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Determination of Genetic Variations of Five Different Indigenous Trout Species in Turkey (*Salmo trutta* sp. L.) by Microsatellite Markers

Saltuk Buğrahan Ceyhun[1,*], Abdulkadir Çiltaş[2]

[1] Atatürk University, Fisheries Faculty, Aquaculture Department, 25240, Erzurum, Turkey.
[2] Atatürk University, Agriculture Faculty, Agricultural Biotechnology Department, 25240, Erzurum, Turkey.

* Corresponding Author: Tel.: +90.442 5112815; Fax: +90.442 5112896;
E-mail: saltukceyhun@hotmail.com

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Abstract

In this study, the genotypic differences of five different trout populations were compared using microsatellite markers. Fish analyzed were; Balıklı Lake (BLK) and Abant Lake (ABN) found in lakes, and Fırat Basin (FRT), Aras Basin (ARS), Çoruh Basin (CRH) found in rivers. Allele numbers in each locus were determined by application of Single Strand Conformation Polymorphism (SSCP) to the microsatellite sections. Totally 62 alleles were observed from 10 microsatellite loci. According to the results from factorial correspondence analysis, it was determined that all of the populations formed different groups from each other. Considering all loci, observed heterozygosity (H_o) and expected heterozygosity (H_e), values changed from 0.602-0.668 and 0.570-0.670, respectively. Ho value was significantly ($P<0.01$) higher than He value of populations of FRT. Fixation index for subpopulations (F_{IS}) values were ranged from 0.069 to -0.173 (BLK to FRT) in populations, and the overall average diversity between populations (F_{ST}) value of the all loci was calculated as 0.0790. According to FST values calculated among populations, genetic distance was found to be significant ($P<0.05$) among four populations. It was determined that four of five populations were at the Hardy-Weinberg equilibrium (HWE). Considering genetic distance in populations, it was observed that the distance was shorter in lake forms compared to River forms.

Keywords: *Salmo trutta*, microsatellite, SSCP, genetic variation.

Türkiye'deki Beş Farklı Yerli Alabalık Türünün Genetik Varyasyonunun Mikrosatelit Markırlar ile Belirlenmesi

Özet

Bu çalışmada beş farklı alabalık poulasyonunun genotipik farklılığı mikrosatelit markırlar kullanılarak karşılaştırıldı. Analiz edilen balıklardan; Balıklı Göl (BLK) ve Abant Gölü (ABN) gölde, ve Fırat Havzası (FRT), Aras Havzası (ARS) ve Çoruh Havzası (CRH) nehirde yaşamaktadırlar. Her bir lokustaki allel sayısı Single Strand Conformation Polymorphism (SSCP) yöntemi vasıtasıyla belirlendi. 10 mikrosatelit bölgede toplam 62 allel gözlendi. Faktöriyel uyum analizi sonuçlarına göre, populasyonların tamamının birbirlerinden farlı gruplar oluşturduğu saptanmıştır. Tüm lokuslar ele alındığında gözlenen (H_o) ve beklenen (H_e) heterozigotluk değerleri sırasıyla 0,602-0,668 ve 0,570-0,670 arasında değişmiştir. FRT populasyonunda H_o değeri *He* değerinden önemli derecede yüksek çıkmıştır ($P<0,01$). popülasyonlar arası fiksasyon indeksi (F_{IS}) değeri 0,069 (BLK) -0,173 (FRT) arasında ve tüm lokuslardaki popülasyonlar arası genel ortalama çeşitlilik (F_{ST}) 0,0790 olarak hesaplanmıştır. Popülasyonlar arası hesaplanan genel ortalama çeşitlilik (F_{ST}) değerine göre dört popülasyon arası genetik mesafe önemli olarak bulunmuştur ($P<0,05$). Beş popülasyondan dördünün Hardy-Weinberg dengesinde (HWE) olduğu belirlenmiştir. Popülasyonlardaki genetik mesafe değerlendirildiğinde mesafenin nehir formlarına göl formlarında daha dar olduğu gözlenmiştir.

Anahtar Kelimeler: *Salmo trutta*, mikrosatelit, SSCP, genetik varyasyon.

Introduction

Many populations and species of plants and animals are in danger of losing significant components of their genetic variation as a result of lowered population sizes and habitat fragmentation leading to restricted gene flow (Hedrick and Miller, 1992). This general problem is also compromising the gene pools of Salmonid fish species.

Brown trout *Salmo trutta* L., have been found in genetically subdivided populations over different geographical areas (Ryman, 1983; Ferguson, 1989;

Apostolidis *et al.*, 1997; Carlsson *et al.*, 1999). On a large geographic scale (between river or lake basins), it was found that the genetic differentiation may be a result of repeated post-glacial colonization (Carlsson *et al.*, 1999). On a smaller scale (within river or lake basins), geological features such as impassable waterfalls may lead to isolated populations that differentiate by genetic drift (Hindar *et al.*, 1991).

