Paternal Lineages in Libya Inferred From Y-Chromosome Haplogroups

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ABSTRACT Many studies based on genetic diversity of North African populations have contributed to elucidate the modelling of the genetic landscape in this region. North Africa is considered as a distinct spatial-temporal entity on geographic, archaeological, and historical grounds, which has undergone the influence of different human migrations along its shaping. For instance, Libya, a North African country, was first inhabited by Berbers and then colonized by a variety of ethnic groups like Phoenicians, Greeks, Romans, Arabs and, in recent times, Italians. In this study, we contribute to clarify the genetic variation of Libya and consequently, of North African modern populations, by the study of Libyan male lineages. A total of 22 Y-chromosome-specific SNPs were genotyped in a sample of 175 Libyan males, allowing the characterization of 18 Y-chromosomal haplogroups. The obtained data revealed a predominant Northwest African component represented by haplogroup E-M81 (33.7%) followed by J(xJ1a,J2)-M304 (27.4%), which is postulated to have a Middle Eastern origin. The comparative study with other populations (~5,400 individuals from North Africa, Middle East, Sub-Saharan Africa, and Europe) revealed a general genetic homogeneity among North African populations (F_{ST} = 5.3 %; P-value < 0.0001). Overall, the Y-haplogroup diversity in Libya and in North Africa is characterized by two genetic components. The first signature is typical of Berber-speaking people (E-M81), the autochthonous inhabitants, whereas the second is (J(xJ1a,J2)-M304), originating from Arabic populations. This is in agreement with the hypothesis of an Arabic expansion from the Middle East, shaping the North African genetic landscape. Am J Phys Anthropol 157:242–251, 2015.

INTRODUCTION

Several studies based on uniparental markers (mtDNA and Y-chromosome) have provided evidence that the North African gene pool has been shaped by the back-migration of Eurasian lineages in Palaeolithic (Olivieri et al., 2006) and Neolithic times (Arredi et al., 2004), and highlighted more recent influences from sub-Saharan Africa (Harich et al., 2010; Fadhlaoui-Zid et al., 2011b), and Mediterranean Europe (Zalloua et al., 2008). In particular, Libya has experienced a variety of human migrations that have modelled the cultural groups inhabiting the area. The history of Libya includes the history of its rich mix of ethnic groups added to the indigenous Berber tribes. Berbers are commonly considered as *in situ* descendants of local Paleolithic and/or Mesolithic populations. Moreover, archaeological data points to a human occupation of North Africa since at least 45,000 years ago, as attested by the Aterian industry (Garcea and Giraudi, 2006), and subsequent cultures until the Neolithic, which began around 5,500 years ago in the region (Camps, 1974, 1982).

By the 5th century B.C.E., Carthage, had extended its hegemony across North Africa, where a distinctive civilization, known as Punic, came into being. The Punics were a group of Western Semitic-speaking peoples who traced their origins to a group of Phoenician settlers, but also to North African Berbers. Punic settlements on the Libyan coast included Tripoli, in an area that came to be known collectively as Tripolis, or "Three Cities." The influence of Punic civilization on North Africa remained deep-seated. After the destruction of Carthage, in 146 B.C.E. Tripolitania became a Roman province. On the other hand, Greek settlements in Libya began in the 7th century B.C.E. in the East of Libya. Throughout the

Additional Supporting Information may be found in the online version of this article.

Electronic-database information: BATWING: (http://www.mas.ncl.ac.uk/~nijw/) and Y chromosome consortium: (http://www.isogg.org/tree/)

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period of Punic and Greek colonization of the coastal plain, the area known as Fezzan was dominated by the Garamantes, a tribal people who entered the region sometime before 1,000 B.C.E. They established a powerful kingdom in the desert astride the trade route between the Western Sudan and the Mediterranean coast.

By the end of the 7th century C.E., Arab armies migrated to expand the Islamic religion and Arabic language into North Africa. In the 11th century C.E., the Fatimid caliph, in Cairo, invited the Bani Hilal and Bani Salim, Bedouin tribes from Arabia known collectively as the Hilalians, to migrate to the Maghreb. The number of Hilalians who moved westward out of Egypt has been estimated as high as 200,000 families, leading to the Arabization of the region by imposing their social organization, values, and language (Metz, 1987; Camps, 1996). In the 16th century C.E., the Ottoman Empire in Constantinople expanded into North Africa. And later, after the Italo-Turkish War (1911–1912 C.E.), Italy turned Libya into colony. However, in 1951 C.E., Libya declared its independence as the United Kingdom of Libya (Ahmida, 1994).

