Deep Into the Roots of the Libyan Tuareg: A Genetic Survey of Their Paternal Heritage

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ABSTRACT Recent genetic studies of the Tuareg have begun to uncover the origin of this semi-nomadic northwest African people and their relationship with African populations. For centuries they were caravan traders plying the trade routes between the Mediterranean coast and south-Saharan Africa. Their origin most likely coincides with the fall of the Garamantes who inhabited the Fezzan (Libya) between the 1st millennium BC and the 5th century AD. In this study we report novel data on the Y-chromosome variation in the Libyan Tuareg from Al Awaynat and Tahala, two villages in Fezzan, whose maternal genetic pool was previously characterized. High-resolution investigation of 37 Y-chromosome STR loci and analysis of 35 bi-allelic markers in 47 individuals revealed a predominant northwest African component (E-M81, haplogroup E1b1b1b) which likely originated in the second half of the Holocene in the same ancestral population that contributed to the maternal pool of the Libyan Tuareg. A significant paternal contribution from south-Saharan Africa (E-U175, haplogroup E1b1a8) was also detected, which may likely be due to recent secondary introduction, possibly through slavery practices or fusion between different tribal groups. The difference in haplogroup composition between the villages of Al Awaynat and Tahala suggests that founder effects and drift played a significant role in shaping the genetic pool of the Libyan Tuareg. Am J Phys Anthropol 145:118–124, 2011.

The genetic diversity of Saharan desert populations is attracting growing interest. The world's largest desert, the Sahara now covers most of northern Africa from the Red Sea to the Atlantic Ocean, but it was even larger during the Last Glacial Maximum, when it extended south beyond its current boundaries (Ehret, 2002; Brooks et al., 2005). About 14,000 years ago (kya), an abrupt shift to a monsoonal climate dramatically changed the Saharan landscape: rainfalls increased, rainforest vegetation spread across the Congo Basin and up to the West African coast, woodland savannah belts shifted farther northward where tropical steppe and grassland vegetation established. Perennial streams flowed out of the Tibesti and Hoggar mountain ranges and the palaeolake Megachad extended over more than 350,000 km^2 in the southern Sahara (Schuster et al., 2005). Regions formerly uninhabited became available to human occupation. Interaction between human activity and the ever-changing Saharan environment during the first half of the Holocene, known as the Holocene Climatic Optimum, can be traced to the various different civilizations that coexisted in this area, e.g., the Agripastoral tradition of the Saharo-Sahelians and the Aquatic tradition of the southern Nilo-Saharan communities in the southern Sahara, and the Capsian tradition of the Erythraites, a pastoral people whose territory extended into the northern Sahara (Ehret, 2002). A rapid shift to drier conditions in the second half of the Holocene led to an irreversible process of desertification, strongly affecting food security and consequently the lifestyle and the economic strategies of the Saharan human communities. In the central Sahara, pastoral groups had to rely more on large-scale mobility in search of water for their livestock. This paved the way to the development of nomadic practices in the Sahara, along with the beginning and growth of trade and contact in the exchange of resources between human groups throughout the Sahara (di Lernia et al., 2002). This economic system laid the basis for the rise of the Garamantian civilization, which is attested in Fezzan, southwestern Libya, and dominated the trans-Saharan trade routes from the 1st millennium BC to the 5th century AD (Liverani, 2000; di Lernia et al., 2002). Increasing water scarcity and closing of the Mediterranean seaports inevitably led to the

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decline of Garamantian polity. It is thought that the modern Tuareg originated out of the fall and fragmentation of the Garamantes (Camps, 1974; Smith, 2003; Sadr, 2004).

Nowadays, the Tuareg occupy northwest Africa, concentrated in the central Sahara and the Sahel regions. They speak a Berber language, the *Tamasheq*, characterized by several dialects spoken in different regions (Greenberg, 1970). Once caravan traders linking the Mediterranean coast and south-Saharan Africa, the Tuareg lived in a stratified society based on tribe-entities and pastoral activities. With early 20th century French colonization, came the decline of the Tuareg socio-political system. Though they have maintained a semi-nomadic or nomadic lifestyle, sedentarization processes can be observed in some instances.