The genomes of animals and plants contain regions that consist of a series of repeated units of DNA–Variable Number Tandem Repeats (VNTR). One type of VNTR microsatellites consist of dinucleotide (e.g. CACACACA), trinucleotide (e.g. GTAGTAGTAGTA) or tetranucleotide (e.g. TAGCTAGCTAGCTAGC) repeats (Beaumont and Hoare, 2003). The number of repeated units contained within a particular microsatellite locus can vary within a population, and this produces variation in the length of the locus (Beaumont and Hoare, 2003). Microsatellite DNA markers have been proved to be useful for assessing the stocked or indigenous status of salmonid fish populations (Nielsen *et al.*, 1997; Poteaux *et al.*, 1999; Hansen *et al.*, 2000).

An individual primer pair amplify only microsatellite locus because the primers are specific. Any variants of microsatellite size have been revealed by gel electrophoresis of the polymerase chain reaction (PCR) products from different individuals. Thus, all size variations of the microsatellite repeat have been detectable. A high proportion of such PCR analyses reveal several marker "alleles", or different-sized strings of repeats (Griffiths *et al.*, 2000).

Common measures of genetic diversity are heterozygosity (the proportion of heterozygous individuals in the population), allelic diversity (number of alleles at a locus in the population), and the proportion of polymorphic loci (Pujolar *et al.*, 2005; Chistiakov *et al.*, 2006).

The main purpose of this study was to analyze genetic variation among five indigenous trout strains using microsatellite markers. To execute this, the genetic differentiations among the strains and amount of genetic variation within strains were quantified. The results were compared to literature data, and found to be in correspondence.

Materials and Methods

Research Regions and Populations

Samples were collected from five different trout populations, two of which are found in lakes and three in rivers. The rivers are the main sources that feed basins where they are located whereas lakes are under national park protection and there is no human activity. All of these brown trout populations are genetically healthy and sustaining, and random mating populations. A description of the five populations used can be found in Table 1 and Figure 1. For identification of the species, population was analyzed to use it as an external control (Geldiay and Balık, 2002).

Table 1. Description of five populations of brown trout studied

Station	Population	Basin	Location	Coordinate	Species
1	BLK	Aras	Balıklı Lake	39° 45′ N 43° 33′ E	*S. t. labrax* natio *lacustris*
2	ABN	West Black Sea	Abant Lake	40° 36′ N 31° 16′ E	*S. t. abanticus*
3	FRT	Karasu/Fırat	Yeşildere River	40° 07′ N 41° 25′ E	*S. t. macrostigma*
4	ARS	Aras	Hamzanlar River	39° 27′ N 41° 15′ E	*S. t. caspius*
5	CRH	Çoruh	Yağlı River	40° 21′ N 41° 04′ E	*S. t. labrax*

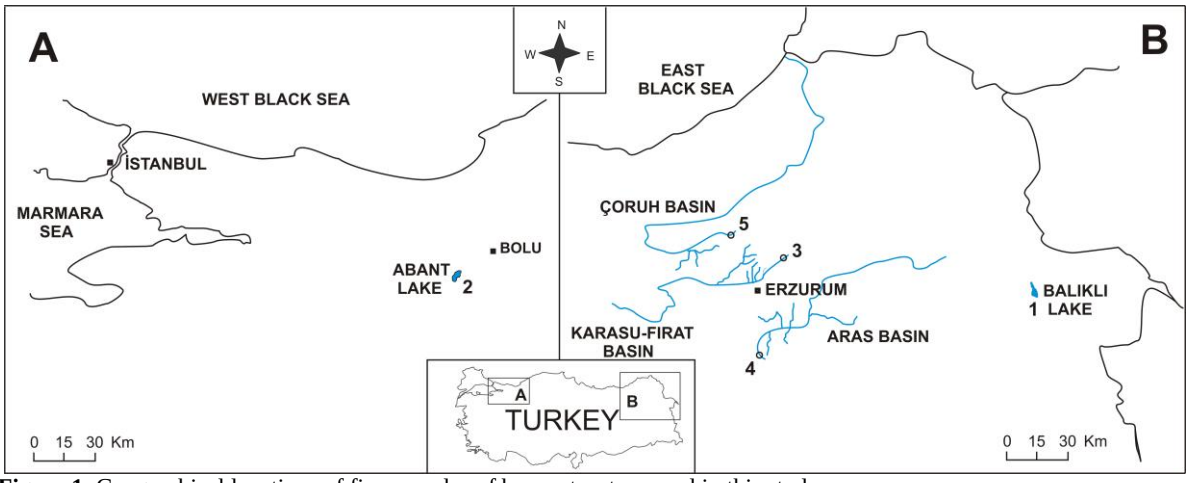

Figure 1. Geographical locations of five samples of brown trout assayed in this study.

