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First Genetic Insight into Libyan Tuaregs: A Maternal Perspective

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Summary

The Tuaregs are a semi-nomadic pastoralist people of northwest Africa. Their origins are still a matter of debate due to the scarcity of genetic and historical data. Here we report the first data on the mitochondrial DNA (mtDNA) genetic characterization of a Tuareg sample from Fezzan (Libyan Sahara). A total of 129 individuals from two villages in the Acacus region were genetically analysed. Both the hypervariable regions and the coding region of mtDNA were investigated. Phylogeographic investigation was carried out in order to reconstruct human migratory shifts in central Sahara, and to shed light on the origin of the Libyan Tuaregs. Our results clearly show low genetic diversity in the sample, possibly due to genetic drift and founder effect associated with the separation of Libyan Tuaregs from an ancestral population. Furthermore, the maternal genetic pool of the Libyan Tuaregs is characterized by a major "European" component shared with the Berbers that could be traced to the Iberian Peninsula, as well as a minor 'south Saharan' contribution possibly linked to both Eastern African and Near Eastern populations.

Keywords: Libyan Tuaregs, mitochondrial DNA, Central Sahara, phylogeny

Introduction

The Tuaregs are a semi-nomadic, pastoralist people of northwestern Africa (southern Algeria, southwestern Libya, Mali and Niger), and in fewer numbers inhabit Burkina Faso, Chad and Nigeria. Their origin is unclear as the scarcity of written chronicles prevents a reliable reconstruction of their history. Most Arabian historians and geographers report that the Tuaregs descend from Arabic or Semitic populations that reached the Maghreb after various military campaigns and progressively entered the southern parts of the region, where they intermingled with the local Berber populations (Lhote, 1955; Hama, 1967). The Tuaregs speak a Berber language, *Tamajaq* (also called *Tamasheq* or *Tamahaq*, according to the region where it is spoken), which appears to have several dialects spoken in different regions (Greenberg, 1970). The Tamajaq writing system is the *Tifinagh* (also called *Shifinagh* and *Tifinar*), whose origins remain unclear. An old version of Tifinagh, also known as Libyco-Berber, dates to between the 3rd century BC and the 3rd century AD in northwestern Africa (Gaudio, 1993).

Despite their sharing a common language and culture, the Tuareg population has always been divided into different groups called confederations. Precolonial organization of Tuareg society was based on rigid division into social classes and reflected tribal separation. French colonization in the early 20th century, long periods of war, and Tuareg group rebellions between 1916 and 1919 severely weakened the Tuareg socio-political system. Under French rule, most of the slaves were set free and the confederations disassembled (Giazzi, 1996). This was accompanied by a significant decline in pastoralism: nomad tribes were confined to areas designated by the new administration, and pastoral activities were restricted to small ranges. Many Libyan Tuaregs came to Libya from Chad, Algeria and Niger, and settled in the south of the country, near Ghat and Ubarj (Fig. 1) (Gaudio, 1993).

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Figure 1 Map of the sampled area. The main villages in Fezzan are shown. The Tuareg individuals analysed in the present study came from the villages of Tahala and Al Awaynat.

Genetic data collected so far on the Tuaregs are quite scarce (Cavalli-Sforza et al., 1994; Watson et al., 1996; Gonzalez et al., 2006; Martinez-Labarga et al., 2007). Nuclear genetic markers show a high genetic affinity between the Tuaregs and Eastern African populations from Ethiopia, and with the Beja in particular (Cavalli-Sforza et al., 1994). Mitochondrial DNA (mtDNA) data collected from the Tuaregs of Mali, Niger and Nigeria show a high affinity of the Tuaregs with western south Saharan populations (Watson et al., 1996; Rando et al., 1998, Gonzalez et al., 2006).

The present work aimed to trace the origin of the Libyan Tuaregs inhabiting the Fezzan, in southwestern Libya (Fig. 1) through analysis of mtDNA lineages in two samples from the region of Tahala near the Acacus massif. Oral traditions in the villages of Fezzan claim that the Tuaregs directly descend from the Garamantes, whose presence in the central Sahara can be dated to between 2700 and 1800 years ago (Liverani, 2000). Nevertheless, the origin of the Garamantes and their relationship with the pastoral peoples inhabiting the Sahara during the second half of the Holocene are largely unknown. The molecular data from the Tuareg sample presented here constitute the first mtDNA genetic study to focus on the Tuaregs and offer an insight into an African region that is genetically almost unknown: the central Sahara. It might be said that the Sahara is nearly uninhabited; nonetheless, we think that the small local ethnic groups, both sedentary and nomadic, that occupy this region represent an important source for collecting information about the dynamics of human migrations in the Sahara and northern Africa as well.

Materials and Methods

DNA Extraction and mtDNA Analysis

Two batches of mouth swab samples from healthy and maternally unrelated individuals of ascertained Tuareg descent were collected from Fezzan (Libya). A total of 129 individuals were genetically analyzed: 111 from the village of Al Awaynat and 18 from the neighbouring village of Tahala (Fig. 1). Both the villages have about 500 inhabitants. Appropriate informed consent to anonymously use their data was obtained from all individuals. Sample collection was carried out as part of the Italian-Libyan Archaeological Mission in the Acacus and Messak (Libyan Sahara) of the University of Rome La Sapienza and the Department of Antiquities, Tripoli, directed by Prof. Savino di Lernia and the late Ebrahim Azzebi.