Genetic data collected so far on the Tuareg have revealed a fairly heterogeneous genetic make-up, particularly with regard to the mitochondrial DNA (mtDNA) pool (Watson et al., 1996; Ottoni et al., 2009; Pereira et al., 2010). Beside a heterogeneous south-Saharan component, a common West Eurasian component (mainly H1 and V) has been identified in the Tuareg from different geographic areas, with proportions ranging from 65% in Libya (Ottoni et al., 2009) to 39% in Sahel (Pereira et al., 2010). These mtDNAs were involved in the post-Glacial expansion from the Iberian refugia toward Europe and Northwest Africa (Achilli et al., 2004, Torroni et al., 2006, Ottoni et al., 2010); it is likely that they subsequently dispersed further in northern Africa during the Holocene Climatic Optimum. Overall, available mtDNA data suggest a North African origin of the Tuareg, who likely spread in distinct migratory events to southern locations across the Sahara during the Holocene. Later, founder effects and drift may have affected the genetic composition of these nomadic people over the centuries. In addition, the widespread geographic dispersion of the Tuareg in northwest Africa could have determined differential patterns of admixture with neighboring populations, particularly in the groups that became sedentary (Pereira et al., 2010). An alternative scenario claiming an Eastern African origin of the Tuareg is supported by nuclear genetic markers, which showed an affinity of the Tuareg with the Beja from Ethiopia (Cavalli-Sforza et al., 1994).

Recent Y-chromosome data from the Tuareg in the Sahel region have revealed a high incidence of lineages that can be traced back to the Neolithic demic diffusion of Afro-Asiatic-speaking pastoralists from the Middle East (Pereira et al., 2010). In the present work, the paternal genetic pool of the Libyan Tuareg from the Fezzan in Libya was characterized by mean of high-resolution investigation of 37 Y-chromosome STR loci and analysis of 35 bi-allelic markers in 47 subjects belonging to the same sample whose maternal contribution has been previously characterized (Ottoni et al., 2009). By integrating the mtDNA data and comparative analyses with other populations from the African and Eurasian continents, we tried to reconstruct the migratory trajectories and the demographic processes which made up the genetic pool of the Libyan Tuareg.

MATERIALS AND METHODS

Genotyping analyses

DNA previously extracted from buccal swab samples (Ottoni et al., 2009) was available for 47 males from 129 subjects sampled in two Libyan villages, Al Awaynat ($n = 38$) and Tahala ($n = 9$), in Fezzan. All the sampled subjects were from families that, according to individual interview, were unrelated for at least three generations. Appropriate informed consent to anonymously use their data was obtained from all individuals. DNA was quantified using real-time PCR (Quantifiler™ Human DNA kit, Applied Biosystems). A total of 37 STR loci were then genotyped for all samples as described previously (Jacobs et al., 2009) based on PowerPlex® Y (Promega, Madison, WI) (DYS19, DYS385, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439) and three novel multiplexes (DYS385, DYS388, DYS390, DYS391, DYS393, DYS426, DYS442, DYS447, DYS448, DYS449, DYS454, DYS455, DYS456, DYS458, DYS459, DYS460, DYS464, DYS570, DYS576, DYS607, DYS724, GATA H4.1, YCAII). Some STRs were included in more than one multiplex as an internal control. The inclusion of DYS464 in two assays facilitated the interpretation of the alleles and peak height ratios (Butler and Schoske, 2005).

