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RESEARCH ARTICLE

Population genetic structure of taimen, *Hucho taimen* (Pall.), in China

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Abstract: *Hucho taimen* (Pall.) is one of the largest salmonid species, and has been classified as an endangered species in China since 1998. In recent years, its wild stocks, habitats, and spawning grounds have been severely damaged by environmental degradation and human intervention. To protect and exploit this treasured species effectively and sustainably, the genetic diversity and structure of taimen populations in China was investigated using AFLP, microsatellite markers, and partial sequences of mtDNA genes. This paper summarizes these studies on the genetic diversity and structure of taimen populations in China.

Keywords: taimen, genetic diversity, genetic structure

Introduction

Taimen, *Hucho taimen* (Pall.), which belongs to Samoniformes, Salmonidea, *Hucho* genus, is one of the largest of the salmonid species (Hočík J. et al. 1988). It is a well-known, highly-prized cold water fish in China, is of high economic value, and it is ranked first among the eight prized fishes in the Heilongjiang River. In the 1960s, taimen was widely distributed in China, and large catches of it were noted in many regions, including in the Mudan, Songhua, Wusuli, Heilong, Burqin, Haba, and Ertix rivers and in Lake Kanas (Li et al. 1966, Dong et al. 1998). In the recent decades, however, the size of inventoried taimen populations has decreased dramatically. Its habitat has become fragmented as a result of environmental degradation, overfishing, and climate change, and another factor contributing to its decline is its reproductive characteristics of later maturity and weak fecundity. Only very small breeding populations exist in the Heilongjiang and Lake Kanas water systems, and it is observed very rarely in other Chinese waters (Hong 2003, Yin et al. 2003). It has been on the Red List of Endangered Species since 1998 (Yue et al. 1998). Thus, it is imperative for researchers to protect this fish with effective methods as soon as possible. While investigations on the population structures, stocks, and artificial production of this species have been conducted, it is also important to learn about genetic structure of natural taimen populations, and to implement measures to restore them. AFLP, SSR, and mtDNA were used to analyze the genetic diversity and structure of taimen populations in China.

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AFLP analysis of genetic structure

An AFLP technology system was created for analyzing taimen (Tong et al. 2008). Twelve primer pairs were used to amplify genomic DNA samples from 20 wild individuals caught in the Huma River. Four-hundred and seventy-seven loci were polymorphic, and the percentage of polymorphism was 84.43 %. The mean Nei genetic diversity index was 0.3867, and the mean Shannon information index was 0.5102, which indicated relatively higher genetic polymorphism. Twenty individuals were divided into two groups according to phylogenetic trees, and these indicated there was serious inbreeding within the taimen populations (Kuang et al. 2007).

Another round of AFLP analysis was conducted with seven pairs of primers on five wild *H. taimen* stocks (HM, HTA, HTB, HQ, ZJ), which included 104 individuals. There were 193 polymorphic sites in total at a rate of 70.18 %. Amplified alleles (*N*a), effective alleles (*N*e), Nei genetic diversity indexes (*H*), and Shannon information indexes (*I*) had the same variation trend of HTA>ZJ>HQ>HTB>HM. The Nei genetic diversity indexes (*H*) were between 0.148 and 0.1953. The Shannon information indexes (*I*) were between 0.2215 and 0.2931. The genetic diversity of the five groups was low, and all five groups had close genetic relationships, which meant that individuals in one stock could be clustered together with phylogenetic analysis. This indicated that serious inbreeding occurred within these five stocks (Tong et al. 2009).

SSR analysis of genetic diversity and structure

Four microsatellite DNA libraries were constructed by enriching (ACA)n, (CAG)n, and (CA)n motifs with magnetic beads from genomic DNA and cDNA. In 2006, a microsatellite DNA library was first constructed by enriching (ACA) motifs with magnetic beads. This library contained 2100 colonies, and 686 positive clones were obtained by screening the library with the radioactive labeling probe P^{32}-(ACA)n. One-hundred and forty positive clones were sequenced, and 149 microsatellite 1oci were characterized (Tong et al. 2006a). Six polymorphic microsatellite markers from this genomic library were identified (Tong et al. 2006b). In 2011, two microsatellite libraries with (CA)n and (CAG)n motifs were constructed with the following six restricted enzymes: Sau3AI, *Eco*RI, *Bfa*I, *CviQ*I, *Tru9*I, *Taq*I. Consequently, one thousand clones were screened using forward primers $M13^{+}$ or $M13^{-}$ and reverse primers $(CA)_{10}$ or $(CAG)_{7}$. After sequencing these clones, about 900 SSRs were characterized, and 700 pairs of primers were designated with the self-developed Perl script. Forty polymorphism markers were identified with wild stock samples. At the same time, twelve polymorphic EST-SSR microsatellite markers with (CA)n motifs were developed from the cDNA library (Wang and Kuang 2011).

