

Announcement of Population Data

Population genetic data of 15 tetrameric short tandem repeats (STRs) in Berbers from Morocco

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Abstract

The allele frequency distribution of 15 short tandem repeats (STR) loci contained in the AmpF/STR IdentifierTM PCR Amplification Kit (Applied Biosystems), was determined in two Berber populations from Asni and Bouhria, in Central and Eastern Morocco, respectively. A total of 209 individuals were typed. No deviations from the Hardy–Weinberg equilibrium were observed for Asni at the 15 STRs loci whereas for the Bouhria samples, two loci (D5S818 and TH01) showed significant departures from Hardy–Weinberg expectations (after Bonferroni's correction). All loci are highly polymorphic and population differentiation tests showed that the Moroccan samples from Asni and Bouhria have significant differences in 4 out of 15 loci (D21S11, D7S820, D16S539 and TPOX). The aim of the study was to obtain accurate allele frequencies relevant for forensic applications. Comparative analyses between our population data and other population samples gathered from the literature are also presented.

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1. Population

We studied 209 unrelated and healthy adult donors of both sexes coming from two Moroccan regions, about 700 km apart (Fig. 1): 105 individuals came from Asni and 104 from Bouhria. The people were subjected to an interview in order to make sure of their ethnic origins and to obtain their informed consent. All the individuals are Berber-speakers and their families have been living in the regions for at least three generations. Asni is located in the Tacheddirt valley in High Atlas Mountains (47 km from Marrakech). The Berbers from Asni speak Chleuh and belong to the Rhiraya tribe. Sidi Bouhria is located in Eastern Morocco (Oujda wilaya). The Berbers from Bouhria belong to the Beni Moussi Roua fraction of the Beni Snassen tribe. The Berbers are considered as the oldest inhabitants in the Maghreb (since the Neolithic). In the course of history, different Mediterranean people (Phoenicians, Vandals, Romans and Byzantines) invaded this region. The most important invasion

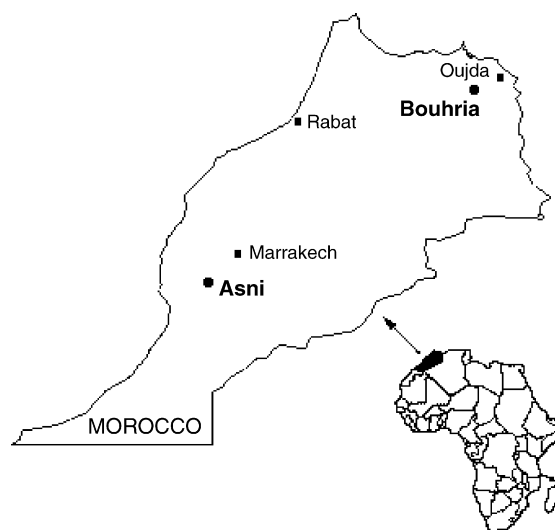


Fig. 1. Geographical position of Asni and Bouhria.

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Table 1

Allelic frequencies and forensic parameters of 15 STR loci in 105 Moroccan Berbers from Asni