Table 2. Description of five populations of brown trout studied

Details of the microsatellites					PCR Conditions						
Microsat. locus	Forward Reverse	Repeat motif	Annealing Temp. (°C)	Allele size range (bp)	$MgCl_2$ (mM)	Denaturation 94 °C	Annealing (Tm)	Extension 72 °C	Final extension 72 °C	Cycles	References
BS131	CACATCATGTTACTGCTCC CAGCCTAATTCTGAATGAG	$(TG)_{6}\ldots(TG)_{18}$	50	160–186	1,5	1 min	45 s	1 min	5 min	30	Estoup *et al.* (1998)
543AE	CTTTCTCTTGCGATAGTACGG GTTTCTACAGTCAGCACAAG TC	$(CT)_{13}$	52	147–175	1,2	1 min	45 s	1 min	5 min	30	Estoup *et al.* (1998)
T3-13	CCAGTTAGGGTTCATTGTCC CGTTACACCTCTCAACAGAT G	$(TG)_{10}CG(TG)_{40}$	54	180–256	1,0	1 min	45 s	1 min	5 min	27	Estoup *et al.* (1998)
85	GGAAGGAAGGGAGAAAGGT GGAAAATCAATACTAACAA	$(CT)_{23}$	54	160–180	1,5	1 min	45 s	1 min	5 min	28	Estoup *et al.* (1998)
43AEU + 43AEL	GTTGTGGGCTGAGTAATTGG CTCCACATGCATCTTACTAAC C	$(TG)_{5}AG(TG)_{4}TA(TG)_{3}AG$ $(TG)_{7}TA(TG)_{7}AG(GT)_{4}$	56	156–166	1,2	1 min	45 s	1 min	5 min	28	Estoup *et al.* (1998)
FGT1	AGATTTACCCAGCCAGGTAG CATAGTCTGAACAGGGACAG	$(GT)_{23}GC(GT)_{5}GC(GT)_{2}$ $GCGTGC(GT)_{2}GC(GT)_{4}$	60	190–246	0,8	1 min	45 s	1 min	5 min	27	Estoup *et al.* (1998)
MST-73	CTATTCTGCTTGTAACTAG CCTA CCTGGAGATCCTCCAGCAG GA	$(GT)_{13}TTATCT(GT)_{3}$	58	148–166	1,5	30 s	30 s	30 s	10 min	30	Poteaux *et al.* (1999)
Strutta-12	AATCTCAAATCGATCAGA AG AGCTATTTCAGACATCACC	$(GT)_{43}$	55	151–231	1,5	45 s	45 s	45 s	10 min	30	Poteaux *et al.* (1999)
Strutta-58	AACAATGACTTTCTCTGAC AAGGACTTGAAGGACGAC	$(GT)_{40}$	55	135–205	1,5	45 s	45 s	45 s	10 min	30	Poteaux *et al.* (1999)
OMM-1338	TTCCCCTCAAACTGATGCA TA TTTAGAATCCTCCGGTTC	ATG	58	230–260	2,5	30 s	30 s	30 s	10 min	36	Palti *et al.* (2002)

Microsatellite Analysis

Fish were captured by cast nets and killed with a sharp blow on the head. Dorsal muscle that obtained from the point between linea lateral and dorsal fin was cut out, and frozen under liquid nitrogen and than transferred to laboratory for extraction. DNA was extracted from the muscle tissue using the phenol/chloroform method according to Asahida *et al.*, (1996) with minor modifications (Aksakal and Erdoğan, 2007). The quality and concentration of DNA from both sources were assessed by 1% agarose gel electrophoresis, and then samples were stored at -20 ºC until use.

Totally 60 specimens from five populations (each population contains 12 individuals) were screened for genetic variation at ten microsatellite loci. PCR reactions (20 µl) contained approximately 50 ng of total DNA, 1 unit of Taq polymerase, 20 pmol of each primer, 200 µM of each dNTP and 10x buffer, 5% w/v dimethyl sulphoxide (Kitade *et al.*, 2003) and $MgCl_2$ (see Table 2 for the concentration of $MgCl_2$). All chemicals used in analyses were provided by Sigma. Primer sequences, annealing temperatures, $MgCl_2$ concentration, PCR conditions and references for all microsatellite loci are shown in Table 2.

For each amplification fragment, 5 µl of PCR product was diluted with 5 µl of the loading buffer (95% formamide, 100 mM NaOH, 0.05% bromophenol blue, 0.05% xylene cyanol). After heating at 95ºC for 10 min, the mixture was immediately placed in ice for denaturation and then loaded on a 6% polyacrylamide SSCP gel (acr : bis, 29 : 1) in 1x TBE buffer for 14 h at 100 V. After etidium bromide staining, the GeneSnap version 6.05 software was used to determine the size of alleles.

The number of alleles at each locus (A), factorial correspondence analysis (FCA), Hardy-Weinberg departure, observed (H_o) and expected (H_e) heterozygosities were estimated for each population using Genetix 4.02 software (Belkhir, 2001). One thousand permutations were generated to assess significance of the value according to Excoffier *et al.* (1992). Observed and expected heterozygosity were calculated, and F_{IS} values were obtained. Genetic differentiation among the strains was estimated using F_{ST} values according to Weir and Cockerham (1984) by means of the same program. Neighbor-Joining dendogram produced by Population software 1.2.28, showing the relationship between five populations by microsatellite data set. Bootstrap value was obtained from 100 replicates. Neighbor-Joining dendrogram was drawn by TreeView software (Page, 1996).

