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Genetic studies of Iranian and Ferench Rainbow trout brood stocks

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ABSTRACT

In study of genetic variation of two stocks of rainbow trout breedes, the 22 sample fish were collated in 2 station of rainbow trout farm. A piece of soft fin (2-3 g) of pectoral were separated and fixed in alcohol etelic of 96 percent. DNA was extracted by phenol-chloroform method. The quality and quantity of DNA were determinate by spectrophotometer and 1 percent gel Agaroz of electrophoresis. Polymerase chain reaction (PCR) by thermocycler using microsatelite primers Omyf, Otsg3, Ots100, Otsg249 showed polymorphs. The number of alleles was studied in 12 variable positions. According to the findings, the maximum number of observed alleles was Ots100 in Iranian stock and Otsg249 in Ferench stock. The maximum and the minimum of expected heterozygosity was calculated 0.864and 0.416 and observed heterosigocity 0.864 and 0.150 respectively. In Hardy Weinberg equilibrium the Iranian sample in locus Omyf while French sample in other locus showed significant deviation. The most genetic differences (Fst=0.064) was between the two stocks. The results showed that, rainbow trout of this two stocks genetically was differ from each other but the difference was not significant (P>0.05).

Key words: *Rainbow trout Microsatelite*, heterozygosity, genetic diversity

Introduction

The rainbow trout is the native fish of North America and introduced to Europe in 1880 and to Iran in 1948 and was adapted in natural waters. The rainbow trout have a good potential for easy propagation therefore the breeders of many farm will be collected from cultured fish of the farm. The breeders are collected base on the growth rate and phenotypic characteristics. The stocks of rainbow trout are from Italian, Denmark, Ferench etc, that are introduced in farms for culture but in some farms they are mixed. The propagation of rainbow trout with small numbers of breeders and without notice its pedigree information, causes inbreeding effect and reduces the quality of brood stocks. For increasing the quality of breeders we must first have study in genetic structure of breeders and progeny test in farm. Recently, the molecular techniques especially microsatellite has been used in fish breeding program. The aim of the present study was investigation of genetic variation and comparison of Iranian and French rainbow trout stocks by using of microsatellite. The low genetic wariation in any brood stocks is harmful in production and reduces the fish adaptation. The previous work on salmo trouta by microsatellite in 8 loci showed a polymorphic structure and in each loci 6-11 alleles that illustrate high polymorph and hetrosigocity in Caspian Sea salmo trouta (Yousefian, 2010).

In another work using microsatellite, DNA variation at six microsatellite loci was examined in approximately 900 sockeye salmon, *Oncorhynchus nerka*, collected between 1987 and 1995 from three stocks on the west coast of Vancouver Island, British Columbia, Canada. Variation in allele frequencies among stocks was, on average, about 12 times greater than temporal variation within stocks (Beacham *et al*., 2004).

Also the study for Artificial Neural Networks (ANN) was applied to microsatellite data to separate genetically differentiated forms of brown trout in south-western France. From a biological point of view, the study enabled evaluation of the genetic composition and differentiation of different river populations and of the impact of stocking (Aurelle *et al*., 1999).

The study of genetic structure of rainbow trout in Iran is rear and the information is not available. In the other hand for management of propagation of fish, genetical background is important, therefore this study was performed as preliminary work in understanding the genetic structure of rainbow trout.

Material and Method

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The fish were sampled from two active farm in north of Iran. 30 fish from a big stock of French brood stocks and 30 from Iranian stock all in average of 2000±100 g and length of 28±5 cm were selected.

DNA was extracted from a part of soft fin that was fixed in alkohol96% according (Hillis and Moritz, 1990). For extraction of DNA with phenol-chloroform (Sambrook &Russell, 2004), 50 mg sample were separated and cut it in small piece. After determining the quality and quantity of DNA, this extract DNA was used for amplification of the sample by the polymerase chain reaction (PCR). Loci amplified by PCR were the dinucleotide repeats Ots100, Otsg3, Omyf and Otsg474. For all primer sets used in this study, PCR was conducted in 25-µL reactions containing 12 pmol (0.48 µM) of each primer, 80 µM of each nucleotide, 20 mM Tris-pH 8.8 buffer. For observation of DNA band, the method of (Hildbrandt and Igarashi, 1999) was used.