Total genomic DNA was extracted from mouth swab samples as previously described in the literature (Budowle et al., 2000). PCR amplification of the first and second hypervariable segments (HVS-I and HVS-II) of mtDNA was carried out in a 25-μl reaction volume. The primers in the amplification reactions allowed us to read clear sequences from nucleotide position (np) 15996 to 16401 and np 00029 to 00408 for HVS-I and HVS-II, respectively (Rickards et al., 1999, 2001). When haplogroup classification could not be properly resolved by sequencing HVS-I and the HVS-II, selected diagnostic markers in the mtDNA coding region were analyzed by RFLP screening and sequencing. Details about the primers and the PCR conditions are reported as Supporting Information (Tables S1-S4 available on the journal's website).

Population Genetic and Phylogeographic Analysis

Each mtDNA sequence was phylogenetically classified according to the literature (Salas et al., 2002; Achilli et al., 2004, 2005; Olivieri et al., 2006; Torroni et al., 2006; Behar et al., 2008). Published HVS-I sequences were used for comparative analysis: a total of 5,757 HVS-I sequences from 92 African population samples were entered into a database and divided into geographical groups: Northern Africa, Eastern Africa, Western Africa, Central Africa, Equatorial Africa, Southern Africa (i.e., populations south of the Equator). Literature references and other details about the samples collected in the database are reported in Table S5 as Supplementary Online Material (available on the journal's website). Database update and file conversion into the appropriate format (i.e., text, fasta, phylip) were done with the mtDNA 2.4 program (kindly provided by E. Fabrini; for details see http://www.doppiovu3.it/mtdna/index.htm). In addition to the comparative survey, which relies mainly on the huge amount of published HVS-I data collected so far, we performed higher

resolution comparison analysis of the L sequences based on the latest tree of complete mtDNA sequences reported in Behar et al., 2008. This comparative survey provided valuable phylogeographic information about the lineages characterizing the individuals in our collection.

We used the Arlequin 2.000 program (Schneider et al., 2000) to calculate the standard diversity indices (i.e., sample size, number of haplotypes, number of polymorphic sites, haplotype diversity) and the molecular indices (mean number of pairwise differences) of the populations in our database on the basis of the HVS-I haplotype (Schneider et al., 2000). For the Libyan Tuareg sample, these parameters were calculated by combining the HVS-I and HVS-II haplotypes.

Bayesian 95% credible regions (CRs) and a χ^2 test were performed on the haplogroup frequencies observed in the two Libyan Tuareg samples. Bayesian 95% CRs were calculated with the Sampling program (kindly provided by V. Macaulay, Department of Statistics, University of Glasgow).

Computation of Slatkin's linearized F_{ST} was done with Arlequin 2.000 software, and HVS-I sequences for each population were used as input data. The Libyan Tuaregs from both Al Awaynat and Tahala were pooled together to calculate the matrix of Slatkin's linearized F_{ST}. A second matrix was calculated after excluding the non-originally African haplotypes from the Libyan Tuaregs. Multidimensional Scaling (MDS) of Slatkin's linearized F_{ST} genetic distances between all database samples was done for each matrix with Statistica 6.0 software (StatSoft, Inc. Tulsa, OK), and the data were represented on a two-dimensional plot.

Median Joining (Bandelt et al., 1999) (MJ, $\varepsilon = 0$) network analysis and Reduced Median (Bandelt et al., 1995) (RM, $r = 2$) network analysis were carried out to locate most of the HVS-I haplotypes observed in the Tuareg sample in the context of other lineages collected from literature in the database. All networks were performed at the haplogroup level using Network 4.5 software (Fluxus Technology Ltd., Clare, Suffolk, UK). Weights were assigned to the polymorphic sites according to their relative mutation rate (Allard et al., 2002). For the H1 lineages, published African, European and Asian haplotype data (Achilli et al., 2004; Loogväli et al., 2004; Coudray et al., 2009) were used together with 169 North African sequences from Algeria, Morocco, Libya and Tunisia (Table S6), which are characterised by the H1 diagnostic substitution G3010A. From this dataset of H1 sequences, HVS-I haplotypes that are shared or closely related to Libyan Tuareg were shown in the network. Furthermore, in order to have a clearer resolution of the network, polymorphic status at np 00073 in HVS-II was considered in the analysis. The original African Tuareg lineages were plotted in the latest phylogenetic tree of complete African mtDNA sequences available in the literature (Behar et al., 2008). When relevant, coalescence time estimation was carried out: the ρ (*rho*) statistic (Forster et al., 1996) and its standard deviation as defined by the parameter σ (*sigma*) (Saillard et al., 2000) were calculated and converted into years according to the rate of 1 synonymous transition/8,006 years (E-L Loogväli, T Kivisild, T Margus, R Villems, in preparation).

