RESEARCH ARTICLE **(Open Access)**

Allelic frequencies of two microsatellite loci in four populations of brown trout (*Salmo trutta*)

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Abstract.

Two microsatellite loci, **Str60Inra** and **Ssa197,** were PCR amplified on 30 individuals for each populations of brown trout (*Salmo trutta*). A total of 120 individuals were selected from rivers of the Florence province (Italy), Valbona and Cen (Albania), Lepenci (Kosovo). There were identified 32 different alleles for Str60Inra and 41 for the locus Ssa197. Mean number of alleles ranged from 9 (Cen) to 20.5 (Florence). The mean observed and expected heterosygosities values were 0.329 and 0.755, respectively. Both microsatellite loci were polymorphic. The highest value of heterozygosity was observed in Lepenci. Significant deviations from Hardy Weinberg were found in both loci.

Keywords: microsatellite, polymorphism, observed and expected heterosygozity.

Introduction

The brown trout (*Salmo trutta*) is an originally European species of salmon fish [8, 12]. The brown trout is one of the genetically most substructures vertebrate species currently known to exist [13]. There is considerable confusion regarding the taxonomy of the brown trout [12]. Maric et al [14] defined the morphological characters from stocked brown trout *Salmo trutta* in Serbia. Morphometric and meristic characters of trouts (*Salmo trutta* and *Salmo platycephalus*) were investigated in the upper basin of Seyhan, Ceyhan and Euphrates rivers [11]. Large-scale patterns of genetic diversity in brown trout have been studied extensively over the last two decades, using microsatellite [1, 3, 6, 7, 10, 16, 17] and mitochondrial (mtDNA) [2, 4, 5, 9, 18]. Microsatellites are considered as good genetic markers for estimating genetic variability between different populations of brown trout. *Salmo trutta* populations in Albania have experienced a strong decline during the past two decades because of overfishing, fragmenting habitats and the illegal fishing. This is the first study for the genetic characterization of Albanian trout populations, using microsatellite markers.

Material and methods.

A total of 120 samples of brown trout were collected from 4 locations, rivers of Florence province, provided by the University of Florence (Italy), Valbona and Cen (Albania), Lepenci (Kosovo). Material was taken from the caudal fin and preserved in the 99% ethanol. DNA was extracted using standard procedure of proteinase K digestion, chloroform extraction and isopropanol precipitation. Two microsatellites loci, Str60Inra and Ssa197, were used for the genetic characterization of four trout populations. PCR conditions comprise 1 x PCR buffer, 0.2mM dNTP, 0.2 unit Taq DNA polymerase. Thermal cycling conditions for both loci were: 3 min at 94 °C, followed by 8 cycles of 94 °C for 30 s, 60^0 for 30s, and 72 °C for 1 min, followed by 25 cycles as mentioned above, but annealing at 55°C. At the end was followed by a final extension step of 72°C for 10 min. Primer sequences for each locus are shown in table 1.

Fragment length of both microsatellites have been analyzed in Licor 4300 DNA Analyzer. PCR products were separated on 6% denaturing polyacrylamide gel electrophoresis, using a size standard 50-350, 700IRDye.

Number of alleles per locus (Na), observed (Ho) and expected (He) heterozygosity were calculated with the program GenAlEx 6.5 [15]. The same software was used also, to test departures from Hardy-Weinberg Equilibrium.

Results and discussions

Both microsatellite loci were polymorphic in four populations (Table 2). A total of 32 different alleles were found for locus Str60. Fragment length ranged from 80 to 334 bp (Figure 1, Table 1), meanwhile for the locus Ssa197, 41 different alleles were identified with fragment length ranging from 82 – 278 bp (Figure 2, Table 1).

Table 1: Data on two microsatellite loci used in this study

Locus	*Primer sequence*	*Size range (bp)*	*Number of Alleles*
Str60 Inra	5'-CGGTGTGCTTGTCAGGTTTC-3' 5'GTCAAGTCAGCAAGCCTCAC-3	80 - 334	32
Ssa197	5'-GGGTTGAGTAGGGAGGCTTG-3' 5'-TGGCAGGGATTTGACATAAC-3	80 - 278	41

