Conspecific relation between two seasonal migratory forms of endangered Caspian trout, *Salmo trutta caspius* Kessler, 1877, revealed by RAPD markers

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Abstract

Random amplified polymorphic DNA (RAPD) markers were used to estimate genetic relationships between two seasonal immigrant forms (namely fall-run and spring-run) of Caspian trout *Salmo trutta caspius*. In this regard, 62 fin tissue samples were collected from southern parts of the Caspian Sea basin and examined with 16 oligodecamer primers to asses their genetic diversity as well as probable specific population bands. The total number of RAPD bands produced in spring and fall-run were 162 bands, of which 69 and 53 bands were polymorphic for each population. Nei's genetic identity and genetic distance between spring-run and fall-run populations were 0.9858 and 0.01430, respectively. The RAPD based data revealed that the two migratory forms of Caspian salmon were categorized in conspecific value.

Keywords: Genetic diversity, RAPD, Caspian trout, Fall and spring run.

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Introduction

Caspian trout, *Salmo trutta caspius* Kessler, is one of the nine subspecies of brown trout *Salmo trutta* in the world and native to the Caspian Sea (Quillet, et al., 1992). In Iran, Caspian trout populations have experienced a strong decline during the past two decades as a result of overfishing pressure, habitat pollution and reduction in spawning areas. This fish migrates to some Southern and Northern Caspian Sea rives for spawning (caspianenvironment.org). Caspian trout forms local stocks confined to certain rivers such as Kura, Samur and Yalama, which differ in morphological features, age and interval of maturity (Kazancheev, 1981).

The most important Iranian rivers for their spawning consist of, Karganrood, Navrood, Astarachiy in Gilan state and Tonekabon (Cheshmekileh), Chaloos, Sardaberood in Mazandaran state. Distribution of subspecies occurs commonly at the Western and Southern coasts, from Terek to Sefid-Rud river and is seldom found in the Northern part (Caspianenvironment.org). Locality of Gilan and Mazandaran states in southern part of Caspian Sea coastline were shown in figure 1. Caspian salmon spawns in Terek, Kura, Sefid-Rud and other minor rivers of the Western and Southern coasts of the Caspian Sea. In respect to its reproductive life cycle, two immigrant forms namely fall-run and spring-run were observed. In local areas, spring-run and fall-run are named Salmon and Tian. Spawning migration to Kura takes place from November till February. The temperature range of salmon migration is rather wide, though the majority (appr.75%, immature fish with undeveloped gonads) enters the rivers at a comparatively low temperature, 8.2 – 12.8^{o}C in November-December.Spawning takes place only a year later, starts in October and completes in early January. Eggs are released in the bottom and buried in substrates. Incubation period continues for 30-50 days. Afterwards, spawners either die (in Kura river), or return to the sea (Kazancheev, 1981). Spring-runs differ from fall-runs by a greater body depth and silver color body; they migrate with unripend gonads to rivers at the end of winter and beginning of spring and stay in rivers until autumn for spawning. Fall-runs migrate with mature gonads and spawn at the end of winter. Among spawning rivers in Iran, the most stock migratory forms of *S. t. caspius* were found in Tonekabon River. There is a local hypothesis that these two immigrant forms may be genetically different and probably belonged to two different populations. Determination of genetic diversity between them could have a great importance for aquacultural strains development, protection of small-endangered populations and biogeographical inferences (Hassanien, et al., 2004). Despite the commercial and conservation importance of this subspecies, there are no published data on their molecular variability, yet. In this regard, Random Amplified Polymorphic DNA technique (RAPD) was applied as a useful tool for their genetic variability. Polymorphisms in RAPD markers are inherited in a Mendelian fashion and can be used as genetic markers (Bardakci et al., 2004). Thus, the genome can be scanned more randomly than with conventional techniques.

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Figure1: Locality of Rasht (city center of Gilan state) and Sari (city center of Mazandaran state) in southern part of the Caspian Sea of coastline states in Iran

The ability of genomic variation examination without previous sequence information, relatively low cost of the technique, and only nanograms of template DNA requirement, are all advantages of RAPD in population and other genetic studies (Tassanakajon et al., 1997).

