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Genetic variability and differentiation of rainbow trout strains in Iran

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ABSTRACT

To determine the genetic structure and differences of rainbow trout, variations in 9 microsatellite loci in four stocks of rainbow trout from Mazandran, Iran were examined. Three samples were introduced from Norwegian, French stocks, and one was from Iranian stocks rearing in North of Iran. Most but not all loci were polymorphic in all stocks. There was substantial genetic differentiation among populations, with an average F_{ST} value of 0.065. The four stocks averaged 5.2–7.4 alleles per locus, with average heterozygosities of around 0.625 to 0.762. A large variation in observed hetrozygocity, averaged over all samples was observed among loci and ranged from 0.015 in OTSG474 to 0.850 in OTSG249. Nine of 20 tests for Hardy – Weinberg expectations gave significant deviations. The cause(s) of these deviations are unknown. The Norway strain was significant deviations from HWE in all 5 polymorphic loci. The degree of differentiation among strain or Nei Genetic Distance compared pairwise accorded well with their likely genetic connectivity. Two of the strains, Iranian and Norway had the highest Nei Genetic Distance (0.213) and were different in all loci except for OTSG3. The level of genetic variation in the domesticated rainbow trout strains that are farmed in European countries (French, Norway) and Iran did not differ significantly neither within nor between the countries, but the highest pronounced difference was comparable with that of the Norway and Iran strains.

Key words: Allele number, Heterozygosity, Microsatellites, *Oncorhynchus mykiss*

The rainbow trout (*Oncorhynchus mykiss*) is native to the West Coast of North America and it is an important species in aquaculture, which is one of the most important cold water fish species for culture. The most of the rainbow trout strains cultured around the world originate from the McCloud River hatchery in California which was established already in 1879 (Gall and Crandell 1992). Since then, numerous strains of rainbow trout have been developed by selective breeding and crossbreeding with the goal of improving economically important traits like growth rate, viability, disease resistance, age at maturity, time of spawning, flesh quality etc. (Gjedrem 2000). A large part of the phenotypic diversity of rainbow trout also can be explained by its local reproductive isolation in different countries, however, not much is known about the genetic structure wild type rainbow trout or the effect of different breeding practices on genetic diversity.

Rainbow trout is one of important commercial species that has been introduced to Iran, first about 50 years ago as a part of sport fisheries and enhancement programs. Afterward, the rapid and widespread establishment of rainbow trout in Iran appears to derive from several major shipments to North of Iran from European country. By rapid expansion of fish farms, rainbow trout rearing decreased the genetic variability present in farmed stocks by breeding related individuals or by the use of small numbers of parents as broodstock. To have their own broodstocks, farms operate a mass selection approach with high selection intensities without considering pedigree records, they selected related individuals as parents and thereby increasing inbreeding. To solve the problem, recently minor parties included eggs and fingerlings of rainbow trout of different country such as French and Norway introduced to fish farm.

The aim of this study was to determine the genetic variability and differentiation among rainbow trout strains of imported and between the country. For this, we used highly variable microsatellite DNA markers. Also it is expected due to the potential of microsatellite markers for 'genetic tagging' of the individuals, we may identify a marker of strain of origin.

Examination of microsatellite variation at several polymorphic loci is a widely accepted method for determining parentage and examining genetic diversity within rainbow trout breeding programs. Genotyping costs are considerable (Johnson 2007), however many scientist prefer to have reliable results and spend more cost for their work.

MATERIALS AND METHODS

Young rainbow trouts (120) were collected from 6 fish farms, the down stream fish farm of Haraz River of Iran,

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representing 4 strains from the French, Norway and Iran. All fish were caught over spawning sites in summer of 2011, as pre mature brood stocks. The characteristics of the fishes, including length (29±6 cm), weight (1990±132 g) at two years old, were recorded.