All haplotypes were submitted to Whit Atheys' Haplogroup Predictor (Athey, 2005, 2006) to infer haplogroups and their probabilities. Based on these results, the samples were assigned to a specific Y-single nucleotide polymorphism (SNP) assay to confirm the inferred haplogroup and to increase the resolution of the classification according to the latest Y-chromosomal tree of the Y Chromosome Consortium, as reported in Karafet et al. (2008) and according to Cruciani et al. (2010a). To screen the main diagnostic sites within haplogroups E1b1, R1a, and R1b, four multiplex systems with a total of 32 Y-SNPs were developed using SNaPshot mini-sequencing assays (Applied Biosystems, Foster City, CA) and analyzed on an ABI3130 Genetic Analyzer (Applied Biosystems) according to a previously published protocol (Caratti et al., 2009). In the sample AW 60, assignation to inferred haplogroup Q-M242 was tested by allele-specific PCR using SYBR green on an ABI 7500 Real-Time PCR System (Applied Biosystems). Affiliation to R1 sub-haplogroups was then tested using the aforementioned multiplex PCRs and additional typing of R-M207 and R1-M173 through singleplex PCR and SNaPshot mini-sequencing reactions. All primer sequences and concentrations are available upon request from the authors.

Statistical and phylogenetic analyses

Computation of Slatkin's linearized F_{ST} distance values (Slatkin, 1995) from 24 Y-SNP sub-haplogroup frequencies in African and Eurasian populations available in the literature (Supporting Information Table S1) was done with ARLEQUIN ver. 3.1 (Excoffier et al., 2005). Slatkin's linearized F_{ST} values were visualized in a two-dimensional plot through non-metric Multidimensional Scaling (MDS) analysis using Statistica 9 (Statsoft, Tulsa, OK).

Median Joining network analyses (Bandelt et al., 1999) were used to investigate the phylogenetic relationships among 25 single-copy microsatellite haplotypes (including DYS389I and DYS389b) assigned to the sub-haplogroups E1b1b1b and E1b1a found in the Libyan Tuareg sample. The amount of accumulated diversity within E1b1b1b and E1b1a was estimated based on the mean microsatellite variance. The networks were created in NETWORK 4.5.1.0. (http://www.fluxus-engineering.com), using the

weighting scheme described by Qamar et al. (2002) due to different mutation rates among the markers. Additionally, a network analysis was performed on 11-microsatellite E1b1b haplotypes and 8-microsatellite R1b1a haplotypes from the Libyan Tuareg, respectively with samples from northern African (Arredi et al., 2004) and from northern Africa, central Africa, and Europe (Cruciani et al., 2010a). To estimate the time to the most recent common ancestor (tMRCA) within relevant star-like nodes of the networks, we applied the average square distance (ASD) method (Thomas et al., 2002) implemented in Ytime ver. 2.08 software (Behar et al., 2003), where the ancestral haplotype was assumed to be the haplotype carrying the most frequent allele at each microsatellite locus. We calculated mean and standard deviation of the father-to-son mutation rates of all genotyped microsatellites according to Vermeulen et al. (2009), and to use them as evolutionary mutation rate we reduced them by a factor 2.4 (Zhivotovsky et al., 2006).

RESULTS

Results of STR genotyping on the 47 Libyan Tuareg revealed 26 different haplotypes in the Al Awaynat and 9 in the Tahala samples (Supporting Information Table S2). One haplotype was found to be shared between Al Awaynat and Tahala (AW 29 and TAH 16). The Y-STR haplotypes were submitted to YHRD (YHRD accession number: YA003669). All haplotypes were tentatively assigned to four haplogroups inferred through Whit Atheys' Haplogroup Predictor (Supporting Information Table S2). Haplogroup assignation was confirmed and resolved to a higher phylogenetic level by SNP assay for all individuals, except for AW 60, in which the SNP analysis (R1-M173) confuted haplogroup prediction (i.e., Q, 78% and R1a, 22%). We observed four branches of the Y-chromosome tree out of the 34 detected by our SNP assays. Figure 1 shows the haplogroup distribution of the two Tuareg villages from Libya. Overall, the two major haplogroups observed in the Libyan Tuareg are the south-Saharan E1b1a8 (43%) and the Northwest African E1b1b1b (49%), which were detected by screening the biallelic markers U175 and M81, respectively. The high incidence of these two haplogroups is responsible for the low genetic diversity values encountered in the Libyan Tuareg (H = 0.200 ± 0.154 in Tahala, and H = 0.602 ± 0.048 in Al Awaynat, Fig. 1), and their contribution to the gene pool of the two villages in Fezzan appears to be different: E1b1b1b was predominant (89%) versus E1b1a8 (11%) in Tahala, whereas its frequency was lower (39%) in Al Awaynat where the main haplogroup was E1b1a8 (50%). In Al Awaynat, the recently described haplogroup R1b1a (R-V88, Cruciani et al., 2010a) was also observed (8%), together with haplogroup R1* (3%).