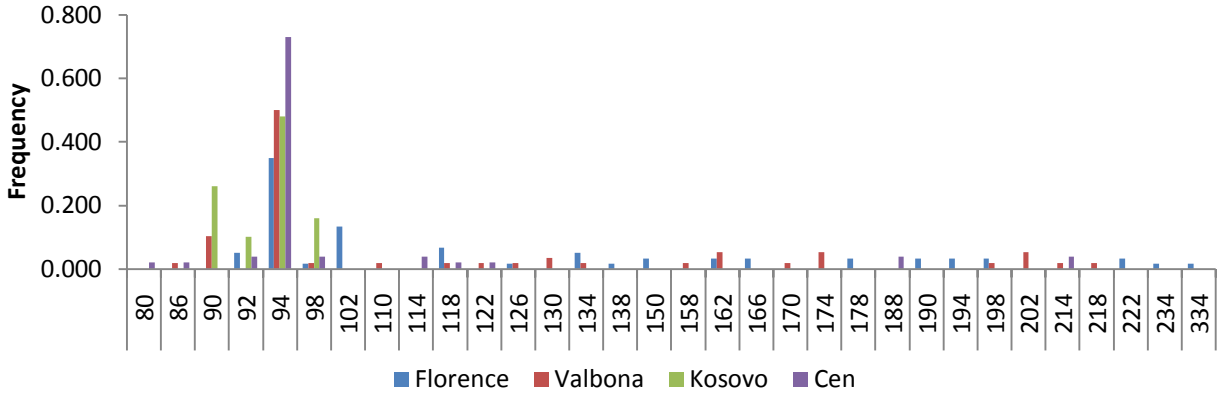

Figure 1: Distribution of allelic frequencies for all populations at Str60Inra

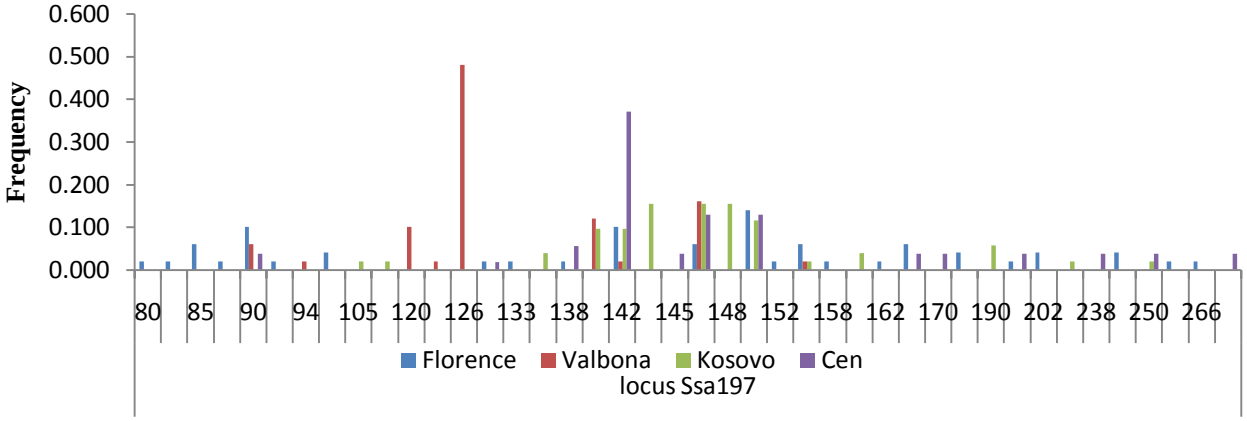

Figure 2: Distribution of allelic frequencies for all populations at Ssa197

Florence population displayed the highest number of alleles in both loci. Cen has the lowest number of alleles for Str60Inra and Lepenci for Ssa197 (Table2). Mean number of alleles for the whole population was 12.5 and 15.0 for Str60Inra and Ssa197 respectively.

Mean number of alleles ranged from 9 (Cen) to 20.5 (Florence). Observed heterozygosity (H_O) ranged from 0.2066 (Valbona) to 0.449 (Lepenci) with a mean value of 0.334, while the expected heterozygosity (H_E) ranged from 0.683 to 0.886, with a mean value of 0.771 (Table 3). Similar values of observed and expected heterozygosities are found for two of Caspian trout, using 5 microsatellite markers [17]. In 27 turkish trout population, analysed by 5 microsatellite markers, the average number of alleles was 7.4 and average observed heterozygosity 0.254 [3]. All the populations displayed a high value of inbreeding (F_{IS}) ranging from 0.391 (Lepenci) to 0.707 (Valbona). As F_{IS} parameter has a high positive value (0.579), it indicates inbreeding and intermixing [19].

Table 2: Genetic variability for both microsatellite loci in 4 trout populations

Locus		*Florence*	***Lepenci***	***Cen***	***Valbone***	*Average across all population*
Str60Inra	**N**	30	29	25	26	27.500
	Na	18	18	4	10	12.500
	Ne	6.250	3.657	2.998	1.842	3.687
	Ho	0.133	0.379	0.160	0.154	0.207
	He	0.840	0.727	0.666	0.457	0.673
	F	0.841	0.478	0.760	0.663	0.686
Ssa197	**N**	25	25	26	27	25.750
	Na	24	9	14	13	15.000
	Ho	0.640	0.520	0.385	0.259	0.451
	He	0.934	0.714	0.889	0.815	0.838
	F	0.315	0.272	0.567	0.682	0.459

Table 3: Genetic variation in four populations across both

Population	H_E	H_O	*MNA*	F_{IS}
Florence	0.886	0.387	20.5	0.576
Lepenci	0.721	0.449	13.5	0.391
Cen	0.792	0.292	9.0	0.642
Valbona	0.683	0.206	11.5	0.707
Mean	0.771	0.334	13.625	0.579

MNA = Mean number of alleles, H_O = mean observed heterozygosity, H_E = mean expected heterozygosity, F_{IS} = inbreeding coefficient

Test of Hardy-Weinberg equilibrium (HWE) shows that the 4 populations are not in equilibrium states for each of the loci. This can be explained maybe by the presence of null alleles in all populations, that might mislead to a deficiency of heterozygosity.

