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Study on the Population Genetics of the Great Sturgeon (*Huso huso*) in the Southern Part of the Caspian Sea Using Microsatellite Loci

Hassan GHADIRNEJAD*[1] Bin ZHU[2] Jianbo CHANG[2]

[1] Golestan Fisheries Research Centre (GFRC), P. O. Box 139, Gorgan, Iran

[2] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

Corresponding Author
e-mail: Ghadirnejad@yahoo.com

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Abstract

Fourteen sets of microsatellites primers were tested on randomly selected individuals of great sturgeon, *H. huso*. Five sets were developed for lake sturgeon (Afu-19, 34, 39, 54, and 68) and seven sets were developed for Adriatic sturgeon (An-0, 1, 16, 20, 40, 76, and 77) and finally two sets were developed for Chinese sturgeon (As-73, and 74). Except two sets of Adriatic sturgeon primers, An-76, and An-16, the rest of primers reproducibly amplified the beluga sturgeon DNA. Five pairs of primers out of 14 have been selected and confirmed to be used for separating the populations. Those primers are; Afu-19, Afu-39, Afu-54 from lake sturgeon; As-73 from Chinese sturgeon, and An-77 from Adriatic sturgeon. The samples from the east region of the southern Caspian Sea did not amplify at Afu-19, Afu-39, Afu-54, and An-77 loci. However, samples from middle and west regions of the Caspian Sea did amplify at Afu-19, Afu-39, Afu-54, An-77 loci in different manners of producing the bands. Samples from all three regions did amplify at As-77 locus. However, the bands at the samples from the east region are distributed differently from the bands at the samples from the middle and west regions. Statistical analysis revealed that the number of alleles per locus ranged from 6 to 11 and one locus had at least six alleles. Population differentiation combination test (Fisher's method) based on both genic differentiation and on genotypic differentiation (G-based) for the two sets of samples demonstrated that the probabilities are highly significant. It means possibly two great sturgeon populations exist in the southern part of the Caspian Sea.

Key words:

INTRODUCTION

The composite stocks of sturgeon species represent, economically, the most valuable fish resource of Iran. Confined to the Caspian Sea, the fishery has a high export value earning significant foreign exchange each year Because of international developments and lack of accord between the littoral States, the sturgeon resource has been subjected to deleterious fishing and environmental pressures over the last decade.

There are five species of sturgeon in the Caspian Sea which are of economic importance to Iran. The various populations of sturgeon species in the Caspian Sea are under threat. Catches throughout the whole area have decreased. Since 1982 production has fallen by more than 90 % to only a little over 1,000 t in two decades and the situation gives cause for much concern.

In addition to indiscriminate malpractices in capture, the sturgeon is particularly vulnerable to pollution. Oil extraction, expansion of urban areas, increasing industrialization, and agricultural waste are beginning to be found in greater concentrations to the detriment of all fish stocks including sturgeon. This is becoming more prevalent and the regimes of various rivers have been seriously affected.

Similarly, the construction of dams, hydro electric schemes and irrigation projects cause considerable disruption and change to the ecology of the river systems. It is known that such work on the River Volga has resulted in the destruction of large areas of sturgeon breeding grounds. Consequently, the natural spawning of various sturgeon species are very low and the recovery of wild stocks from the fishing activities mostly dependent on the fingerling production and release into the Caspian Sea.

The technology for the propagation of sturgeon in Iran has been well developed and a continuing program for the enhancement, or more correctly, rehabilitation of the resource established since 1973. The practices required for propagation are well established. However, there is a need to define where the brood stocks should be taken from. In another word how many populations of each species of sturgeon are there in the southern part of the Caspian Sea?

It means that the juvenile production should come from genetically selected brood stocks. The Caspian Sea sturgeon fishery is almost entirely dependent on culture based fish; however, there is little attempt to preserve the biodiversity and gene pools of the native stocks or to place a high priority on research and training in fish genetics or brood stock management.

Interest in the restoration of sturgeon, as part of ecosystem rehabilitation of the Caspian Sea, has become more clearly defined and continues to expand at the Iranian Fisheries Research Organization (IFRO) and the Iranian Fisheries Organization (IFO). Sound conservation of fishery resources requires a fundamental understanding of how populations are structured genetically and the effects of anthropogenic forces on partitioning of genetic diversity. Therefore, genetics issues are at the forefront of sturgeon enhancement efforts.

