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Original scientific paper

Genetic Diversity of 15 STR Loci in a Population of Montenegro

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A B S T R A C T

Genetic diversity and forensic parameters based on 15 AmpFlSTR Identifiler short tandem repeat (STR) loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) were evaluated in a sample of 101 unrelated, autochthonous adults from Montenegro. After applying Bonferroni correction, the agreement with Hardy-Weinberg equilibrium (HWE) was confirmed for all loci with the exception of D5S818 (χ^2 test) and D21S11 (exact test). The combined power of discrimination (PD) and the combined power of exclusion (PE) for the 15 studied loci were 0.9999999999999999844 and 0.99999382, respectively. According to measures of within-population genetic diversity, D2S1338, D18S51 and FGA may be considered as the most variable and most informative markers for forensic testing and population genetic analyses out of the 15 analysed loci in a population of Montenegro. D5S818 showed to be the least variable and together with TPOX, the least informative. Interpopulation comparisons were carried out and levels of genetic differentiation between population of Montenegro and five South-eastern European populations (Kosovo Albanians, Serbians from Vojvodina province, Macedonians, Bosnians and Croatians) were evaluated. The most differentiated population in relation to Montenegro is a population of Kosovo Albanians as suggested by both AMOVA and coefficients of genetic differentiation (F_{ST} and R_{ST}).

Key words: *STRs, AmpFlSTR Identifiler, population genetics, genetic diversity, forensic genetics, Montenegro*

Introduction

Short tandem repeats (STRs) are also known as microsatellites with repeat motifs 2 to 6 base pairs long. They are highly polymorphic markers, whose importance in forensic testing[1–2], studying mutation patterns[3] and genetic microdifferentiation[4] among various populations is widely approved and well documented.

The Republic of Montenegro is the smallest country in South-eastern European region and according to 2003 census data; its population totals 620,145 people, what makes it also the smallest population size in the region.

The population of Montenegro has been influenced and shaped by some particular traditional customs through the history. This population had been divided in clans and practiced complementary endogamy. This term defines some kind of marital consensus in which men from one clan would take the bride from another particular clan and vice versa[5]. This traditional custom might contributed in some extent to genetic isolation of this population trough the history.

This population study was carried out in order to establish a forensic database for the population of the Republic of Montenegro as well as to evaluate genetic diversity based on 15 STR loci and to asses genetic differentiation between this one and other five South-eastern European populations.

We report allele frequency distribution and forensic parameters for 15 highly polymorphic STR loci included in the AmpF*l*STR Identifiler PCR amplification kit (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA). As measures of within-population genetic diversity we used number of alleles, heterozygosity as well as allele-size variance, which is a reliable measure of genetic diversity for loci conforming to a stepwise mutation model (SMM)[6], as STR loci usually do[7].

Population comparisons were carried out among population of Montenegro and five South-eastern European populations from previously reported similar studies:

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Kosovo Albanians[8], Serbians (Vojvodina province)[9], Macedonians[10], Bosnians[11] and Croatians[12]. This included locus-by-locus analysis of molecular variance (AMOVA) and estimation of genetic differentiation between populations through pair-wise coefficients of genetic differentiation based on gene diversity (F_{ST}) as well as on allele size variance (R_{ST}).

Sample and Methods

Sample

Autochthonous, unrelated (not from the same nuclear family), 101 adults from central part of the Republic of Montenegro participated in this study after giving informed consent. Whole blood samples were obtained by venipuncture, collected into EDTA tubes and stored at –20 °C. DNA was extracted from whole blood (7.5 mL) by the salting-out procedure[13].