Network analysis showed a very different pattern of microsatellite variation of haplogroups E1b1a8 and E1b1b1b within the Libyan Tuareg (Fig. 2a,b), as also indicated by the values of microsatellite variance (0.082 and 0.202, respectively). The network of E1b1a8 showed a very homogenous pattern of variation, with a star-like topology notwithstanding two distantly related haplotypes (i.e., TAH 4 and AW 12), (Fig. 2a, Supporting Information Table S2). The age of microsatellite variation was found to be 1,283 years (95% bounds, 923–1,758 years), nevertheless after excluding the two distantly related haplotypes, which may likely represent "erratic" outliers introduced through sporadic events (e.g., recent migration), the age significantly decreased (i.e., 336 years; 95% bounds, 172–602 years).

Conversely, a high degree of diversification was observed in E1b1b1b (Fig. 2b). When the relationships between the Tuareg E1b1b1b haplotypes and other Northern African chromosomes were investigated in the network of Supporting Information Figure S1 at lower resolution (11 microsatellites), most of the Libyan Tuareg located in two branches departing from the central node. Specifically, nine Tuareg out of the total of 23 E1b1b1b (39%) were found together with a Tunisian chromosome in a star-like node from which, in turn, seven Tunisian, one Algerian and four Tuareg haplotypes descend. Coalescence age of this cluster was found to be 2,581 years (95% bounds, 1,682–3,982).

Multidimensional Scaling analysis of Slatkin's linearized F_{ST} distances based on available Y-SNP sub-haplogroup frequencies data from Eurasian and African populations resulted in a fairly clear geographic structure, with most south-Saharan and European populations positioned at opposite sides of the two-dimensional plot (see Fig. 3). Within the African samples, clustering of some populations can be noted: at the center of the plot the north-eastern African populations from Egypt cluster together with two Middle Eastern samples, close to the East-Mediterranean European populations from the Balkans. Another small cluster is composed of the North African Arab populations from Algeria and Tunisia, including one Algerian Berber sample. The more marginal positions in the plot are occupied by the Moroccan Berbers followed by the Tuareg samples. The Libyan Tuareg from Tahala were found to cluster together with the Tuareg samples from the Sahel region, though it should be mentioned that this result may be affected by the small sample size. Differently, the Tuareg from Al Awaynat are located close to other south-Saharan populations, in particular the Daba and mixed Nilo-Saharan groups from Cameroon.

DISCUSSION

The Y-chromosome pool of the Libyan Tuareg is characterized by two major haplogroups: the North African E1b1b1b (E-M81) and the south-Saharan E1b1a8 (E-U175). E1b1b1b is the most common haplogroup in North Africa, with cline frequencies decreasing eastward from Morocco (76%) to Egypt (10%), and is particularly spread among Berber-speaking groups (Cruciani et al., 2004). The pattern of genetic variation within this haplogroup points to a demic expansion from the Middle East which is likely related to the spread of Afro-Asiatic pastoral people during the Neolithic (Arredi et al., 2004). E1b1a is predominant in many south-Saharan areas, particularly in the central and west region (Rosa et al., 2007, Chiaroni et al., 2009) and high frequency of its subclade E1b1a8 have been found in west-central Africa, particularly in the Cross River region (38%), in Nigeria (Veeramah et al., 2010).