Because of this rich mix of ethnic groups which superimposed over the indigenous Berber tribes, many studies have attempted to describe the genetic structure of the Libyan population using different autosomal markers: polymorphic Alu insertions (Cherni et al., 2011, Halima et al., 2014), the human leukocyte antigen (HLA) polymorphism (Galgani et al., 2013), autosomal STRs (Immel et al., 2006; Khodjet-El-Khil et al., 2012; Elmrghni et al., 2012b;) and polymorphisms of the COL1A2, CYP1A1 and HS1,2 Ig enhancer genes in the Tuaregs from Libya (Martinez-Labarga et al., 2007). Uniparental markers were also investigated in Libya through the mitochondrial DNA polymorphisms (Ottoni et al., 2009, 2010; Fadhlaoui-Zid et al., 2011b) and Y-STRs (Elmrghni et al., 2012a; Triki-Fendri et al., 2013). These studies support the important role that migratory movements have played in the North African gene pool, at least since the Neolithic period leading to an admixture between the original Berber inhabitants and neighboring and more distant populations, even though a strong Berber genetic substratum remains.

However, even with the increase of population genetic studies in Libya during the last few years, the Y-chromosome haplogroups remain poorly studied. In fact, only one investigation on the Y-SNP diversity was performed in Libya but in a particular population: Libyan Tuaregs from Fezzan (Ottoni et al., 2011). Thus, we report here, for the first time, Y-haplogroup genetic data obtained through the high-resolution investigation of 22 Y-SNPs in a sample of 175 Libyans from Tripoli. Indeed, we have conducted a comparative study between our results and the existing genetic data on other populations from North Africa, Middle East, Sub-Saharan Africa, and Europe. The aim of this work is to assess the robustness of the hypothesis produced by previous studies such as the genetic homogeneity among North African populations, the direction of the human population movements across the region, and the high level of genetic isolation of the Libyan Tuaregs compared with the general population of Libya. We, also, intended to determine the genetic components of Libya and consequently of North Africa, in general, knowing the rich mix of ethnic groups that have inhabited the region added to the indigenous Berber tribes.

Fig. 1. Map of Libya.

MATERIAL AND METHODS

Population samples

A total of 175 unrelated males from Libya (Tripoli region) (Fig. 1), previously typed for 17 Y-STR (Triki-Fendri et al., 2013), were analyzed after informed consent. DNA extraction was done using a standard phenol-chloroform procedure.

Y-SNP genotyping

Y haplogroups were determined through the analysis of 22 Y-SNPs (SRY10831, M213, M9, M22, Tat, 92R7, M173, P25, M269, M70, M96, M35, M78, M81, M123, M34, M201, M170, M26, M304, M62, and M172). The selection of these Y-SNPs was performed based on both the Y-SNP tree topology (Karafet et al., 2008; Cruciani et al., 2010; Trombetta et al., 2011; Van Oven et al., 2014) and the information in literature concerning the Y-haplogroups that have been found in North African populations until present (Robino et al., 2008; El-Sibai et al., 2009; Fadhlaoui-Zid et al., 2011a). The selected method for allele discrimination was a single base extension reaction with the use of the SNaPshot multiplex kit (Applied Biosystems, Foster City, USA) according to Blanco-Verea et al. (2008). To determine the haplogroup distribution in Libya, the Y-SNPs were hierarchically typed in three multiplex reactions as previously reported in the literature (Brión et al., 2005; Brisighelli et al., 2012). These multiplexes were implemented in such a way that avoids genotyping of unnecessary Y-SNPs to define the final haplogroup, saving effort and cost, as we only need to genotype one single multiplex in the best case, and two in the worst case.

Statistical analysis

The haplogroup frequencies were estimated by direct counting. Arlequin software version 3.5 (http://cmpg.unibe.ch/software/arlequin35/; Excoffier et al., 2005) was used to perform the Analysis of Molecular Variance (AMOVA) and to calculate the pairwise F_{ST} genetic distances between Libya and 28 populations issued from North Africa, Sub-Saharan Africa, Middle East, and Europe (Table 1). The Y-SNPs used in this work are the same as those used for comparisons with other populations. Genetic distances were visualized in two-dimensional space using the multidimensional scaling (MDS) method included in the Statistical Package for the Social Sciences (SPSS) software 17.0 (SPSS).