RAPD has been used in several fish genomic studies including, identification of largemouth bass subspecies and its intergrades (Williams, et al., 1998), genetic relationships in Salmonidae species including brown trout and Atlantic salmon (Elo et al., 1997), hybrid identification (Jamshidi, et al., 2009) and genetic variation of *Rutilus rutilus caspius* (Keyvanshokohet al., 2006). The main objective of the present study was to investigate the genetic diversity and similarity between two migratory forms (spring-runs and fall-runs) of endangered Caspian salmon in Iran coastlines by using RAPD as a useful and efficient marker; for better broodstocks management and conservation of this valuable endemic fish of the Caspian Sea.

Material and methods

Fish sampling and DNA extraction

Fall-run fin samples (31 individuals) of Caspian salmon were obtained from Shahidbahonar center of breeding which were collected from Tonekabon and some other rivers in Mazandaran state and 31 spring-run individuals were obtained from Caspian basin local rivers by a morphological detection of spring and autumn migratory forms. Fin tissue samples were preserved in 96% ethanol until DNA extraction.

Total DNA was extracted from 50 mg of fin tissue using Roche DNA Extraction Kit (Roche, Germany). Resting of DNA extraction was done according to Roche manual kit on the basis of DNA extraction method of Hillis et al. (1996). The quality and concentration of DNA from samples were assessed by 1% agarose gel electrophoresis (Sambrook and Russel, 2001). The extracted DNA was stored at 4ºC until use.

PCR-RAPD analysis

Sixteen commercially available oligodecamers random primers from Bioneer Company (Table 1) were used for detection of polymorphism in this study.

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Table 1: Sequences of used RAPD primers (Bioneer company) for genetic analysis of migratory forms of Caspian salmon

Primer code	Oligonuclotide sequences
P-1	5'-GTAGCACTCC-3'
P-2	5'-TCGGCACGCA-3'
P-3	5'-CTGATACGCC-3'
P-4	5'-GTGTCTCAGG-3'
P-5	5'-CCCCGGTAAC-3'
P-6	5'-GTGGGCTGAC-3'
P-7	5'-GTCCATGCCA-3'
P-8	5'-ACATCGCCCA-3'
P-9	5'-GTGGTCCGCA-3'
P-10	5'-TCCCGCCTAC-3'
OPA-5	5'-AGGGGGCTTG-3'
OPA-7	5'-GAAACGGGTG-3'
OPA-8	5'-GTGACGTAGG-3'
OPA-12	5'-TCGGCGATAG-3'
OPA-17	5'-GACCGCTTGT-3'
OPC-5	5'-GATGACCGCC-3'

RAPD-PCR was performed in 25 µl reaction volumes containing 25 ng of *S. t. caspius* DNA, 20 pmoles of primer (1 µl), 0.1 each dNTP(0.5 µl), 4 mM $MgCl_2$(0.75 µl), 2.5 µl PCR buffer and 1.25 unit Taq DNA polymerase (Cinnagen). DNA was amplified by Eppendorff thermalcycler (Germany). It was programmed to provide a first denaturation of 5 min at 94□C, followed by 45cycles of 1 min at 94°C, 1 min at 36°C and 3 min at 72°C and finally one cycle at 72□C for 6 min. Amplification products were resolved by electrophoresis in 2% agarose gels with TAE buffer (40 mMTris-Acetate, 1 mM EDTA pH 8.0) containing ethidium bromide. A 100 bp ladder Fermentas and marker II Roche (Germany) were used as molecular standard size markers.

Statistical analysis

The genotypes were detected by scoring the presence (1) or absence (0) of distinct reproducible bands and faint bands were neglected. The index of similarity (Band Sharing Frequency) between individuals was calculated according to Nei and Li (1979) formula:

$F = 2N_{XY} / (N_X + N_Y)$

Where N_{XY} is the number of bands shared in common between individuals X and Y, and N_X and N_Y are the total numbers of bands scored for individuals X and Y respectively. Thus, *F* reflects the proportion of bands shared between two individuals and ranges from 0 (no common bands) to 1 (all bands identical). The genetic distance (d) was calculated as:

$d = 1 - F$

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The analysis of the data was carried out using the POPGENE version 2.6.2 program.

