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Genetic Structure of the Azores Islands: a Study using 15 Autosomal Short Tandem Repeat Loci

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A B S T R A C T

The Azores archipelago (Portugal), located in the Atlantic Ocean, 1,500 km from the European mainland, is formed by nine islands of volcanic origin. The relative position of these islands allows the definition of three geographical groups: Eastern, Central and Western. Previous studies of the Azores using Short Tandem Repeats (STRs) have highlighted differences in the frequencies of several loci, when compared to Mainland Portugal or Madeira Island. Furthermore, linkage disequilibrium (LD), described for Azorean samples has been tentatively explained as reflecting the presence of genetic sub-structuring in the archipelago. To provide information concerning the genetic profile of the Azores Islands and to evaluate the presence of substructuring we have determined the allelic frequencies of 15 autosomal STR loci, using the AmpFlSTR® Identifiler™ Kit, in representative samples from the Azorean Islands. Either considering the Azores as a whole, or analysing by island all the loci were in conformity with Hardy-Weinberg equilibrium. Average gene diversity ranged from 0.7669 in Corvo to 0.7972 in Terceira Island. Allelic independence between loci, tested for the global sample, detected significant LD (after correction for multiple tests) for pairs D21S11/D7S820 and D3S1358/D5S818. The exact test of population differentiation, combining the information of the 15 markers analysed, revealed significant differences between the three groups of islands, and between islands. Inter-island analysis reinforces the previous data that suggested the existence of sub-structuring in the Azores archipelago. Moreover, the data generated by this study can be used in a future forensic genetic database of the Azores after the appropriate enlacement of sample size by island, preventing, in that way, misinterpretations caused by population substructuring and small sample sizes.

Key words: *autosomal STRs, Azores, genetic differentiation*

Introduction

The Azores archipelago (Portugal), located in the Atlantic Ocean, 1,500 km from the European mainland, is formed by nine islands of volcanic origin. The relative position of these islands allows the definition of three geographical groups: Eastern (S. Miguel and Sta. Maria), Central (Terceira, Faial, Pico, Graciosa and S. Jorge) and Western (Flores and Corvo) (Figure 1). With a total area of 2,344 km^2, the Azores have presently a total population of 237,315 inhabitants, distributed in a very asymmetric way among islands. Uninhabited when first discovered by the Portuguese in 1432, the settling of the archipelago was a slow process that lasted almost a century, starting by the islands of Sta. Maria and São Miguel, and latter being completed by the islands of Flores and Corvo. According to historical records, the first settlers came mainly from various regions of mainland Portugal and from Madeira Island. Moreover, people of different origins, such as Spanish, French, Italian, English, German and Flemish, whose important presence is always referred to in accounts of the peopling of the archipelago (namely of the Central group), also made up part of the early settlers. There is clear evidence, both from historical as well as molecular data that individuals of Jewish extraction and African and Moorish slaves also contributed to the peopling of the archipelago.

Research conducted so far, based namely on the monoparental genomes – mitochondrial DNA (mtDNA) and non-recombining portion of the Y chromosome (NRY) –

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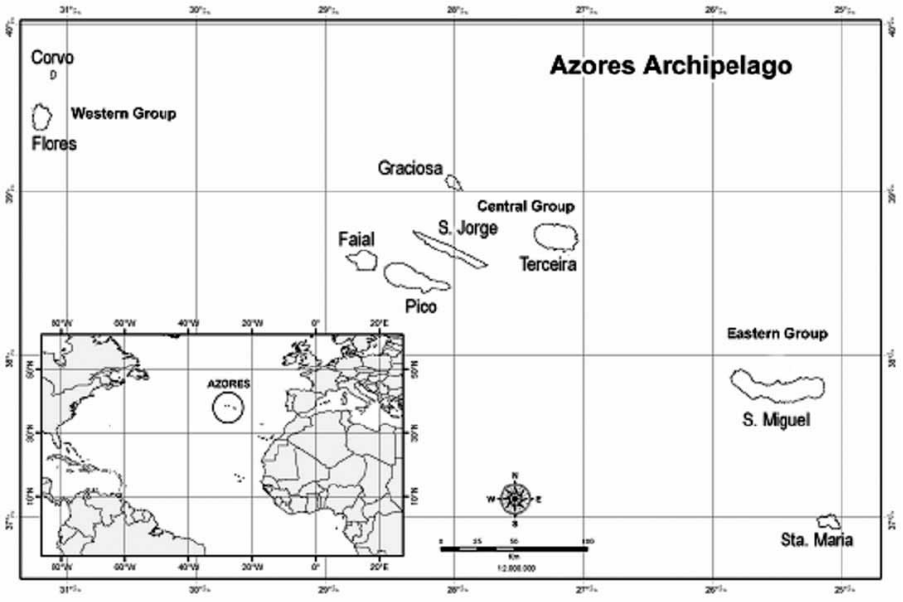

Fig. 1. Geographic location of the Azores. The archipelago is formed by three groups of islands: Western, Central and Eastern. Source: Geography Section, Department of Biology, University of the Azores.

(i.e.) have highlighted the fact that the archipelago cannot be considered a true homogeneous population, since the existence of genetic sub-structuring between groups of islands has been demonstrated. Also, data gathered by our group, using surnames as genetic markers, corroborates the importance that this factor might have on the genetic make-up of the Azorean populations.