TABLE 1. Populations included in the comparative study

Group	Abbreviation	Full name	N	Reference
North Africa	Lib	Libya	175	Present study
	Trg	Libyan Tuaregs	47	Ottoni et al. (2011)
	Tun	Tunisia	159	Fadhlaoui-Zid et al. (2011b)
	Alg	Algeria	102	Robino et al. (2008)
	MA	Moroccan Arab	44	Arredi et al. (2004)
	MB	Moroccan Berber	102	
	Egp	Egypt	116	El-Sibai et al. (2009)
Middle East	Kw	Kuwait	40	
	SA	Saudi Arabia	157	Abu Amero et al. (2009)
	Qt	Qatar	72	Cadenas et al. (2008)
	UAE	United Arab Emirates	164	
	Yem	Yemen	62	
	Irq	Iraq	203	Al-Zahery et al. (2003)
	Syr	Syria	356	El-Sibai et al. (2009)
	Leb	Lebanon	916	Zalloua et al. (2008)
	Anat	Anatolia	523	Cinnioğlu et al. (2004)
Sub-Saharan Africa	Ug	Uganda	118	de Filippo et al. (2011)
	Tz	Tanzania	237	
	Ken	Kenya	89	
	Cam	Cameroon	55	
	Bfaso	Burkina Faso	335	
	Eth	Ethiopia	98	
	Som	Somalia	201	Sanchez et al. (2005)
	Sud	Sudan	445	Hassan et al. (2008)
Europe	Port	Portugal	87	Nogueiro et al. (2010)
	Hng	Hungary	215	Völgyi et al. (2009)
	It	Italy	162	Onofri et al. (2007)
	Sp	Spain	169	López-Parra et al. (2009)
	Ukr	Ukraine	152	Mielnik-Sikorska et al. (2013)

N: Number of samples.

The R environment for statistical computing and graphics (v2.15.2; http://www.r-project.org/) was used to perform the Principal Component Analysis based on haplogroup frequencies as well as the Mantel tests, to check for correlation between geographical and genetic distances. Chi-square association tests were implemented with Clump (v2.4; http://www.smd.qmul.ac.uk/statgen/dcurtis/software.html) (Sham et al., 1995). Ten thousand simulations were performed to achieve a satisfactory accurate estimate of the true significance. These tests were performed to assess the difference between haplogroup distributions of Libya compared with eight other neighbor or adjacent populations (Tuaregs from Libya, Tunisia, Algeria, Egypt, Moroccan Arabs, Kuwait and Sudan) at the pairwise level.

A Bonferroni correction for all multiple testing was used (Hochberg 1988). Genetic relationships between Y-STR haplotypes within specific haplogroups were analyzed using Network 4.6.1.0 software available at (http://www.fluxus-engineering.com) (Bandelt et al., 1999), applying an STR variance-based weighting as described by Qamar et al. (2002). For network construction, the number of repeats at DYS389II was calculated after subtracting the number of repeats at DYS389I. DYS385 was not considered for statistical analysis.

RESULTS AND DISCUSSION

A sample of 175 unrelated males from Libya (Tripoli region) (Fig. 1), previously studied for 17 Y-STRs (Triki-Fendri et al., 2013), was genotyped for a set of 22 Y-SNPs. Both SNP and STR data are shown in ESM_1. These data were submitted to YHRD (http://www.yhrd.org/) receiving the following accession number: YA003752.

Y-SNP haplogroup variation

A haplogroup diversity of 0.7988 was found in the Libyan population, that is higher than those previously observed in other North African populations: 0.4072 in Tunisia (Fadhlaoui-Zid et al., 2011a), 0.6709 in Algeria (Robino et al., 2008), and 0.7035 in Egypt (El-Sibai et al., 2009) and, however, similar to that observed in Sudan 0.8033 (Hassan et al., 2008).

The 22 SNPs genotyped in this work allowed the discrimination of 18 different Y-SNP haplogroups and sub-haplogroups, which are presented in Figure 2 along with their respective frequencies in the Libyans from Tripoli. The haplogroup with the highest frequency observed in Tripoli was E-M81 (33.7%) followed by J-M304 (27.4%) and E-M78 (8%). The remaining haplogroups occurred with much lower frequencies representing in some cases only one individual.

Phylogeographic analysis

Five sub-haplogroups were detected inside clade E (Fig. 2) in our population sample. The most frequent sub-haplogroup was E-M81 (33.7 %), which is also the most common in North Africa accounting for 42% of the total samples analyzed in North Africa (Arredi et al., 2004). E-M81 shows the highest concentrations in North Western Africa (76% in Morocco) with cline frequencies decreasing eastward (45% in Algeria (Robino et al., 2008)), and it is particularly spread among Berber-speaking groups (Bosch et al., 2001; Cruciani et al., 2002, 2004; Arredi et al., 2004; Fadhlaoui-Zid et al.,