Results

A total of 162 bands were produced from 16 primers. The number of fragments generated per primer varied between 5 and 17. All primers gave RAPD patterns, (Fig.2, 3, 4) the average number of bands per primer was 10.125. The bands ranged in molecular size from approximately100 to 2500 bp. No band was population specific. Primer OPA-05 produced the highest number of fragments among the primers used with an average of 17 fragments, and primer P-07 produced the lowest number of fragments with an average of 5 bands. The total number of RAPD bands produced in spring and fall-run were 162 bands, of which 69 and 53 bands were polymorphic for each population. (42.59% and 32.72%, respectively).Nei's genetic identity and genetic distance between spring and fall-run were 0.9858 and 0.01430, respectively.

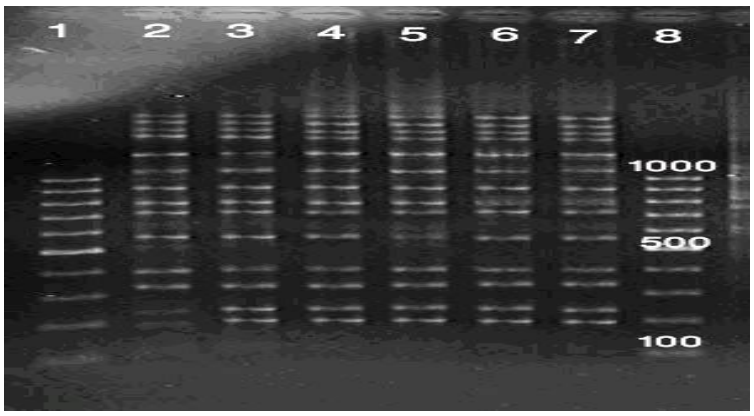

Fig2: RAPD pattern of primer OPA8 in 2% Agarose gel,15 reproducible bands were generated which none of them werepopulation specific. From left to right 2-4 (Spring-run), 5-7 (Fall-run). In these samples none of the generated bands showed any polymorphism.Lanes 1and 8 are 100 bp Ladder Fermentas Company.

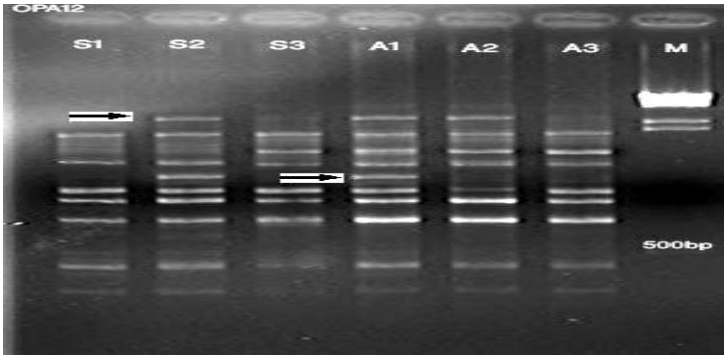

Fig3: RAPD pattern of primer OPA12 in 2% Agarose gel,10 reproducible bands were generated which none of them were population specific. From left to right S1-S3 (Spring-run), A1-A3 (Fall-run). Two bands which were shown have polymorphism in two spring and fall-run populations, but not population specific .M: marker II Roche Company.

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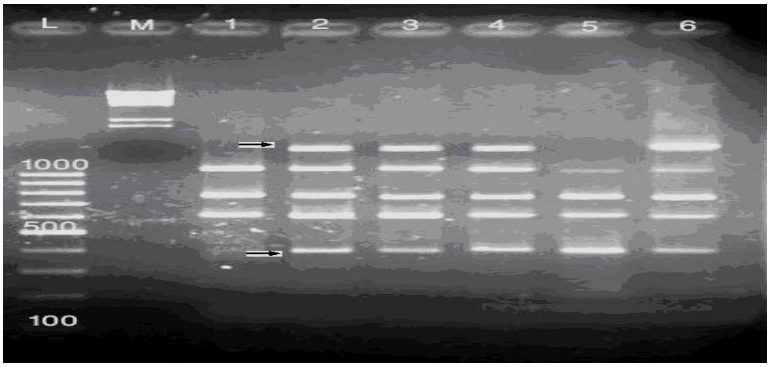

Fig4: RAPD pattern of primer P07 in 2% Agarose gel, 5 reproducible bands were generated which none of them werepopulation specific. From left to right L: 100 bp ladder Fermentas Company, M: marker II Roche, 1-3 (Spring-run), 4-6 (Fall-run). In these samples, two indicated bands have polymorphisms in two populations.