Previous reports, for the Azores, using Short Tandem Repeats (STRs) have highlighted differences in the frequencies of several loci, when compared to Mainland Portugal or Madeira Island thus justifying the need, in forensic studies, for previously generated population data base of autochthonous people. Furthermore, autosomal STR linkage disequilibrium (LD), described for Azorean samples, has been tentatively explained as reflecting the presence of genetic sub-structuring in the archipelago. However, the lack of samples for all the islands, has limited the investigation of this hypothesis.

To provide information concerning the genetic profile of the Azores Islands and to evaluate the presence of substructuring we have determined the allelic frequencies of 15 autosomal STR loci in samples from the nine Azorean Islands.

Materials and Methods

Samples

Two-hundred and ninety-four unrelated individuals from the Azores Islands were used in the study (58 from S. Miguel; 23 from Sta. Maria; 46 from Terceira; 25 from Graciosa; 34 from S. Jorge; 37 from Pico; 35 from Faial; 24 from Flores and 12 from Corvo). All subjects signed an informed consent form and filled in a genealogical questionnaire which confirmed their Azorean ancestry until the third generation. Samples were distributed considering the island of origin of the grandparents of the individuals sampled.

DNA extraction and typing

DNA from buccal cells was extracted using the JET-QUICK Blood and Cell DNA Purification Kit (Genomed), according to the manufacturer's specifications. DNA from blood samples was extracted using standard protocols. Multiplex polymerase chain reaction for fifteen STR markers (Table 1) was performed using the AmpFlSTR® Identifiler™ Kit of Applied Biosystems according to the kit manual specifications. The amplified products were analyzed using the ABI PRISM 3100 (AB) Genetic Analyzer with 3100 Data collection software (Version 1.0.1). The identification of the alleles was performed using the GeneScan (Version 3.7).

Data analysis

Allele frequencies were estimated for all loci. Forensic parameters (Power of Discrimination and Chance of Exclusion) were calculated for each locus using Powerstatsv12.xls software (http://www.promega.com/geneticidtools/powerstats/). Hardy-Weinberg equilibrium (HWE) was tested using an exact test, based on a Markov chain approach, followed by a Bonferroni correction for multiple tests. For marker yielding p-values lower than 0.05, a score test was performed to evaluate the hypothesis of homozygotes excess. An unbiased estimate of heterozigosity was computed according to Nei. Allelic independence between loci was tested performing pairwise comparisons between loci. Population differentiation exact tests, using allelic frequencies, were carried out to