DNA Analysis

Multiplex PCR amplification was performed in a total reaction volume of 25 μl consisting of 1 μl of diluted genomic DNA samples, 9.0 μl sterile water, 9.5 μl AmpF*l* STR Identifiler PCR reaction mix, 5.0 μl of AmpF*l*STR Identifiler primer set (Applied Biosystems, Foster City, CA) and 0.5 μL of AmpliTaq Gold DNA polymerase. Amplification was carried out in a 2700 Thermal Cycler (Applied Biosystems, Foster City, CA) performing 28 cycles under the following conditions (after an initial denaturation step of 11 min at 95 °C): 94 °C for 1 min, 59 °C for 1 min, 72 °C for 1 min and final extension 60 °C for 60 min. For electrophoresis, 1 μL of the PCR product was combined with 24 μL of formamide and 0.5 μL of GeneScan 500 LIZ size standard. Electrophoresis, detection of PCR products and genotyping were carried out on the ABI PRISM 310 Genetic Analyzer using the ABI PRISM 310 Data Collection Software and Genescan 3.7 Analysis Software (Applied Biosystems, Foster City, CA).

Data analysis

Allele frequencies, observed and expected heterozygosities, and forensic parameters were calculated using the software package PowerStats[14]. The agreement of genotype frequencies with Hardy-Weinberg equilibrium (HWE) was determined using the χ^2-test based on the number of observed and expected heterozygotes and the exact test based on the number of observed and expected genotypes[15], as implemented in a software developed at the Institute for Anthropological Research, Zagreb, Croatia. Allele size variance and rank correlations[16] were calculated trough the standard formulas. Intrapopulation fixation indices (f) were calculated using FSTat program[17]. Interpopulation comparisons were carried out using locus-by-locus AMOVA and F_{ST} and R_{ST} coefficients of genetic differentiation, as implemented in the Arlequin package version 2.000[18]. The Bonferroni correction[19] was applied while testing for Hardy-Weinberg equilibrium as well as interpopulation comparisons.

Results and Discussion

Genetic variation

The observed allele frequency distributions, summarized in Table 1, indicate that each STR locus is moderately to highly polymorphic in a population of Montenegro. This is confirmed by summary measures of within-population genetic variation, namely the number of alleles, allele size variance and heterozygosity, which are presented in Table 2, together with statistical parameters for forensic testing, based on 15 AmpF*l*STR Identifiler loci in a population of Montenegro.

The agreement with Hardy-Weinberg equilibrium, based on the exact test, was confirmed for 9 of the studied loci, while it was rejected for D21S11, D7S820, CSF1PO, D19S433, TPOX and D5S818. After applying Bonferroni correction (testing on significance level of 0.003), only departure for D21S11 remained significant. Based on the χ^2-test, only D5S818 showed departure from HWE.

The average observed (0.754) and expected heterozygosity (0.790) are comparable to other European populations[1]. However, the excess of homozygotes at some of the loci, leading to a certain deviation from HWE (six loci on the level of $p=0.05$) and causing the significant values of fixation indices at the three loci: D21S11, D13S317, D5S818, is notable. The value for D5S818 remains significant even after Bonferoni correction is applied. Fixation index across 15 loci is 0.051 ($p=0.000$). However, the fixation index is considerably different for different loci ranging from –0.031 to 0.220, and pointing to the existence of population subdivision[20] in this population from Montenegro. In accordance with population subdivision is also the finding of excess of homozygotes, which could be explained by Wahlund principle. However, this observation could also be due to sample size, having in mind highly polymorphic nature of these loci.

According to measures of within-population genetic diversity, D2S1338, D18S51 and FGA loci are the most variable, showing the highest level of observed (0.842–0.881) and expected (0.850–0.870) heterozygosity, as well as among the lowest values of fixation index, which is close to zero in D18S51 and FGA. These loci also have the highest allele size variances: 10.252 (D2S1338), 5.283 (D18S51) and 3.688 (FGA). On the other side is locus D5S818 that showed to be the least variable according to the lowest observed heterozygosity (0.545) and allele size variance (1.057), and significantly high value of fixation index ($f=0.220$; $p=0.002$).

Significant positive correlations among the three measures of variation, heterozygosity, number of alleles and allele size variance (Var & H_{exp}: $=0.486$, $p=0.012$; Var & N_a: $=0.536$, $p=0.005$ and N_a & H_{exp}: $=0.557$, $p=0.004$) were observed using rank correlations. That observation is consistent with the general SMM model with mutation as the main factor affecting the within-population variability[21].