Microsatellite DNA: The fish representing *Oncorhynchus mykiss* strain were first screened at 9 loci to examine the potential of corresponding primers before being developed for this study. Five primers producing microsatellite bands that could be unambiguously determined were then selected for analysis the microsatellite locus. A total of 2 to 3 g tissue from dorsal fin was separated. The fin samples were stored in 1.5 ml Eppendorf tubes with 96% ethanol. The fin tissue was crushed and then 300 µl STE, 50 ml sodium dodecyl sulphate (SDS, 20%), and 3 µl proteinase-k was added to the crushed tissue and incubated at 55 to 60°C overnight. Then, 500 µl phenols were added to fin extract, centrifuged at 8500 for 5 min. Total genomic DNA extracted by phenol-chloroform according to Hillis and Moritz (1990). The quality and quantity of DNA were assessed by 1% agarose gel electrophoresis and spectrophotometer (model Cecil DE2040), and then stored at –20°C till it was used.

The quality of extracted DNA was checked by TBE buffer on 0.1% agaros gel (Gross and Nilsson 1999). Polymerase chain reaction (PCR) conditions, especially the annealing temperatures, for each of the primer sets were optimized for rainbow trout. The PCR reactions were performed in a 25 µl reaction volume containing 100 ng of the template DNA, 0.5 to 1 pµ of primer, 200 mM of dNTPs, 1U Taq DNA polymerase, 1.5 µl reaction buffer (10X), 1 to 2.5 mM $MgCl_2$ and distilled water to final volume of 25 µl. The samples were subjected to an initial denaturation step at 95°C for 3 min, followed by 30 cycles of 95°C for 30 sec, at annealing temperature for 45 sec, 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were electro-phoresed on 8% polyacrylamide gel at 150 V for 3 h with ladder marker pBR322 DNA/AluI Marker, 20, MBI Fermentas) and the DNA fragments were stained by silver nitrate.

Reaction products were analyzed by 8% polyacrylamide gel (Gross and Nilson 1999). The PCR products were separated by polyacrylamid gel electrophoresis. Three micro litter of the PCR product was loaded with gel-loading buffer (Sambrook *et al.* 1989). Gel was run for 2 h until front bands reached the end of the gel. After electrophoresis, the DNA fragments were visualized by silver staining according to Klinkicht and Tautz (1992).

Data analysis: Microsatellite alleles were sized by using UVI DOC Version V.99.04 software. In order to calculate allelic and genotype frequencies, observed (Ho) and expected (He) heterozygosity, deviations from Hardy-Weinberg expectations (HWE), F_{ST} value, analysis of molecular variance (AMOVA) were conducted by using the GenAlex 6.2 software (Peakall and Smouse 2006). The genetic distance and the genetic identity between the populations was estimated from Nei standard genetic distance and genetic similarity index (Nei 1972), unweighted pair-group mean analysis (UPGMA) computed in TFPGA version 1.3 and the presence of null alleles was checked by using Microcheker (Version 2.2.3).

RESULTS AND DISCUSSION

Genetic variability of microsatellite DNA: All 9 microsatellite DNA primers originally developed for other species successfully amplified apparently homologous loci. Four of these were monomorphic, while the others displayed moderate to very high levels of polymorphism. Five primers amplifying microsatellite alleles that could be unambiguously determined were then selected for subsequent analysis. Allele frequencies for the five microsatellite loci of rainbow trout strain are shown in Table 1.

Fifty seven alleles were found in the population, ranging from 3 for locus Ostg478 to 12 for locus Ostg100. The Ostg100 microsatellite loci were highly polymorphic in the all strains.

The degree of variability was different at each locus. Otsg100 had the highest number of allele, 12 in Norway strain while the lowest number of polymorphic alleles was in Otsg3 with 2 allele in French. The mean number of alleles per locus detected in French, Turkey, Iran and Norway was 5.2, 5.4, 6 and 7.4 respectively.

High significant differences in allele frequencies were observed among samples in all loci and within strain samples ($P<0.001$). Allele composition shows difference between samples strains. Thus some alleles which appears in Norway strain e.g. Ostg474 may not be found in the other strain.

The expected and observed hetrozygosity are given in Table 2. A large variation in observed hetrozygocity, averaged over all samples was observed among loci and ranged from 0.015 in OTSG474 to 0.850 in OTSG249. The mean expected hetrozygocity in four microsatellite was 0.625 in Norway and 0.762 in the Iranian strains. Significant differences in expected hetrozygocity was detected among the two groups ($P<0.05$-Table 2). The He were higher than Ho in almost all loci except in OMYF and compared to the He, the Ho were significantly reduced in all the four strains ($P<0.05$).