TABLE 1
ALLELIC FREQUENCIES FOR ALL THE MAKERS FOR THE TOTAL AZOREAN SAMPLE AND DISCRIMINATED BY ISLAND

Locus	Allele	Azores (N=294)	Corvo (N=12)	Flores (N=24)	Faial (N=35)	Pico (N=37)	S. Jorge (N=34)	Graciosa (N=25)	Terceira (N=46)	S. Miguel (N=58)	St. Maria (N=23)
D8S1179	8	0.014	0.042	0.021	0.014	0.014	0.029	0.020	0.011	0.000	0.000
	9	0.015	0.042	0.000	0.014	0.041	0.015	0.000	0.033	0.000	0.000
	10	0.083	0.083	0.125	0.086	0.081	0.044	0.100	0.044	0.121	0.065
	11	0.064	0.083	0.021	0.057	0.054	0.059	0.060	0.109	0.060	0.065
	12	0.149	0.042	0.104	0.229	0.108	0.235	0.240	0.120	0.103	0.152
	13	0.331	0.333	0.354	0.229	0.365	0.382	0.240	0.337	0.319	0.413
	14	0.209	0.292	0.229	0.229	0.189	0.132	0.260	0.174	0.276	0.109
	15	0.122	0.083	0.146	0.143	0.135	0.059	0.080	0.152	0.112	0.174
	16	0.012	0.000	0.000	0.000	0.014	0.029	0.000	0.022	0.009	0.022
	17	0.002	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000
D21S11	24.2	0.005	0.042	0.021	0.000	0.000	0.015	0.000	0.000	0.000	0.000
	25.2	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
	27	0.015	0.000	0.000	0.014	0.041	0.015	0.000	0.022	0.017	0.000
	28	0.160	0.250	0.313	0.114	0.095	0.162	0.120	0.152	0.138	0.239
	29	0.255	0.292	0.292	0.229	0.216	0.353	0.200	0.261	0.250	0.217
	29.1	0.003	0.000	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000
	29.2	0.002	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000
	30	0.204	0.167	0.146	0.214	0.243	0.118	0.220	0.152	0.267	0.261
	30.2	0.051	0.042	0.042	0.057	0.014	0.059	0.080	0.076	0.043	0.044
	31	0.075	0.000	0.021	0.100	0.081	0.074	0.080	0.044	0.095	0.130
	31.2	0.107	0.042	0.063	0.143	0.122	0.132	0.120	0.109	0.112	0.044
	32	0.010	0.000	0.021	0.014	0.014	0.000	0.020	0.011	0.009	0.000
	32.2	0.077	0.125	0.042	0.086	0.054	0.059	0.080	0.152	0.052	0.044
	33	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
	33.2	0.027	0.042	0.042	0.029	0.068	0.015	0.080	0.011	0.000	0.000
	34.2	0.005	0.000	0.000	0.000	0.014	0.000	0.000	0.011	0.000	0.022
D7S820	7	0.022	0.000	0.021	0.086	0.000	0.029	0.000	0.033	0.009	0.000
	8	0.155	0.083	0.167	0.071	0.203	0.147	0.240	0.054	0.216	0.196
	9	0.151	0.333	0.229	0.171	0.081	0.162	0.120	0.217	0.086	0.109
	10	0.301	0.250	0.313	0.214	0.432	0.265	0.260	0.217	0.353	0.370
	11	0.201	0.125	0.104	0.257	0.149	0.221	0.200	0.261	0.207	0.174
	12	0.145	0.167	0.146	0.143	0.095	0.162	0.180	0.174	0.121	0.152
	13	0.024	0.042	0.000	0.057	0.041	0.015	0.000	0.044	0.009	0.000
	14	0.002	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CSF1PO	7	0.003	0.000	0.000	0.000	0.014	0.000	0.000	0.011	0.000	0.000
	8	0.009	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.035	0.000
	9	0.020	0.000	0.021	0.014	0.014	0.015	0.000	0.044	0.026	0.022
	10	0.279	0.458	0.333	0.243	0.270	0.324	0.140	0.304	0.302	0.174
	11	0.308	0.208	0.333	0.286	0.405	0.309	0.480	0.217	0.267	0.304
	12	0.304	0.125	0.167	0.329	0.203	0.279	0.320	0.370	0.328	0.500
	12.1	0.002	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000
	13	0.054	0.208	0.063	0.100	0.041	0.059	0.020	0.044	0.043	0.000
	14	0.020	0.000	0.083	0.029	0.054	0.000	0.020	0.011	0.000	0.000
D3S1358	13	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
	14	0.116	0.042	0.042	0.114	0.068	0.147	0.200	0.152	0.121	0.087
	15	0.247	0.167	0.188	0.257	0.351	0.294	0.220	0.261	0.172	0.283
	16	0.281	0.417	0.229	0.200	0.243	0.221	0.340	0.272	0.353	0.304
	17	0.202	0.292	0.396	0.257	0.230	0.177	0.180	0.141	0.164	0.109
	18	0.145	0.083	0.146	0.157	0.095	0.147	0.040	0.174	0.172	0.217
	19	0.009	0.000	0.000	0.014	0.014	0.015	0.020	0.000	0.009	0.000
TH01	6	0.214	0.042	0.188	0.271	0.284	0.221	0.240	0.250	0.147	0.196
	7	0.145	0.208	0.125	0.157	0.149	0.088	0.060	0.098	0.250	0.109
	8	0.134	0.125	0.146	0.143	0.095	0.191	0.080	0.207	0.086	0.130
	9	0.225	0.083	0.208	0.271	0.162	0.206	0.200	0.294	0.233	0.239
	9.3	0.279	0.542	0.333	0.157	0.284	0.294	0.420	0.152	0.285	0.326