Allele	D3S1358	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	D8S1179	FGA	TH01	VWA	TPOX	D19S433	D2S1338
6	–	–	–	–	–	–	–	–	–	–	0.167	–	0.005	–	–
7	–	–	–	–	–	0.005	–	0.010	–	–	0.295	–	–	–	–
8	–	–	–	0.048	0.081	0.076	0.019	0.024	–	–	0.157	–	0.438	–	–
9	–	–	–	0.038	0.014	0.100	0.090	0.010	–	–	0.248	–	0.190	–	–
9.3	–	–	–	–	–	–	–	–	–	–	0.076	–	–	–	–
10	–	–	0.010	0.062	0.014	0.314	0.081	0.300	0.090	–	0.057	–	0.086	0.005	–
11	–	–	–	0.257	0.362	0.348	0.243	0.281	0.105	–	–	–	0.267	0.019	–
12	–	–	0.138	0.371	0.371	0.133	0.310	0.338	0.148	–	–	–	0.010	0.176	–
13	–	–	0.124	0.200	0.129	0.024	0.238	0.038	0.238	–	–	–	0.005	0.290	–
13.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.033	–
14	0.038	–	0.138	0.024	0.019	–	0.019	–	0.167	–	–	0.119	–	0.200	–
14.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.062	–
15	0.257	–	0.095	–	0.010	–	–	–	0.243	–	–	0.257	–	0.081	–
15.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.029	–
16	0.271	–	0.181	–	–	–	–	–	0.010	–	–	0.190	–	0.033	0.057
16.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.048	–
17	0.281	–	0.133	–	–	–	–	–	–	0.005	–	0.257	–	0.024	0.300
18	0.133	–	0.105	–	–	–	–	–	–	0.010	–	0.124	–	–	0.129
19	0.019	–	0.033	–	–	–	–	–	–	0.057	–	0.043	–	–	0.076
20	–	–	0.029	–	–	–	–	–	–	0.138	–	0.010	–	–	0.181
21	–	–	0.010	–	–	–	–	–	–	0.157	–	–	–	–	0.095
22	–	–	0.005	–	–	–	–	–	–	0.195	–	–	–	–	0.019
23	–	–	–	–	–	–	–	–	–	0.176	–	–	–	–	0.067
24	–	–	–	–	–	–	–	–	–	0.095	–	–	–	–	0.043
24.2	–	–	–	–	–	–	–	–	–	0.005	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–	0.090	–	–	–	–	0.024
26	–	0.029	–	–	–	–	–	–	–	0.057	–	–	–	–	0.010
27	–	0.010	–	–	–	–	–	–	–	0.014	–	–	–	–	–
28	–	0.086	–	–	–	–	–	–	–	–	–	–	–	–	–
29	–	0.257	–	–	–	–	–	–	–	–	–	–	–	–	–
30	–	0.238	–	–	–	–	–	–	–	–	–	–	–	–	–
30.2	–	0.024	–	–	–	–	–	–	–	–	–	–	–	–	–
31	–	0.029	–	–	–	–	–	–	–	–	–	–	–	–	–
31.2	–	0.124	–	–	–	–	–	–	–	–	–	–	–	–	–
32.2	–	0.086	–	–	–	–	–	–	–	–	–	–	–	–	–
33.2	–	0.057	–	–	–	–	–	–	–	–	–	–	–	–	–
34	–	0.005	–	–	–	–	–	–	–	–	–	–	–	–	–
34.2	–	0.005	–	–	–	–	–	–	–	–	–	–	–	–	–
35	–	0.052	–	–	–	–	–	–	–	–	–	–	–	–	–
Ho	0.810	0.781	0.886	0.752	0.705	0.733	0.848	0.638	0.819	0.848	0.800	0.838	0.686	0.838	0.829
He	0.762	0.839	0.874	0.748	0.707	0.746	0.773	0.714	0.815	0.863	0.790	0.800	0.693	0.828	0.835
PD	0.936	0.950	0.895	0.871	0.892	0.913	0.864	0.900	0.950	0.944	0.926	0.856	0.964	0.893	0.961
PIC	0.790	0.821	0.707	0.660	0.721	0.759	0.658	0.738	0.818	0.808	0.771	0.642	0.860	0.709	0.848
PE	0.635	0.564	0.482	0.339	0.617	0.599	0.436	0.690	0.653	0.672	0.672	0.407	0.766	0.514	0.690
p	0.758	0.179	0.630	0.096	0.288	0.158	0.308	0.329	0.327	0.712	0.927	0.523	0.589	0.706	0.523

Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphism information content; PD: power of discrimination; PE: power of exclusion; p: p-values of the exact test for Hardy–Weinberg equilibrium (with a 5% significance level).

Table 2

Allelic frequencies and forensic parameters of 15 STR loci in 104 Moroccan Berbers from Bouhria

Allele	D3S1358	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	D8S1179	FGA	TH01	VWA	TPOX	D19S433	D2S1338
5	–	–	–	–	–	–	–	–	–	–	0.005	–	–	–	–
6	–	–	–	–	–	–	–	–	–	–	0.207	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	0.183	–	0.014	–	–
8	–	–	–	0.014	0.077	0.202	0.005	0.005	0.005	–	0.154	–	0.447	–	–
9	–	–	–	0.029	0.038	0.072	0.149	0.034	–	–	0.293	–	0.053	–	–
9.3	–	–	–	–	–	–	–	–	–	–	0.101	–	–	–	–
10	–	–	–	0.058	0.034	0.313	0.087	0.370	0.058	–	0.058	–	0.091	–	–
11	–	–	0.043	0.207	0.293	0.279	0.346	0.288	0.096	–	–	0.005	0.365	0.010	–
12	–	–	0.173	0.361	0.433	0.111	0.178	0.279	0.173	–	–	–	0.029	0.144	–

Table 2 (Continued)