Results

MtDNA Gene Pool in the Libyan Tuaregs

Haplogroup affiliations and HVS-I and HVS-II sequences were determined for 129 Tuareg individuals. In the two samples collected from the villages of Al Awaynat and Tahala (Table 1), the haplogroup frequencies were not dissimilar, as suggested by 95% Credible Regions (data not shown). Very similar frequencies were noted for H1, which comprised the main component in both samples (Table 1), while the frequency of haplogroup L1b1 was unexpectedly higher in the Tahala sample. These results were statistically confirmed by χ^2 analysis which provided non-significant p values ($p > 0.01$) when the L1b1 haplogroup was excluded. For this reason, and considering that the main objective of this study was not to determine the genetic relationship between the neighbouring villages of Al Awaynat and Tahala, the two samples were subsequently analysed together.

A total of 79 mtDNAs (61%) were characterised by the diagnostic RFLP markers of −7025 *Alu*I, and −14766 *Mse*I, and by the transition at np 3010. This pattern of mutations allowed us to assign these mtDNAs to H1, the main H subhaplogroup. Within the H1 haplotypes, 68 shared the same HVS-I/HVS-II pattern (CRS/263). The HVS-I transition at 16298 and the HVS-II transversion at np 72, together with the RFLP marker −4577 *Nla*III and the mutation at np 15904,

Table 1 Absolute and relative haplogroup frequencies in the Tuareg samples from the villages of Al Awaynat and Tahala (Fezzan, Libya) analysed in the present study.

	Al Awaynat		Tahala		Tuareg Libya[a]	
	N	%	N	%	N	%
H1	68	61	11	61	79	61
V	4	4	1	6	5	4
L0a1a	8	7	-	-	8	6
L1b1	2	2	-	-	2	2
L1b1a	-	-	3	17	3	2
L2a1	11	10	1	6	12	9
L2a1a	1	1	-	-	1	1
L2b	2	2	-	-	2	2
L3e1	4	4	-	-	4	3
L3e2	1	1	-	-	1	1
L3e2b	1	1	-	-	1	1
L3e3	4	4	-	-	4	3
L3f1b	-	-	2	11	2	2
L3i2	3	3	-	-	3	2
M1	2	2	-	-	2	2
total	111		18		129	

[a]Individuals from Al Awaynat and Tahala pooled in one sample.

allowed classification of five additional mtDNAs (4%) into haplogroup V. In contrast, haplogroup M1 and south Saharan lineages within L0, L1, L2 and L3 were observed at a lower frequency (35%) than the typically Western Eurasian H1 and V (65%) (Table 1). L2a1 (9%) and L0a1a (6%) were the most frequent originally African haplogroups. Transitions at nps 143 and 12693 allowed classification of 12 Tuareg mtDNAs as L2a1. Among these, 11 were characterised by a reversion at np 16309 (16309A!). According to the latest L2a1 phylogeny (Behar et al., 2008), a reversion to 16309A was observed in at least seven L2a1 clusters of full mtDNAs, while motif 16189–16192-16309A!, which characterizes the Libyan Tuaregs, was observed in two different L2a1 sub-clades. One individual was classified as L2a1a due to the presence of a reversion at np 143 and a mutation at np 16286. The L0a1a Tuareg lineages were defined by the transition at np 200; more particularly, the presence of a transition at np 64 might allow us to group them in a specific L0a1a sub-clade. The five L1b1 lineages were characterised by a mutation at np 2768; specifically, two individuals from Al Awaynat were assigned to L1b1a because of a mutation at np 5393. The L3e haplotypes were characterised by the RFLP marker +2349 *Mbo*I; their assignment to the L3e sub-haplogroups was based on the HVS-I and HVS-II patterns. The mutation at np 7645, together with 152 and 16260, made it possible to assign three individuals to L3i2, while motif 189–200-16292-16311 observed in two individuals was diagnostic for L3f1b. Haplotype L2b was defined by motif 204–16114-16129–16213, together with the RFLP marker +4157 *Alu*I. Finally, two individuals were affiliated to M1 due to the −10397 *Alu*I. A complete list of mutations observed in each individual is reported in Table S7 as Supporting Information (available on the journal's website).

As shown in Table 2, despite the relatively large sample size, the Libyan Tuareg sample showed the lowest number of different haplotypes, followed by two Pygmy populations, the Mbuti and the Mbenzele. The high homogeneity at the haplotype level was reflected by the lowest value of haplotype diversity. This is also true when considering only the South-Saharan component in the Libyan Tuareg sample ($H = 0.909 \pm 0.025$). The Tuaregs were also characterised by the lowest mean number of pairwise differences.

Comparisons with other African Populations

The results of the MDS suggest that geographical criteria can explain the grouping of populations on the two-dimensional plot. Almost all North African populations located at one end of the first dimension, with the remaining populations (i.e., Western, Eastern, Equatorial and Southern African samples) at the other end (Fig. 2). Berber groups and some isolates (i.e., Açores islanders, Canary islanders, Madeira islanders) from North Africa occupied the most marginal position on the left of our two-dimensional plot, together with the Libyan Tuareg sample. A further geographical partition into East-Central-West might be observed according to the second dimension. African populations south of the Equator located at the top-right fringe of the plot (e.g., Khwe, Mozambique, and Southeast Bantu). The analysis was repeated after removing all the 'extra-African' haplotypes (i.e., the haplotypes classified as H1 and V) from the Libyan Tuareg sample. Interestingly, the two-dimensional representation showed a shift of the Libyan Tuaregs from nearby the other northern African populations toward the Eastern African samples (Fig. 2).