Locus	Allele	Azores (N=294)	Corvo (N=12)	Flores (N=24)	Faial (N=35)	Pico (N=37)	S. Jorge (N=34)	Graciosa (N=25)	Terceira (N=46)	S. Miguel (N=58)	St. Maria (N=23)
	10	0.003	0.000	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000
D13S317	8	0.134	0.083	0.167	0.100	0.108	0.147	0.200	0.130	0.121	0.152
	9	0.060	0.000	0.063	0.071	0.081	0.074	0.100	0.044	0.052	0.022
	10	0.061	0.208	0.042	0.057	0.054	0.059	0.020	0.065	0.043	0.109
	11	0.308	0.250	0.333	0.300	0.311	0.250	0.280	0.413	0.319	0.196
	12	0.286	0.333	0.250	0.343	0.297	0.265	0.320	0.217	0.302	0.283
	13	0.104	0.083	0.083	0.100	0.122	0.118	0.080	0.087	0.112	0.130
	14	0.046	0.042	0.042	0.029	0.027	0.088	0.000	0.044	0.043	0.109
	15	0.002	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D16S539	8	0.026	0.000	0.021	0.043	0.041	0.029	0.020	0.011	0.035	0.000
	9	0.114	0.167	0.146	0.143	0.108	0.044	0.100	0.152	0.095	0.109
	10	0.049	0.000	0.042	0.043	0.041	0.059	0.080	0.011	0.052	0.130
	11	0.299	0.458	0.333	0.357	0.284	0.250	0.200	0.391	0.233	0.283
	12	0.287	0.167	0.208	0.214	0.378	0.294	0.380	0.228	0.336	0.283
	13	0.192	0.167	0.250	0.157	0.108	0.294	0.220	0.141	0.216	0.196
	14	0.032	0.042	0.000	0.043	0.041	0.029	0.000	0.065	0.035	0.000
D2S1338	15	0.003	0.000	0.000	0.000	0.000	0.015	0.000	0.011	0.000	0.000
	16	0.058	0.042	0.083	0.100	0.054	0.029	0.040	0.076	0.052	0.022
	17	0.286	0.125	0.333	0.329	0.284	0.294	0.280	0.272	0.293	0.261
	18	0.075	0.208	0.083	0.057	0.095	0.059	0.040	0.022	0.095	0.109
	19	0.092	0.208	0.146	0.086	0.122	0.044	0.080	0.054	0.078	0.130
	20	0.138	0.167	0.042	0.129	0.149	0.265	0.040	0.120	0.164	0.109
	21	0.026	0.042	0.021	0.014	0.041	0.000	0.040	0.033	0.009	0.065
	22	0.032	0.000	0.021	0.014	0.014	0.044	0.040	0.044	0.052	0.022
	23	0.097	0.000	0.104	0.157	0.068	0.059	0.120	0.130	0.086	0.087
	24	0.100	0.042	0.083	0.071	0.095	0.088	0.220	0.152	0.078	0.044
	25	0.082	0.125	0.063	0.043	0.081	0.088	0.100	0.076	0.078	0.130
	26	0.007	0.042	0.021	0.000	0.000	0.015	0.000	0.000	0.009	0.000
	27	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.009	0.000
	28	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022
D5S818	8	0.012	0.000	0.021	0.000	0.027	0.015	0.000	0.011	0.000	0.044
	9	0.026	0.000	0.021	0.000	0.041	0.000	0.040	0.044	0.035	0.022
	10	0.058	0.167	0.063	0.029	0.027	0.088	0.060	0.076	0.052	0.022
	11	0.332	0.542	0.271	0.314	0.338	0.353	0.300	0.359	0.319	0.283
	12	0.407	0.208	0.354	0.443	0.419	0.324	0.520	0.348	0.474	0.435
	13	0.162	0.083	0.271	0.214	0.135	0.221	0.080	0.152	0.112	0.196
	14	0.005	0.000	0.000	0.000	0.014	0.000	0.000	0.011	0.009	0.000
FGA	17	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
	18	0.005	0.000	0.000	0.014	0.014	0.000	0.000	0.011	0.000	0.000
	19	0.071	0.042	0.021	0.043	0.081	0.147	0.120	0.044	0.086	0.022
	19.3	0.002	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000
	20	0.155	0.208	0.146	0.157	0.189	0.191	0.200	0.109	0.103	0.196
	21	0.197	0.208	0.146	0.200	0.162	0.162	0.180	0.239	0.198	0.283
	22	0.179	0.167	0.188	0.186	0.122	0.177	0.120	0.207	0.190	0.239
	22.3	0.005	0.000	0.021	0.000	0.000	0.015	0.000	0.011	0.000	0.000
	23	0.140	0.250	0.104	0.157	0.149	0.132	0.140	0.087	0.172	0.109
	23.3	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000
	24	0.141	0.083	0.313	0.143	0.189	0.059	0.140	0.163	0.121	0.044
	25	0.063	0.000	0.063	0.071	0.041	0.088	0.080	0.076	0.052	0.065
	26	0.022	0.042	0.000	0.000	0.000	0.015	0.020	0.033	0.043	0.044
	27	0.012	0.000	0.000	0.029	0.027	0.015	0.000	0.011	0.009	0.000
	28	0.003	0.000	0.000	0.000	0.014	0.000	0.000	0.011	0.000	0.000
D19S433	10	0.002	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000
	11	0.007	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.009	0.022
	12	0.104	0.083	0.208	0.114	0.081	0.074	0.120	0.087	0.112	0.065
	13	0.253	0.417	0.146	0.271	0.270	0.338	0.260	0.261	0.233	0.130
	13.2	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000