Allele	D3S1358	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	D8S1179	FGA	TH01	VWA	TPOX	D19S433	D2S1338
12.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.005	–
13	–	–	0.144	0.288	0.106	0.014	0.139	0.019	0.260	–	–	–	–	0.264	–
13.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.029	–
14	0.053	–	0.149	0.043	0.019	0.010	0.096	0.005	0.216	–	–	0.139	–	0.308	–
14.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.130	–
15	0.192	–	0.139	–	–	–	–	–	0.159	–	–	0.135	–	0.038	–
15.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.029	–
16	0.361	–	0.139	–	–	–	–	–	0.034	–	–	0.207	–	0.019	0.058
16.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.014	–
17	0.279	–	0.091	–	–	–	–	–	–	–	–	0.269	–	0.005	0.346
17.2	–	–	–	–	–	–	–	–	–	–	–	–	–	0.005	–
18	0.115	–	0.058	–	–	–	–	–	–	–	–	0.212	–	–	0.106
19	–	–	0.034	–	–	–	–	–	0.082	–	–	0.029	–	–	0.130
20	–	–	0.024	–	–	–	–	–	0.115	–	–	0.005	–	–	0.144
21	–	–	–	–	–	–	–	–	0.226	–	–	–	–	–	0.053
21.2	–	–	0.005	–	–	–	–	–	0.019	–	–	–	–	–	–
22	–	–	–	–	–	–	–	–	0.144	–	–	–	–	–	0.038
23	–	–	–	–	–	–	–	–	0.111	–	–	–	–	–	0.024
23.2	–	–	–	–	–	–	–	–	0.005	–	–	–	–	–	–
24	–	–	–	–	–	–	–	–	0.163	–	–	–	–	–	0.077
25	–	–	–	–	–	–	–	–	0.072	–	–	–	–	–	0.024
26	–	–	–	–	–	–	–	–	0.048	–	–	–	–	–	–
27	–	0.005	–	–	–	–	–	–	0.010	–	–	–	–	–	–
28	–	0.168	–	–	–	–	–	–	0.005	–	–	–	–	–	–
29	–	0.303	–	–	–	–	–	–	–	–	–	–	–	–	–
30	–	0.231	–	–	–	–	–	–	–	–	–	–	–	–	–
31	–	0.048	–	–	–	–	–	–	–	–	–	–	–	–	–
31.2	–	0.077	–	–	–	–	–	–	–	–	–	–	–	–	–
32.2	–	0.139	–	–	–	–	–	–	–	–	–	–	–	–	–
33	–	0.005	–	–	–	–	–	–	–	–	–	–	–	–	–
33.2	–	0.019	–	–	–	–	–	–	–	–	–	–	–	–	–
36	–	0.005	–	–	–	–	–	–	–	–	–	–	–	–	–
Ho	0.654	0.837	0.817	0.683	0.721	0.740	0.740	0.702	0.885	0.885	0.731	0.750	0.635	0.721	0.750
He	0.739	0.799	0.873	0.768	0.707	0.766	0.790	0.700	0.817	0.861	0.801	0.802	0.654	0.794	0.817
PD	0.890	0.927	0.967	0.871	0.863	0.905	0.924	0.847	0.933	0.956	0.923	0.931	0.832	0.928	0.937
PIC	0.696	0.770	0.859	0.695	0.663	0.729	0.762	0.642	0.792	0.846	0.772	0.772	0.593	0.766	0.798
PE	0.361	0.669	0.632	0.402	0.462	0.493	0.493	0.431	0.764	0.764	0.477	0.510	0.334	0.462	0.510
p	0.180	0.845	0.444	0.000*	0.791	0.543	0.265	0.272	0.875	0.619	0.002*	0.649	0.719	0.331	0.068

Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphism information content; PD: power of discrimination; PE: power of exclusion; p: p-values of the exact test for Hardy–Weinberg equilibrium (with a 5% significance level).

* Bonferroni's correction (0.05/15 = 0.0033).

was the Arab conquest (seventh century A.D.) when autochthonous North African Berbers had to adopt a new religion and a new language. The people who resisted arabization – the ancestors of the present day Berbers – were forced to live in isolated groups.

2. DNA extraction

Genomic DNA was isolated from the blood samples using a standard proteinase-K digestion followed by phenol–chloroform extraction and ethanol precipitation.

3. PCR

DNA amplification was carried out in a GeneAmp 2700 Thermal cycler (Applied Biosystems) from 1 ng target DNA in a final volume of 25 µl following the manufacturer's

instructions for the AmpF/STR Identifier™ PCR Amplification Kit (Applied Biosystems). The samples were amplified in single reactions for all 15 loci using fluorescently labelled primers.