Results and Discussion

Biodiversity includes genetic variation within species, the variety of species in an area, and the variety of habitat types within a landscape. The assessment of biodiversity in a given ecosystem depends on making detailed inventories of species and varieties. Thus, the current study aims in determination of the genotypic variation of five different native trout populations using ten microsatellite loci. Microsatellites have been increasingly used as molecular markers, because their polymorphisms have shown high efficiency for many studies (King *et al.*, 2012; Meier *et al.*, 2011). In present study, all selected microsatellites were polymorphic, and sixty-two different alleles were observed among all populations. The mean number of alleles per locus within populations ranged from 2.0 to 9.0 (Table 3). The lowest allele number within populations was 2, and the highest was 11 (Table 3). In general, there was significant correlation between genetic diversity and the number of alleles. Therefore the number of alleles can be used for the evaluation of genetic diversity (Huang *et al.*, 2002). Average observed heterozygosity (H_o) and expected heterozygosity (H_e) within populations varied from 0.570 to 0.670 and from 0.602 to 0.668 respectively as shown in Table 3. We observed that $H_e > H_o$ for BLK population which spent their whole life in Ağrı Balıklı Lake. However, although the situation was different in ABN population, endemic form in Abant Lake, the values (0.640 and 0.662) were not far from each other (Table 3). The highest difference between H_e (0.570) and H_o (0.656) was found in FRT population which has the greatest genetic variety inter population (Figure 2 and Table 3).

DeWoody and Avise (2000) reported that overall expected heterozygosity calculated from microsatellites falls within the average found for 48 species of no piscine animals (0.58±0.25). They suggested that this level of diversity appears to be the result of high levels of population structure, however, retention of diversity from a larger historic population size cannot be discounted.

A decrease in levels of genetic variability often leads to an increase in homozygosity and inbreeding in the successive selected generations as fewer and genetically less diverse individuals contribute to the next breeding generation (Romano-Eguia *et al.*, 2004). Low heterozygosity then indicates inbreeding and fitness may be lost through increased expression of deleterious recessive alleles (Hoffman *et al.*, 2006).

In the current study, FCA was used which allows observation of the genetic distance of inter-population and intra-population on a three dimensional graph. According to the graph, short distance between the individuals belonging to the same population indicates low inter-population genetic distance. It was clear from our data that genetic distance was closer in lake populations (BLK and ABN), rather than brook populations (FRT, ARS and CRH). Similarly, Beacham *et al.* (2004) observed that river-type sockeye salmons were more genetically diverse than lake-type sockeye salmon.

As shown in figure 2, BLK and FRT populations are the farthest populations. But BLK is the closest to

Table 3. Number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e) and F_{IS} by Population (GENETIX 4.02) for 10 microsatellites for five populations of *Salmo trutta* L

Microsatellite Locus		Populations					
		BLK	ABN	FRT	ARS	CRH	Main
BS131	He	0.700	0.600	0.600	0.600	0.700	0.640
	Ho	0.545	0.480	0.650	0.480	0.695	0.570
	A	3	2	4	2	5	3.2
	F_{IS}	0.221	0.2	-0.083	0.2	0.007	
543AE	He	0.700	0.700	0.700	0.600	0.700	0.680
	Ho	0.495	0.565	0.635	0.480	0.565	0.548
	A	2	3	3	2	3	2.6
	F_{IS}	0.293	0.193	0.093	0.200	0.193	
T3-13	He	0.600	0.700	0.400	0.700	0.700	0.620
	Ho	0.700	0.815	0.640	0.685	0.815	0.731
	A	6	6	4	5	7	5.6
	F_{IS}	-0.167	-0.164	-0.600	0.021	-0.164	
85	He	0.400	0.500	0.500	0.500	0.400	0.460
	Ho	0.480	0.620	0.485	0.495	0.480	0.512
	A	2	3	3	2	2	2.4
	F_{IS}	-0.200	-0.240	0.030	0.010	-0.200	
43AEU+43AEL	He	0.700	0.600	0.700	0.600	0.500	0.620
	Ho	0.495	0.605	0.595	0.420	0.495	0.522
	A	2	3	3	2	2	2.4
	F_{IS}	0.293	-0.008	0.150	0.300	0.010	
FGT1	He	0.800	0.600	0.600	0.500	0.700	0.640
	Ho	0.810	0.840	0.795	0.790	0.795	0.806
	A	6	8	6	6	6	6.4
	F_{IS}	-0.013	-0.400	-0.325	-0.580	-0.136	
MST-73	He	0.700	0.600	0.500	0.500	0.600	0.580
	Ho	0.495	0.480	0.495	0.495	0.480	0.489
	A	2	2	2	2	2	2
	F_{IS}	0.293	0.200	0.010	0.010	0.200	
Strutta-12	He	0.800	0.800	0.600	0.800	0.800	0.760
	Ho	0.825	0.880	0.795	0.875	0.880	0.851
	A	8	10	6	10	11	9
	F_{IS}	-0.031	-0.100	-0.325	-0.094	-0.100	
Strutta-58	He	0.700	0.800	0.600	0.600	0.666	0.673
	Ho	0.745	0.830	0.745	0.615	0.845	0.756
	A	7	7	5	3	9	6.4
	F_{IS}	-0.064	-0.037	-0.242	-0.025	-0.269	
OMM-1338	He	0.600	0.500	0.500	0.600	0.600	0.560
	Ho	0.560	0.505	0.720	0.685	0.625	0.619
	A	3	3	4	4	3	3.4
	F_{IS}	0.067	-0.010	-0.440	-0.142	-0.042	
Main	He	0.670	0.640	0.570	0.600	0.637	
	Ho	0.615	0.662	0.656	0.602	0.668	
	A	4.1	4.7	4.0	3.8	5.0	
	F_{IS}	0.069	-0.037	-0.173	-0.010	-0.050	
	P (He;Ho)	P<0.05	ns	P<0.01	ns	P<0.05	