The data provided here are only preliminary, since the number of loci is quite low. This is the first genetic study to deal with the trout of Albanian and Kosova based on microsatellite markers. This study demonstrate that microsatellite are powerful markers for monitoring the genetic condition of trout populations.

References:

1. Apostolidis A, Karakousis Y, Triantaphyllidis C: **Genetic divergence and phylogenetic relationships among Salmo trutta L.(brown trout) populations from Greece and other European countries**. *Heredity* 1996, **76**(6), 551 - 560.
2. Apostolidis AP, Triantaphyllidis C, Kouvatsi A, Economidis PS: **Mitochondrial DNA sequence variation and phylogeography among Salmo trutta L.(Greek brown trout) populations**. *Molecular Ecology* 1997, **6**(6): 531-542.
3. Arslan S, Bardakci F: **Genetic Structure of Brown Trout (Salmo trutta) Populations from Turkey Based on Microsatellite Data**. *Biochemical genetics* 2010, **48**(11-12): 995-1014.
4. Bernatchez L, Guyomard R, Bonhomme F: **DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout Saltno trutta populations**. *Molecular Ecology* 1992, **1**(3): 161-173.
5. Bernatchez L: **The evolutionary history of brown trout (Salmo trutta L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation**. *Evolution* 2001, **55**(2): 351-379.
6. Borroni I, Trasforini S, Bardazzi M, Gentili G: **Taratterizzazione fenotipica e genetica della popolazione di trota del Rio Baracca (Bacino dell'Orba)**. 1999.
7. Garcia-Marin JL, Pla C: **Origins and relationships of native populations of Salmo trutta (brown trout) in Spain**. *Heredity* 1996, **77**(3), 313 - 323.
8. Gibertoni PP, Pensierini M: : **Trote native degli Appennini.**: Università di Parma. Italia; .
9. Giuffra E, Bernatchez L, Guyomard R: **Mitochondrial control region and protein**

coding genes sequence variation among phenotypic forms of brown trout Salmo trutta from northern Italy. *Molecular Ecology* 1994, **3**(2): 161-171.

10. Jug T, Berrebi P, Snoj A: **Distribution of non-native trout in Slovenia and their introgression with native trout populations as observed through microsatellite DNA analysis**. *Biological Conservation* 2005, **123**(3): 381-388.

11. Kara C, Alp A, Gurlek ME: **Morphological variations of the trouts(Salmo trutta and Salmo platycephalus) in the rivers of Ceyhan, Seyhan and Euphrates, Turkey**. *Turkish Journal of Fisheries and Aquatic Sciences* 2011, **11**(1): 79-87.

12. Ladiges W, Vogt D: **Guida dei pesci d'acqua dolce d'Europa**, II, edn: Muzzio, ed, Padova; 1979.

13. Laikre L: **Conservation genetic management of brown trout (Salmo trutta) in Europe**: Sl; 1999.

14. Maric S, Nikolic V and Simonovic P: **Pilot study on the morphological identity of wild brown trout (Salmo trutta) stocks in the streams of the Danube river basin (Serbia)**. *Folia Zoologica* 2004, **53**(4): 411-416.

15. Peakall R, Smouse PE: **GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update**. *Bioinformatics* 2012, **28**(19): 2537-2539.

16. Santos NP, Fontainhas-Fernandes AA, Faria R, Torres-Castro LF, Anjos MR, Cortes RM, Alexandrino P: **Genetic evidence for limited introgression between wild and stocked individuals in Portuguese brown trout, Salmo trutta populations**. *Folia Zoologica-Praha-* 2006, **55**(4): 433.

17. Shirangi SA, Kalbassi MR, Dorafshan S: **Microsatellite Polymorphism Reveals Low Genetic Differentiation between Fall and Spring Migratory Forms of Endangered Caspian Trout, Salmo trutta caspius (Kessler, 1870)**. *Caspian J Env Sci* 2011, **9**(1): 9-16.

18. Weiss S, Antunes A, Schlotterer C, Alexandrino P: **Mitochondrial haplotype diversity among Portuguese brown trout Salmo trutta L. populations: relevance to the post-Pleistocene recolonization of northern Europe**. *Molecular Ecology* 2000, **9**(6): 691-698.

19. Wright S: **The genetical structure of populations**. *Annals of eugenics* 1949, **15**(1): 323-354.