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The relation between growth hormone (GH) gene and Cytochrome b gene in three *salmon* types

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ABSTRACT

In the present study, growth hormone (GH) gene and Cytochrome b gene in *Salmo trutta caspius* and *Salmo trutta fario* were discussed, the rate of relationships between *salmonids* were analyzed by GH and Cytochrome b gene. The GH gene is a genetics marker in nuclear DNA that expressed paternal traits in *salmons*, furthermore, Cytochrome b gene also is genetics marker that expressed maternal DNA in mitochondrial genomics. With two genes we documented that there were high homology between sequences of GH gene and Cytochrome b gene, hence the *salmonids* types, specially *salmo trutta caspius*, *salmo salar* and *salmo trutta fario* probably had similar ancient in bony fishes.

Keywords: *Salmons*, mitochondrial, cytochrome b, growth hormone gene, genetics

INTRODUCTION

The *salmo* types including, *salmo trutta caspius* (S. t. caspius), *salmo salar* (S. Salar) and *Salmo trutta fario* (S. t. fario), is important for economic aquaculture industry. They live in the part of rivers and exhibit homing behavior. Therefore the difference of population related to migration of them (sometimes connected to the sea). There are studies related to physiological and genetic on the *salmons*, confirmed marker genetic differentiation (Fergusen, 1989, Guyomard, 1989).

The research on the marker of genetics most concentrated on the paternal and maternal traits in fishes. There are some genes for paternal traits specially growth hormone (GH) gene, that associated with various quantitative traits (reviewed in Peter and Marchant, 1995). However, GH gene is an important trait in other animals, like cattle that regulate somatic growth in muscle and the skeletal body (Harvey *et al.*,1995; Rocha *et al*., 1991; Hoj *et al*., 1993; Pilla *et al.,* 1994; Schlee *et al*., 1994a, b; Lagziel *et al.,* 1996), and pigs (Casas-Carrillo *et al*.,1994; Nielsen *et al.,* 1995).

Salmonid fishes have two GH genes (GH1 and GH2) resulting from their polyploid ancestry (Agellon *et al.,* 1988; Rentier-Delrue et al., 1989; Forbes *et al.,*1994). The polymorphism of GH genes has been detected and compared between *solmonids*(Gross and Nilsson, 1995).

The full length of GH gene in *salmonids* sequenced and deposited in Genebank. In *salmo trutta caspius* was found 2048 bp. (Accession number, JN241634.1), containing, five exons and six introns in the length. (Rezaei and Akhshabi, 2011 a and b; Rezaei and Akhshabi, 2012). The result of sequence in *Salmo Salar* (Johansen *et al*., 1989), however there were not report regards sequence of GH gene in *S. t. fario*, but also we can conclude the variation is low

between *S. t. fario* , *S. Salar* and *S. t. Caspius*, because the result of mitochondrial DNA that related to maternal traits had been high homology between *salmonids*. The mitochondrial genomic in *salmonids* including 13 protein coding genes, 22 transfer tRNA genes, and 2 ribosomal RNAs genes corresponding to the 12S and 16S transcripts, that was followed between the species of *salmonids* including, the *O. mykiss* (Zardoya *et al*., 1995), *S. salar* (Hurst *et al*., 1999), and other *salmonids* such as *S. alpinus* (Doiron *et al.,* 1999), *C. Lavaretus* (Miya and Nishida, 2000) and *S. fontinalis* (Doiron *et al*., 2002).

Recently, in *S. t. Caspius* for the full length of cytochrome b gene in mitochondrial DNA was found and deposited by (Jamshidi and Kalbasi, 2009) in Genebank (Accession number, JN995186), this gene has one exon in full length, the result of alignment with another sequence of cytochrome b in *S. t. Capius* and *S. Salar*, there were high homology between sequences. In this study we have two aims, first, is their relationship between someone's or *salmon* types has been common ancestor? Second, how much relationship between genetic markers engaged in paternal and maternal traits?