Locus	Allele	Azores (N=294)	Corvo (N=12)	Flores (N=24)	Faial (N=35)	Pico (N=37)	S. Jorge (N=34)	Graciosa (N=25)	Terceira (N=46)	S. Miguel (N=58)	St. Maria (N=23)
	14	0.313	0.083	0.333	0.271	0.351	0.309	0.380	0.239	0.388	0.304
	14.2	0.015	0.000	0.000	0.014	0.014	0.000	0.000	0.011	0.043	0.022
	15	0.197	0.292	0.188	0.243	0.162	0.162	0.100	0.250	0.129	0.370
	15.2	0.039	0.000	0.042	0.014	0.000	0.029	0.060	0.065	0.052	0.065
	16	0.046	0.125	0.042	0.029	0.108	0.044	0.040	0.044	0.026	0.000
	16.2	0.014	0.000	0.021	0.000	0.014	0.029	0.020	0.022	0.000	0.022
	17	0.005	0.000	0.021	0.014	0.000	0.000	0.000	0.011	0.000	0.000
	17.2	0.003	0.000	0.000	0.000	0.000	0.000	0.020	0.011	0.000	0.000
vWA	13	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000
	14	0.100	0.000	0.125	0.157	0.135	0.088	0.160	0.098	0.043	0.087
	15	0.124	0.042	0.104	0.143	0.162	0.103	0.040	0.130	0.138	0.174
	16	0.231	0.208	0.354	0.200	0.189	0.221	0.180	0.272	0.241	0.196
	17	0.231	0.333	0.250	0.214	0.176	0.206	0.220	0.250	0.259	0.217
	18	0.243	0.375	0.083	0.243	0.297	0.309	0.340	0.163	0.224	0.261
	19	0.060	0.042	0.083	0.043	0.027	0.044	0.060	0.065	0.086	0.065
	20	0.009	0.000	0.000	0.000	0.014	0.029	0.000	0.011	0.009	0.000
TPOX	6	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.009	0.000
	7.3	0.002	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000
	8	0.507	0.458	0.667	0.514	0.662	0.456	0.400	0.391	0.509	0.522
	9	0.087	0.125	0.000	0.086	0.054	0.088	0.060	0.130	0.095	0.130
	10	0.065	0.000	0.042	0.029	0.041	0.088	0.120	0.087	0.095	0.000
	11	0.296	0.333	0.146	0.343	0.216	0.338	0.340	0.348	0.267	0.348
	12	0.039	0.083	0.146	0.029	0.014	0.029	0.060	0.033	0.026	0.000
	13	0.002	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
D18S51	10	0.015	0.000	0.063	0.000	0.014	0.044	0.020	0.011	0.000	0.000
	11	0.010	0.000	0.000	0.000	0.014	0.015	0.020	0.011	0.000	0.044
	12	0.158	0.250	0.125	0.243	0.189	0.103	0.160	0.120	0.112	0.239
	13	0.143	0.000	0.167	0.143	0.162	0.191	0.080	0.174	0.103	0.196
	14	0.157	0.250	0.188	0.186	0.122	0.132	0.080	0.120	0.216	0.130
	15	0.129	0.167	0.104	0.157	0.176	0.118	0.160	0.163	0.069	0.087
	16	0.128	0.125	0.146	0.100	0.095	0.118	0.220	0.087	0.164	0.109
	17	0.100	0.125	0.021	0.057	0.068	0.132	0.100	0.087	0.155	0.130
	18	0.075	0.000	0.083	0.071	0.081	0.103	0.060	0.098	0.069	0.044
	19	0.043	0.083	0.042	0.029	0.054	0.044	0.020	0.065	0.043	0.000
	20	0.026	0.000	0.042	0.014	0.000	0.000	0.060	0.044	0.035	0.022
	21	0.005	0.000	0.000	0.000	0.000	0.000	0.020	0.011	0.009	0.000
	22	0.012	0.000	0.021	0.000	0.027	0.000	0.000	0.011	0.026	0.000

compare data obtained for groups of islands and by island and also the Azores populations with Mainland Portugal. The genetic distances matrix resulting from the F_{st} Slatkin transformation was represented in a bi-dimensional space using the Multidimensional Scaling procedure. Statistical packages used were Genepop on the web, Arlequin ver. 3.0 and SPSS 15.0.

Results and Discussion

Allelic frequencies for all makers, discriminated by island, are presented in Table 1. Either considering the Azores as a whole, or analyzing each island separately, and after performing the Bonferroni correction for multiple tests, all the loci were in conformity with Hardy-Weinberg equilibrium expectations (Table 2). The power of discrimination (PD) was higher than 0.808 for all the loci. The chance of exclusion (CE) had the highest value in D21S11 system and the lowest in CSF1PO and TPOX (Table 2). On what concerns levels of expected heterozygosity, the reported values are similar to that observed in mainland Portugal, with the highest value being registered for TPOX and the lowest for D18S51 (Table 2). The pairwise comparisons between loci showed linkage disequilibrium (after correction for multiple tests) for D21S11/D7S820 and D3S1358/D5S818 (Table 3). However, the tests for detection of homozygous excess showed a borderline p value only for marker D13S317.

Average gene diversity, considering all loci, ranged from 0.7669 in Corvo to 0.7972 in Terceira Island (Table 4). Diversity values obtained from STRs were compared with those derived from mono-parental markers and surname data (Table 4). The Western group displays the lowest values of diversity derived both from mtDNA sequences and from STR data obtained in the present study. The lowest values of diversity based on NRY mark-

TABLE 2
VALUES OF OBSERVED AND EXPECTED HETEROZYGOSITY, P VALUE FOR HW TEST AND RESPECTIVELY STANDARD DEVIATION (SD), POWER OF DISCRIMINATION (PD) AND CHANCE OF EXCLUSION (CE), ARE PRESENTED FOR ALL LOCI, IN THE TOTAL AZOREAN SAMPLE (N=294)

Locus	Obs. Het.	Exp. Het.	p-value	SD	PD	CE
D8S1179	0.77551	0.80079	0.14804	0.00098	0.93	0.554
D21S11	0.86395	0.84246	0.91143	0.00040	0.954	0.723
D7S820	0.80612	0.80166	0.09152	0.00082	0.928	0.610
CSF1PO	0.67007	0.73214	0.73745	0.00083	0.887	0.383
D3S1358	0.76190	0.78648	0.81356	0.00124	0.922	0.530
TH01	0.83333	0.78828	0.53626	0.00136	0.915	0.662
D13S317	0.79932	0.78673	0.03206	0.00050	0.918	0.598
D16S539	0.78231	0.77508	0.99500	0.00022	0.914	0.567
D2S1338	0.83673	0.85557	0.58193	0.00104	0.964	0.669
D5S818	0.68027	0.69573	0.68290	0.00103	0.857	0.398
FGA	0.84694	0.85755	0.64955	0.00069	0.961	0.689
D19S433	0.77551	0.78537	0.94949	0.00039	0.925	0.554
vWA	0.78231	0.80613	0.76583	0.00100	0.935	0.567
TPOX	0.67007	0.64343	0.18168	0.00088	0.808	0.383
D18S51	0.85034	0.87998	0.69259	0.00104	0.972	0.696

ers, were observed in the Western group. Globally, values of diversity obtained with STRs are less discrepant between islands than those reported for mono-parental markers. Notwithstanding, these differences in diversity must be interpreted with caution due to small sample size available for the small islands of the Western group.