To better guide sturgeon restoration and enhancement efforts in the southern Caspian Sea, resource managers need a better understanding of the genetic structure among sturgeon populations. Some Iranian Fisheries Research Organization (IFRO) personnel from Gilan, Mazandaran, and Golestan provinces are all collecting tissue samples for genetic analysis; however, there is currently limited applicable genetic

information to support sturgeon recovery efforts within the southern basin of the Caspian Sea.

Scientists have developed species-specific DNA markers to aid in the understanding of gene mixing among sturgeon sub-populations. This is vital to proper management of genetic diversity. Specific nuclear DNA markers dubbed "microsatellites" are among the most useful that researchers have developed. These markers have identified a significant amount of previously undetected genetic variability that has proven useful in identifying subpopulations in sturgeon species.

Advantages of microsatellites as molecular markers include their high mutation rate, co-dominant inheritance, easy scoring of the alleles, reproducibility, and accessibility to laboratories lacking highly sophisticated analysis equipment. In addition, microsatellite loci can be scored from tissues non-destructively sampled (e.g., muscle, fin, hair, blood, feces, scale, feather) and preserved by freezing, drying, or stored in alcohol or lysis buffer. Microsatellite markers designed for one species could often be amplified successful with other related species [3].

Here we present a preliminary attempt to find out how many great sturgeon populations exist in the southern region of the Caspian Sea. Finding answer for this fundamental question would certainly help the conservation biologists as well as enhancement program managers at the IFC and IFRO to receive updated knowledge about the genetic population structure of the great sturgeon, *H. huso*, in the southern part of the Caspian Sea.

Consequently that would improve the brood stock management for artificial propagation and would save the gene pool and biodiversity of this valuable endangered species. Moreover, it would certainly increase the quality of the sturgeon rehabilitation program, including the great sturgeon, which currently runs in the region by the IFC and monitored by IFRO, via introducing the number of populations and the brood stocks which must be taken from each population for the artificial propagation and release of the fingerlings accordingly. Finally, to answer the question of the present study would save all the sturgeon conservation endeavors, time, and capital put in, not to be wasted to some extend.

MATERIALS and METHODS

Samples

One hundred specimens of great sturgeon *H. huso* from 3 landing areas along the southern coasts of the Caspian Sea from 2005-2006 fishing season used as samples in the present study. Portions (1cm^2) of dorsal fin from each specimen was collected and fixed in absolute alcohol (ethanol 100%) and kept in the refrigerator at 4°C. Morphometric and meristic as well as other biometrical data for each specimen were recorded for further analysis. Each landing area covers some of sturgeon fishing stations from the west to the east coast of the southern shoreline. Namely from the west, Anzali port landing area (W), located in the Gilan Province, which covers 24, Babolsar landing area (M), which is in the Mazandaran Province, that includes 22, and Ashooradeh landing area (E), that located in the Golestan Province, which covers 5 fishing stations. All samples replaced in the small Gama counter test tubes and transferred to the molecular biology laboratory of the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China, for further genetic analysis.

Amplification of microsatellite loci

Genomic DNA was extracted with 3S Spin Genomic DNA Miniprep Kit V3.0 (Shenergy Biocolor Bioscience & Technology Company). Quality and quantity of DNA were examined by agarose gel. Twenty one samples out of hundred were chosen as to be the best for further microsatellite experiments. The samples renamed as E04, E09, and so on. It means sturgeon sample number four from east region, of the Golestan province's shoreline. Samples from middle, and west region were renamed in the same manner. For example; M01, means sample number one from the middle region, of the Mazandaran Province's shoreline; and W04 means sample number four from the west region, of the Gilan Province's shoreline. Fourteen sets of microsatellite primers were tested on randomly selected individuals of great sturgeon, *H. huso*. Five sets were developed for lake sturgeon (Afu-19, 34, 39, 54, and 68; [1 1]) and seven sets were developed for Adriatic sturgeon (An-0, 1, 16, 20, 40, 76, and 77; [18]) and finally two sets were developed for Chinese stugeon (As-73, and 74; [20]). Amplifications were performed in a PE 2400 GeneAmp PCR System using a 10-μl reaction mixture. Each reaction mixture contained 0.5 unit of *Taq* DNA polymerase (Biostar), 0.6 μM of each primer, 5-7 ng template DNA, 150-175 μM dNTPs, 1.5mM $MgCl_2$ and 1-μl of reaction buffer. Cycling conditions were as follows: denaturation at 94°C for 4 min; 30 cycles of 30 s at 94°C, 30 s at 52°C, 30 s at 72°C; and final extension at 72°C for 5 min for lake sturgeon primers; denaturation at 94°C for 5 min; 30 cycles of 40 s at 94°C, 40 s at 60°C, 50 s at 72°C; and final extension at 72°C for 5 min for Chinese and Adriatic sturgeon primers. Following amplification, 4-6 μl of PCR product was mixed with 2 μl loading dye buffer and electrophoresed in a 10% nondenaturing polyacrilamide gel electrophoresis (PAGE) in 1×TBE running at 200 V for 3 h. Allele sizes were estimated in relation to the pBR322/Msp I ladder (Promega). After electrophoresis, gels were stained and visualized with ethidium bromide bath for 20 minutes and then scanned and photographed with an Ultraviolet Gel Document System (Biolab).