MATERIALS AND METHODS

Samples and DNA isolation: The samples are included, *S. t.caspius* and *S. t. fario* were obtained from the rivers of Tonekabon - Iran, the fishes both male and female had three years age old.

DNA isolation: Total genomic DNA from *S.t.caspius* and *S.t.fario* was isolated from powdered tissue has taken from muscle body following described by Sambrook et al., (1998). Briefly, the samples was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (24:25: 1). Vortexed 10x is then centrifuged at 3000 x g for 5 min at room temperature. DNA was precipitated overnight at 4°C with then washed with 2 vol. 100% ethanol. Then the DNA genomics amplified cytochrome b gene was separated by 1.5 % agarose gel electrophoresis. After electrophoresis, the DNA full length was visualized ethidium bromide and then was taken photos by gel DOC Bio RAD Company.

Designing of primers and PCR amplification: There were not any report regards GH gene in *S.t. Caspius* in Genebank, hence we used other sequences specially *S. Salar* and *S. t. fario* for designing primers, so were designed three pair of primers from first to end of the GH gene, we used a DNAMAN program (USA) and also the BLAST NCBI Network system for designing primers (Table 1). The primers could amplify three different sizes of fragments including, 910, 312 and 819 bp. The primers has also able cross amplified, means a forward primer of first fragment can match with a reverse primer of the second or third fragment, hence, we could amplify that a full length of GH gene in *S.t. Caspius.*

Table 1: the primers for synthesis of GH gene in *S. t. Caspius*

Primer	Sequence (5′ to 3′)	Product Size
Fwd Primer(SsGH1):	ACATACTCAACCGACCACCGCACTTTCAAG	910 bp.
Rev Primer (SsGH2):	GTGACAGGTCCACTCTGCTATTCA	
Fwd Primer(SsGH3):	GTAAATAGGGAATCTCAAGCTGT	312 bp.
Rev Primer(SSGH4):	CTCAAATACTTCTAGTAAGTTGA	
Fwd Primer(SsGH5):	CATCACTAATATTGACTATATCAG	819bp.
Rev Primer (SsGH6):	CAGATTAGGCCTTGCCCTGCACTGA	
Sequence Primer:	*ATCTGGTAGAGCCTGACTCCA*	

Designing primers of GH gene for *S.t. fario*:

Were designed primers of GH gene in *S. t. fario*, according to reports of sequences of GH gene in *salmonids* in Genebank, by DNAMAN program (USA) and the BLAST NCBI Network system, because we thought probably there is a high homology between *S.t. fario* and other *salmonids*. The primers could amplify three fragments, including, 1495, 1500 and 1493 bp. These primers also could amplify by crossly, means, first forward primer with third reverse primer could amplify a fragment, also these results confirmed regards other forward and reverse primer in GH gene (Table 2).

Table 2: Primers were designed for amplified of GH gene in S. t. fario.

Product size: 1495 bp.
Primer set I-Forward: AATCATCCTTGGCAATTAAGAG
Primer set I-Reverse: CCTTAGTTGAAGGCACTGAGGT
Product size: 1500 bp.
Primer set II-Forward: GCATGTTATGCCCTTTAAAACC
Primer set II-Reverse: CAGTCCTGTGGCCTTCAAGT
Product size: 1493 bp.
Primer set III – Forward: TGAACTCAAAGTCAATGAAAAGTCA
Primer set III – Reverse: AACCCTGGAGACAGGCTCTT

Primers of cytochrome b in *S. t. fario*: Primers designed to specifically amplify the cytochrome b gene based on conserving sequences from regions identified by the alignment of all the available sequence data from several salmonid species. These primers can amplify from first to end of cytochrome b gene including 1191 bp. These primers including:

Forward Primer 5' GACTTGAAAAACCACCGTTG 3'