Interestingly, the North African E1b1b1b appears to constitute a common paternal genetic matrix in the Tuareg populations since it was encountered at high frequency also in some samples from the Sahel region (Pereira et al., 2010). Specifically, E1b1b1b was found to be dominant in Tahala (89%) and in the Tuareg samples from Burkina Faso (Gorom-Gorom, 78%) and Mali

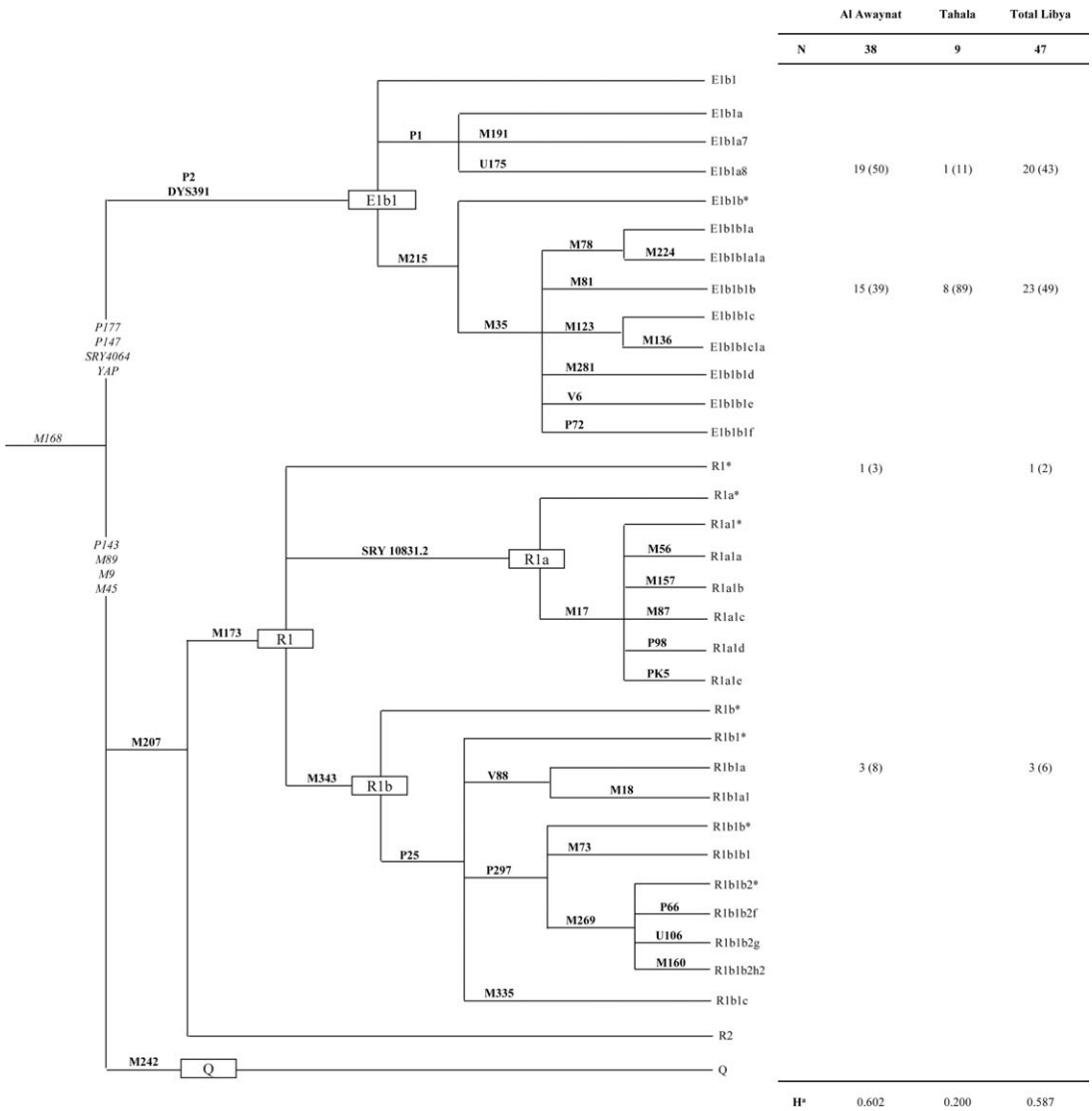

Fig. 1. Schematic representation of the phylogeny of Y-chromosomes haplogroups and their absolute and relative (%) frequencies in the Tuareg samples from the villages of Al Awaynat and Tahala, in Libya. The markers typed in this study are given in bold. Inferred markers are given in italics. *Paragroups: Y-chromosomes not defined by any downstream-examined mutation. [a]Intrapopulation haplogroup diversity.

(Gossi, 82%), which explains the cluster observed in the MDS analysis. Differently, in Al Awaynat, as well as in the Tuareg sample from Niger (Tanut), the south-Saharan haplogroup E1b1a was found to be predominant, with frequencies of 50 and 44%, respectively.

Analysis of the microsatellite variation in E1b1a8 and E1b1b1b (Fig. 2a,b) provides more clues about the history of these haplogroups in the Libyan Tuareg. The high diversity of E1b1b1b as opposed to the sharp homogeneity of E1b1a points to a more complex history of E1b1b1b in the Libyan Tuareg, suggesting that this might represent the original paternal genetic matrix of the Tuareg villages in Fezzan. More information about the origin of the Tuareg E1b1b1b chromosomes is given by the analysis of STR variation in Northern African populations (Supporting Information Fig. S1). It is likely that most of the Tuareg E1b1b1b Y-chromosomes (i.e., 13 out of 23, 57%) are related to an expansion event that took place about 2.6 kya in an ancestral population inhabiting a region between Tunisia and the Central Sahara. This event may have coincided with an expansion that led to the formation of derived Tunisian and Central Saharan populations, with the latter, in turn, contributing to the paternal genetic pool of the Tuareg villages in Fezzan. Interestingly, a maternal genetic link with Tunisia, the time estimates of which are compatible with a population split that may have occurred in the second half of the Holocene, was also observed at the level of the African mtDNA H1 phylogeny (Ottoni et al., 2010). This suggests that the same ancestral population may have contributed to the paternal and maternal genetic pool of the Libyan Tuareg. However it should be noted that the issue of a Tunisian genetic link in the Libyan Tuareg needs to be corroborated by further genetic survey in Northwest Africa, particularly in regions which still appear to be underrepresented (e.g., Algeria).

The Tuareg E1b1a8 Y-chromosomes were found to be highly homogenous with an overall age of 1.3 kya. Under