CRH, because, BLK population (*S. t. labrax natio lacustris*) is lake ecotype of CRH (*S. t. labrax*). When river populations are compared to each other, CRH and FRT are the closest populations. Similarly, ARS population is closer to FRT than CRH. Also, according to the expected and observed heterozygoty values, it is clearly seen from the graph that the inter-population genetic distance is the farthest in FRT population. Samuiloviene *et al.* (2009) reported that significant population differentiation was found both between river systems and between population pairs within the same river system in sea trout (*Salmo trutta*) populations in Lithuanian rivers. They suggested that even geographically adjacent tributaries exhibited significant genetic differences.

According to our results, a phylogenic analysis was plotted and shown in Figure 3. The phylogenic analysis was in correspondence with FCA results. BLK and CRH populations belong to the same linkage group at the ratio of 55%, because the BLK population is a form of the lake ecotype of CRH population. FRT and ARS also belong to the same linkage group at the ratio of 63%. The other population, ABN, belongs to different linkage group from the others, because the population is endemic to Abant Lake.

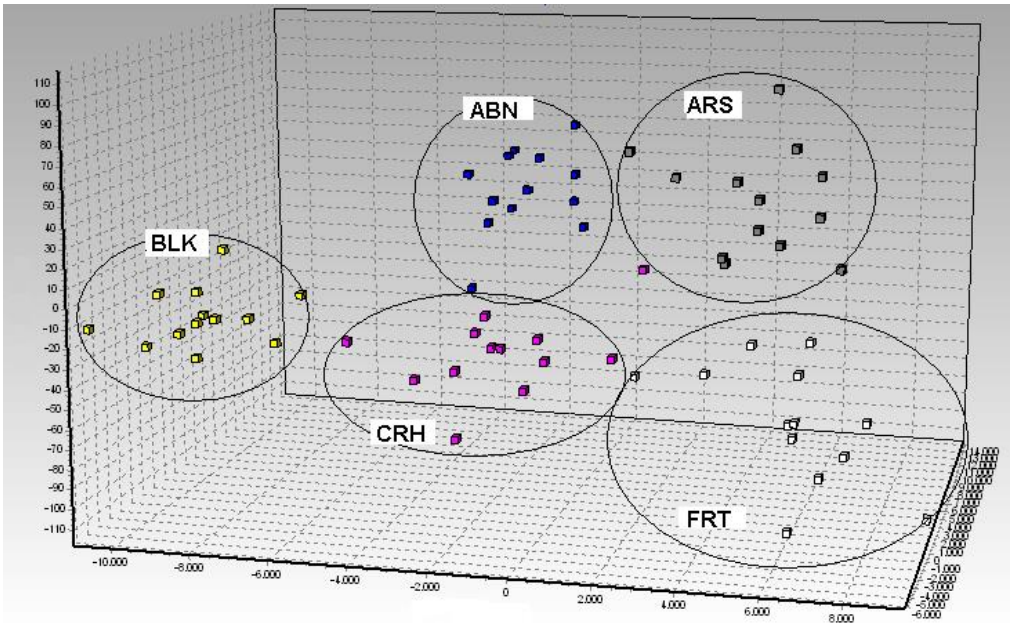

Figure 2. Factorial Correspondence Analysis (FCA) for populations.

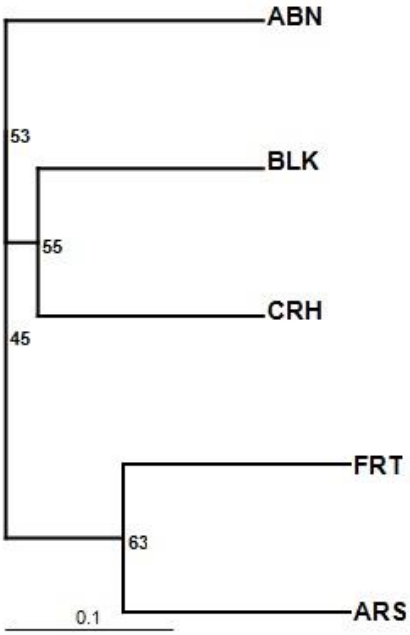

Figure 3. Neighbor-Joining dendogram produced by population software 1.2.28, showing the relationship between 5 populations by microsatellite data set. Bootstrap value obtained from 100 replicates. Tree generated by TREEVIEW Software (Page, 1996).