The exact test of population differentiation (combining the information of the 15 markers) revealed significant differences between the three groups of islands (Western group/Central group: $p=0.00183$; Western group/Eastern group: $p<0.00001$; Central group/Eastern group: $p=0.00025$). Moreover significant differences between the genetic profiles obtained for each of the nine islands was also observed: Corvo appears to be the most differentiated island, since it presents significant differences with all the remaining. The less differentiated islands are S. Jorge and Faial. The differentiation between islands seems to be related with the allelic distribution of six of the 15 markers analysed, namely: D7S820, CSF1PO, D3S1358, TH01, D19S433 and TPOX.

To better access the relation between the nine Azorean islands the Slatkin's genetic distance matrix, derived from pairwise F_{st} values, was represented in a bi-dimensional space using the Multidimensional Scaling procedure (Figure 2). With the exception of Flores and Corvo the Azorean Islands clustered together, and Graciosa appears to be the more differentiate island from the cluster formed by the Central and Eastern group of islands. The differentiation of the two islands of the Western group (Flores and Corvo) from the other islands of the archipelago and between each other is evident, however, this result could be related to the small sample size available for these islands, that is limited by the small number of inhabitants (especially in the case of Corvo island).

The results for the analysis of differentiation between groups of islands and between islands are in accordance to that previously reported for mtDNA and NRY: the Western group of islands, once again, appears as the most differentiated. Moreover, there are evidences for differentiation between islands of the same geographical group, as it was also pointed out by Santos et al. in a recent work based on surname analysis.

Allele frequencies obtained were also compared with data available for mainland North Portugal. Considering the global Azorean sample significant differences were only found for FGA (Table 5). However, when the nine islands were individually compared with Mainland Portugal (Table 5), significant differences were found for TPOX, TH01, D8S1179, D7S820, CSF1PO, FGA, D13S317,

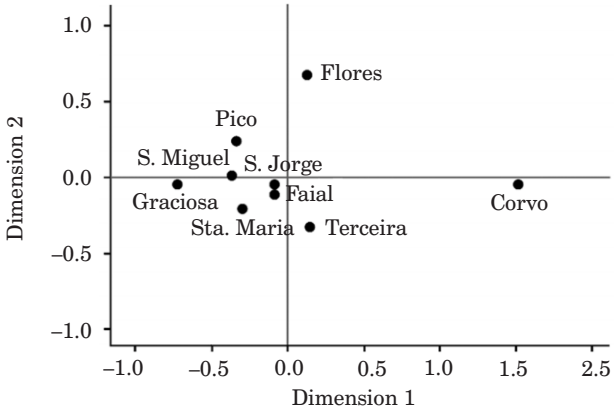

Fig. 2. Multidimensional Scaling analysis using Slatkin genetic distance between the nine islands of the Azores.