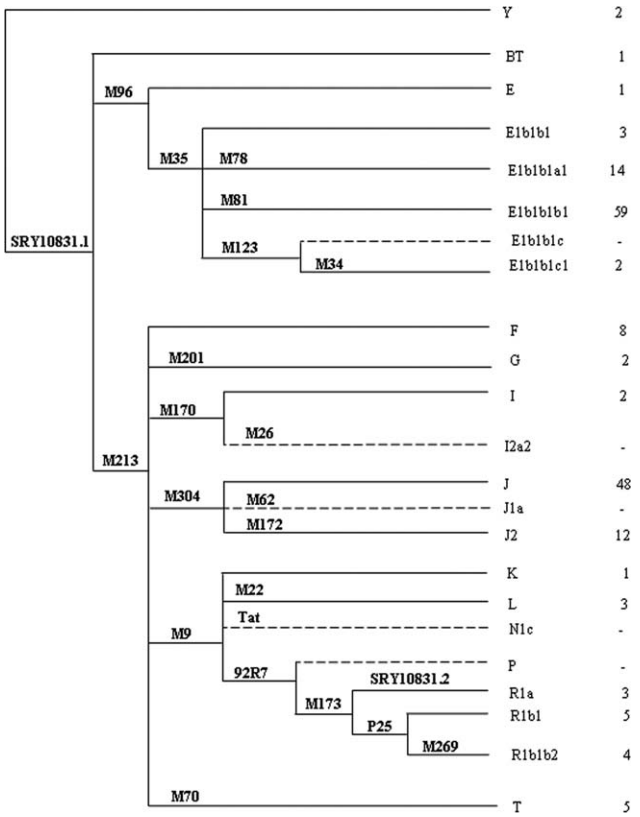

Fig. 2. Phylogenetic tree of Y chromosome haplogroups searched in this study. The analyzed Y-SNPs are shown in each branch and the corresponding haplogroup and observed absolute frequencies are shown at the end of each branch according to Karafet et al. (2008), Cruciani et al. (2010), and Trombetta et al. (2011). *Broken line branches* correspond to haplogroups not found in our sample and *solid line branches* to those found here.

2011a; Ottoni et al., 2011). The distribution of E-M81 chromosomes in Africa closely matches the present area of Berber-speaking population's allocation on the continent, suggesting a close haplogroup-ethnic group parallelism.

Outside Africa, E-M81 is almost absent in Europe (with the exception of Iberia and Sicily) and the Middle East. The presence of E-M81 in the Iberian Peninsula (12% in southern Portugal) (Cruciani et al., 2004) has been attributed to possible introgressions linked to the Islamic influence, because it is typically Berber (Bosch et al., 2001; Semino et al., 2004; Beleza et al., 2006; Alvarez et al., 2009).

Despite haplogroups shared at low frequency, suggesting limited gene flow, it has been proposed that North African populations have a genetic history largely distinct from both Europe and sub-Saharan Africa, over the time scales needed for the Y-chromosomal differentiation to develop (Arredi et al., 2004). Hence, the Libyan sample analyzed here is characterized by a major Northwest African paternal component, which is shared with several Berber-speaking groups in North Africa.

As Y-STR mutation rates are exceptionally high relative to binary mutations, network analysis provides a useful technique to help disentangle complex multilocus haplotype data and potentially identify chromosomes with distinctively different evolutionary histories (Myres et al., 2007). To evaluate the relationship among E-M81 lineages between Libya (this work) and other North African populations (such as Libyan Tuaregs and Tunisia) available in literature (Ottoni et al., 2011; Fadhlaoui-Zid et al., 2011a), two networks were constructed: ESM_2a and ESM_2b using 13 and 10 Y-STR haplotypes, respectively. According to ESM_2a, including Tripoli and Tuaregs, Libyan E-M81 lineages from Tripoli are clearly separated from those from Libyan Tuaregs. When adding data from Tunisia (ESM_1b), we obtained that the most frequent haplotypes inside E-M81 are shared by individuals from the three analyzed populations.

In addition to the common E-M81 lineage, E-M78 is also frequent in Tripoli (17.7 %). This haplogroup is widespread in Somalia (77.6%), Sudan (25.6%), and Ethiopia (22.7%) (Underhill et al., 2000; Sanchez et al., 2005; Hassan et al., 2008). In fact, it has been reported that North eastern Africa seems to be the place from where E-M78 chromosomes started to disperse into other African regions and outside Africa (Cruciani et al., 2007). E-M78 is also present in Europe and in the Middle East (Semino et al., 2004).

J-M304, the second most important haplogroup in Tripoli, is 34.3%, of which 7% are M172. Haplogroup J has been considered to represent the signature of the Neolithic demic diffusion associated with the spread of agriculture (Semino et al., 1996). It has been postulated to have a Middle Eastern origin (Semino et al., 2004) and its presence in North Africa attests for a gene flow from the Middle East. Previous studies on haplogroup J1-M267 have revealed a decreasing frequency moving from Southern Arabia northwards: Yemen (72.6%), Qatar (58.3%), Iraq (56.4%), Oman (38%), Egypt (20%), Lebanon (12.5%), and Turkey (9%) (Semino et al., 2000; Cinnioğlu et al., 2004; Luis et al., 2004; Cadenas et al., 2008; Al-Zahery et al., 2011). J2-M172 lineages display a decreasing frequency gradient from the Near East toward Western Europe and strongly contribute to the overall gradient of haplogroup J (Semino et al., 2004). Alternatively, J(xJ1a,J2) chromosomes are believed to be probably spread by the Arab people (Semino et al., 2004). In fact, Zalloua et al. (2008) has suggested that many J(xJ1a,J2) chromosomes were introduced into Lebanese Muslims by the Muslim expansion from the Arabian Peninsula. Furthermore, a review of the frequency data concerning Europe, the Caucasus, Iran, Iraq, and Northern Africa reveals that, in the Mediterranean, this haplogroup is mainly confined to coastal areas (Di Giacomo et al., 2004). Remarkably, J(xJ1a,J2) is widespread in Tripoli (27.4%) and in other North African populations like in Tunisia (Fadhlaoui-Zid et al., 2011a) indicating an important paternal gene flow from the Middle East, and particularly from the Arab speaking populations toward North Africa (Semino et al., 2004; Cadenas et al., 2008; Zalloua et al., 2008; Tofanelli et al., 2009). This result is in agreement with historical data such as the migration, during the 11th Century of Arabic tribes like the Hilalians, estimated as high as 200.000 families, leading to the Arabization of the region by imposing their social organization, values, and language (Metz, 1987; Camps, 1996).

