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Preliminary studies on evolutionary of genetic markers in the *salmonid* species

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ABSTRACT

This study deals with evolutionary genetics of *salmonids* populations, with the special emphasis on the roles of migration, random genetic drift, mutation, and natural selection affecting the patterns of molecular variation across contemporary and historical time scales.

Studies of nuclear DNA and mitochondrial genomic variation supported the hypothesis that *salmonid* populations differ from the geographical regions, indicating for genetic diversity between populations. This study were used some genes for nuclear DNA genomic and mitochondrial DNA genomic for evaluation of the rate diversity. We suggest that the region of geographically is important to rate of diversity between and within populations. Were used marker genetic techniques such as the microsatellites markers, SNPs, RFLP, and some genes from mitochondrial genomic that engaged on the rate of diversity in populations of *salmonids*. Between and within population of *S. salar* and *S. trutta* were found single mutation by SNPs technique. RFLP analysis by nuclear DNA genomic such as microsatellites and growth hormone gene and also mitochondrial DNA genomic as cytochrome b and 12S rRNA gene and markers also showed the low variation between and within *salmonids* populations.

Keywords: *Salmonids* populations, Evolutionary genomic, Mitochondrial genomic.

INTRODUCTION

Salmon types and its life cycle

Salmonids family constitutes one of the most manipulated fish in temperate countries. Moreover, they are living from Eurasia to North Africa that their behaviors are migrated from Sea to rivers that including specialization for anadromous, fluviatile and lacustrine ecological modes of life (Behnke, 1968; Hamilton *et al*., 1989; Hindar *et al*., 1991). According to theory of Professor Berg (1948), *Salmonids* belongs to the phylum Chordata (the chordates), the class Osteichthyes (the bony fishes), the order Salmoniformes, the family *Salmonidae* and genus of *Salmon*. In the *Salmonid* species , there are specially fishes such as *Salmo Salar* (Atlantic salmon) lives in Europe and North America can live up to 15 years and can reach a length of 150 cm and a weight up to 50 kg (Klemetsen *et al*. 2003).

S. salar exhibit a remarkable phenotypic plasticity and variations of its life cycle that allow it to adapt to the varied temperate biogeography and seasonal climate. *S. salar* exists in both anadromous (migratory) and landlocked (freshwater) forms. Together with the Rainbow trout (*Oncorhynchus mykiss*) it is one of the most intensively studied fish species in a wide range of research areas (Thorgaard *et al*.,

2002; Rise *et al*., 2004). As most *salmonid* species, *S. salar* is divided into geographically distinct units that are more or less isolated from each other.

Numerous studies have demonstrated that *salmonids* species have distinct populations on the river or tributary basis (McConnell *et al*., 1995a; Sanchez *et al*., 1996; Koljonen *et al*., 1999; Verspoor *et al*., 1999; Spidle *et al*., 2001; King *et al*., 2001). *Brown trout, Salmo trutta fario* and *Salmo trutta caspius* belong to the nine subspecies of *Salmo trutta* in the world (Quillet *et al*., 1992). These species live in the North of Europe and America, the Southern Caspian Sea and its tributaries respectively (Quillet *et al*., 1992). This species is critically endangered anadromous. Hence, *salmonid* species have more importance in industry of aquaculture for studies of their behavior and genetics distant. Hence, these species are very rare in the world. In this study we aimed to discuss about relationship, origin ancient and the rate of the variation genetics between *salmonids.*

Studies of the evolution of DNA genomic in the *salmonids*:

Studies on the evolution of genomic DNA have been investigated on the nuclear genomic DNA and mitochondrial DNA genomic markers. The DNA genomic marker is exclusively parental traits and mtDNA sequences are almost exclusively maternally inherited (Gyllensten *et al*., 1985). There is a lot of sequence marker for the investigation of an ancient inheritance that reported by scientists. They defined as a change in allele frequencies over time. A fundamental goal of evolutionary biology is to better understand how natural selection operates in interaction with other evolutionary processes such as mutation, migration, and random genetic drift. Determination of the relative roles of single evolutionary forces that affect the genetic variation across genomes or populations is a challenging task, particularly because researchers usually depend on observations of the patterns of genetic variation within and between populations or species, to infer the *dynamic* processes that could not be directly observed (Lewontin, 2002). In *salmonids* we had very rapid growth of sequence information (Bayne *et al*., 2001; Davey *et al*., 2001; Thorgaard *et al*., 2002; Martin *et al*., 2002; Rise *et al*., 2004; Tsoi *et al*., 2004). We offer an exciting possibility to evaluate the relative roles of different evolutionary factors. Here we discussed about some molecular markers that engaged in the evolution of *salmonids*.

Polymorphism in *Salmonids*:

Mini and microsatellites: Mini and microsatellites in fact are tandemly repeated DNA sequences are 1-6 and 10-100 base pairs in the length respectively and are common throughout the nuclear genomes of eukaryotes (Jarne & Lagoda 1996). Microsatellite markers can be used to determine the population structure within and among populations (Wang *et al*., 2009a). Evaluations of population differentiation permit the estimation of the migration rate between populations, assuming that these populations are in equilibrium (e.g., no selection, identical mutation rates and generation time) (Weising *et al*., 2005).