When the study evaluated in terms of using microsatellite loci, less variation was detected in MST-73 which has two alleles, and the highest variation was detected in Strutta-12 which has eleven alleles. It was also pointed out that the highest average allele number was 9.0 in Strutta-12 locus, and the lowest average allele number was 2.0 in MST-73 locus.

Poteaux *et al.* (1999) found that average allele numbers of Strutta-12 and Strutta-58 loci ranged from 11 to 16 and 12 to 18 respectively and MST-73 locus was 4 in Mediterranean brown trout. In our study, it was found that the average allele number was 6.4 in Strutta-58 locus. In another study, Estoup *et al.* (1993) found seven alleles for MST-73 locus in brown trout (*Salmo trutta* L.). OMM-1338 locus was characterized for rainbow trout (*Oncorhynchus mykiss*) and the number of alleles was 4 in different trout species (Palti *et al.* 2002). We also confirmed that the average allele number in the same locus was 3.4. The allele numbers of BS131, 543AE, T3-13, 85, 43AEU+43AEL and FGT1 were compared to those of

Table 4. Genetic differentiation (F_{ST}) between pairs of populations calculated by Populations software

	ABN	FRT	ARS	CRH
BLK	0.0926 *	0.1201**	0.1447**	0.0731 *
ABN		0.0645 *	0.0837 ^{ns}	0.0342 ^{ns}
FRT			0.0700 ^{ns}	0.0509 ^{ns}
ARS				0.0510 *

Estoup *et al.* (1998) in French brown trout (*Salmo trutta* L.). Our allele numbers lay between Estoup's allele numbers limitation.

Seventeen of observed sixty-two alleles were unique. These unique alleles were; two of them belong to the BLK population, one to ABN, four to FRT, three to ARS and seven to CRH. The highest number of alleles was found to be 11 in Strutta-12 locus in CRH population concerning locus and population together (Table 3).

The lowest F_{IS} (-0.173) and the highest F_{IS} (0.069) were calculated in FRT and BLK populations, respectively. It can be expressed that whole populations were in Hardy-Weinberg equilibrate except for FRT. It can be mentioned that heterozygoting was higher in BLK population which has the higest F_{IS} value, opposite to other populations. While the closest population to HW equilibrate was ARS, the farthest was FRT. Ward *et al.* (2003) reported that investigated six of forty tests for HW expectations gave significant deviations which cause(s) are unknown in rainbow trout (*Oncorhynchus mykiss*) introduced to Western Australia. Three of these gave heterozygote excesses; all were from the one population. They suggested that this naturally recruiting population is maintained by small numbers of spawners, giving some HW deviations in descendants. This naturally recruiting situation is maybe the same for our FRT population.

F_{ST} values, which represent genetic distance among populations, changes between 0.0731 and 0.1447. According to these data, the closest populations are BLK and CRH (Table 4). In Carlsson *et al.* (1999)'s study, five microsatellite loci revealed a pronounced genetic structure in research area, and global multilocus F_{ST} was estimated at 0.031 (0.011 to 0.071) in brown trout. In another study, Corujo *et al.* (2004) estimated F_{ST} values as 0.066 and 0.0423 in Deuro and Sil basin, respectively in brow trout.

Conclusion

In order to prevent depletion of biodiversity due to man-made efforts or otherwise, it is necessary to understand how the diversity of life particularly at the genetic level is maintained under natural conditions (Narain, 2000). It is equally necessary to know how the terminal extinction of species takes place under natural conditions. The protection of biodiversity is expected to be both crucial and continuing, with conservation genetics being of primary importance for avoiding the extinction of most endangered species alongside the ecological, political and economic aspects of biodiversity protection. In addition to there are a lot of studies related to biochemistry, genetic and molecular biology (Ekinci *et al.*, 2011a; 2011b; Ceyhun *et al*,. 2011a; Ceyhun *et al.*, 2011b; Erdogan *et al.*, 2011; Aksakal *et al.*, 2011) the application of molecular techniques, including genome approaches, to conservation genetics has made possible the examination of the genetics of species in danger of extinction and genetic analysis has become widely used in conservation research (Oliveira *et al.*, 2006).

Our results not only show that there was a genetic variation according to their living habitats and geographic region, but also how genetic variation changed, intra and inter relative variation among population using 10 microsatellites loci.

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References

Aksakal, E. and Erdoğan, O. 2007. Use of PCR-RFLP Analysis of mtDNA cytochrome-b gene to determine genetic differences in *Capoeta* spp. The Israeli Journal of Aquaculture–Bamidgeh, 59(4): 206-211. doi: 10.11/j.1365-2007.a01-176

Aksakal, E., Ekinci, D., Erdoğan, O., Beydemir, Ş., Alım, Z. and Ceyhun, S.B. 2011. Increasing stocking density causes inhibition of metabolic-antioxidant enzymes and elevates mRNA levels of heat shock protein 70 in rainbow trout. Livestock Science, 141: 69-75. doi: 10.1016/j.livsci.2011.07.006

Apostolidis, A.P., Trianthaphyllidis, C., Kouvatsi, A. and Economidis, P.S. 1997. Mitochondrial DNA sequence variation and phylogeography among *Salmo trutta* L. (Greek brown trout) populations. Molecular Ecology, 6: 531–542. doi: 10.1046/j.1365-294X.1997.d01-176

Asahida, T., Kobayashi, T., Saitoh, K. and Nakayama, I. 1996. Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. Fisheries Science, 62: 727-730. doi: 10.2108/zsj.19.601

Beacham, T.D., Mcintosh, B., and Macconnachie, C. 2004. Population structure of lake-type and river-type sockeye salmon in transboundary rivers of northern British Columbia. J. Fish Biol., 65: 389–402. doi: 10.1111/j.0022-1112.2004.00457

Beaumont, A.R. and Hoare, K. 2003. Biotechnology and Genetics in Fisheries and Aquaculture, Blackwell, London: 35-38.