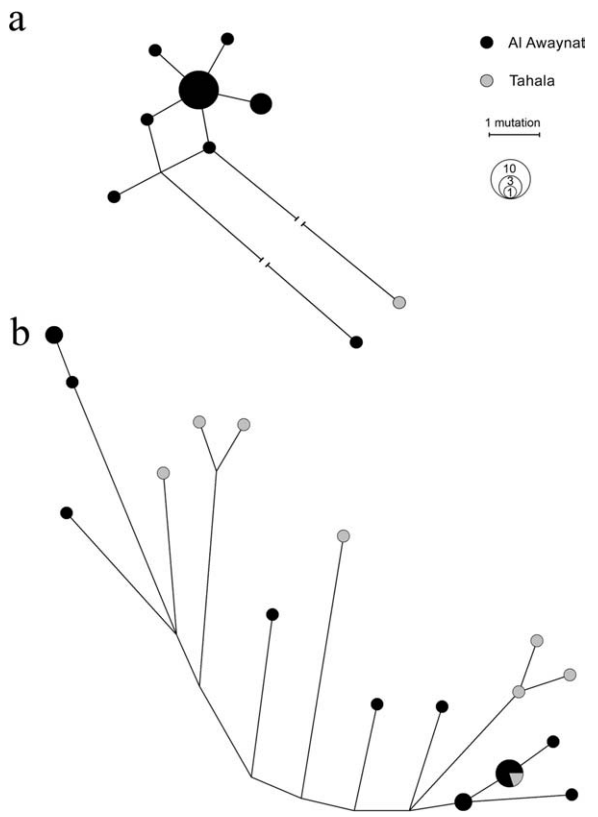

Fig. 2. Median Joining Network of 25-microsatellite E1b1a (**a**) and E1b1b1b (**b**) haplotypes in two Libyan Tuareg villages, Al Awaynat and Tahala. Node size is proportional to frequency. In (b), interrupted lines indicate distantly related haplotypes, characterized by 11 and 12 mutational steps, respectively, for the haplotype from Tahala and Al Awaynat (b).

the assumption of a sporadic introduction of two "erratic" outlier haplotypes, the E1b1a8 Y-chromosomes in El Awaynat were found to derive from an even more recent founder event (336 years). It is thus likely that South-Saharan lineages may have blended into the North African Libyan Tuareg paternal genetic pool through posterior introduction.

As an additional issue, it is worth noting that drift and founder events, together with a certain degree of isolation regardless of the genetic proximity between Al Awaynat and Tahala, may have played a significant role in shaping the paternal genetic pool of such isolated small groups, leading to different haplogroup frequencies in the two Libyan Tuareg villages. Supporting this hypothesis, which however should be interpreted cautiously due to the small sample size of Tahala, is the peculiar village-specificity of some mtDNA H1 subclades observed in the same Tuareg villages (Ottoni et al., 2010).

A possible source for the introduction of south-Saharan lineages in the Libyan Tuareg could have been the Arab trans-Saharan slave trade, which started in the 7th century AD and continues today in Mauritania and Sudan. This is compatible with the young age calculated for E1b1a8 and is supported by historical data attesting to the existence of slavery practices in the Tuareg until the beginning of the 20th century (Giazzi, 1996). Furthermore, a recent genetic study has pointed to the significant genetic impact the Arabic slave trade had on the maternal genetic pool of North African populations. Two trans-Saharan slave trade routes linked the Fezzan with Central-West African regions, e.g., Lake Chad (Harich et al., 2010), and some of the maternal lineages which mark the slave trades were observed in the mtDNA pool of the Libyan Tuareg, e.g., L3e and L3f (Ottoni et al., 2009).

Nevertheless, an alternative scenario of recent fusion of different tribal groups cannot be excluded, given the events of the colonial era and the subsequent dismantling of the Tuareg socio-political system.

The haplogroup R1b1a (R-V88) was found with a frequency of 8% in the village of Al Awaynat. Generally, haplogroup R and its subsets are spread in Eurasia as far as Siberia (Karafet et al., 2008; Chiaroni et al., 2009; Lancaster, 2010). Nevertheless, R1b1a has been observed at high frequencies in Northwest Africa (27% in the Egyptian Berbers), with peaks in the Chadic-speaking populations from Central Africa, ranging from 29 to 96% in Cameroon, and very rarely is found outside Africa (Cruciani et al., 2010a,b). This haplogroup has been proposed to represent the paternal genetic signature of the mid-Holocene migration of proto-Chadic Afro-asiatic speakers across the Central Sahara to Lake Chad (Ehret, 2002; Cruciani et al., 2010a); this suggests a link between Chadic speakers and other Afro-Asiatic speakers to the north of the Sahara.

In the eight-microsatellite Network analysis of R1b1a chromosomes from Northern and Central Africa (Fig. S2), the Libyan Tuareg R1b1a Y-chromosomes were found to belong to a branch characterized exclusively by haplotypes from Central Africa, more particularly from the Chad area (Cruciani et al., 2010a). This may be likely explained by recent introduction through the slavery practices mentioned above. Nonetheless, the hypothesis that the Libyan Tuareg R1b1a haplotypes may be relics of the migration of Pastoral proto-Chadic speakers, as hypothesized by the "trans-Saharan" hypothesis (Ehret, 2002; Cruciani et al., 2010a), cannot be ruled out.