Phylogeography of Lineages H1, L2a and L0a1

A detailed phylogeographic analysis of the H1 lineage and the African haplogroups observed in the Tuareg samples (L and M1) was carried out. Only the results of the most represented lineages (H1, L2a and L0a1) are reported here.

The network of H1 haplotypes (Fig. 3) shows that the CRS-3010 haplotype, which is the central node of the network, is widely distributed in northern African populations, including the Berbers and the Tuaregs, while the Eurasian samples show much more diversity. In order to include in our comparative analysis other north African populations that have not been typed for the H1 marker (i.e., the transition at np 3010), a comparison of the HVS-I H-CRS haplotype frequencies among the African populations was carried out (Table 3). Again, results show that this haplotype is well spread in northwestern Africa, especially among some Berber groups, where it may account for more than 15% of their mtDNA pool. The coalescence age of H1 variation in the Tuareg sample was estimated to be 1800 years (SE 1550). More remarkably, after focusing exclusively on the H1-CRS, we calculated that with a 95% probability the age of a clade that shows 72 times no mutations is not older than 850 years.

Phylogeographic investigation of the L2a1 lineages was limited to the comparison with the tree of fully sequenced mtDNAs from Behar et al., 2008. The major concern when using HVS-I haplotypes was that L2a1 could have been misclassified as L2a because of the lack of 16309G, as described by old classification schemes (Salas et al., 2002). The three Tuareg haplotypes clustered in a specific clade defined by transition A3203G. Results of the investigation of the variable positions in the coding region and HVS-I motif 16189–16192-16309A! (see Table S7) suggested that the Tuareg haplotypes could be assigned to the sub-clade that included three sequences from the Arabian peninsula and one from Israel (samples L430, L442, L584 and L313 in Behar et al., 2008). Coalescence age

Table 2 Indices of molecular diversity in some African populations. All indices were calculated from HVS-I haplotypes of population collected in the database. Abbreviations are as follows: N, sample size; K, number of haplotypes; S, number of polymorphic sites; H, haplotype diversity; π, mean number of pairwise differences.

POPULATION[a]	N	K	S	H	π	Reference
Libyan Tuareg	129	20	41	0.677 ± 0.046	4.398 ± 2.186	Present study
Libyan Tuareg[b]	129	21	61	0.678 ± 0.046	7.769 ± 3.642	Present study
Algerians	47	27	50	0.956 ± 0.014	5.681 ± 2.772	Plaza et al., 2003
Arabs Chad	27	20	37	0.963 ± 0.023	7.242 ± 3.500	Cerny et al., 2007
Arabs Shuwa	38	28	44	0.979 ± 0.012	6.345 ± 3.075	Cerny et al., 2007
Asni Berbers	53	36	50	0.963 ± 0.016	5.603 ± 2.733	Coudray et al., 2009
Bouhria Berbers	70	36	54	0.957 ± 0.012	5.686 ± 2.758	Coudray et al., 2009
Buduma	30	22	44	0.968 ± 0.021	8.252 ± 3.934	Cerny et al., 2007
Chenini Douiret Berbers	53	23	41	0.939 ± 0.017	6.822 ± 3.264	Fadhlaoui-Zid et al., 2004
Egypt	126	103	98	0.994 ± 0.003	7.710 ± 3.616	Krings et al., 1999
El Alia Berbers	48	28	44	0.962 ± 0.016	5.826 ± 2.835	Cherni et al., 2008
El Andalous Berbers	29	21	29	0.970 ± 0.018	5.192 ± 2.588	Cherni et al., 2008
Figuig Berbers	94	29	53	0.937 ± 0.014	6.312 ± 3.023	Coudray et al., 2009
Fulani	185	57	61	0.938 ± 0.008	7.215 ± 3.396	Cerny et al., 2007
Hide	23	22	49	0.996 ± 0.014	9.292 ± 4.431	Cerny et al., 2007
Kesra Berbers	43	30	53	0.960 ± 0.020	6.888 ± 3.305	Cherni et al., 2008
Matmata	49	29	56	0.946 ± 0.021	5.050 ± 2.494	Fadhlaoui-Zid et al., 2004
Mauritania	64	46	45	0.984 ± 0.007	6.697 ± 3.201	Gonzalez et al., 2006
Mbuti	20	9	19	0.858 ± 0.054	6.205 ± 3.077	Vigilant et al., 1991
Mbenzele	57	12	22	0.805 ± 0.037	4.986 ± 2.460	Destro Bisol et al., 2004
Moroccan Berbers	64	42	51	0.968 ± 0.013	4.497 ± 2.243	Rando et al., 1998, Pinto et al., 1996
Moroccans	50	43	67	0.992 ± 0.007	7.029 ± 3.357	Rando et al., 1998, Pinto et al., 1996
Mozabiti Berbers	85	30	37	0.943 ± 0.010	4.821 ± 2.377	Corte-Real et al., 1996
Nubia	80	53	72	0.976 ± 0.008	8.351 ± 3.908	Krings et al., 1999
Saharawi	80	52	57	0.982 ± 0.006	5.473 ± 2.661	Rando et al., 1998
Sened Berbers	53	37	64	0.975 ± 0.011	7.526 ± 3.570	Fadhlaoui-Zid et al., 2004
Siwa Berbers	78	31	48	0.927 ± 0.015	5.556 ± 2.698	Coudray et al., 2009
Skira Berbers	20	14	27	0.937 ± 0.043	4.753 ± 2.345	Cherni et al., 2008
Slouguia Berbers	28	20	36	0.971 ± 0.018	5.397 ± 2.681	Cherni et al., 2008
Souss Berbers	50	34	38	0.961 ± 0.018	4.604 ± 2.298	Brakez et al., 2001
Sudan	60	52	68	0.994 ± 0.005	8.546 ± 4.006	Krings et al., 1999
Testour Berbers	50	36	59	0.958 ± 0.021	6.236 ± 3.013	Cherni et al., 2008
Tunisians	47	42	61	0.990 ± 0.009	6.150 ± 2.977	Plaza et al., 2003
Tunis Berbers	51	44	62	0.992 ± 0.062	7.013 ± 3.349	Cherni et al., 2008
Western Tuareg	24	22	40	0.993 ± 0.014	6.989 ± 3.402	Watson et al., 1996
Zriba Berbers	35	38	19	0.931 ± 0.028	6.198 ± 3.017	Cherni et al., 2008