In 2004, 30 microsatellite markers derived from rainbow trout, *Oncorhynchus mykiss* (Walbaum), were obtained from NCBI and used to analyze the genetic diversity of taimen from the Wusuli River. Ten microsatellite markers were used to asses the polymorphism and six markers were used to assess the genetic diversity of 17 individuals. The allelic frequency ranged from 0.0455 to 0.7857, the polymorphic information content (PIC) values ranged from 0.2801 to 0.6351, and the heterozygosity values from 0.3368 to 0.6563. The genetic diversity of the taimen was not great at this time (Liang et al. 2004).

In 2009, 135 individuals from four wild stocks of the Huma (HM), Hutou (HT), Haiqing (HQ), and Zhuaji (ZJ) populations in the Heilong River were analyzed using 17 microsatellite markers. Genetic parameter (*Ao*, *Ae*, *Ho*, *He*, *PIC*) statistics showed that the genetic diversity of each population in terms of the value of each parameter was at a medium level as follows: HT>HQ>ZJ>HM. More specifically, the mean effective alleles (*Ae*) for all the populations were 3.2581, 3.2072, 2.9906, and 2.7580, respectively; the mean expected heterozygosity (*He*) was 0.5735, 0.5570, 0.5532, and 0.5091, respectively; and the mean polymorphic information content (*PIC*)

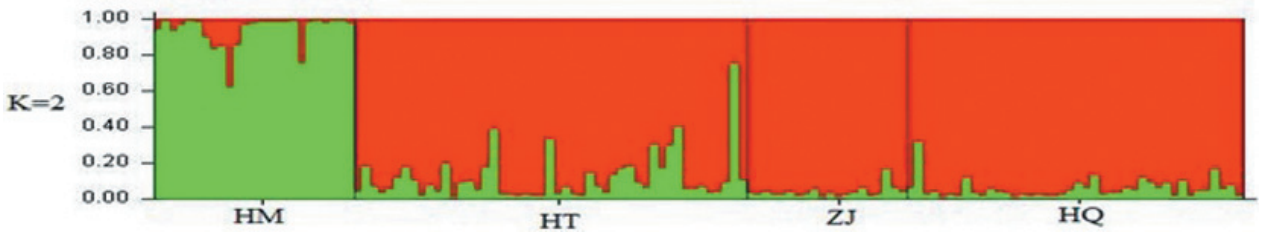

Figure 1. Analysis of the genetic diversity and structure of taimen (*H. taimen*) populations.

was 0.5228, 0.5094, 0.4973, and 0.4607, respectively. Genetic diversity was on the decline in the HT populations as indicated by the comparison of two data sets that were sampled separately in 2002 and 2006. The genetic diversity of taimen in Heilongjiang River decreased annually, and genetic differentiation was remarkable among the populations. The taimen in Heilongjiang River was divided into two genetic types, HM and WSL (Fig. 1), and the WSL type was further subdivided. Strong inbreeding stress was incurred because of the small size of the effective population. In the HM population, up to 80 % of tested samples were sibs, which resulted in this population having the largest number of siblings. The immigrant and emigrant rates in each population were asymmetrical, and the large population migrated to the small population (Kuang et al. 2009a).

In 2011, nine wild taimen populations (HH, BJ, BH, LG, HM, XK, HQ, ZJ, HT) were investigated using 20 microsatellite markers. The heterozygosity observed ranged from 0.0994 to 0.8882, the expected heterozygosity ranged from 0.2005 to 0.8759, and the PIC index ranged from 0.3432 to 0.5261. The HM population had the lowest genetic diversity. There was a high inbred pressure, and the risk of bottlenecks within most groups was similar to the findings of Kuang et al. (2009b). All individuals from the Huma and Wusuli rivers were clustered as one clade, whereas all individuals from the upper reaches of the Heilongjiang River were clustered as another clade. According to the two studies with SSRs, it can be concluded that decreased taimen stocks had affected gene exchange among populations, and recent gene flow has been lesser than long-term gene flow. Gene exchange has been blocked because of drastic environmental degradation and decreased populations determined during inventories (Liu et al. 2011).