Another test for determining the polymorphism, is the score of effective allele in each microsatellite loci. The number of effective allele are determined and presented in Table 2. In average, effective allele in Norway (0.506) are much lower than French (0.523), Turkish (0.586) and Iranian (0.610).

Test for departure from Hardy–Weinberg (H.W) expectations yielded significance across all loci sampled. According to the Table 3, no deviation from Hardly-Weinberg equilibrium was observed for the loci OMYF in Turkish and Iran samples and OTSG249 and Omyf in Turkish strain. The H.W test showed interesting result in case of French and Norway samples. All loci showed significant deviation from HWE in Norway strain and it showed opposite in French (except locus OMYF).

Table 1. Allele frequencies of five microsatellite loci of rainbow trout

Locus	Allele	Norway	France	Turkish	Iran	Locus	Allele	Norway	France	Turkish	Iran
OTSG3	142	0.069	0.295	0.381	0.250	OTSG249	115	0.085	0.000	0.000	0.000
	146	0.000	0.000	0.024	0.000		120	0.000	0.000	0.000	0.175
	150	0.923	0.705	0.429	0.475		136	0.000	0.000	0.000	0.225
	151	0.008	0.000	0.000	0.000		137	0.008	0.000	0.000	0.000
	154	0.000	0.000	0.000	0.100		139	0.138	0.000	0.333	0.000
	158	0.000	0.000	0.167	0.175		143	0.208	0.000	0.024	0.000
OTS100	115	0.031	0.000	0.000	0.000		147	0.254	0.523	0.167	0.000
	165	0.008	0.045	0.024	0.025		148	0.000	0.000	0.000	0.125
	168	0.015	0.023	0.000	0.000		149	0.000	0.023	0.000	0.000
	169	0.200	0.091	0.143	0.100		151	0.008	0.000	0.000	0.000
	176	0.008	0.023	0.000	0.000		152	0.000	0.000	0.000	0.075
	177	0.238	0.000	0.119	0.000		155	0.031	0.091	0.024	0.000
	181	0.000	0.114	0.000	0.150		156	0.000	0.000	0.000	0.125
	185	0.085	0.068	0.119	0.300		160	0.000	0.000	0.000	0.050
	188	0.008	0.068	0.000	0.025		161	0.031	0.000	0.000	0.000
	189	0.023	0.023	0.000	0.000		163	0.200	0.091	0.167	0.000
	193	0.008	0.068	0.095	0.025		165	0.000	0.023	0.048	0.000
	196	0.008	0.000	0.000	0.000		167	0.008	0.000	0.071	0.000
	197	0.369	0.477	0.500	0.300		175	0.000	0.250	0.071	0.000
	200	0.000	0.000	0.000	0.075		179	0.000	0.000	0.024	0.000
OMYF	149	0.038	0.091	0.024	0.025		184	0.000	0.000	0.000	0.100
	161	0.338	0.273	0.310	0.050		191	0.000	0.000	0.071	0.000
	165	0.262	0.114	0.238	0.350		196	0.000	0.000	0.000	0.050
	177	0.077	0.182	0.167	0.250		197	0.031	0.000	0.000	0.000
	181	0.169	0.182	0.190	0.275		200	0.000	0.000	0.000	0.050
	185	0.077	0.023	0.048	0.025		252	0.000	0.000	0.000	0.025
	195	0.015	0.000	0.000	0.000	OTSG474	156	0.008	0.000	0.000	0.000
	197	0.023	0.136	0.024	0.025		160	0.985	1.000	1.000	1.000
							164	0.008	0.000	0.000	0.000

Table 2. Sample sizes (N), number of alleles (Na), number of effective alleles (Ne), information index (I), observed (Ho) and expected heterozygosity (He), and fixation index (F)