[a]The data is from the present study as well as from literature. For further details about all the samples collected in the database see Table S5 in Supplementary Information.
[b]Calculations from the HVS-I and HVS-II combined haplotypes.

was calculated in this cluster of full mtDNAs (excluding the partial Tuareg mtDNAs) and a value of 16,012 yrs (SD 5661) was observed.

The eight identical L0a1a haplotypes were characterised by a reversion at np 16223 and HVS-II mutations at nps 146 and 150. They could be possibly attributed to L0a1a-64T clade encompassing mainly Eastern and Central Africa (2 haplotypes in Egypt, 1 in Sudan and 1 in Chad) and, more interestingly, one Israeli mtDNA (samples L407, L408, L259p and L553 in Behar et al., 2008). Coalescence time in this clade was 14,678 years (SD 4,811). A similar geographic distribution was observed in the MJ network of 221 HVS-I L0a1 haplotypes (Fig. S1 Supporting Information), in which the eight Libyan Tuaregs departed through 16223C! from a root that is widely distributed in eastern Africa (44% of the haplotypes).

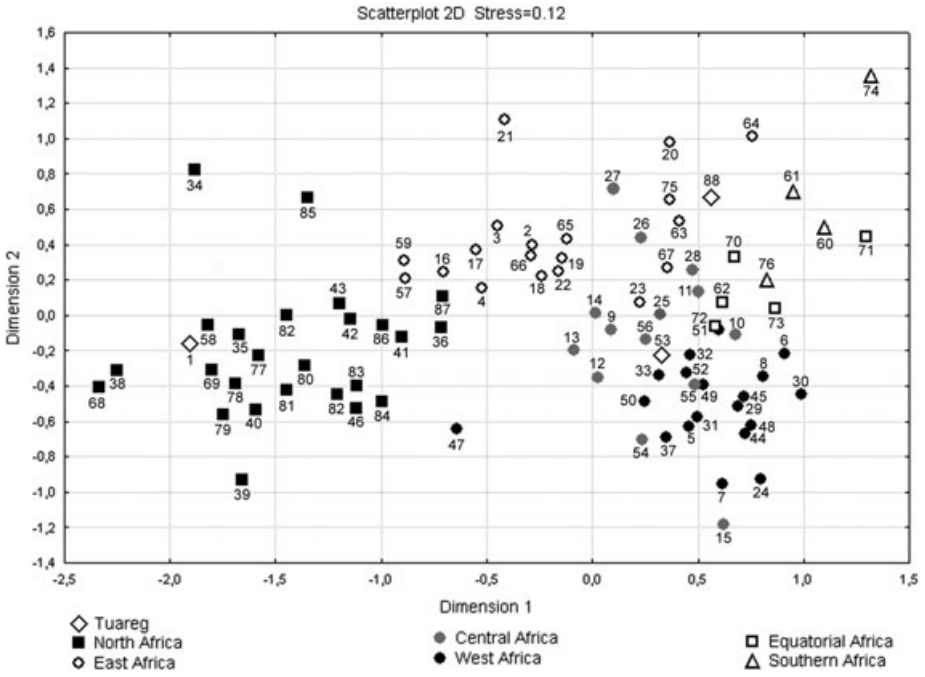

Figure 2 Multidimensional Scaling. Two-dimensional MDS plot of Slatkin's linearized Fst between African populations. Note that 3 points describe the position of the Tuaregs: Libyan Tuaregs with full data (1), Libyan Tuaregs with H1 and V haplotypes excluded (88), and Western Tuaregs taken from literature (53; Watson et al., 1996). The numeric code is as follows: 1 Libyan Tuaregs; 2 Oromo_a; 3 Amhara_a; 4 Fayum Egypt; 5 Fon; 6 Dendi; 7 Berba; 8 Bariba; 9 Kanuri_a; 10 Kanembu; 11 Fali; 12 Buduma; 13 Arabs Shuwa; 14 Arabs Chad; 15 Fulani; 16 Yemen; 17 Tigrai; 18 Oromo_b; 19 Gurage; 20 other Ethiopians; 21 Eritreans; 22 Amhara_b; 23 Afar; 24 Mandenka; 25 Hide; 26 Mafa; 27 Kotoko; 28 Masa; 29 Mende; 30 Loko; 31 Limba; 32 Temne; 33 Guineans; 34 Chenini-Douiret Berbers; 35 Matmata Berbers; 36 Sened Berbers; 37 Senegal; 38 Moroccan Berbers; 39 Mozabiti Berbers; 40 Souss Berbers; 41 Moroccans; 42 Tunisians; 43 Algerians; 44 Wolof; 45 Serer; 46 Saharawi; 47 Mauritania; 48 Mali; 49 Barbara; 50 Capo Verde; 51 Yoruba; 52 Songhai; 53 Western Tuareg; 54 Fulbe; 55 Hausa; 56 Kanuri_b; 57 Egyptians; 58 Canary islanders; 59 Libyans; 60 Mozambicans; 61 Southeast Bantu; 62 Sao Tomè/Bioko; 63 Kikuyu; 64 Turkana; 65 Somalia; 66 Nubia; 67 Sudan; 68 Açores islanders; 69 Madeira islanders; 70 Bamileke; 71 Angolares; 72 Forros; 73 Tonga; 74 Khwe; 75 Kenya; 76 Angola; 77 Bohuria Berbers; 78 Slouguia Berbers; 79 El Alia Berbers; 80 Asni Berbers; 81 Skira Berbers; 82 Zriba Berbers; 83 Testour Berbers; 84 Figuig Berbers; 85 Siwa Berbers; 86 Kesra Berbers; 87 Tunis Berbers; 88 Libyan Tuaregs (after excluding H1 and V haplotypes). Note that in addition to Libyan Tuaregs, the Western Tuareg sample of 24 individuals (code 53) from Nigeria, Niger and Mali, from a study by Watson et al., 1996 was used in the analysis. Literature references are given in Table S5 in the Supporting Information available on the journal's website.

Discussion

The Libyan Tuareg sample as a whole appears to be extremely homogenous, as indicated by the low estimate of haplotype diversity. Its value is the lowest ever observed in African populations analysed so far (Table 2). Only 20 different HVS-I haplotypes were found in a total of 129 Tuareg individuals. The reason for this low genetic diversity, at least as regards mtDNA, may be explained by high genetic drift.

Furthermore, the structure of Tuareg society might have reduced the diversity of maternally inherited mtDNA due to matrilocality phenomena (Oota et al., 2001; Bolnick & Smith 2003).

The European Component

A high fraction of HVS-I CRS sequences were present in the Libyan Tuareg sample (56%). Screening of the single