Reverse Primer 5' CTCCGATCTCCGGATTACAAGAC 3'

The PCR programs: The PCR reaction used 10 microgram PCR reactions contained: 1 µl template DNA, 2 µl forward primer (100 ng/µl), 2 µl reverse primer (100 ng/µl), 2 µl dNTP mix (2.5mM each), 5 µl 10X ChromTaq Assay buffer, 0.5 µl ChromTaq enzyme (3U/µl), Water 37.5 µl, in a total volume, 50 µl. 94° of 5 min, 35 cycles of 94°C 30 Sec., 55°C 30 Sec., and 72°C 1 min. Two to ten µl of each PCR reaction were run on 1.5% agarose gels in TAE buffer containing ethidium bromide. One µl 500bp, DNA ladder (Gibco-BRL) was used as a size standard. Then the PCR products after purification by the Chromous kit purification were sent to the Choromous Geni Company-India for doing sequence.

Sequencing of the GH gene in *S.t.caspius*, and cytochrome b gene in *salmo trutta fario*: For sequencing of GH gene and cytochrome b gene we designed one set of primer for per gene, that process of the sequencing including:

PCR Purification: Amplified PCR product was purified using QIA quick PCR Purification Kit Protocol:

A. Added 5 volumes of Buffer PB to 1 volume of the PCR sample and mixed. **B**. Placed a QIAquick spin column in a provided 2 ml collection tube. **C**. Centrifuged at 8000 rpm for 30–60 s. **D**. Discarded flow-through. Placed the QIA quick column back into the same tube. **E**. Washed with 0.75 ml Buffer PE to the QIA quick column and centrifuged for 30–60 s. **F**. Discarded the flow-through and placed the QIA quick column back in the same tube. **G**. Centrifuged the column for an additional 1 min at maximum speed. **H**. Placed QIA quick column in a clean 1.5 ml microcentrifuge tube. **K**. To elute PCR product, added 40 µl of B H2O to the center of the

QIAquick membrane and centrifuged the column for 1 min.

Sequencing of Amplified GH gene and Cytochrome b gene: Sequencing was performed along with the Forward and reverse primers in ABI 3730XL high throughput sequencer machine. Forward and reverse sequences were assembled and edited.

RESULTS

Study variations at the DNA level contribute to the genetic characterization of *Salmons*. We used GH of gene and cytochrome b gene . According to the annotation GH genes, these are genes linked to economic traits and polymorphism genetics which are governed by many genes, Following to the sequences of the *salmon* GH gene and cytochrome b were published in the BLASTn on the National Centre for biotechnology information (NCBI) network service, was designed a fragment of almost 3kb. for *S.t.caspius* and also *S.t.fario* (Figure 1). Regarding cytochrome b gene a full length (1191 bp.) were amplified and are shown in Figure 2.

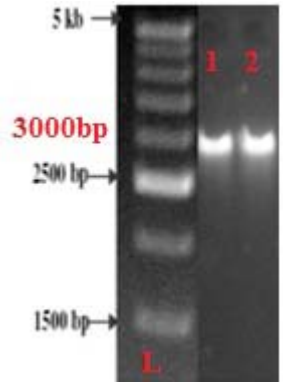

Fig.1: PCR amplification of high quality *salmo t. caspius* using the primers of designed Samples were separated by electrophoresis in 1.5% gel electrophoresis. M. Size marker 500 bp.

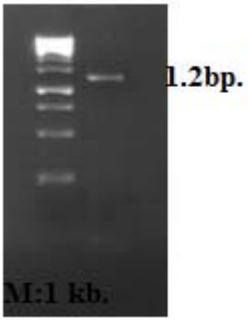

Fig. 2: PCR amplification of high quality *s. t. fario* using the primers of designed Samples were separated by electrophoresis in 1.5% gel electrophoresis. M. Size marker 1000bp.