4. Typing

Capillary electrophoresis was conducted in an ABI PRISM 310 Genetic Analyzer using the separation medium Performance Optimized Polymer (POP) 4 and 47 cm capillaries (Applied Biosystems). The LIZ labelled ladder (GeneScan 500 LIZ) was used for sizing the determinations of the amplified fragments in combination with GeneScan Software (vs.3.7, Applied Biosystems). Numerical allele designations of the profiles were obtained by comparison to the allelic ladder using Genotyper Software (vs.3.7, Applied Biosystems).

Table 3
Genetic distances (F_{ST}) between Asni and Bouhria

STR	D3S1358	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	D8S1179	FGA	TH01	VWA	TPOX	D19S433	D2S1338
F_{ST}	0.00386	0.00648	0.00173	0.00329	0.00228	0.00963	0.02473	0.00218	0.00278	0.00666	0.00582	0.00989	0.01665	0.01974	0.00251
p -Value	0.13477	0.05273	0.19141	0.17578	0.20801	0.02148	0.00000 *	0.21680	0.13281	0.02832	0.06641	0.01367	0.00488	0.00098 *	0.14160

In bold: significant F_{ST} p -values.

* The p -values that remain significant after Bonferroni's correction.

Table 4
Population differentiation tests between Asni and Bouhria

STR	D3S1358	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	D8S1179	FGA	TH01	VWA	TPOX	D19S433	D2S1338
p -Value	0.08455	0.00000	0.02690	0.14460	0.27565	0.00085	0.00000	0.08600	0.11135	0.03275	0.23580	0.02070	0.00000	0.00615	0.07430
S.E.	0.0127	0.000000	0.0111	0.0222	0.0335	0.0006	0.000000	0.0162	0.0193	0.0092	0.0476	0.0079	0.000000	0.0016	0.0132

In bold: p -values that remain significant after Bonferroni's correction. S.E.: standard error.

5. Results

See Tables 1–5.

6. Analysis of data

The frequency of each allele for each locus and the observed heterozygosity (Ho) were calculated from the number of observed genotypes in the sample (direct gene counting). Expected heterozygosity (He) was estimated as $1 - \sum_i p_i^2$, where p_i is the frequency of the i th allele in the locus. The Hardy–Weinberg equilibrium exact test (dememorization value 10,000, and 5000 iterations per batch) was assessed by means of GENEPOP (1.2) statistical package [1]. The Bonferroni correction was applied on loci showing a departure from Hardy–Weinberg equilibrium [2]. In order to assess the relationship between the populations analyzed, the F_{ST} genetic distances [3] were calculated, locus by locus, using the Arlequin program package [4]. Population differentiation was tested for each locus by exact tests [5] as implemented in the Arlequin program [4]. Forensic parameters (polymorphism information content, PIC; power of discrimination, PD; and power of exclusion, PE) were calculated using Microsoft Excel-PowerStats program [6] downloaded from Promega corporation website at <http://www.promega.com/geneticidtools/powerstats/>.

7. Access to the data

Complete data can be obtained from the authors on request to: dugoujon@cict.fr.

8. Other remarks

Population data for the 15 STRs are shown in Tables 1 and 2 for Asni and Bouhria, respectively. Based on the results of the exact tests, no deviations from Hardy–Weinberg equilibrium were observed for Asni at the 15 STRs but, for the Bouhria samples, the D5S818 and TH01 loci showed departures from the Hardy–Weinberg expectations. When using the Bonferroni correction for the number of loci analyzed, the two deviations remain significant ($p = 0.000$ for D5S818 and $p = 0.002$ for TH01). Possible reasons for the deviations observed include: statistical artefacts due to multiple testing, population size, inbreeding, population substructure or selection. Given the structure of the Moroccan population with contributions to the genepool mainly from both Middle Eastern and African ancestry, population sub-structure might be a likely explanation.

In Moroccans from Asni, the observed heterozygosity (Ho) ranges from 0.638 (CSF1PO) to 0.886 (D18S51). The power of discrimination (PD) varies between 0.856 (VWA) and 0.964 (TPOX) and the probability of exclusion (PE) varies between 0.339 (D5S818) and 0.766 (TPOX). For the 15 loci, the combined power of discrimination and the combined probability of exclusion are 2.467×10^{-17} and 0.9999988, respectively.