Forensic parameters

Out of the 15 analyzed loci in the population of Montenegro and based on the heterozygosity and the poly-

morphism information content as measures of informativeness, D2S1338, D18S51 and FGA may be considered the most informative markers for population genetic analyses and forensic testing.

The least informative markers according to heterozigosity, power of exclusion (PE) and polymorphism information content (PIC) are D5S818 and TPOX. These loci have the lowest observed (0.545 and 0.624, respectively)

TABLE 1
OBSERVED ALLELE FREQUENCIES AT THE 15 STR LOCI IN A POPULATION OF MONTENEGRO

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	VWA	TPOX	D18S51	D5S818	FGA
6						0.267	0.005								
7			0.025			0.119						0.010		0.005	
8	0.015		0.158	0.005		0.158	0.109	0.025				0.564			
9	0.020		0.168	0.020		0.218	0.059	0.163				0.119		0.025	
9.3						0.213									
10	0.069		0.233	0.307		0.025	0.084	0.050				0.050	0.010	0.054	
11	0.035		0.252	0.282			0.347	0.238		0.005		0.218	0.005	0.287	
12	0.079		0.104	0.297			0.252	0.307		0.099		0.035	0.074	0.436	
12.2										0.020					
13	0.371		0.040	0.084			0.109	0.178		0.134	0.010	0.005	0.094	0.173	
13.2										0.069			0.005		
14	0.233		0.020	0.005	0.104		0.030	0.040		0.297	0.139		0.193	0.015	
14.2										0.094			0.020		
15	0.158				0.292		0.005		0.005	0.109	0.139		0.173	0.005	
15.2										0.050					
16	0.020				0.262				0.059	0.064	0.153		0.183		
16.2										0.035					
17					0.193				0.272	0.020	0.292		0.074		
17.2										0.005					
18					0.134				0.059		0.223		0.084		
19					0.015				0.084		0.030		0.020		0.114
20									0.114		0.015		0.035		0.069
21									0.035				0.020		0.173
22									0.010				0.005		0.228
23									0.149						0.124
23.2															0.005
24									0.079				0.005		0.163
25									0.104						0.074
26		0.005							0.025						0.050
27		0.010							0.005						
28		0.134													
28.2		0.025													
29		0.233													
29.2		0.015													
30		0.193													
30.2		0.025													
31		0.054													
31.2		0.129													
32		0.035													
32.2		0.089													
33		0.005													
33.2		0.025													
34		0.005													
34.2		0.020													

and expected (0.616 and 0.694, respectively) heterozygosities, the lowest PE (0.230 and 0.320, respectively) and also the lowest PIC (0.644 and 0.573, respectively).

Regardless the measures of heterozygosity, forensic parameters such as power of discrimination (PD) and PIC (with the exception of the above mentioned decreased value in TPOX and D5S818) are high for all loci. Even relatively reduced levels of genetic variation at some of the loci provide discrimination power between 79.0% and 96.3% (average of 91.5%).

TABLE 2
MEASURES OF GENETIC DIVERSITY AND STATISTICAL PARAMETERS FOR FORENSIC TESTING AT THE 15 STR LOCI IN A POPULATION OF MONTENEGRO