The final picture that we can draw from the paternal and maternal genetic pool of the Libyan Tuareg from Fezzan is that of a North African origin, as evidenced by the predominant haplogroups E1b1b1b for the Y-chromosome and H1 for the mtDNA (Ottoni et al., 2009, 2010). With this regard, a genetic link with Berbers seems likely, as also supported by the fact that Tuareg speak a Berber language. In agreement with the data collected from the Tuareg in Sahel (Pereira et al., 2010), analyses of the uniparental markers (mtDNA and Y-chromosome) in the Libyan Tuareg seem to exclude the hypothesis for an Eastern African origin of the Tuareg, as advanced on the basis of nuclear genetic markers (Cavalli-Sforza et al., 1994).

The North African paternal lineages found in the Libyan Tuareg likely originated in the second half of the Holocene, when irreversible desertification of the Sahara triggered human migration and population splits associated with local small-scale expansion events. A significant portion of both the maternal and the paternal genealogies may have arisen in the same ancestral population which contributed to the genetic pool of the modern Tuareg in Fezzan. A younger south-Saharan paternal contribution to the Libyan Tuareg which may have been recently introduced through slavery practices was also observed; however, alternative hypotheses such as the mere fusion of different tribal groups cannot be totally ruled out.

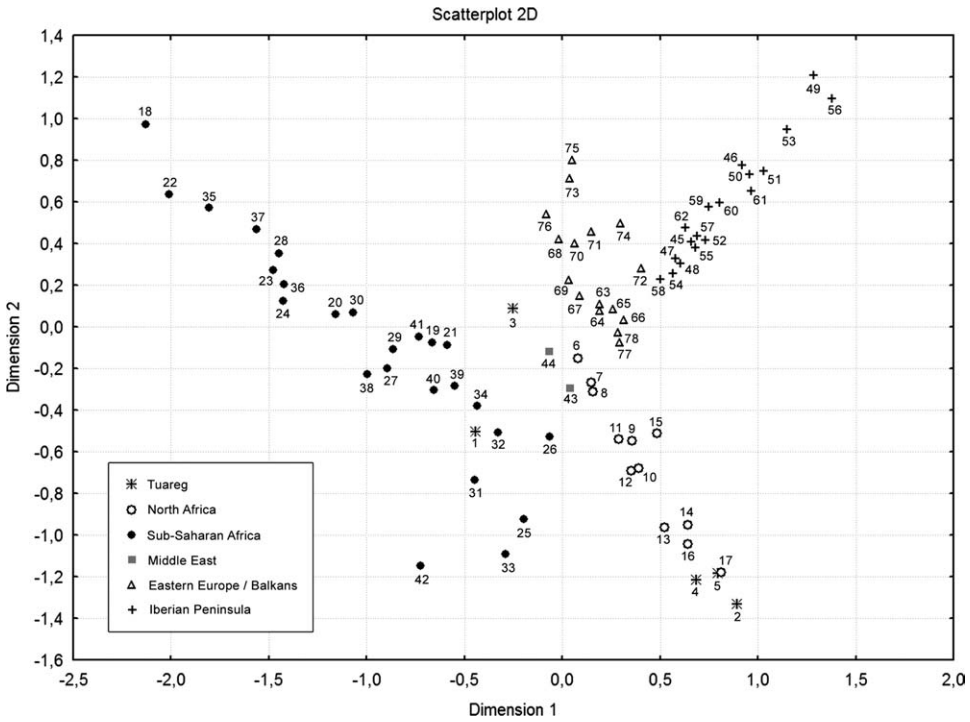

Fig. 3. Two-dimensional MDS plot of F_{ST} genetic distances calculated from Y-chromosomes haplogroup frequencies in 78 population samples from Africa and Eurasia. List of populations, code numbers, and references are given in Supporting Information Table S1. Stress = 0.15.

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