Discussion

Caspian trout *Salmo trutta caspius* is one of the endemic fishes in the Caspian Sea and attains the greatest size, weight and growth rate of all brown trout complex (Sedgwick, 1995). Caspian trout natural populations have declined drastically in recent decades as a result of over-fishing pressure and poaching, river pollution and destruction of natural spawning areas. This fish was once caught in commercial quantities in the south, west and southwest of the Caspian Sea but now barely survives in extremely small numbers and is critically endangered according to IUCN criteria (Coad, 2000).

Although previous studies based on hatchery stocks, confirm considerable population diversity between fall-run forms (Rafiee, 2006), but there is no study on populations genetic diversity between two migratory forms in Iranian coastlines and this study is the first report on two seasonal migratory forms of Caspian trout based on RAPD –PCR marker.

RAPD technique is suggested to be more useful in closely related populations (Borowskyet al., 1995). This technique has been used as a molecular tool for detecting genetic variation and genetic similarity in several fish species including Tilapia species and subspecies identification (Bardakci and Skibinski, 1994). Brown Trout and Atlantic Salmon (Elo, et al. 1997) Largemouth Bass (Williams, et al., 1998) Spanish Barbus (Callejas and Ochando, 2002) Common Carp (Bartfai, et al., 2003) Indian carps (Barman, et al., 2003) and Tilapia (Hassanien et al., 2004), Keyvanshokoh and Kalbassi (2006) for finding molecular markers in two different geographical populations of *Rutilus rutilus caspicus* , Jamshidi et al. (2009) for finding specific bands in molecular determining hybrid of *Ctenopharyngodon idella* ♀×*Hypophtalmichthys nobilis* ♂and Keyvanshokoh et al.(2007) for sex determination in *Huso huso*. Applied RAPD markers and no specific bands were observed in these studies. Charles et al., (2005) by genotyping *S. trutta* in Oir River (tributary of River Selune in France) concluded that the resident and anadromous *S. trutta* form a single naturally reproducing unit although they

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differ by their morphological, demographical and ecological characteristics. Because the RAPD markers show dominant markers, therefore it couldn't differentiate heterozygous and homozygous fish (Weising, et al., 1995) and this could be the disadvantage of RAPD marker.The randomly amplified polymorphic DNA technique is one of the most frequently used molecular methods for taxanomic and systematic analyses of various organisms. (Bartish et al., 2000; Callejas and Ochando, 2002) concluded RAPD technique is a very advantageous and useful tool for studying the genetic structure and phylogenetics of the species and population levels of *Barbus*. Javanrouh Aliabad et al., (2004) applied RAPD markers in order to show genetic variation among six Iranian goats and they showed that RAPD technique is a useful tool for evaluating genetic variation among domesticated animals**.** Although spring-run and fall-run populations of Caspian trout differ in observed forms (body depth and color) but our analysis of the RAPD data showed very low genetic variation.

In this study, we didn't find any population-specific markers between two populations and Nei's genetic distance (Nei 1972; Lynch, 1991) for the populations was very low (0.01430). Thorpe and Sol-Cave (1994) showed that average genetic distance for conspecific populations is 0.05 (range: 0.002- 0.07) and for congeneric species is 0.30 (range: 0.03-0.61). Therefore the genetic distance of our study revealed that the two migratory forms of the Caspian trout were categorized in conspecific value. Artificial breeding of Caspian trout adults and subsequent release of juveniles into the wild is commonly performed in conservation programs to avoid extinction, because this subspecies of *Salmo trutta* is an endangered fish. Kiabi et al. (1999) identified *Salmo trutta caspius* as endangered species of the South Caspian basin.

Probably no selective programs in their artificial breeding, cause low genetic variation between each ther. Also, in conservation genetics programs this subspecies is being bred and released into the Caspian Sea Rivers for restocking management, but exact monitoring of genetic similarity and genetic distance among bred fishes is essential to avoid inbreeding problems.

In conclusion, this study represents a first step towards the initial assessment of genetic variation between two migratory forms of *Salmo trutta caspicus* from Iranian coastline of the Caspian Sea. Other genetic markers such as microsatellites, mtDNA and AFLP may serve as a better marker to detect genetic variation among them.

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