Pop	Locus	N	Na	Ne	I	Ho	He	F
Norway	OTSG	65	3.000	1.167	0.296	0.138	0.143	0.032
	OTS100	65	12.000	4.126	1.686	0.708	0.758	0.066
	OTSG249	65	11.000	5.652	1.913	0.785	0.823	0.047
	OMYF	65	8.000	4.431	1.689	0.785	0.774	–0.013
	OTSG474	65	3.000	1.031	0.090	0.015	0.030	0.494
France	OTSG	32	2.000	1.713	0.607	0.318	0.416	0.236
	OTS100	32	10.000	3.752	1.766	0.682	0.733	0.070
	OTSG249	32	6.000	2.830	1.294	0.818	0.647	–0.265
	OMYF	32	7.000	5.531	1.797	0.864	0.819	–0.054
	OTSG474	32	1.000	1.000	0.000	0.000	0.000	0.000
Turkish	OTSG	31	4.000	2.800	1.118	0.476	0.643	0.259
	OTS100	31	6.000	3.243	1.444	0.667	0.692	0.036
	OTSG249	31	10.000	5.378	1.941	0.667	0.814	0.181
	OMYF	31	7.000	4.546	1.642	0.619	0.780	0.206
	OTSG474	31	1.000	1.000	0.000	0.000	0.000	0.000
Iran	OTSG	30	4.000	3.042	1.235	0.150	0.671	0.777
	OTS100	30	8.000	4.545	1.708	0.750	0.780	0.038
	OTSG249	30	10.000	7.339	2.127	0.850	0.864	0.016
	OMYF	30	7.000	3.774	1.495	0.650	0.735	0.116
	OTSG474	30	1.000	1.000	0.000	0.000	0.000	0.000

Pair wise test between samples indicated that all samples were significantly genotypically differentiated from each others by P2 test: $P<0.05$). Pairwise Population Matrix of Nei Genetic Distance are presented in Table 4, and differentiation index of Genetic Distance described as Pairwise Population F_{ST} (via Frequency) Values are

Table 3. Chi-square tests for Hardy-Weinberg equilibrium

Strain	Locus	DF	Chi squre	Prob	Signif
Norway	OTSG3	3	13.770	0.003	**
	OTS100	66	251.838	0.000	***
	OTSG249	55	97.477	0.000	***
	OMYF	28	59.823	0.000	***
	OTSG474	3	130.000	0.000	***
France	OTSG3	1	1.223	0.269	ns
	OTS100	45	59.383	0.074	ns
	OTSG249	15	11.270	0.733	ns
	OMYF	21	42.503	0.004	**
	OTSG474	Monomorphic			
Turkish	OTSG3	6	15.827	0.015	*
	OTS100	15	29.741	0.013	*
	OTSG249	45	52.024	0.219	ns
	OMYF	21	24.314	0.278	ns
	OTSG474	Monomorphic			
Iran	OTSG3	6	36.283	0.000	***
	OTS100	28	58.333	0.001	***
	OTSG249	45	71.140	0.008	**
	OMYF	21	31.893	0.060	ns
	OTSG474	Monomorphic			

Table 4. Pairwise population matrix of Nei genetic distance

Norway	France	Turkish	Iran	
0.000				Norway
0.104	0.000			France
0.121	0.111	0.000		Turkish
0.213	0.204	0.152	0.000	Iran

Table 5. F_{ST} estimated (degree of genetic differentiation) of strain

Norway	France	Turkish	Iran	
0.000				Norway
0.042	0.000			France
0.049	0.041	0.000		Turkish
0.065	0.064	0.042	0.000	Iran

F_{ST} (via frequency) values below diagonal.

illustrated in Table 5. Fst between strain are between 0.042 to 0.065 that shows low to moderate genetic variations between the strains.

It was identified that the application of microsatellite technology to stock identification can provide the most reliable and cost effective results (Beacham and Dempson 1998). Five of nine microsatellite loci chosen for analysis were polymorphic in all four strains. The genetic variation of five microsatellite loci proved to be extensive, as the genetic variability indices in terms of the mean number of allele per locus ranged from 5.2 to 7.4. This was obviously higher than those estimated.

For six loci studied in common in Western Australia and North America, average heterozygosities were 0.56 and 0.75, and average numbers of alleles per locus were 3.8 and 10.5, respectively (Ward *et al.* 2003). Comparing to the results of our study, allele variable was higher than Western Australia samples but lower than North America.