Statistical analysis

The data at the first attempt were analyzed by direct observation of the PAGE gel images. Selected gel images then being observed and confirmed that whether special bands are exist on the electrophoresis gels in order to use them for differentiation of potential populations or not. Moreover, statistical analysis on data carried out using genetic analysis software; GeneTool, and Genepop.

RESULTS

Applicability of primers

A total 14 sets of primers from lake sturgeon (LS), *A. fulvescens*, (5 pairs; Afu-19, Afu-34, Afu-39 Afu-54, Afu-68), Chinese sturgeon, *A. sinensis*, (2 pairs; As-73, As-74), and Adriatic sturgeon, *A. naccarii*, (7 pairs; An-0, An-1, An-16, An-

20, An-40, An-76, and An-77), have been tested for amplifying the great sturgeon, *H. huso*, genomic DNA. Except two sets of Adriatic sturgeon primers, An-76, and An-16, the rest of primers reproducibly amplif ed the beluga sturgeon DNA.

Gel pattern of the samples

Five pairs of primers out of 14 have been selected and conf rmed to be used for separating the populations. Those primers are; Afu-19, Afu-39, Afu-54 from lake sturgeon; As-73 from Chinese sturgeon, and An-77 from Adriatic sturgeon

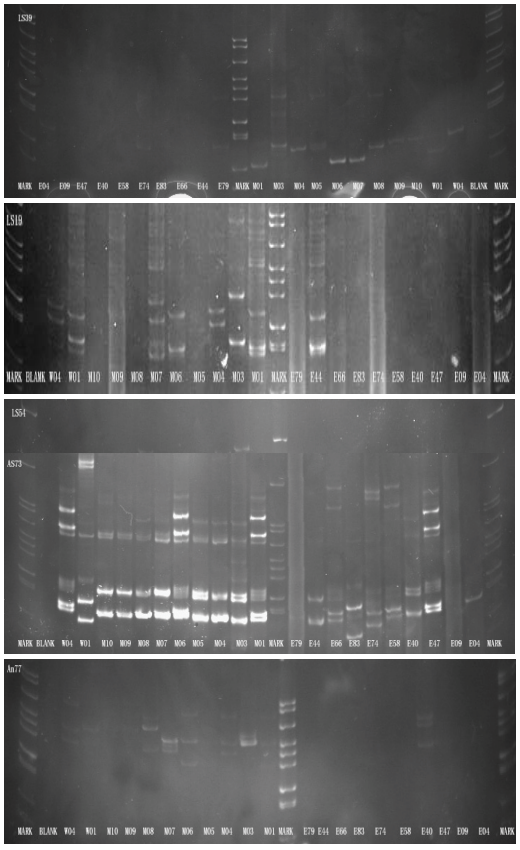

Figure 1. Genotype pattern at microsatellite loci in the *H. huso* from the southern Caspian Sea.

The samples form the east region of the southern Caspian Sea did not amplify at Afu-19, Afu-39, Afu-54, and An-77 loci. However, the samples from middle and west regions of the Caspian Sea did amplify at Afu-19, Afu-39, Afu-54, An-77 loci in dif ferent manner of producing the bands. The samples from all three regions did amplify at As-77 locus. However , the bands at the samples from the east region are distributed differently from the bands at the samples from the middle and west region.