Locus	D8S11	D21S11	D7S82	CSF1P	D3S135	TH01	D13S3	D16S5	D2S13	D19S4	VWA	TPOX	D18	D5S81	FGA	Average (SE)
N_a	9	16	8	7	6	6	9	7	13	13	8	7	16	8	9	9.467 (0.795)
Var	2.577	2.570	2.368	1.100	1.577	1.862	2.472	2.205	10.252	1.527	2.287	1.925	5.283	1.057	3.688	2.850 (0.436)
H_{obs}	0.752	0.802	0.782	0.683	0.723	0.762	0.703	0.782	0.842	0.802	0.762	0.624	0.871	0.545	0.881	0.754 (0.224)
H_{exp}	0.770	0.859	0.815	0.730	0.780	0.796	0.781	0.786	0.858	0.850	0.802	0.616	0.870	0.694	0.850	0.790 (0.230)
χ^2	0.085	2.304	0.533	0.918	1.587	0.510	3.144	0.000	0.102	1.467	0.759	0.003	0.010	9.915	0.532	/
Exact	0.136	0.001	0.006	0.024	0.734	0.239	0.112	0.578	0.095	0.009	0.695	0.009	0.342	0.020	0.723	/
f	0.027	0.072	0.046	0.070	0.078	0.047	0.105	0.010	0.024	0.061	0.054	–0.007	0.003	0.220	–0.031	/
p	0.324	0.049	0.190	0.136	0.080	0.182	0.024	0.463	0.306	0.074	0.162	0.587	0.537	0.002	0.817	/
PIC	0.739	0.845	0.790	0.681	0.745	0.765	0.752	0.754	0.844	0.836	0.774	0.573	0.856	0.644	0.833	0.762 (0.226)
PM	0.093	0.047	0.080	0.125	0.082	0.079	0.077	0.088	0.046	0.050	0.069	0.210	0.037	0.140	0.049	0.085 (0.075)
PD	0.907	0.953	0.920	0.875	0.918	0.921	0.923	0.912	0.954	0.950	0.931	0.790	0.963	0.860	0.951	0.915 (0.247)
PE	0.514	0.603	0.566	0.403	0.464	0.531	0.433	0.566	0.678	0.603	0.531	0.320	0.737	0.230	0.757	0.529 (0.188)

N_a – number of alleles, Var – allele size variance, H_{obs} – observed heterozygosity, H_{exp} – expected heterozygosity, χ^2-test – for HWE, Exact – p-value of exact test for HWE, f – fixation index, p – p-value of fixation index, PM – probability of match, PD – power of discrimination, PE – power of exclusion, PIC – polymorphism information content

TABLE 3
LOCUS-BY-LOCUS AMOVA (P-VALUE) AND COEFFICIENTS OF GENETIC DIFFERENTIATION (F_{ST}, R_{ST}) BETWEEN POPULATION OF MONTENEGRO AND FIVE SOUTH-EASTERN EUROPEAN POPULATIONS

Locus	Montenegro vs. Kosovo	Montenegro vs. Vojvodina	Montenegro vs. Macedonia	Montenegro vs. Bosnia	Montenegro vs. Croatia
D8S1179	0.5074	0.3241	0.0860	/	/
D21S11	0.1537	0.6803	0.5430	/	/
D7S820	0.0316	0.0445	0.5341	0.8456	0.2522
CSF1PO	0.0029	0.2495	0.5385	0.4888	0.6402
D3S1358	0.0840	0.9802	0.6551	0.1378	0.0498
TH01	0.8167	0.2668	0.6695	0.8690	0.0430
D13S317	0.1112	0.7589	0.3414	0.3773	0.6744
D16S539	0.3429	0.5128	0.2283	/	/
D2S1338	0.3666	0.4649	0.2658	/	/
D19S433	0.0346	0.0667	0.0054	/	/
vWA	0.4076	0.4783	0.1606	0.9726	0.0791
TPOX	0.0040	0.5025	0.1141	0.7566	0.2179
D18S51	0.1700	0.1364	0.1537	/	/
D5S818	0.3666	0.5909	0.5909	0.3685	0.3646
FGA	0.0356	0.3706	0.1591	0.3343	0.2766
F_{ST}	0.0039	0.0004	0.0017	–0.0011	0.0023
p	0.0010	0.3336	0.0476	0.8274	0.0495
R_{ST}	0.0051	–0.0004	0.0032	–0.0030	–0.0007
p	0.0496	0.4423	0.1157	0.9279	0.4869

The combined power of discrimination (PD) and the combined power of exclusion (PE) for the 15 studied loci were 0.9999999999999999844 and 0.99999382, respectively.