Hence, two main genetic components characterize the population of Libya. The first one is originated from the Berbers, the autochthonous inhabitants of the region, whereas the second is derived from the Arab-speaking populations.

TABLE 2. AMOVA results based on Y-SNP data

Source of variation	4 groups	2 groups	North Africa	North Africa[a]
Among groups	12.44	13.75	0	—
Among populations within groups	10.91	4	7	5.28
Within populations	76.65	82.25	93	94.72
F_{ST}	23.35%	17.75%	7%	5.28%
P-value	0.0000	0.0000	0.0000	0.0000

4 groups: North Africa, Middle East, Sub-Saharan Africa, and Europe.
2 groups: North Africa and Middle East.
[a]without Tuaregs.

Furthermore, it is noteworthy the constant association of intermediate alleles (17.2, 18.2, 19.2, and 21.2) at locus DYS458 (Triki-Fendri et al., 2013) with haplogroup J(xJ1a,J2)-M304 in the present Libyan sample and *vice versa*, all the Libyan individuals belonging to haplogroup J(xJ1a,J2)-M304 have the DYS458*.2 allelic variants. In fact, such association has already been observed in other populations like Tunisia (Fadhlaoui-Zid et al., 2011a), Algeria (Robino et al., 2008), Yemen, UAE and Qatar (Cadenas et al., 2008), Jordan, Oman and Palestine (Myres et al., 2007).

To illustrate the relationship between haplotypes carrying the DYS458*.2 allelic variants inside of J(xJ1a,J2)-M304, a Median Joining Network was constructed with data from three populations: Libya (this study), Tunisia (Fadhlaoui-Zid et al., 2011a) as North African countries and Saudi Arabia (Abu-Amero et al., 2009) as a population from the Middle East. As the Network software does not accept intermediate alleles, allelic variants were pondered adding 10 repeats to the biggest observed allele, so that the probably distance in mutation terms of *.2 variants may be reflected. In ESM_2c, two sub-lineages inside J(xJ1a,J2)-M304 are clearly distinguished by the presence/absence of the *.2. In fact, Y-chromosomes from Tunisia (blue nodes) and Libya (red nodes), with DYS458*.2 allelic variants, were clustered together apart from those from Saudi Arabia (black nodes), with no DYS458*.2. This result is in accordance with the non-significant pairwise R_{ST} genetic distance between Libya and Tunisia (R_{ST}= 0.00133, *P*-value= 0.27195) and the significant distance between Libya and Saudi Arabia (R_{ST}=0.08040, *P*-value=0.00000) based on Y-STR analysis (Triki-Fendri et al., 2013).

About 7% of the Libyan Y chromosomes belonged to the R1-M173. The origin of R1-M173 is believed to predate the Last Glacial Maximum. This haplogroup is thought as dispersed from East to West, possibly 30 kya, along with the Aurignacian culture (Semino et al., 2000). It is likely that M173 arose initially in Central Asia, and those subpopulations carrying M173 migrated westward into Europe soon thereafter. The extremely high frequency of this haplotype in Western Europe is probably the result of drift, consistent with an inferred population bottleneck during the Last Glacial Maximum (Wells et al., 2001). The sub-haplogroup R-P25 was found in 2.5% of the Libyan chromosomes. Phylogenetic evidence and coalescence time estimates suggest that R-P25* chromosomes may have been carried to Africa by an Asia-to-Africa mid-Holocene back migrations (Cruciani et al., 2010).

Population relationships and genetic structure

To explore potential correlation between genetic diversities and geographic partitioning, an AMOVA was conducted over biallelic markers data. The Y-SNP profile of the Libyan population was first compared with 28 other populations (Table 1) pooled into four groups (North Africa, Middle East, Sub-Saharan Africa, and Europe). AMOVA results are shown in Table 2. Highly significant difference was observed when four groups were compared (F_{ST}=23.3 %; *p*-value $<$ 0.0001). This F_{ST} was about 4.5 times higher than the value obtained when AMOVA was applied to the North African populations (except the Tuaregs of Libya) considered as a single group (F_{ST} = 5.3 %; *P*-value $<$ 0.0001). Indeed, 5.3 % of the variation in the North African populations (one group only), which is due to the variation among populations, was the half of that obtained among populations within groups when four groups were compared (10.9%). These results show a remarkable genetic homogeneity among North African populations.