Statistical analysis

The number of alleles per locus ranged from 6 to 11 (mean value: 4.8), and one locus had at least six alleles. Microsatellite loci have shown dif ferent numbers of alleles at the two sets of samples that are as follows; Afu-19, 1; Afu-39, 0; Afu-54, 4; As-73, 8; and An-77, 3 alleles in the east samples and Afu-19, 7; Afu-39, 6; Afu-54, 8; As-73, 3; and An-77, 6 alleles in the middle and west samples. Microsatellite loci also have shown different genotypes at the two sets of samples which are as follows; Afu-19, 1; Afu-39, 0; Afu-54, 2; As-73, 8; and An-77, 1 genotypes in the east samples and Afu-19, 7; Afu-39, 11; Afu-54, 7; As-73, 11; and An-77, 5 genotypes in the middle and west samples. Linkage Disequilibrium test for each pair loci in each set of samples was not possible. Hardy Weinberg Exact Tests Probability for all loci and for all samples (Fisher's method) revealed that it is highly signi f cant. Population differentiation combination test (Fisher's method) based on both genic differentiation and on genotypic dif ferentiation (G-based) for the two sets of samples demonstrated that the probabilities are highly signif cant.

DISCUSSION

12 sets of microsatellite primers out of 14 produced replicable amplicons in *H. huso*. These results suggest that there is evolutionary conservation of the f anking regions for these loci among related taxa. The cross amplif cation between lake stur geon, Chinese stur geon, Adriatic stur geon, and great stur geon is consistent with earlier f ndings that primers developed in one species often work in other related species [11; 4; 16; 19].

Some individuals for the f ve loci we used exhibited banding patterns with more than two bands and asymmetry in band intensities. The multiple banding patterns observed at some loci are consistent with tetrasomy in *H. huso* as has been shown for *A. fulvescens* [14; 12] and for *A. sinensis* [19] and the single banding patterns observed at some other loci are also consistent with disomy in *H. huso* [10]. The debate is still going on about the ploidy level of species with ~120 chromosomes including *H. huso*. Some authors believe that all species with ~120 chromosomes are tetraploid [13; 5; 1; 2]; others call them functionally diploid [6; 7, 8; 9; 17].

If great stur geon is considered as tetraploid stur geon species it would have important consequences for the study of population structure. First it would be dif f cult to determine whether speci f c bands represent one, or more, copies of the relevant allele, especially in individuals with one or two bands. Secondly, it would be diff cult to test for the occurrence of null alleles in *H. huso* from population data because it is diff cult to determine the difference between 2: 1 and 3: 1 intensity rations in two banded individuals. Thirdly, observed vs expected heterozygosity values are not possible to calculate, limiting the types of analyses can be performed [15; 19].

Microsatellite loci have shown different numbers of alleles at the great sturgeon samples, of the present study, meaning that we possibly can assume there are two different populations. On the top of that microsatellite loci also have shown dif ferent genotypes at the great stur geon samples which support our assumption. Moreover, population dif ferentiation combination test (Fisher's method) based on both genic dif ferentiation and on genotypic dif ferentiation (G-based) demonstrated that the probabilities are highly signi f cant, which again approve and conf rm that, there seems to be two great stur geon populations in the southern part of the Caspian Sea.

With these highly polymorphic genetic markers, it is also possible to distinguish the arti f cially and naturally propagated individuals among juvenile samples of great stur geon in the

estuaries of S f d Roud River and Gor gan Roud River in the further studies. Artif cial breeding practices may inadvertently decrease the genetic variation of the great stur geon population by breeding related individuals or by the use of small numbers of parents as brood stocks. The high levels of variation in microsatellite loci make them very useful for the estimation of relatedness between potential breeding pairs, and more intensive breeding regimes based on these polymorphic markers should be employed to avoid loss of genetic variation within the great sturgeon population.

CONCLUSION

This study indicates the moderate to high levels of polymorphism in *H. huso* detectable with microsatellite primers developed from Adriatic stur geon, lake stur geon and Chinese sturgeon. Results of the present study demonstrate that; we may assume the samples of east region are from dif ferent population than the middle and west part. It means possibly two great sturgeon populations exist in the southern part of the Caspian Sea.

This is just a preliminary study and it is necessary to continue the study in order to increase the certainty of the assumption of having two populations in the southern part of the Caspian Sea. However, based on the preliminary results we can suggest to the IFO that for the great stur geon rehabilitation and enhancement program it seems to be necessary and wise to use brood stocks for the artif cial propagation from both populations.

Microsatellite loci have co-dominant expression, high genetic variation and mutation rate, and also the ability to use non lethal sampling, they have the potential to be of great use in monitoring changes in genetic variation and investigation of population structure, specially in threatened and endangered species including the great stur geon, *H. huso* from the Caspian Sea.

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