Belkhir, K. 2001. GENETIX, logiciel sous WindowsTM

pour la génétique des populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier, France. pp.

Carlsson, J., Olsén, K.H., Nilsson, J., Overli, O. and Stabell, O. B. 1999. Microsatellites reveal fine-scale genetic structure in stream-living brown trout. Journal of Fish Biology, 55(6): 1290-1303. doi: 10.1111/j.1095-8649.1999.tb02076.x

Ceyhun, S.B., Şentürk, M., Yerlikaya, E., Erdoğan, O., Küfrevioğlu, Ö.I. and Ekinci, D. 2011a. Purification andcharacterization of carbonic anhydrase from the teleost fish *Dicentrarchus labrax* (European Sea bass) liver and toxicological effects of metals on enzyme activity. Environmental Toxicology and Pharmacology, 32: 69–74. doi: 10.1016/j.etap.2011.03.013.

Ceyhun, S.B., Aksakal, E., Ekinci, D., Erdoğan, O. and Beydemir, Ş. 2011b. Influence of cobalt and zinc exposure on mRNA expression profiles of metallothionein and cytocrome P450 in rainbow trout. Biological Trace Element Research, 144: 781–789. doi: 10.1007/s12011-011-9068-z.

Colihueque, N., Vergara, N. and Parraguez, M. 2003. Genetic characterization of naturalized populations of brown trout (*Salmo trutta* L.) in southern Chile using allozyme and microsatellite markers. Aquaculture Research, 34: 525–533. doi:10.1046/j.1365-2109.2003.00847.x

Corujo, M., Blanco, G., Vâzquez, E. and Sánchez, J.A. 2004. Genetic structure of northwestern Spanish brown trout (*Salmo trutta* L.) populations, differences between microsatellite and allozyme loci. Hereditas, 141:258–271. doi: 10.1111/j.1601-5223.2004.01860.x

Chistiakov, D.A., Hellemans, B. and Volckaert F.A.M. 2006. Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. Aquaculture, 155: 1-29. doi:10.1016/j.aquaculture.2005.11.031

DeWoody, J.A. and Avise, J.C. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J. Fish Biol., 56: 461–473.

Ekinci, D., Ceyhun, S.B., Şentürk, M., Erdem, D., Erdoğan, O., Küfrevioğlu Ö.I. and Supuran, C.T. 2011a. Characterization andanions inhibition studies of an α-carbonic anhydrase from the teleost fish Dicentrarchus labrax. Bioorganic and Medicinal Chemistry, 19: 744-748. doi: 10.1016/j.bmc.2010.12.033.

Ekinci, D., Ceyhun, S.B., Aksakal, E. and Erdoğan, O. 2011b. IGF and GH mRNA levels are suppressed upon exposure to micromolar concentrations of cobalt and zinc in rainbow trout white muscle. Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology, 153: 336-341. doi: 10.1016/j.cbpc.2010.12.004.

Estoup, A., Presa, P., Krieg, F., Vaiman, D. and Guyomard, R. 1993. (CT)n and (GT)n microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity, 71(5): 488–496. doi:10.1038/hdy.1993.167

Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J.M., Adriamanga, M. and Guyomard, R. 1998. Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). Molecular Ecology, 7: 339-353. doi: 10.1046/j.1365-294X.1998.00362.x

Erdoğan, O., Ceyhun, S.B., Ekinci, D. and Aksakal, E. 2011. Impact of deltamethrin exposure on mRNA expression levels of metallothionein A, B and cytochrome P450 1A in rainbow trout muscles. Gene, 484: 13-17. doi: 10.1016/j.gene.2011.05.026.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131: 479-491.

Ferguson, A. 1989. Genetic differences among brown trout, *Salmo trutta*, stocks and their importance for the conservation and management of the species. Freshwater Biology, 21: 35–46. doi: 10.1111/j.1365-2427.1989.tb01346.x

Geldiay, R. and Balık, S. 2002. Turkey Freshwater Fish. Ege University Aquaculture Faculty Publications, Turkey.