Based on both pairwise F_{ST} genetic distances and Chi-square tests of association (χ^2), no significant differences were obtained between Libya (Tripoli) and two neighboring populations: Algeria (F_{ST} = 0.00633, *P*-value = 0.011) (χ^2 *P*-value = 0.011), and Egypt (F_{ST} = 0.00309, *P*-value = 0.19869) (χ^2 *P*-value = 0.044), as well as with Moroccan Arabs (F_{ST} = 0.03948, *P*-value = 0.00851) (χ^2 *P*-value = 0,033), and Kuwait (F_{ST} = 0.05817, *P*-value = 0.00178) (χ^2 *P*-value = 0.005) (Table 3). In addition, Algeria has already been reported to be not significantly different from Libya, based on the pairwise R_{ST} genetic distances calculated on the basis of Y-STR haplotypes data (Triki-Fendri et al., 2013). However, the difference between paternal and maternal lineages when comparing Libya with Egypt is noteworthy. In fact, based on both Y-haplogroups (F_{ST}) (present work) and Y-haplotype (R_{ST}) distances (Fadhlaoui-Zid et al., 2012), these populations were not significantly different, whereas a genetic discontinuity was found in the Libyan/Egyptian border, based on mtDNA lineages (Fadhlaoui-Zid et al., 2011b) suggesting, probably, different patterns of gene flows between males and females during the past. Such contrast between Y-chromosomes and mtDNA genetics was also reported by Badro et al. (2013) suggesting that females did not always accompany male migrants, especially into North Africa.

Nevertheless, significant differences were obtained between Tripoli and other neighboring populations like Tunisia (F_{ST} = 0.07870, *P*-value $<$ 0.0001) and Sudan (F_{ST} = 0.03206, *P*-value $<$ 0.0001) despite the low geographic distances. This result is in agreement with the significant *P*-values obtained with Chi-square tests of association applied to Libya against Tunisia and Sudan separately (Table 3). In fact, the dissimilarity between Libya and Tunisia could be due to sampling, as 60% of the Tunisian samples are Berbers (Fadhlaoui-Zid et al., 2011a). Indeed, a significant genetic distance was found

TABLE 3. Results of Chi-square tests of association (χ^2) between populations

	Algeria	Egypt	Moroccan Arabs	Kuwait	Tunisia	Sudan	Tuaregs	North Africa	Italy
Libya									
Chi-square	14.03	12.48	13.05	18.24	35.22	59.12	80.54	34.37	120.005
P-value	**0.01100**	**0.04400**	**0.03300**	**0.00500**	0.00100	0.00100	0.001000	0.00100	0.00100

In bold are indicated the nonsignificant *P*-values after applying the Bonferroni adjustment (α = 0.01/9=0.00111).

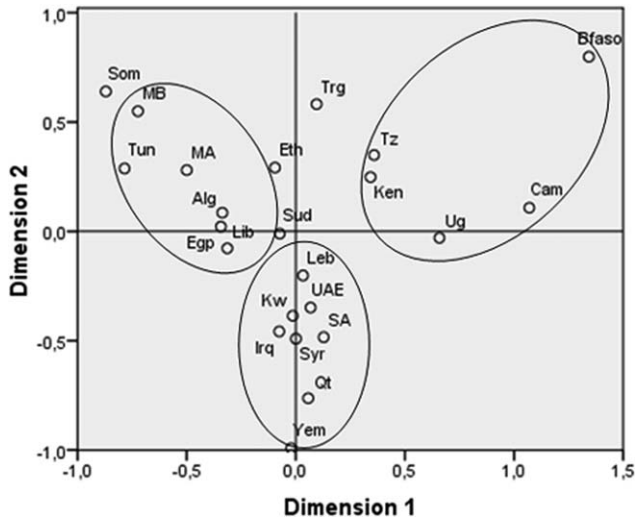

Fig. 3. Multidimensional Scaling (MDS) using pairwise F_{ST} genetic distances based on Y-haplogroup frequency data from the populations described in Table 1 (the abbreviations are mentioned in Table 1) excepting European populations (Stress value = 0.026 and DAF = 0.987).