Comparison of the GH gene and cytochrome b in the *s. t. caspius* and *S. t. fario* with other *salmons*: There are high homology between bony fishes, such as *s. t. fario*, *s. salar*, *s.* t. *caspius* and *rainbow trout* regarding marker genetics as mithocondrial genetics (cytochrome b) (Rezaei and Akhshabi, 2012) , In related to, GH gene were compared between *s. t. caspius* was recorded in Genebank (JN241634.1) with *s.salar* was recorded in Genebank (AY614010), the result showed there were high homology between sequences, in figure 3, however the number of exons of GH in *s.salar* more than *s. t. caspius* (6 exon in *s. salar* and 5 exons in *s. t. caspius*) but was similar almost the position of exons in *s.salar* and *s. t. caspius.* Moreover the number of introns in *s. salar* and *s. t. caspius* was 5 numbers that was same between

sequences, also the position of introns were similar between together of sequences.

comparisons between the cytochrome b gene in the *S. t. fario* and other *Salmons*, including *S. Salar*, *S. t. fario* and *S. t. caspius* populations by the DNA gene program. Therefore the results are shown; there is a high homology between sequences. In figure 4. A 99% DNA sequence similarity was observed, but there were five nucleotides differences from the first of the two sequences and end of two sequences between a sequence of *S. t. fario* and *S. t. caspius*. But regarding *S. salar* there was 93 percent homology between sequences. However there are some different the shape of s*almons, S. t. caspius* and *S. Salar* with s. *t. fario* but the analysis are shown homology was 93 percent, especially with *s. salar*. Moreover these results innovated that *S. t. fario* in Iran is originated from *S. Salar* that the ancestor of *S. t. fario* had migrated from the Atlantic Ocean to White Sea and then to Caspian Sea, because we collected samples has been connected from Rivers to Caspian Sea.

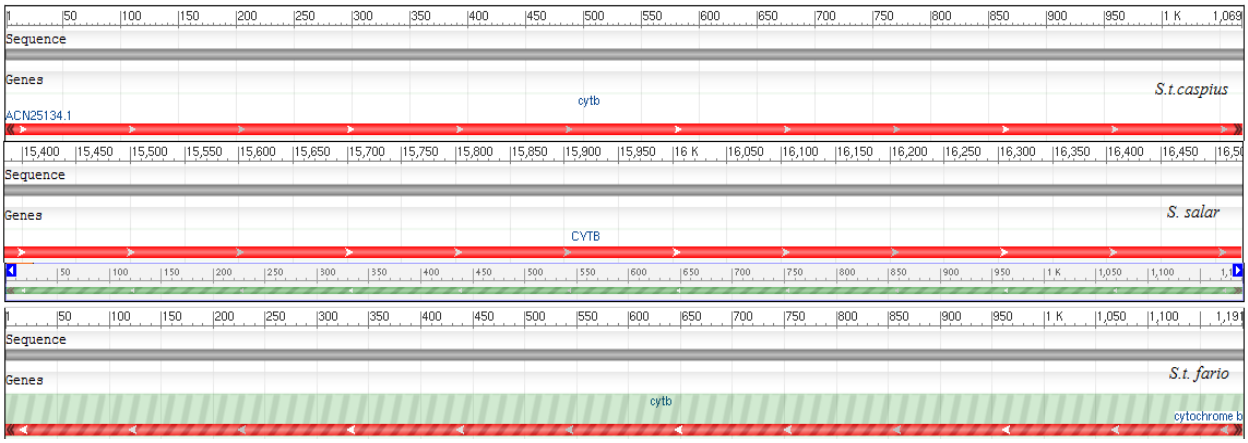

Fig. 3: Comparison of sequences of the Cytochrome b gene between *S.t. caspius*, *s.salar* and *s. t. fario*. The maximum identity is shown, there is a high homology between sequences.