TABLE 3
RESULTS OF THE LINKAGE DISEQUILIBRIUM TEST BETWEEN PAIRS OF LOCI

Locus pair	χ^2	df	p-value
D8S1179 & D21S11	0.006	2	0.997
D8S1179 & D7S820	0.243	2	0.885
D21S11 & D7S820	Infinity	2	Highly sign.
D8S1179 & CSF1PO	0.415	2	0.813
D21S11 & CSF1PO	1.546	2	0.462
D7S820 & CSF1PO	0.305	2	0.859
D8S1179 & D3S1358	0.753	2	0.686
D21S11 & D3S1358	1.153	2	0.562
D7S820 & D3S1358	2.406	2	0.300
CSF1PO & D3S1358	1.844	2	0.398
D8S1179 & TH01	1.953	2	0.377
D21S11 & TH01	6.420	2	0.040
D7S820 & TH01	0.571	2	0.752
CSF1PO & TH01	5.351	2	0.069
D3S1358 & TH01	0.343	2	0.842
D8S1179 & D13S317	2.777	2	0.249
D21S11 & D13S317	2.466	2	0.291
D7S820 & D13S317	0.269	2	0.874
CSF1PO & D13S317	2.770	2	0.250
D3S1358 & D13S317	2.540	2	0.281
TH01 & D13S317	3.224	2	0.199
D8S1179 & D16S539	2.107	2	0.349
D21S11 & D16S539	7.335	2	0.026
D7S820 & D16S539	4.729	2	0.094
CSF1PO & D16S539	5.422	2	0.066
D3S1358 & D16S539	0.550	2	0.760
TH01 & D16S539	0.337	2	0.845
D13S317 & D16S539	0.140	2	0.933
D8S1179 & D2S1338	0.911	2	0.634
D21S11 & D2S1338	2.557	2	0.279
D7S820 & D2S1338	0.000	2	1.000
CSF1PO & D2S1338	1.311	2	0.519
D3S1358 & D2S1338	0.799	2	0.671
TH01 & D2S1338	2.156	2	0.340
D13S317 & D2S1338	5.135	2	0.077
D16S539 & D2S1338	1.850	2	0.396
D8S1179 & D5S818	0.236	2	0.889
D21S11 & D5S818	2.668	2	0.263
D7S820 & D5S818	0.401	2	0.818
CSF1PO & D5S818	1.167	2	0.558
D3S1358 & D5S818	Infinity	2	Highly sign.
TH01 & D5S818	0.144	2	0.931
D13S317 & D5S818	4.218	2	0.121
D16S539 & D5S818	4.423	2	0.110
D2S1338 & D5S818	2.462	2	0.292
D8S1179 & FGA	0.887	2	0.642
D21S11 & FGA	1.452	2	0.484
D7S820 & FGA	3.577	2	0.167
CSF1PO & FGA	0.777	2	0.678
D3S1358 & FGA	3.852	2	0.146
TH01 & FGA	0.346	2	0.841
D13S317 & FGA	4.135	2	0.127
D16S539 & FGA	4.584	2	0.101
D2S1338 & FGA	3.083	2	0.214
D5S818 & FGA	2.432	2	0.296
D8S1179 & D19S433	0.130	2	0.937
D21S11 & D19S433	1.008	2	0.604
D7S820 & D19S433	0.900	2	0.638
CSF1PO & D19S433	7.819	2	0.020
D3S1358 & D19S433	1.894	2	0.388
TH01 & D19S433	0.189	2	0.910
D13S317 & D19S433	0.018	2	0.991
D16S539 & D19S433	4.212	2	0.122
D2S1338 & D19S433	1.598	2	0.450
D5S818 & D19S433	0.755	2	0.686
FGA & D19S433	2.045	2	0.360
lparD8S1179 & VWA	2.368	2	0.306
D21S11 & VWA	0.243	2	0.885
D7S820 & VWA	1.201	2	0.549
CSF1PO & VWA	1.429	2	0.489
D3S1358 & VWA	1.298	2	0.523
TH01 & VWA	0.562	2	0.755
D13S317 & VWA	1.017	2	0.602
D16S539 & VWA	4.014	2	0.134
D2S1338 & VWA	0.302	2	0.860
D5S818 & VWA	6.760	2	0.034
FGA & VWA	1.645	2	0.439
D19S433 & VWA	1.755	2	0.416
D8S1179 & TPOX	0.458	2	0.795
lmult1D21S11 & TPOX	0.841	2	0.657
D7S820 & TPOX	1.755	2	0.416
CSF1PO & TPOX	0.043	2	0.979
D3S1358 & TPOX	5.136	2	0.077
TH01 & TPOX	0.599	2	0.741
D13S317 & TPOX	1.922	2	0.382
D16S539 & TPOX	1.218	2	0.544
D2S1338 & TPOX	0.094	2	0.954
D5S818 & TPOX	2.359	2	0.307
FGA & TPOX	7.520	2	0.023
D19S433 & TPOX	0.680	2	0.712
VWA & TPOX	0.347	2	0.841
D8S1179 & D18S51	0.341	2	0.843
D21S11 & D18S51	0.535	2	0.765
D7S820 & D18S51	0.708	2	0.702
CSF1PO & D18S51	0.987	2	0.611
D3S1358 & D18S51	0.992	2	0.609
TH01 & D18S51	1.588	2	0.452
D13S317 & D18S51	6.807	2	0.033
D16S539 & D18S51	1.810	2	0.405
D2S1338 & D18S51	3.238	2	0.198
D5S818 & D18S51	2.680	2	0.262
FGA & D18S51	0.076	2	0.963
D19S433 & D18S51	2.335	2	0.311
VWA & D18S51	5.480	2	0.065
TPOX & D18S51	1.335	2	0.513

TABLE 4
NUMBER OF INHABITANTS, VALUES OF DIVERSITY BASED ON SURNAMES (HETERONYMY), GENE DIVERSITY BASED ON NRY HAPLOGROUP FREQUENCIES AND ON MTDNA SEQUENCES

Group of Islands	Island	No. inhabitants[a]	Heteronymy (Het)[b]	Gene diversity NRY (N)[c]	Gene diversity mtDNA (N)[d]	Gene diversity STR (N)[e]
Western group	Flores	3995	0.9845	0.8571 (21)	0.9080 (40)	0.7788 (24)
	Corvo	425	0.9750	–	0.8530 (17)	0.7669 (12)
Central group	Faial	15063	0.9762	0.8143 (21)	0.9480 (31)	0.7913 (35)
	Pico	14806	0.9795	0.7333 (6)	0.9370 (23)	0.7758 (37)
	S. Jorge	9674	0.9760	0.6818 (12)	0.9170 (25)	0.7922 (34)
	Graciosa	4780	0.9288	0.3182 (12)	0.9160 (29)	0.7820 (25)
	Terceira	55833	0.9874	0.6471 (17)	0.9650 (50)	0.7972 (46)
Eastern group	S. Miguel	131609	0.9851	0.5818 (64)	0.9510 (51)	0.7813 (58)
	Sta. Maria	5578	0.9712	0.3202 (23)	0.9290 (26)	0.7773 (23)

a. INE , b. Santos et al., c. Montiel et al. and Fernando et al. , d. Santos et al. and Santos et al., e. Present study

