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Genetic structure of Caspian populations of stellate sturgeon, *Acipenser stellatus* (Pallas, 1771), using microsatellite markers

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Abstract

Information about genetic relationships between populations of the stellate sturgeon *Acipenser stellatus* is scarce, despite its being commercially the most important and ecologically the most unique species in the Caspian Sea. We conducted a large-scale genetic analysis on diversity at the molecular level of stellate sturgeon populations at three sites (the estuaries of the Rivers Ural, Kura and Sefidrud) around the Caspian Sea, by tests on the genomic DNA with 15 sets of microsatellite markers (*LS-19, 34, 39, 54, 57, 62, 68, 69, Spl-105, 104, 163, 168, 170, 173, 113*). Ten primer sets were used to analyze the genetic variation in adults of the three populations. The analyses revealed that the average of alleles per locus was 12.1 and all the sampled regions contained private alleles. The observed and expected heterozygosity averaged 0.674 and 0.860, respectively. The F_{ST} values ranged between 0.050 to 0.055, showing a significant difference between the three sites ($P < 0.01$). The genetic distance between populations was 0.530, thus indicating that the genetic difference among populations is pronounced. These results, combined with the highly significant R_{ST} of genotypic difference between pairs of collection sites, provide useful information on the genetic variation and differentiation among the Caspian Sea populations of the stellate sturgeon.

Keywords: *Acipenser stellatus*, Genetic structure, Microsatellite markers, Caspian sea

Introduction

The stellate sturgeon plays an important role in biodiversity and commercial harvest of the Caspian Sea, being also listed as endangered species by IUCN (Pourkazemi 2006). Since April, 1998, its trade is regulated under the Convention on International Trade in Endangered Species Fauna and Flora (CITES). Overfishing and illegal catches are the main causes of decrease in stocks of this species and of the number of adult spawners (Pourkazemi 2006).

Despite its commercial and ecological importance, information on the genetic relationships among populations and the diversity at the molecular level in stellate sturgeon is scarce. This has been so far limited to a few studies using RFLP methods (Pourkazemi 2001; Shabani et al. 2006), which showed low genetic variation while no significant differences in haplotype frequency were stated.

Microsatellites are frequent in stellate sturgeon genome and can easily be amplified with PCR; they denote high levels of allele polymorphism. These features, taken together, offer the basis for a successful analysis in a wide range of fundamental and applied sectors of fisheries and aquaculture (Sekar et al. 2009).

Microsatellite genotypes are particularly helpful for detecting the structure of closely related populations, regardless of whether they are in an evolutionary equilibrium. Additionally, primers designed for one species can often be used with other related species (Chistiakov et al. 2005).

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The development of management plans and the implemention of actions to restore stellate sturgeon within its native stocks can benefit from an understanding of the genetic diversity of its populations. This information is helpful in choosing donor populations to use as sources of reintroduction, as well as in formulating restoration goals regarding the population structure. This study was aimed at analyzing inter-population genetic diversity in stellate sturgeon by the analysis of microsatellites.

Materials and methods

Sampling

The fishes were caught from three different regions, including 43 samples from Ural (Kazakhstan), 49 samples from Kura (Azerbaijan) and 43 samples from Sefidrud estuary (Iran) (Fig.1). Fin tissue samples were prepared from 135 fishes of each location and preserved in 95% ethanol and stored at room temperature.

Fig. 1. Map showing the sampling sites of stellate sturgeon at Ural, Kura, and Sefidrud estuaries.

DNA extraction and microsatellite analysis

The genomic DNA was extracted following the method described by Pourkazemi et al. (1999). The quality and concentration of DNA were assessed by 1% agarose gel electrophoresis and then stored at -20 °C until use.