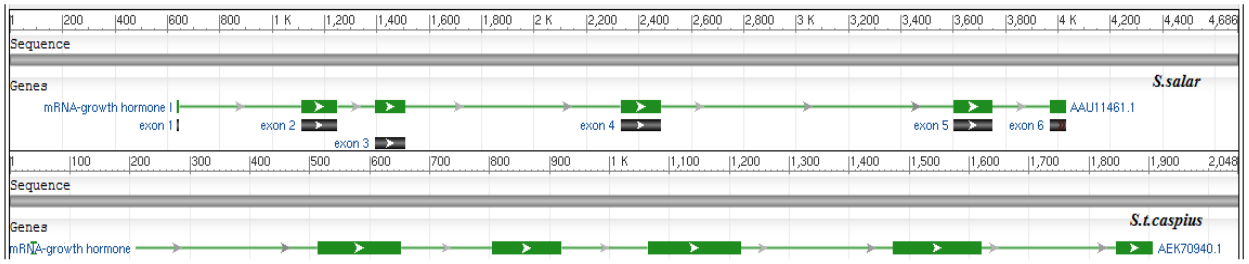

Fig. 4: Comparison of sequences of the GH gene between *S.t. caspius* and *s.salar*. The maximum identity is shown, there is a high homology between sequences.

DISCUSSION

In the present study the phylogenetic analysis of *Salmons* showed that there were a high homology between *salmons* such as *S.t. caspius*, *s. t. fario* and *s. salar* according to some genes that had cited marker genes in *salmons*, the genes such as a cluster gene from mitochondrial genomic, as Cytochrome b, Cytochrome C oxidase (I, II, III), (reviewed by Bihington & Hebert, 1991; Beckenbach, 1991; Carr and Marshall, 1991; McVeigh and Davidson, 1991; Bernatchez *et al.*, 1992; Whitmore *et al.*, 1992; Ovenden *et al.*, 1993). GH genes (GH1 and GH2), in *Atlantic salmon* (Johansen *et al.*, 1989; Male *et al.*, 1992), *rainbow trout* (Agellon *et al.*, 1988a;

Rentier-Deirue *et al.,* 1989), and *common carp* (Chiou *et al.,* 1990). Mini-satellite DNA (Fields *et al*., 1989; Taggart and Ferguson, 1990, 1991; Turner *et al*., 1991; Bentzen *et al.,* 1993; Stevens *et al*., 1993), random amplified polymorphic DNA (RAPD) markers amplified with single primers of arbitrary nucleotide sequence (Elo & Vuorinen, 1993).

However the nuclear DNA will express paternal traits and mithocondrial DNA will express maternal DNA in *salmons* but also together can have been similar aims for studies of phylogenetics analysis. In this study we sequenced both Cytochrome b and GH genes in *s. t. fario* and *s. t. caspius*, the results were compared between sequences of reported in Genebank – NCBI Network system, there were high homology between sequences of Cytochrome b and GH genes, however there is some variation in the shape of *s. t. fario* with *s. t. caspius*, and *S. salar*. in similarity the Atlantic populations there are four black stripes and variable number of small irregular black, spots white halos on the body sides. In *S. t. fario*, in hatchery trout has a bluish gray body color and no black strips, However, they are larger than *Atlantic salmon*, *s. t. caspius* but more regular in shape, and less intensively pigmented, moreover there are red spots are always observed in populations of *s. t. fario*. Nevertheless our studies showed the among of variation between *salmons* is very less(around 2-3 %) regarding Cytochrome b, GH gene, however we have to do more studies on mini and microsatellites, RFLP, RAPD and other genetics marker for getting better results. But the more reports indicated that GH gene is influence on the growth of the body and genetics marker that is part of nuclear DNA, expressed paternal traits. Furthermore, Cytochrome b and C oxidase expressed from mithocondrial DNA genomic that penetrated on the maternal traits, that's important for studies of phylogenetics and evolution of *salmons*.

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