TABLE 5
RESULTS OF THE EXACT TEST OF POPULATION DIFFERENCIATION, FOR EACH STR MARKER, BETWEEN MAINLAND NORTH PORTUGAL (MNP) AND THE AZORES ISLANDS

	MNP / Corvo	MNP / Faial	MNP / Flores	MNP / Graciosa	MNP / Pico	MNP / S. Jorge	MNP / S. Miguel	MNP / Sta. Maria	MNP / Terceira	MNP / Azores
CSF1PO	0.01985	0.18295	0.00490	0.01575	0.00480	0.48905	0.10430	0.15165	0.21150	0.09755
D3S1358	0.34910	0.80905	0.16195	0.06270	0.58350	0.79020	0.08560	0.72385	0.75030	0.43995
D5S818	0.12165	0.46930	0.50880	0.48300	0.75520	0.34315	0.52040	0.52405	0.96640	0.67125
D7S820	0.47805	0.04655	0.11590	0.64375	0.00610	0.99795	0.02845	0.57520	0.11670	0.46140
D8S1179	0.34340	0.26395	0.54100	0.38185	0.44930	0.00415	0.65380	0.63330	0.40205	0.15785
D13S317	0.09985	0.78505	0.00000	0.28460	0.89990	0.62860	0.60750	0.11370	0.79505	0.23180
D18S51	0.32800	0.64685	0.30070	0.48575	0.14660	0.53295	0.10015	0.24810	0.32080	0.43680
D21S11	0.49330	0.94835	0.15200	0.65855	0.06175	0.32040	0.42495	0.58375	0.42570	0.39015
FGA	0.91970	0.84770	0.19030	0.86825	0.10940	0.04810	0.45400	0.47595	0.42380	0.00435
TH01	0.09820	0.34330	0.85950	0.16650	0.47460	0.45165	0.22430	0.79080	0.01425	0.10670
TPOX	0.56140	0.88635	0.00375	0.12575	0.07505	0.81115	0.64025	0.49235	0.27485	0.81700
vWA	0.24095	0.75725	0.46840	0.22980	0.27470	0.48320	0.59445	0.89630	0.78460	0.77100

these last two, also reported previously by Velosa et al. to produce significant differences between the Azores and Mainland Portugal.

Conclusions

The results obtained allow to detect genetic substructuring within the Azores archipelago, reinforcing the results previously obtained using other markers. However, to obtain definitive conclusions, it would be desirable to increase the sample size by island. Moreover, the data generated by this study can be used in a future forensic genetic database after the appropriate enlacement of sample size by island, preventing, in that way, misinterpretations caused by population substructuring and small sample sizes.

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GENETIČKA STRUKTURA AZORSKOG OTOČJA: STUDIJA 15 AUTOSOMNIH KRATKIH PONAVLJAJUĆIH SLIJEDOVA (STR)

SAŽETAK

Azorski arhipelag (Portugal) smješten u Atlanskom oceanu, 1500 km od europskog kontinenta, sastavljen je od 9 otoka vulkanskog porijekla. Relativna pozicija ovih otoka definirana je u tri geografske grupe: Zapadni, Centralni i Istočni otoci. Prethodna istraživanja Azora koristeći kratke ponavljajuće slijedove (STR) rasvjetlila su razlike u frekvenciji nekoliko lokusa, u odnosu na kontinentalni Portugal i otok Madeiru. Nadalje, »linkage disequilibrium« (LD) se, kakv je opisan za azorske uzorke, pokušao objasniti kao odraz prisutstva genetičke podstrukture arhipelaga. Kako bi dobili informacije o genetičkom profilu Azorskih otoka i procjenili prisutstvo podstruktura, odredili smo frekvencije alela 15 autosomnih STR loksa, koristeći AmpFlSTR® Identifiler™ Kit, u reprezentativnom uzorku sa otočja. Uzimajući Azore kao cjelinu, ili analizirajući otoke same za sebe, kod svih lokusa nalazili smo Hardy-Weinbergovu ravnotežu. Prosječno gensko razlikovanje rangiralo je od 0,7669 na otoku Corvo do 0,7972 na otoku Terceira. Alelska neovisnost među lokusima, testirana za cijelokupni uzorak, detektirala je značajni LD (nakon korekcije višestrukim testovima) za parove D21S11/D7S820 i D3S1358/D5S818. Egzaktni test populacijske diferencijecaije, kombinirajući informacije iz 15 analiziranih markera, otkrio je značajne razlike između sve tri grupe otoka i među otocima. Međuotočna analiza učvrstila je prethodne podatke koji su pretpostavljali prisutnost podstruktura Azorskog arhipelaga. Štoviše, podaci sakupljeni ovom analizom mogu se koristiti u budućim forenzičkim genetičkim bazama podataka Azora, nakon prihvatljivog zaokruživanja veličine uzorka po otoku, sprečavajući tako krivu interpretaciju uzrokovanu populacijskim podstrukturama i malim uzorkom.