Interpopulation comparisons

Locus-by-locus AMOVA interpopulation comparisons (Table 3) based on analysis of molecular variance between population of Montenegro and five South-eastern European populations revealed significant differences (at the level p=0.05) in 4 (Kosovo Albanians), 1 (Serbians, Vojvodina province) and 1 (Macedonians) out of 15 loci, as well as in 2 out of 9 loci with Croatians. However, after applying Bonferroni correction, only 1 locus (CSF1PO) between Montenegrins and Kosovo Albanians remained significantly different.

Table 3 also displays the values of the coefficients of genetic differentiation, F_{ST} and R_{ST} for the 15 STR loci in the five population pairs. The estimated F_{ST} values across all corresponding loci between population of Montenegro and five South-eastern European populations are lower than those observed across Europe (F_{ST} = 0.0028)[1] except vs. Kosovo Albanians, where F_{ST} was 0.0039 (p=0.001), nevertheless still smaller than the value of 0.01 recommended by the National Research Council (1996)[22]. However, there is considerable heterogeneity in the degree of differentiation at different loci and only F_{ST} values for loci CSF1PO, TPOX and D7S820 in comparison with Kosovo Albanians were significant (data not shown). Borderline significant values of F_{ST} were observed between Montenegro vs. Croatia (due to loci D3S1358 and TH01) and vs. Macedonia (due to locus D19S433). Since there is a positive correlation between allele size variance and heterozygosity it was appropriate to estimate genetic differentiation among populations also through R_{ST}, an analogue of F_{ST} based on allele size variance adapted to microsatellite loci by assuming an SMM model[6]. Values of R_{ST} coefficient of genetic differentiation were also lower than 0.01. Significant difference (on the borderline of statistical significance) was only observed between Montenegro and Kosovo Albanians, also due to loci CSF1PO, TPOX and D7S820 (data not shown).

In summary, based on presented allelic frequencies and statistical parameters for forensic testing for the AmpF*l*STR Identifiler detection system, the combination of these 15 STR loci represents an effective strategy for individual identification and kinship analyses in the population of Montenegro. Furthermore, these loci showed to be useful for population genetic studies of microdifferentiations between geographically close populations. Deeper insights into the population structure of Montenegro will be obtained by analysis of mitochondrial DNA and Y chromosome markers.

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GENETIČKA RAZNOLIKOST 15 STR LOKUSA U POPULACIJI CRNE GORE

S A Ž E T A K

Genetička raznolikost i forenzički parametri određeni su na uzorku od 101 odrasle osobe podrijetlom iz Crne Gore na temelju 15 AmpF*l*STR Identifiler (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) kratkih uzastopno ponavljajućih sljedova DNA (STR). Nakon primjene Bonferronijeve korekcije, slaganje s Hardy-Weinberg-ovom ravnotežom (HWE) potvrđeno je za sve lokuse osim za D5S818 (χ^2 test) i D21S11 (exact test). Zajednička snaga diskriminacije (PD) i zajednička snaga isključivanja (PE) za 15 ispitanih lokusa iznosila je 0.9999999999999999844 odnosno 0.99999382. Na temelju mjerila unutarpopulacijske raznolikosti, lokusi D2S1338, D18S51 i FGA su najvarijabilniji, te najinformativniji za forenzičke i populacijsko – genetičke analize u populaciji Crne Gore, dok se lokus D5S818 pokazao najmanje varijabilnim, a uz TPOX i najmanje informativnim. Provedene su i međupopulacijske usporedbe te je izračunata razina genetičke različitosti između populacije Crne Gore i pet populacija jugoistočne Europe (kosovski Albanci, Srbi iz Vojvodine, Makedonci, Bosanci i Hrvati). Na temelju F_{ST} i R_{ST} koeficijenata genetičke raznolikosti i rezultata AMOVE, u odnosu na populaciju Crne Gore najrazličitija je populacija kosovskih Albanaca.