Data analysis:

The observed and expected genotype was tested by χ^2. The genetic distance, allele frequency, observed and estimated heterosigocity real and effective alles and liklyhood matrix all were used based on Nei, 1978. Each stock at each locus was tested for departure from Hardy-Weinberg equilibrium by using χ^2.

Gene flow, and genetic variation based on AMOVA was determined with Gene Alex. 6 (Peakall and Smouse, 2006). Tests of genetic differentiation with three pairwise comparisons among the populations were based on Nei 1978. Fst estimates for each locus were calculated with Gene Alex.6.

Results:

In present work the PCR with 5 microsatelite were analysed Otsg474 was monomorph and Omyf, Ots100, Otsg249 and Otsg3 showed polimorfic pattern {Fig.1).

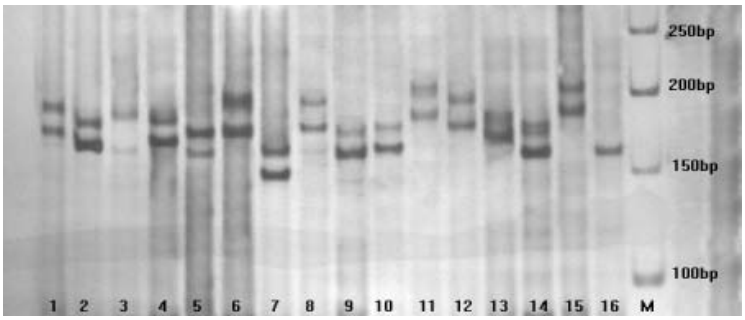

Fig. 1: Genomic pattern of Rainbow trouta by Omyf primer

Polymorphic allele:

All loci except for Otsg474 were polymorphic for Iranian and Ferench rainbow trout. Number of allele and frequency for Otsg3, Otsg249, Omyf and Ots100 for Ferench was 2, 6, 7 and 10 and for Iranian it was 4, 10, 7 and 8 respectively. The highest allele frequency for French sample was 0.705 and for Iranian 0.475 in OTSG3 locus

Real and effective allele:

The highest and minimum real allele in Iranian sample was in Otsg249 and Otsg3 loci. The highest and minimum effective allele number was in loci Otsg249 and Otsg478 respectively (Table 1)..

Table 1: The real and effective allele in in French and Iranian rainbow trout

Alleles	French samples N=22		Iranian Sample N=21	
	Ne	Na	Ne	Na
Otsg 3	1.713	2.0	3.042	4.0
Ots 100	3.752	10.0	4.545	8.0

Otsg 249	2.830	6.0	7.339	10.0
Omyf	5.531	7.0	3.774	7.0

Genetic Variation:

The genetic variation were determined for each stocks and the range of Ho was between 0.154-0864. The range of He was between 0.416-0.864 (Table 2.). The minimum He was in otsg3 and highest in Otsg 249.

Table 2: Observed heterosigocity (Ho) and expected (He) in two stocks

Alleles	French samples N=22		Iranian Sample N=21	
	He	Ho	He	Ho
Otsg 3	0.416	0.318	0.671	0.150
Ots 100	0.733	0.682	0.780	0.750
Otsg 249	0.647	0.818	0.864	0.850
Omyf	0.819	0.864	0.735	0.650

Hardy-Weinberg Equilibrium:

The result showed in French Rainbow trout at Omyf and in Iranian sample except Omyf in all loci the deviation with H-W equilibrium was significant (P<0.001). In table 3 the result of $\chi 2$ test for each locus of Iranian and French sample are presented. The highest $\chi 2$ shows higher deviation fromHWE.

Table 3: Hardy-Weinberg Equilibrium of loci in French and Iranian rainbow trout stocks

Stocks	Loci	DF	χ^2	Prob.	Sig.
French	Otsg 3	1	1.233	0.269	ns
French	Ots 100	45	59.383	0.074	ns
French	Otsg 249	15	11.270	0.733	ns
French	Omyf	21	42.503	0.004	***
Iranian	Otsg 3	6	35.283	0.000	***
Iranian	Ots 100	28	58.333	0.001	***
Iranian	Otsg 249	45	71.140	0.008	**
Iranian	Omyf	21	31.893	0.060	ns

* significant at 0.05; ** significant at 0.01; *** significant at 0.001.

Stock differentiation:

Based on Fst test the both stocks showed highly significant differences (P<0.01) that indicate variation between two group of sampling. High genetic differentiation (Fst=0.065) between French and Iranian stocks were determined.