Gross *et al.* (2007) in study of genetic variability and differentiation of rainbow trout (*Oncorhynchus mykiss*) with the 12 European strains analysed, by 10 microsatellite loci, using different marker with present study, indicated a similar range of mean number of alleles per sample (3.2–7.2) for 12 European strains.

In a study of three domesticated rainbow trout strains in USA, Silverstein *et al.* (2004) reported mean number of alleles per locus per sample of 8.1–9.6, with 9 microsatellite loci. In another study of two hatchery strains maintained at the National Centre for Cool and Cold Water Aquaculture in USA (NCCCWA) (Johnson *et al.* 2007) with the same number of loci, an average number of alleles per strain was from 11.1–13.6, which in both cases is much higher than the values of the present work.

Nine of twenty tests for Hardy–Weinberg expectations gave significant deviations. The causes of these deviations are unknown. The Norway strain was significant deviations from HWE in all 5 polymorphic locus. It is possible that the broodstock sample is maintained by small numbers of spawners, giving some HW deviations in descendants. However, the French sample showed no significant deviations from Hardy–Weinberg expectations despite coming from only 32 broodstock. The heterozygote deficiencies were at almost all locus of Norway strain, which could be explained if there was a non-amplifying or null allele at this locus. The number of allele and estimation of heterozigosity is mainly due to sampling size that can cover all possible allele information and the locus of microsatellite that are analysed for genetic variation. Heterozygote deficiencies have been recorded for rainbow trout and mixed population microsatellite loci in some earlier studies (Gross *et al.* 2007, Narum *et al*. 2004, Ward *et al*. 2003).

The degree of differentiation among strain or Nei Genetic Distance compared pairwise accorded well with their likely genetic connectivity. Two of the strains, Iranian and Norway had the highest Nei Genetic Distance (0.213) and differed in all locus except for OTSG3. This divergence was expected, as the Iranian sample consisted of descendants of 30 broodstock from the 65 broodstock that made up the Norway sample. 1–3 alleles in 4 locus of Norway sample were absent from the Iranian samples. The Nei Genetic Distance also is recorded in Norway samples compared with French samples. The same pattern of allele reduction occurred in French samples. The Norway sample is quite distinct from all three strain samples based on morphological characteristics that are more elongated compared to three other investigated strain (personal observation), this deviation is expected and is rather related to genetic structure of Norway fish, than we refer it to sample size.

There was a moderate level of genetic differentiation among the four strains, with a highly overall F_{ST} value of 0.065 between Norway and Iran samples. Therefore only 7% of the observed genetic variation could be attributed to inter-population differences and as described before the

most variation that exist within the strains. Also moderate level of genetic differentiation is among Norway and French samples.

The degree of genetic differentiation observed among the samples in this study (highest pair-wise F_{ST} = 0.065) is less and far from the range of values observed in most other studies of rainbow trout throughout the world. The degree of genetic differentiation observed in the present study was very low indicating similarity in rainbow trout of different origin, while in analyzing of the genetic of wild population it seems to be very high. Deiner *et al.* (2007) reported pair-wise F_{ST} values as high as 0.385 among 20 land-locked populations in California, whilst, among 24 anadromous samples in California, Aguilar and Garza (2006) reported the large pair-wise F_{ST} value of 0.17 and Among 12 European strains, Gross *et al*. (2007) reported an average F_{ST} of 0.14 among them when looking specifically among Danish and Finnish strains however, these authors reported a mean F_{ST} of 0.1 and 0.05 respectively.

In the other hand, the degree of genetic differentiation is within the range of values observed in hatchery strain. Johnson *et al.* (2007) reported an F_{ST} of 0.0481 between 2 hatchery strains of rainbow trout in West Virginia, whilst Silverstein *et al*. (2004) reported an average F_{ST} of 0.089 among three distinct hatchery strains in USA. Therefore the degree of genetic differentiation observed among the samples in this study was coincident with those of American hatchery samples.

The difference between strains can be due to the method and goal in selective breeding work that might be carried out in Norway and French fish farms. Progeny from French grew faster and exhibited greater tolerance to high water temperature than progeny from Norway. However the Norway progeny may exhibit faster growth in other environmental condition such as tolerance in brackish water, while the rainbow trout broodstocks in most farms in Iran is a mixed population of different lines.

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