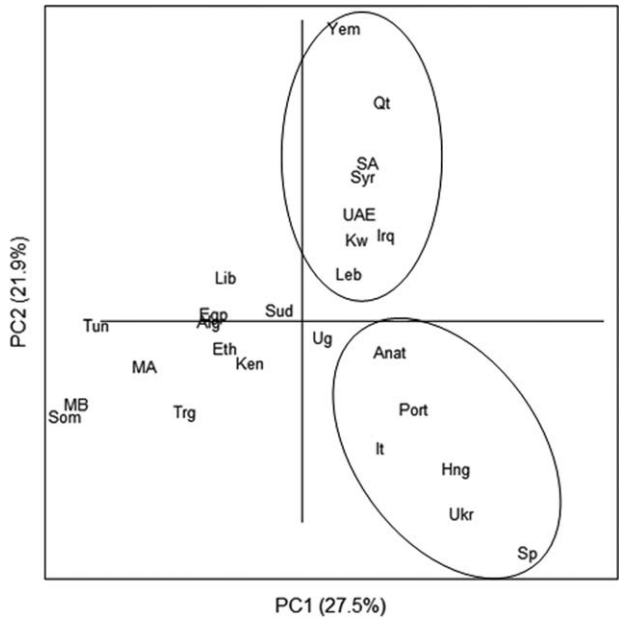

Fig. 4. Principal Component Analysis (PCA) based on Y-chromosome haplogroup frequency data of 26 populations from Table 1 (all the abbreviations are indicated in Table 1).

between Tripoli and Libyan Tuaregs (F_{ST} = 0.15173, *P*-value<0.0001) (χ^2 *P*-value=0.001) (Table 3), which is also underscored by the two clearly separated clusters obtained in the Network of E-M81 haplogroup (ESM_2a). It is noteworthy that the pairwise F_{ST} genetic distances between the Tuaregs of Libya on the one hand and the populations of both Kenya and Tanzania on the other hand were nonsignificant. This could be due to a south-Saharan paternal contribution to the Libyan Tuaregs that may have been recently introduced through slavery practices (Ottoni et al., 2011). In fact, the Tuaregs are a semi-nomadic pastoralist people which are characterized by a low genetic diversity, possibly due to genetic drift and founder effect associated with the separation of Libyan Tuaregs from an ancestral population (Ottoni et al., 2009). Moreover, the observed significant genetic distances between Y-haplogroups from Libya and neighboring countries from Europe like Italy (Table 3) are concordant with R_{ST} genetic distances calculated on the basis of 5 Y-STR haplotype data (Triki-Fendri et al., 2013).

The F_{ST} genetic distance matrix was depicted by an MDS plot (Fig. 3) to assess the phylogenetic relationships among the populations included in Table 1, except the European ones. In the MDS, a fairly clear geographic structure can be detected with three clusters corresponding to North Africa, Middle East, and Sub-Saharan Africa. It showed that Tripoli population belongs to the North African cluster, as also was previously supported by 17 Y-STR haplotype data (Triki-Fendri et al., 2013), data from Alu insertion and apolipoprotein E gene polymorphisms (Bahri et al., 2008) and a set of 119 binary markers and 15 microsatellites from the Y chromosome (Arredi et al., 2004).

Furthermore, we compared the haplogroup frequencies observed in Libya with those found in previous analyses of other populations using the Principal Components Analysis (PCA). The first two components of the PCA are shown in Figure 4, revealing substantial geographical clustering. They account for 49.4 % of the genetic variance. African descent populations are separated by PC 1, having high frequencies of E haplogroup. PC 2 separates two groups: European descent populations characterized by high frequencies of R haplogroup and Middle East populations, characterized by high frequencies of J haplogroup.

Mantel test

Mantel tests showed strong positive correlations between genetic and geographic distances when either all the populations are included (r = 0.36, *P*-value = 0.001) or only populations from North Africa and Middle East (r = 0.704, *P*-value = 0.001) (Fig. 5a). However, this correlation is not significant when only the populations from North Africa were considered in the calculations (r = −0.0139, *P*-value = 0.423) (Fig. 5b), which seems to demonstrate that the North African parental lineages could share the same genetic landscape. This similarity could be, likely, maintained by the current