The advantage of these tandem repeat markers stems for their very high variability which enables to efficiently apply them both at individual and population level analysis (Waser & Strobeck 1998; Manel *et al*., 2003; Hedrick, 1999; Balloux *et al*., 2000; Moss, Piertney & Palmer, 2003). Usually, tandem repeat markers are considered as evolutionary neutral DNA markers (Li *et al*., 2002). However, selection can affect the nearby flanking neutral variation, known as genetic hitch-hiking (Maynard-Smith & Haig, 1974).

In *Salmonids*, specially *S. Salar*, Terauchi and Knuma, 1994; Watkins *et al*, 1995, had reported that the level of heterozygosity observed in the four microsatellite loci the higher may be attributed to a much larger number of alleles at each locus

(0.829) and the mean heterozygosity were observed (0.69), it was observed that were a significant variation between and within populations (Devlin *et al*., 1993). Hence we can conclude in *salmonids*, especially *S. Salar* populations there were high degree polymorphism and genetic variability at the alleles of microsattelites between and within populations, however in other species of *salmonids*, individually *S.trutta* no genetic differentiation was found between populations (Todd *et al*. 2004).

Single nucleotide polymorphism (SNPs):

The vertebrate genome there is a variation polymorphism by SNPs, and their application as genetic markers are known for studies of the molecular genetic variation within and between populations. Recently were used the application of SNPs in studies of molecular variation in *salmonids*. Studies on the *salmonids* were used especially on the *S. Salar, S. trutta* and *Oncorhynchus mykiss*. Hayes *et al*. (2005) proposed that the sequences of SNPs, could in fact be a consequence of ancient duplication events in the *salmonids* genome that actually could sequence difference between ancestral duplicates. In fact the result of this study found high variation about SNPs polymorphism between and within *salmonids.* However the rate of variation between *salmonids* is different, for ex. in *S. salar* populations a lower nucleotide diversity showed (Hayes *et al*., 2005), in opposite that, Bernatchez 2001 proposed that the highest level of diversity were observed in *S. trutta* populations. Hence is difficult to explain as all individual analysis originates from the same evolutionary lineage (the Atlantic lineage). However the population of Finnish *S. trutta* was assessed in the study of Bernatchez *et al*., 2001.

Restriction fragment length polymorphism (RFLP):

RFLP from nuclear DNA genomic has been used for identity of the ancient *salmonids*. There were some genes for studies of the RFLP techniques, individually growth hormone (GH) gene. The GH gene has been studied by (Agellon *et al*., 1988; Rentier-Delrue *et al*., 1989; Gross and Nilsson 1995; Rezaei *et al*., 2011). The *salmon* types including *S. Salar, S. trutta* and *S.t. caspius*, that selected from 1825 bp. from full length of *S. salar* and 2048 bp. from *S.t. caspius*. The results showed, there were high homology between sequences of GH genes in the *Salmonids*.

Mitochondrial DNA genomic variation:

The complete DNA mitochondrial genomic contains thirteen coding gene, twenty two transfer RNAs (tRNA), two ribosomal RNAs (rRNAs) and one non-coding region that commonly known as the displacement loop (D-loop) in vertebrates. The complete sequence of mitochondrial genomic in *S. Salar, S. trutta* and *Oncorhynchus mykiss* was around 16600 bp. in the length, had deposited in GenBank database, that including sense and nonsense coding region. Studies on the mitochondrial genomic had done one by one gene of mitochondrial and also by RFLP methods for genes of the mitochondrial.

Futoshi Aranishi *et al*., 2005, proposed that the PCR-based technique developed in the study of economically important species of *salmonids* (*Alaska pollack, Pacific cod and Atlantic cod*). RFLP analysis of cytochrome b gene by direct double enzyme digestion of the unpurified PCR products proved to be a simple, reliable and rapid method showed no nucleotide mutation was found at the recognized sites of both restriction enzymes. mtDNA lineages also studied by (Bernatchez *et al*., 1992) on the *brown trout* by using of the Alu I restriction enzyme could cut the mtDNA control region at a length of about 1050 bp for two different restriction profiles. In all samples Alu I cut the control region in four places and formed five fragments of lengths of 464, 311, 252, 37 and 4 bp. The results showed that there were low genetic diversity between populations.

The cytochrome b and 12s ribosomal RNA was designed based on consensus sequences detected on different fish mitochondrial genomes extracted from the GenBank database. Following digestions, distinct single restriction enzyme patterns or mtDNA haplotypes were used by specific restriction enzymes. Were identified that 4 different native *brown trout* genotypes. All these hatchery genotypes were distributed across tributaries under restocking act. The genetic diversity associated with their populations (Bernatchez *et al*., 1992).

Cytochrome b in *s. t. fario* also were sequenced (1191 bp) by Rezaei, 2011, He had reported that there were high homology between *s. t. fario* and other *salmonids* such as *s. salar* and *s. t. caspius* populations (Rezaei and Akhshabi, 2011; Rezaei *et al*., 2011; Rezaei and Akhshabi, 2012; Rezaei *et al*., 2012; Rezaei 2012a; Rezaei 2012b).

CONCLUSION

The present study was used differences in trait association of the genetic markers may exist in different populations of *salmonids.* More tests are needed in other populations of bony fishes to variety-associated effects of genetic marker, on the ancient of the *salmonids* in the mitochondrial and nuclear DNA genome. However, the mitochondrial gene traits and nuclear genes in *salmonids* have been varied between and within populations but also studies on the phenotypic and geography is necessary. Hence these parameters are affected on polymorphism of the *salmonids.* We suggest that the region of geographically is important for rate of diversity between and within populations however the species of *salmonids* were same.

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