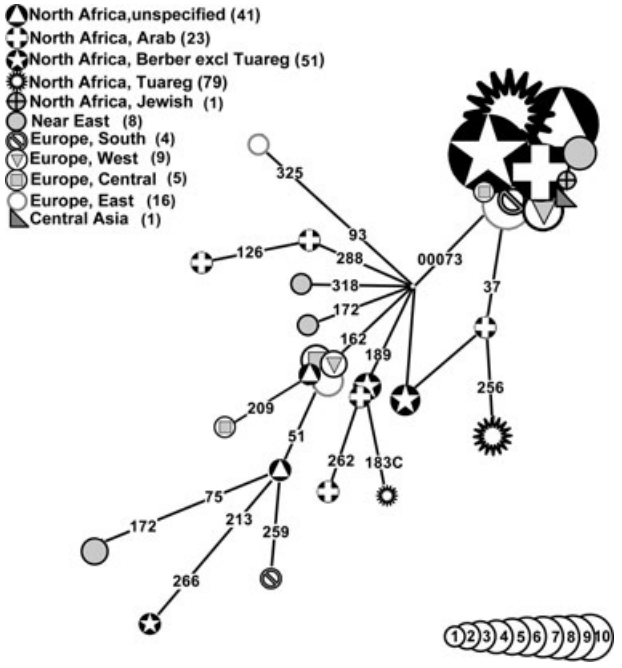

Figure 3 RM network of H1. The full dataset of 390 HVS-I sequences that belong to haplogroup H and have A at np 3010, is from the following sources: 79 Libyan Tuareg (present study); 76 Tunisian, 9 Libyan, 73 Moroccan, 11 Algerian (Table S6); 9 Italian, 1 Iraqi, 1 Georgian and 1 Berber from Achilli et al., 2004; 19 Estonian, 13 Russian, 7 Ukrainian, 13 French, 9 Slovak, 2 Croatian, 3 Greek, 1 Albanian, 8 Turkish, 4 Iranian, 2 Jordanian, 1 Syrian, 2 Kirghiz and 1 Tadjik from Loogväli et al., 2004; 45 Berbers from Coudray et al., 2009. Samples were divided into 7 regions: North Africa, South Europe (Italian, Greek, Albanian, Croatian), West Europe (French), Central Europe (Slovak), Eastern Europe (Estonian, Russian, Ukrainian), Near East (Turkish, Jordanian, Syrian, Iranian, Iraqi, Georgian) and Central Asia (Kirghiz, Tadjik). Haplotypes that are shared or closely related to Libyan Tuareg were selected from the dataset and shown in the network, for a total of 238 sequences. Position numbers are given minus 16,000 except for 00073. The node sizes are proportional to the number of individuals in each node. The network keys to population symbols and node sizes are shown. Number of haplotypes shown in the network for each region is given in brackets. Unless the new nucleotide is specified, all mutations are transitions.

Table 3 HVS-I H-CRS haplotype and haplogroup V frequencies (%) in African populations. * indicates the samples in which H-CRS haplotypes were assigned to H1.

POPULATION[a]	H-CRS	V
Libyan Tuareg*	56	4
Matmata Berbers	20	16
Figuig Berbers*	19	3
Souss Berbers	18	10
El Andalous Berbers*	17	3
Zriba Berbers*	17	0
Moroccan Berbers	16	6
Slouguia Berbers	14	21
Tunisians	11	0
El Alia Berbers*	10	4
Testour Berbers*	10	2
Asni Berbers*	9	8
Sened Berbers	9	0
Algerians	8	0
Western Tuareg	8	0
Mozabiti Berbers	8	8
Moroccans	8	4
Mauritania	6	3
Bouhria Berbers*	6	1
Egypt	6	1
Chenini Douiret Berbers	6	0
Saharawi	4	19
Tunis Berbers*	4	4
Hide	4	0
Buduma	3	0
Kesra Berbers*	2	7
Fulani	1	1
Nubia	1	0
Skira Berbers*	0	5
Sudan	0	5
Arabs Chad	0	0
Siwa Berbers*	0	0
Arabs Shuwa	0	0

[a]Data sources for the populations other than Libyan Tuaregs are in Table 2.

nucleotide polymorphisms (SNPs) in the coding region allowed us to classify all these HVS-I CRS sequences as H1. As observed in the network of the H1 lineages, this haplotype is widely diffused in northern Africa, both in Berber and Arab communities. This is confirmed by the frequency data in Northern African populations shown in Table 3. Haplogroup H1, together with haplogroups H3, V and U5b, marked the population expansion that occurred after the Last Glacial Maximum from the Iberian Peninsula and led Late-Pleistocene hunter-gatherers to repopulate