In Moroccans from Bouhria, the observed heterozygosity (Ho) ranges from 0.635 (TPOX) to 0.885 (D8S1179 and

Table 5
Population differentiation tests between Asni, Bouhria and other published population data

STR	D3S1358	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	CSF1PO	D8S1179	FGA	TH01	VWA	TPOX
Asni vs. Moroccan Arabs	0.16685	0.09305	0.12565	0.40350	0.18560	0.00000	0.04955	0.85790	0.00850	0.19405	0.60645	0.04205	0.19770
Asni vs. Tunisian Berbers	0.00900	0.00000	0.05860	0.03610	0.00370	0.00000	0.03255	0.32295	0.06975	0.29550	0.30850	0.60665	0.03020
Asni vs. Tunisian Arabs	0.00080	0.31055	0.00000	0.00415	0.00000	0.02365	0.00000	0.00000	0.00605	0.00000	0.02710	0.31745	0.00010
Asni vs. Mozabites	0.79585	0.03400	0.66880	0.03240	0.21285	0.03865	–	0.06585	0.00255	0.00050	0.00085	0.00000	0.94675
Asni vs. Andalousians	0.19875	0.00440	0.10805	0.58760	0.53650	0.00410	–	0.14330	0.02155	0.39690	0.00000	0.00090	0.09995
Bouhria vs. Moroccan Arabs	0.00120	0.00000	0.04285	0.00100	0.00725	0.01170	0.05205	0.04890	0.02960	0.43435	0.61125	0.11660	0.00000
Bouhria vs. Tunisian Berbers	0.01650	0.00000	0.06500	0.00785	0.20600	0.03390	0.24520	0.00495	0.77130	0.06495	0.35980	0.05980	0.00000
Bouhria vs. Tunisian Arabs	0.00000	0.01865	0.00000	0.00330	0.02065	0.03400	0.02615	0.00035	0.01945	0.00000	0.04715	0.09780	0.00000
Bouhria vs. Mozabites	0.12880	0.01300	0.05420	0.01990	0.85135	0.00405	–	0.00290	0.00000	0.01660	0.08750	0.01150	0.03930
Bouhria vs. Andalousians	0.52795	0.00355	0.02870	0.69780	0.47190	0.00455	–	0.04750	0.50415	0.20265	0.00215	0.22790	0.08540

In bold: p -values that remain significant after Bonferroni's correction. References for the population data used: Asni and Bouhria (this study); Moroccan Arabs [7,8]; Tunisian Berbers and Arabs [13]; Mozabites [8]; Andalousians [7].

FGA). The power of discrimination (PD) varies between 0.832 (TPOX) and 0.967 (D18S51) and the probability of exclusion (PE) varies between 0.334 (TPOX) and 0.764 (D8S1179 and FGA). For the 15 loci, the combined power of discrimination and the combined probability of exclusion are 6.124×10^{-17} and 0.9999908, respectively.

Then, the two Moroccan populations were compared (Tables 3 and 4). After Bonferroni's correction, only two F_{ST} genetic distances remain significant for the D16S539 ($p = 0.00000$) and D19S433 ($p = 0.00098$) loci (Table 3). The exact tests of population differentiation reveal that 4 out of 15 loci, namely D21S11, D7S820, D16S539 and TPOX, have statistically significant differences in the two population samples (Table 4).

Present analysis of the two Berber groups from Asni and Bouhria was made in order to complete STR database for Moroccan populations. Different works were previously published [7–12] on the two major ethnic groups (Berbers and Arabic-speakers) but this is the first study of D19S433 and D21S1338 allele frequency distribution in Morocco.

We have compared our data with other published data [7,8,13] on geographically related population samples, using the available sets of loci (Table 5). After Bonferroni's correction, population differentiation tests showed that the Berbers from Asni had significant differences with Moroccan Arabs in 1 out of 13 loci (D7S820), with Tunisian Berbers in 2 out of 13 loci (D21S11 and D7S820), with Tunisian Arabs in 7 out of 13 loci (D3S1358, D18S51, D13S317, D16S539, CSF1PO, FGA and TPOX), with Algerian Mozabites in 4 out of 12 loci (D8S1179, FGA, TH01 and VWA), and with Andalousians in 2 out of 12 loci (TH01 and VWA). About the Berbers from Bouhria, they had significant differences with Moroccan Arabs in 4 out of 13 loci (D3S1358, D21S11, D5S818 and TPOX), with Tunisian Berbers in 2 out of 13 loci (D21S11 and TPOX), with Tunisian Arabs in 5 out of 13 loci (D3S1358, D18S51, CSF1PO, FGA and TPOX), with Algerian Mozabites in 2 out of 12 loci (CSF1PO and VWA), and with Andalousians in 1 out of 12 loci (TH01).

This paper follows the guidelines for publication of population data requested by the journal [14].

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