Griffiths, A.J.F., Miller, J.H., Suzuki, D.T., Lewontin, R. C. and Gelbart, W.M. 2000. An Introduction to Genetic Analysis. 7rd Edition, New York

Hamilton, K.E., Ferguson, A., Taggart, J.B., Tómasson, T., Walker, A. and Fahy, E. 1989. Post-glacial colonization of brown trout, *Salmo trutta* L.: Ldh-5 as a phylogeographic marker locus. Journal of Fish Biology, 35: 651–664. doi: 10.1111/j.1095-8649.1989.tb03017.x

Hansen, M.M., Ruzzante, D.E., Nielsen, E.E. and Mensberg, K.-L.D. 2000. Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery trout and wild brown trout (*Salmo trutta* L.). Molecular Ecology, 9: 583–594. doi: 10.1046/j.1365-294x.2000.00898.x

Hedrick, P.W. and Miller, P.S. 1992. Conservation genetics: techniques and fundamentals. Ecological Applications, 2: 30-46. doi:10.2307/1941887

Hindar, K., Jonsson, B., Ryman, N. and Stahl, G. 1991. Genetic relationships among landlocked, resident, and anadromous brown trout, *Salmo trutta* L. Heredity, 66: 83–91. doi: 10.1038/hdy.1991.11

Hoffman, J.I., Forcada, J. and Amos, W. 2006. No relationship between microsatellite variation and neonatal fitness in Antarctic fur seals, *Arctocephalus gazella*. Molecular Ecology, 15: 1995–2005. doi: 10.1111/j.1365-294X.2006.02894.x

Huang, X.Q., Börner, A., Röder, M.S. and Ganal, M.W. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet., 105: 699–707. doi: 10.1007/s00122-002-0959-4

King, T.L., Lubinski, B.A., Burnham-Curtis, M.K., Stott, W. and Morgan II, R.P. 2012. Tools for the management and conservation of genetic diversity in brook trout (*Salvelinus fontinalis*): tri- and tetranucleotide microsatellite markers for the assessment of genetic diversity, phylogeography, and historical demographics. Conser. Gen. Res., 4(3): 539-543. doi: 10.1007/s12686-012-9603-z

Kitade, Y., Ootsuka, S., Iitsuka, O. and Saga, N. 2003. Effect of DMSO on PCR of *Porphyra yezoensis* (Rhodophyta) gene. J. Appl. Phycol., 15: 555–557. doi: 10.1023/B:JAPH.0000004460.36849.13

Meier, K., Hansen, M.M., Bekkevold, D., Skaala, Ø. and Mensberg, K-L.D. 2011. An assessment of the spatial scale of local adaptation in brown trout (*Salmo trutta*

L.): footprints of selection at microsatellite DNA loci. Heredity, 106: 488–499. doi: 10.1038/hdy.2010.164.

Narain, P. 2000. Genetic diversity – Conservation and assessment. Curr. Sci., 79: 170-175.

Nielsen, E.E., Hansen, M.M. and Loeschcke, V. 1997. Analysis of microsatellite DNA from old scale samples of Atlantic salmon: a comparison of genetic composition over sixty years. Mol. Ecol., 6: 487–492. doi: 10.1046/j.1365-294X.1997.00204.x

Oliveira, E.J., Pádua, J.G., Zucchi, M.I., Vencovsky, R. and Vieira, M.L.C. 2006. Origin, evolution and genome distribution of microsatellites. Gen. Mol. Biol., 29(2): 294-307. doi: 10.1590/S1415-47572006000200018

Page, R.D.M. 1996. Tree view: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci., 12: 357–358. doi: 10.1093/bioinformatics/12.4.357

Palti, Y., Fincman, R. and Rexroad III, C.E. 2002. Characterization of 38 polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology Notes, 2: 449–452. doi: 10.1046/j.1471-8286.2002.00274.x

Poteaux, C., Bonhomme, F. and Berrebi, P. 1999. Microsatellite polymorphism and genetic impact of restocking in Mediterranean brown trout (*Salmo trutta* L.). Heredity, 82: 645–653. doi: 10.1046/j.1365-2540.1999.00519.x

Pujolar, J.M., Maes, G.E., Vancoillie, C. and Volckaert, F.A.M. 2005. Growth rate correlates to individual heterozygosity in European eel, *Anguilla anguilla* L. Evolution, 59: 189–199. doi: dx.doi.org/10.1554/04-377

Romana-Eguia, M.R.R., Ikeda, M., Basiao, Z.U. and Taniguchi, N. 2004. Genetic diversity in fanned Asian Nile and red hybrid tilapia stocks evaluated from microsatellite and mitochondrial DNA analysis. Aquaculture, 236: 131–150. doi: 10.1016/j.aquaculture.2004.01.026

Ryman, N. 1983. Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture, 33: 1–21. doi: 10.1038/hdy.1991.11

Samuiloviene, A., Kontautas, A. and Gross, R. 2009. Genetic diversity and differentiation of sea trout (*Salmo trutta*) populations in Lithuanian rivers assessed by microsatellite DNA variation. Fish Physiol. Biochem,. 35: 649–659. doi:10.1007/s10695-009-9310-1

Ward, R.D., Jorstad, K.E. and Maguire, G.B. 2003. Microsatellite diversity in rainbow trout (*Oncorhynchus mykiss*) introduced to Western Australia. Aquaculture, 219: 169–179. doi: 10.1016/S0044-8486(02)00569-0

Weir, B.S. and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358–1370.