central and northern Europe (Torroni et al., 1998, 2001, 2006; Achilli et al., 2004; Loogväli et al., 2004) at the same time as another population movement is thought to have spread southward into northwest Africa (Achilli et al., 2005; Cherni et al., 2008; Coudray et al., 2009). Haplogroup V was found in the Libyan Tuareg sample at a frequency (4%) comparable to that of other Berber populations, and its presence often coincides with the occurrence of HVS-I H-CRS in the Berber samples (Table 3). So, it is possible that the H-CRS component reflects the presence of H1 in the samples which were not defined at the sub-haplogroup level. The high incidence of H1 in the Libyan Tuaregs, particularly its H-CRS pool, points to a genetic

relationship between the Libyan Tuaregs and the Berbers. This is also apparent from the MDS plot, where the Libyan Tuareg sample locates together with Berber populations (Fig. 2). Of note is that the other Tuareg sample described in the literature (Watson et al., 1996) (Western Tuaregs) did not show a close genetic relationship with the Libyan Tuaregs, implying a genetic heterogeneity of the Tuaregs. This difference appears to be primarily caused by the low frequency (8%) of the European component in the Western Tuaregs, characteristic of northern African populations. After the removal of the H and V haplotypes, the Libyan Tuaregs showed a strong affiliation with the Eastern populations, while the Western Tuaregs associated more with the Central and Western African populations (Fig. 2). A scenario can be hypothesized in which the continuously changing Saharan environment, particularly during the second half of the Holocene coinciding with the start of the arid phase (Hassan, 1996, 2002), was responsible for human migratory dynamics that led different Tuareg groups to mix and to separate from one another. In this connection, the hypothesis that the Libyan Tuaregs originated through a founder effect from an ancestral Tuareg population seems likely. Moreover, the coalescence age of the H1 variation confirmed that it might have occurred in the second half of the Holocene, about 1,800 years ago, despite the fact that a more recent event seems likely as indicated by the age of the H1-CRS clade. A Berber origin is supported by linguistic data that characterise the Tuareg language as a proto-Berber language (Greenberg, 1970; Gaudio, 1993). Founder effect coupled to subsequent genetic drift might be the explanation of such a high frequency of H1 as well as of the absence of other typically North African haplogroups that are indeed frequent in the Berbers (e.g. U6).

The South Saharan Component

Besides the European genetic component, a minor but more heterogeneous African component was observed in our samples.