Discussion:

In artificial propagation and for production management, it is necessary to understand the genetic structure of the rainbow trout brood stocks, because in small population, the fishes are relative and in unfavorite condition this increase the risk of high mortality or reduction of production due to inbreeding.

In the present study, the microsatellite marker that is not environment dependent were used to detect the genetic structure of rainbow trout. 5 loci were investigated that except Otsg 474 the other loci Otsg3, Otsg249, Omyf and Ots100 were polymorph. The importance of genetic marker is due to its allelic number and frequency. The allelic number of OTSG 3 and OTSG100 was 4 as minimum and 10 as maximum that is good for genetic study.

Genetic Variation in different species and different population is not the same. The variation among marine fish usually is much higher than fresh water. However DeWoody and Avise (2000) in study of fresh water fish found the genetic variation of 0.46 and mean allelic number of 7.5 in fresh water fish. In the other hand, Beacham and Dempson (1998) in study of *salmo salar* by microsatellite reported that expected heterosigocity was 0.69 and mean allele number of 6. In the present result the mean allele number was 5.6 and expected heterosigocity was 0.508 that was about the two studies mentioned above.

Adams and Hutching in 2003 in study of brook charr (*Salvelinus fontinalis*) by microsatellite in Indian bay, found that this fish have high heterosigocity (0.27-0.6) that was the same as rinbow trout

of the present study. They denoted that heterosigocity in the range of 0.3 to 0.6 is very high. In study of genetic structure of *Oncorhynchus masou* by microsatellite in 6-23 alleles the heterosigocity was 0.66-0.73 (Kitanishi *et al*., 2009). In fact the level of heterosigocity is depending on the sample, fish species, place of sampling, primer and number of sampling.

Hardy-Weinberg Equilibrium:

The genotyoe prediction of progeny based on the genotype of parents is explained by HWE. In study of Sokeye salmon (*Oncorhinchus nerka*) by microsatellite, there was significant deviation to HWE ($P<0.05$), in some locus investigated by Beacham *et al*., 2004.

Dahle *et al*. 2006) in study of costal cod (*Gadus morpha*), stated that several factor such as increasing homozigosit, genetic drift, selection, low sample size and null alleles is possibly cause of the Hardy-Weinberg disequilibrium. The mating of male and female was based on a systematic breeding method, therefore a deviation from Hardy-Weinberg Equilibrium may result from non-random mating, null alleles, frequently found in microsatellite loci as well as low sample size.

In Hardy Weinberg Equilibrium the Iranian sample in locus Omyf showed significant deviation ($P<0.05$), while in French sample the other loci showed significant deviation ($P<0.05$). The reason for this unequivalent in imported breeders is the cases that they are genetically different.

Population structure:

Fresh water fish comparing with marine fish have lower genetic variation therefore we shoul expected low genetic variation in rainbow trout. In the present study maximum 12 alleles were identified in 43 samples.

The amount of Fst between the different samples in present study was 0.031 to 0.139 that shows low and medium genetic difrentiation. The mean variation and differentiation (0.065) in ferench and Iranian rainbow trout shows medium difrentiation. In Sub-population of brook charr *(Salvelinus fontinalis*) in 5 lake the Fst was between 0.32 to 0.084 ($P<0.01$) and the author suggested 5 different population in sampling sites (Anger *et al*., 1995).

Microsatellite DNA variation at six microsatellite loci (Omy77, Ots3, ts100, Ots103, Ots107, and Ots108) was examined in approximately 900 sockeye salmon, *Oncorhynchus nerka*, from three stocks on the west coast of Vancouver Island, British Columbia, Canada. Variation in allele frequencies among stocks was, on average, about 12 times greater than temporal variation within stocks. Individual locus Fst estimates ranged from 0.013 to 0.107 among stocks, with an overall value of 0.056. Analysis of simulated mixed-stock samples indicated that data from four to six of the microsatellite loci surveyed would enable relatively accurate and precise estimates of stock composition for mixtures composed of fish from the three stocks.

Conclusion:

In present study for investigation of Iranian and Ferench stocks by analyzing allele frequency, heterosigocity, HWE, Fst and AMOVA test, was determined and showed that the two stocks are quite different. The presence of 4 allele in locus Ogst3 in Iranian sample and two alleles in French sample is may be the key differences of these two stocks. The difference is may be due to mutation in Iranian rainbow trout or selection program in French stocks.

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