The nuclear DNA was amplified using 15 microsatellite primers designed for *Acipenser* and *Scaphirhynchus* (*LS-19, 34, 39, 54, 57, 62, 68, 69,* May *et al*. 1997; *Spl-104, 105, 113, 163, 168, 170, 173*, McQuown et al. 2000. Polymerase Chain Reaction (PCR) conditions for each primer set was optimized for stellate sturgeon. The annealing temperatures were: 56 °C for *LS-19* and *Spl-113*, 58 °C for *LS-34* and *Spl-163 /170*, 59 °C for *LS-54* and *Spl-105*, 61.2 °C for *LS-68*, 57 °C for *Spl-104*, and 58.5 °C for *Spl-173*. Polymerase chain reaction was performed in 20 µl volume containing 100 ng of template DNA, 0.5-1 pmol of each primer, 200 mM each of the dNTPs, 0.5 U of *Taq* DNA polymerase and 1-2.5 mM $MgCl_2$. PCR products were separated on 6% polyacrylamide gels (29:1 acrylamide: bis-acrylamide; 1X TBE buffer) and followed by silver-staining. The gels were run at 170 W for 2h and 30 min. Alleles were sized using BioCapt software, and each gel contained an allelic ladder (50bp) to assist in consistent scoring of alleles.

Allelic frequencies, observed and expected heterozygosities, genetic distance (Nei 1972), genetic identity (Nei 1972), F_{ST} and R_{ST} value, *Nm*, Hardy-Weinberg (*HW*) tests of equilibrium, and AMOVA (Analysis of Molecular Variance) were computed in GeanAlex 6.0 software (Peakall and Smouse 2005). The UPGMA dendrograms of genetic distance (Nei 1972) were obtained in TFPGA (version 1.3).

Results

Overall 10 loci were investigated; four sets (*LS-69, 57, 62* and *Spl-168*) did not show any flanking sites on stellate sturgeon genome and one set (*LS-39*) showed a monomorphic pattern. The allele frequencies at all loci are given in Table 1. The average number of alleles found *per* site was 12.1, and in each locus the range was from 13 (*LS-34*) to 17 (*Spl-173*) alleles. Out of 165 observed alleles, 110 alleles occurred at frequencies of less than 0.05 in all samples. *LS-54* showed the maximum variability, ranging in frequency from 0.051 to 0.360. All the populations sampled contained private alleles at a significant level ($P < 0.05$). For example, marker *Spl-105* identified four private alleles (one at the frequency of 0.151, the others at the frequencies of 0.116, 0.093, and 0.058) in Ural samples; marker *Ls-34* identified one private allele (at the frequency 0.122) in Kura samples and *Spl-105* three private alleles in Sefidrud estuary samples (at the frequency of 0.276 and the other at the frequencies of 0.209 and 0.058), neither of which was found in the other sites (Table 2).

The observed and expected heterozygosity averaged 0.674 and 0.860, respectively; the observed heterozygosity ranged from 0.349 in Sefidrud estuary to 1 in Kura (Table 1). In all cases, deviations from Hardy-Weinberg equilibrium were significant ($P < 0.01$), except for *LS-68* in Sefidrud and *Spl-104* in Ural (Table 1).*The* $F_{ST,}$ R_{ST}, and gene flow, as analyzed with *AMOVA*, showed a significant genetic differentiation among sites ($P < 0.01$), which suggested that the populations diverged from each other. Values pair wise of R_{ST} among samples were consistently much higher (as much as an order of magnitude) than equivalent F_{ST} values (Table 3) but the difference was not significant ($P > 0.05$). The genetic distance computed by Nei (1972) between the Ural and Kura populations was 0.547, Ural and Sefidrud 0.522, and Sefidrud and Kura 0.519. The mean genetic distance was 0.530 (±0.08) between the populations.

Table 1. Numbers of allelles observed within 3 sampling sites using 10 sets of microsatellite primers. Number of studied samples (*n*), Observed (*Ho*) and expected (*He*) heterozygosities, number of alleles (*Na*), effective allele (*Ne*) at 10 loci in three sampling sites. Loci in accordance with *H-W* equilibrum **P* < 0.05; ***P* < 0.01; ****P* < 0.001; ns= not significant.