Phylogeographic analysis of L0a1a highlighted a genetic affinity of the Libyan Tuaregs with the Northeast African and the Near Eastern populations. More particularly, this holds true when the Libyan Tuareg L2a1 lineages were grouped with the 16189–16192-16309A! sub-branch. Interestingly, the coalescence age calculated in the typically Near Eastern 16189–16192-16309A! cluster of full mtDNAs (16,012 yrs, SD 5,661) was very close to the values observed in the L0a1a cluster (i.e., 14,678 yrs, SD 4,811). Noteworthy is that similar coalescence ages and geographic distributions were observed in the Y-chromosome haplogroup E-V12* (Cruciani et al., 2007), which is related to the movement of people from East Africa northward through the Nile Valley and spreading also into the Central Sahara and the Arabian peninsula. Accordingly, a relationship between the L2a1 and L0a1a mtDNA lineages and this migration flow is proposed. More interestingly, the genetic closeness of the Libyan Tuareg lineages to the haplotypes from Saudi Arabia and Israel can be interpreted as the result of the arrival of pastoral groups from the Near East into North Africa in the early middle Holocene, which is documented by the appearance of sheep and goats in the archaeological records of Egypt and in the Sahara as well (Vermeersch et al., 1994; Wendorf & Schild, 1994; Bradley et al., 1996; Close 2002; Kuper & Kropelin, 2006)

All other south Saharan lineages were represented at very low frequencies (1–3%). That a sporadic introduction of these lineages into the Libyan Tuareg population may have taken place perhaps through the slave trade is confirmed by the typical south Saharan morphological traits of the slaves' descendants.

A remarkable genetic affinity with the Eastern African populations (particularly with the Beja) was observed for autosomal markers by Cavalli-Sforza (Cavalli-Sforza et al., 1994). From an analysis of a sample of individuals from many Tuareg populations in Western and Central Africa, he proposed that the Tuaregs originated through a population split from an ancestral pastoral group in the area between the Nile and the Red Sea in the middle Holocene. Despite some affinity with Eastern African mtDNA lineages, our data differ apparently from Cavalli-Sforza's survey, as he found no close relationship with Berber groups. This might suggest that the geographic connotation is particularly strong in the Tuaregs, so that groups from different areas are genetically different. This has been confirmed by mtDNA data from another Tuareg sample (Western Tuareg) (Watson et al., 1996).

It is worth noting the low haplotype diversity value of the south Saharan mtDNA pool, which pointed out that genetic drift affected this component in the Tuaregs as well as the European one. An early introduction of south Saharan lineages into the main European matrix could be plausible; however, the hypothesis that both mtDNA components were present in the same founder population cannot be ruled out.

Final Remarks

The mtDNA analysis helped to characterise the Libyan Tuaregs as a mixed group in which two main components are present. A European component, marked by haplogroups H1 and V, is strongly predominant and is shared with some Berber groups and other north African populations as well. Also present is a typically south Saharan component that shows a genetic relationship with Eastern African populations. The L2a1 and L0a1a lineages could be related to the movement of people from Eastern Africa approximately 15,000 years ago and subsequently via the Near East during pastoral movements. Additional studies are needed to collect more data

from various African populations in order to improve our understanding of the genetic roots of the Tuaregs, as well as those of other Saharan peoples.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Figure S1 MJ network of L0a1 lineages. The root node is represented by the symbol '#', and all mutated positions reported in the branches are to be read as +16,000 (e.g., 223 stands for 16,223), as they belong to the HVS-I region. State of nucleotide is reported only when transversions occurred, while all other mutations are transitions. Node sizes are proportional to the absolute haplotype frequencies, which are indicated in each node. Geographic provenance of the haplotypes, as reported in the list of populations collected in the database (Supporting Information available on the journal's website) is indicated by different symbols, as follows: open

circle, Western Africa; grey circle, Africa south of the Equator; black circle, Libyan Tuaregs; open square, Eastern Africa; grey square, Central Africa; open rhombus, Equatorial Africa; grey rhombus, Northern Africa (including other Tuaregs from the literature).

Table S1 Primers used for the amplification reactions of the D-Loop and some coding regions of the mtDNA in the Tuareg samples. The resulting PCR products were submitted to sequencing analysis.

Table S2 Primers used for the amplification reactions of the coding regions of mtDNA submitted to RFLP analysis in the Tuareg samples.

Table S3 Cycles conditions used for the amplification of the HVS-I, HVS-II and some coding regions in the mtDNA of the Tuareg samples. All the amplification products were submitted to sequencing reaction.

Table S4 Concentrations of each primer (Forward and Reverse), and PCR conditions used for the amplification of coding regions in the mtDNA of the Tuareg. All the amplification products were submitted to RFLP analysis. For the amplification reactions the 9700/2700 Thermocyclers by Applied Biosystems were used.

Table S5 Complete list of African samples used in the present study for comparative analyses. References are also shown.

Table S6 North African H1 sequences used for comparative analysis in the present study.

Table S7 Polymorphic sites observed in the HVS-I, HVS-II, and some coding regions submitted to sequencing and RFLP analysis, in 129 Tuareg from Fezzan (Libya).

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