show a genetic relationship network that appears to be in correspondence with the geography coupled to the influence of historical patterns of gene flow and genetic drift. This accurate anthropological picture, reinforced by the obtained F_{ST} values, shows that Rh haplotype markers can be considered among powerful genetic and molecular markers used in the study of human evolutionary relationships.

RECOMMENDATIONS

Although the use of molecular markers is currently the preferred method to study human genetic variation, the deepened use of highly polymorphic classical markers, such as Rh haplotypes, might provide complementary explanations at the fundamental and anthropological levels

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