	Ural			Kura			Sefidrud		
n	*43*			*49*			*43*		
Locus	*Na/Ne*	*Ho*	*He*	*Na/Ne*	*Ho*	*He*	*Na/Ne*	*Ho*	*He*
LS-19	14/ 8.405	0.977***	0.881	13/ 7.11	1***	0.859	12/ 8.95	1***	0.888
LS-34	8/ 6.0	0.442***	0.833	9/ 5.3	0.551***	0.801	9/ 5.32	0.767***	0.812
LS-54	11/ 5.11	0.488***	0.804	13/ 8.03	0.694***	0.880	10/ 4.61	0.605***	0.783
LS-68	12/ 9.1	0.698***	0.891	11/ 7.0	0.633**	0.857	12/ 5.03	0.581ns	0.801
Spl104	14/ 10.0	0.744ns	0.900	13/ 7.83	0.857**	0.823	14/ 10.5	0.860***	0.905
Spl105	11/ 7.33	0.372***	0.864	9/ 5.66	0.878***	0.872	8/ 4.65	0.349***	0.785
Spl113	17/ 9.5	0.488***	0.895	13/ 9/78	0.551***	0.872	11/ 6.0	0.465***	0.869
Spl163	14/ 8.7	0.935***	0.885	12/ 7.82	0.980*	0.891	11/ 6.0	0.535***	0.833
Spl170	14/ 10.35	0.465***	0.903	15/ 9/16	0.735***	0.870	13/ 8.9	0.977***	0.888
Spl173	14/ 7.67	0.442***	0.870	13/ 9.72	0.551***	0.551	13/ 7.0	0.581***	0.857
Average	12.9/ 8.22	0.607	0.873	12.1/ 7.5	0.743	0.863	11.3/ 6.8	0.672	0.842

Table 2. Number of private alleles at microsatellite loci in three sampling sites

	Locus					
Site	*LS-68*	*LS-34*	*Spl105*	*Spl163*	*Spl170*	*Spl173*
Ural	1	0	4	0	4	1
Kura	0	1	0	1	1	4
Sefidrud	0	1	3	0	0	0

Table 3. Pairwise estimates of genetic differentiation detected at 10 loci in stellate sturgeon samples, using R_{ST} values (above diagonal) and F_{ST} (below diagonal) and gene flow in parenthesis (*Nm*)($P < 0.01$)

		Rst (Nm)		
	Samples	Ural	Kura	Sefidrud
Fst (Nm)	Ural	-	0.473(0.279)	0.433(0.328)
	Kura	0.050(4.760)	-	0.309(0.559)
	Sefidrud	0.053(4.482)	0.055(4.278)	-

The dendrogram according to the Nei (1972) genetic distance revealed two major clusters. One cluster consists of Sefidrud, the second consists of Kura and Ural together (Fig. 2). Sefidrud showed a separate cluster while Kura and Ural were genetically more similar.

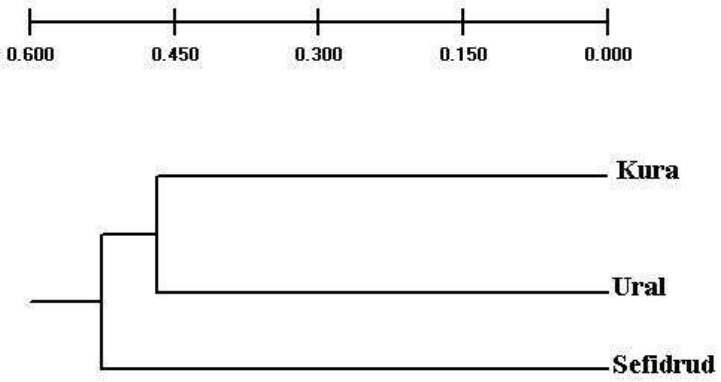

Fig. 2. UPGMA dendrogram according to the Nei (1972) genetic distance among three *ccipenser stellatus* populations using microsatellite DNA analysis.