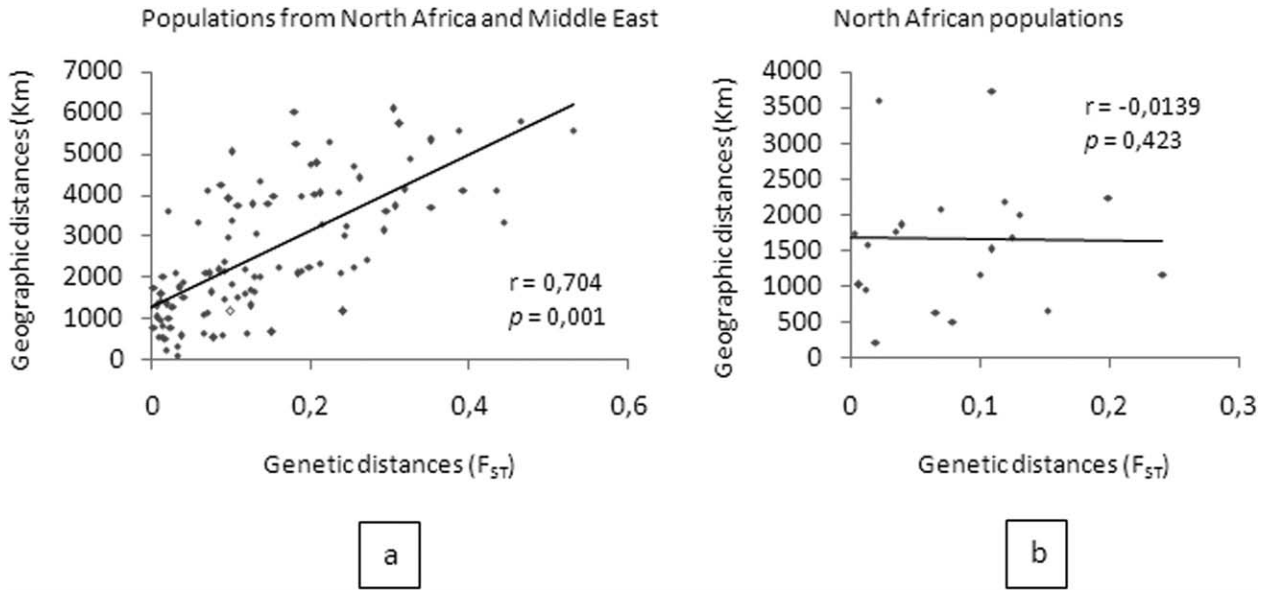

Fig. 5. Correlation analysis of genetic distance (F_{ST} values calculated on the basis of Y-SNP haplogroups) and geographic distance (km) for (**a**) populations from North Africa and the Middle East and (**b**) populations from North Africa only.

physical barriers, like the Mediterranean Sea to the north and Sahara Desert to the South that could have provided genetic barriers leading to the separate evolutionary paths of the region (Arredi et al., 2004).

CONCLUSIONS

Reconstructing the genetic history of African populations is a particularly challenging task. In this way, North Africa constitutes a big region apart from the rest of the continent, which was influenced by several human movements at different times that have generated a particular genetic diversity on the populations of the area.

We have carried out the first analysis on Y-haplogroup diversity of a population from Libya, by examining 22 Y-SNPs in 175 male subjects from Tripoli. This allows us to clarify the genetic landscape of Libya and to contribute in the study of the history of North African populations. So, we compare Libyan Y-haplogroup diversity with that previously reported in world populations.

The male genetic background of the Libyan population is characterized by the high frequency of the specific North African haplogroup E-M81 (33.7%), typical of Berber population (Semino et al., 2004), and the haplogroup J(xJ1a,J2) (27.5 %) probably spread by the Arab people into North Africa (Semino et al., 2004; Zalloua et al., 2008; Badro et al., 2013). The presence of this dual component (Berber and Arab) in Libya is in agreement with previous genetic studies on the same population (Elmrghni et al., 2012a) and in neighboring populations (Robino et al., 2008; Fadhlaoui-Zid et al., 2011a). Likewise, Arredi et al. (2004) have already reported that just two haplogroups predominate within North Africa together accounting for almost two-thirds of the male lineages (E-M81 and J-M304).

The results obtained in AMOVA, together with those of the pairwise F_{ST} genetic distances between Libya and neighboring populations, the MDS and PCA plots and the Mantel test (giving non-significant correlation between geography and genetics) are in favour of a genetic homogeneity among Libya and North African populations, including Egypt. Thus, the hypothesis of different patterns of gene flow between males and females during the past in the Libyan/Egyptian border is corroborated (Fadhlaoui-Zid et al., 2011b).

The genetic divergence between Libya and populations from Europe and Sub-Saharan Africa, based on the Y-haplogroup distribution, is consistent with the hypothesis that human population movements in North Africa have been mostly restricted to an East-West direction because of the geographical barriers imposed by the Sahara Desert and the Mediterranean Sea (Fadhlaoui-Zid et al., 2011b). Moreover, the significant differences found here between Libyans from Tripoli and Libyan Tuaregs (Ottoni et al., 2011) are consistent with the results obtained on mtDNA (Fadhlaoui-Zid et al., 2011b) where the differentiation of both paternal and maternal lineages in the Libyan Tuaregs is explained by drift given the level of isolation of this population.

In conclusion, our results show that the Y-haplogroup diversity in Libya, consistent with the genetic landscape in North Africa in general, is characterized by two genetic components, a typical Northwest African one shared with Berber-speaking people, the autochthonous inhabitants of the region, and a second signature which is likely linked to Arabic populations, in agreement with the hypothesis of an expansion from the Middle East, such as the migration of Bani Hilal and Bani Salim during the 11th century, shaping the North African Y-chromosomal landscape.

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