mtDNA analysis of genetic structure and population evolution

The Cox1 and ND1 gene sequences of mtDNA were used to analyze the genetic structures and evolution of nine taimen populations (HH, BJ, BH, LG, HM, XK, HQ, ZJ, HT) in the Heilongjiang River. The size of 1500 bp fragment was amplified with the Cox1 gene, and ten haplotypes were identified in 30 samples. The average distance of sequence divergence was 0.0013, and the mean haplotype diversity and the mean nucleotide diversity were 0.5185 and 0.0012, respectively. Meanwhile, the size of the 1000 bp fragment was amplified with the ND1 gene, and seven haplotypes were identified in 30 samples. The average distance of sequence divergence was 0.0006, and the mean haplotype diversity and the mean nucleotide diversity were 0.4556 and 0.0009, respectively. Nine populations were of a medium level of genetic diversity. Although nine populations were divided into four sub-populations based on the values of the pairwise *F*st, these nine geographic stocks shared one common haplotype (BH11) identified with haplotype network analysis (Fig. 2), which demonstrated that gene flows existed in these nine stocks, and that they had evolved from one ancestor. A 41.67% share of the Wusuli River population had common haplotypes, and they retained the original taimen haplotype (Kuang et al. 2009b).In summation, the results of these three different analytic

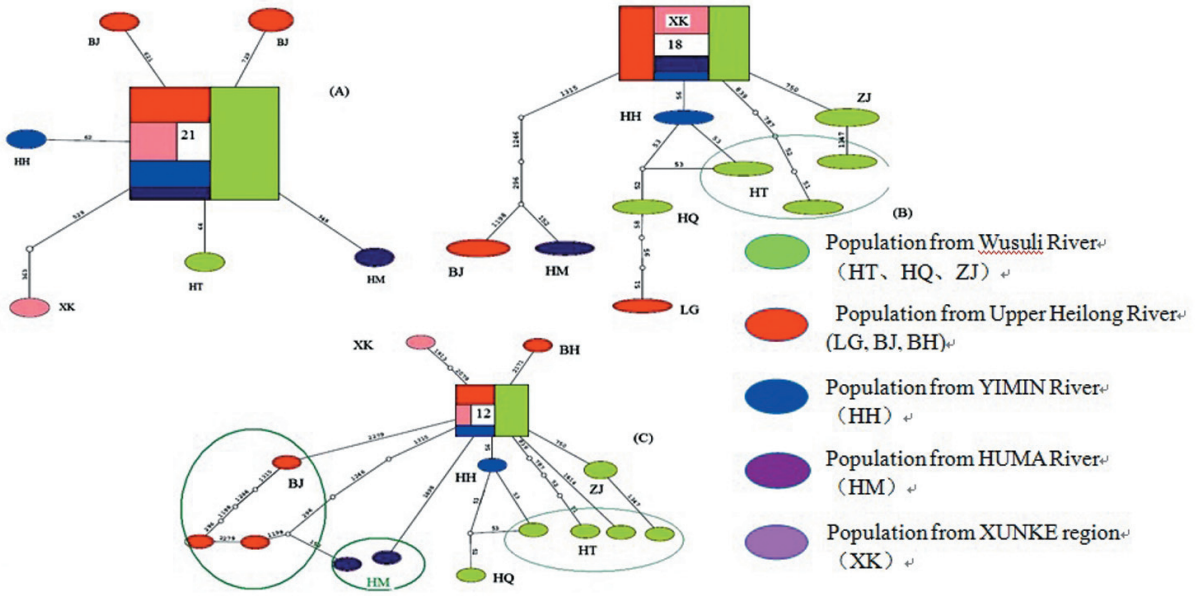

Figure 2. Parsimony networks of Cox, ND1, and combined data haplotypes observed in taimen (*H. taimen*) from Heilong River Basin. Note: Haplotypes are indicated by squares and ellipses, size denotes haplotype distribution frequencies, the small circle represents putative steps between haplotypes. Different colors represent different geographic regions. The populations from the Wusuli River are HT, HQ, and ZJ; populations from the upper Heilong River – LG, BJ, BH; population from the Yimin River – HH; population from the Huma river – HM; population from the Xunke region – XK.

methods produced similar results indicating that the genetic diversity of taimen in China is presently at a medium level, and that it is under serious inbreeding pressure. The small size of the effective population has resulted mainly from habitat degradation, overfishing, and climate change as well as from the reproductive characteristics of this species of later maturity and weak fecundity instead of low levels of genetic diversity. Taimen will remain endangered if measures to protect its wild populations are not implemented. By 2001, artificial taimen breeding had become successful in China (Xu et al. 2003), and large-scale production and comprehensive taimen farming technology had been developed in China (Xu et al. 2008). In 2005, a stocking program was initiated, and to date, about 50,000 juvenile fish have been released to historical taimen habitats. Because of the specific genetic structure of the taimen in Heilongjiang River, the genetic divergence of populations is avoided by stocking geographical populations in order to increase gene flow among populations.

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