Discussion

Despite the importance of stellate sturgeon of the Caspian Sea as unique species, its populations are endangered. It is pivotal for their conservation and sustainable use to have more information, but unfortunately, knowledge on the molecular genetics and on the genetic structure of this species is not extensive.

In this study, we have used 15 microsatellite loci to assess the genetic relationship among the populations of stellate sturgeon collected from three sites in the Caspian Sea. Of the 15 pairs of primers, 4 were not amplified by the PCR reaction, due to the lack of flanking sites in these primers, possibly as the result of the high genetic distance between stellate sturgeon and the species used as source of these primers. The proportion of polymorphic loci among the markers that did amplify decreased with the increasing genetic distance (Cui et al. 2005).

The average number of alleles per locus and the observed heterozygosity were comparable in the populations from the north and south of the Caspian Sea, as reported previously using RFLP on these same populations (Shabani et al. 2006). In fact, although these populations do not differ in the amount of genetic variation expressed as heterozygosity or alleles *per* loci, they differ highly for the nature of their genetic variation, which depends on the private alleles and genotypes. The losses of alleles and heterozygosity may increase with bottlenecking and inbreeding through time in the artificial propagation center stocks. Regular monitoring of genetic variability among the progenies is essential to avoid the loss of current polymorphism due to inbreeding and outbreeding.

In this study, a deviation from the *H-W* equilibrium was observed in most loci and no significant differences were found between the observed and the expected heterozygosities among the populations. The significant deviations from *H-W* equilibrium can be due to a bias in our samples or because we have not used species-specific primers or as the result of null alleles occurring in the studied populations. Heterozygotes with a null allele could be erroneously recorded as homozygotes for the variant allele. Similar results have been reported in lake and white sturgeons (Rodzen and May 2002; McQuown et al. 2003; Welsh and May 2006), Chinese sturgeon (Zhao et al. 2005) and it may also be related to sampling from mixtures of migrating populations.

In our study, the F_{ST} in all sampling sites was low but significant, suggesting that at least three populations are genetically differentiated and not part of a single panmigric population. In fact, in the great majority of cases, the F_{ST} is low, because the effect of polymorphism (due to mutations) drastically deflates F_{ST} expectations (Balloux et al. 2002). In fish species, a negative correlation has been demonstrated between F_{ST} values and dispersal capability (Waples 1987). According to this, *A. stellatus* might show a high dispersal capability presumably due to the absence of physical or ecological barriers. Feeding and spawning migrations are the result of a continuous movement from one part of the sea to another (Keyvan 2003). However, the loss of genetic variability might also be caused by sampling errors. Additionally, released fingerlings with hatchery-origin that return to rivers to spawn may contribute to the loss of regional genetic differentiation (Vasemägi et al. 2005).

Shaklee et al. (1982) and Thorpe and Sol-Cave (1994) showed that the Nei (1972) genetic distance values averaged 0.05 (range: 0.002-0.07) for con-specific populations and 0.30 (range: 0.03-0.61) for con-generic species. The distance value obtained in the present study (Table 4) falls within the average value of con-generic species, which indicates that the genetic difference among the studied populations was pronounced.

In summary, this study provides preliminary evidence for the existence of at least three differentiated populations in the Caspian Sea, including the Ural, Volga, Sefidrud and Gorganrud populations. Probably in each river more than one population exists, suggesting that more samples from each river should be investigated. Characterizing the genetic structure of *A. stellatus* currently being used in the aquaculture industry will aid for future breed stock development and will improve management plans aimed at conserving diversity and minimizing inbreeding in artificial propagation.

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