Amplification of microsatellites in the Persian sturgeon (*Acipenser persicus*)

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Persian sturgeon (*Acipenser persicus*) is a benthic species occurring primarily on sandy bottoms in the mid and southern Caspian Sea, especially along the shores of Iran (Berg, 1948; Holcik, 1989). The Persian sturgeon in the Caspian Sea is anadromous, with at least eighteen groups that segregate spatially and temporally for spawning. These populations are supported by some spawners, each of which considered to be of two seasonal races and divided into two or four biological groups (Perevaryukha, 2001). Accurate species, population and stock identification is a major prerequisite for each conservation and management activity, especially for sturgeons which have been commercially utilized for centuries (Ludwig, 2006, 2008). In addition to biological, morphological and meristic studies of the Persian sturgeons from the Caspian Sea, molecular genetic studies utilizing allozyme, mtDNA, RAPD and cycle sequencing were carried out in these fishes from different parts of the Caspian Sea (Keyvanfar *et al*., 1987; Pourkazemi *et al*., 2000; Rezvani Gilkolaei, 2000; Perevaryukha, 2001; Ataei *et al*., 2004).

Scoring of polymorphic genetic markers is a prerequisite for determining the number of discrete stocks and also for assessing the degree of spatial and temporal variations among these stocks. Microsatellites DNA are ideal markers for this role because they are biparentally inherited and can be scored using polymerase chain reaction (PCR) and thus can be sampled non-invasively. Microsatellites are inherited in a Mendelian

fashion, allowing very powerful statistical analyses. While they are expensive to develop initially, micro-satellite primers developed in one species may often be used in closely related species too. To date there has been no species specific microsatellite primer pairs developed for the Persian sturgeons, but microsatellites have been developed for a number of other sturgeon species including the shovelnose sturgeon, *Scaphirhynchus platorhynchus* (McQuown *et al*., 2000), Atlantic sturgeon, *A. oxyrinchus* (King *et al*., 2001), white sturgeon, *A. transmontanus* (Rodzen & May, 2002) and lake sturgeon, *A. fulvescens* (May *et al*., 1997; Welsh *et al*., 2003). These primers have shown considerable cross-species utility. For example, Welsh *et al*. (2003) found that many micro-satellites developed for lake sturgeon amplified polymorphic loci in green sturgeon (*A. medirostris*) while Tranah *et al*. (2001) found that primers developed in Atlantic sturgeon and Lake sturgeon often amplified diploid loci in shovelnose sturgeon.

One potentially serious problem with the use of microsatellites in sturgeons is that markers may exhibit polyploid (e.g., tetraploid or octoploid) inheritance. Birstein *et al*. (1997) classified all sturgeons as being polyploids (e.g., 4n, 8n) based on their DNA content. But Ludwig *et al*. (2001) assessed allele numbers at six microsatellite loci and classified species ~120 chromosomes as functional diploid species, species with ~250 chromosomes (including Persian sturgeon) as functional tetraploid species and species ~500 chromo-somes as functional octaploids.

However, the process of functional genome reduction is still active in species with ~250 and ~500 chromosomes. Welsh *et al*. (2003) found that the fraction of loci developed in white sturgeon that exhibited polyploid inheritance was 79.1% in white sturgeon and 64.5% in green sturgeon. Welsh and May (2006) reported that only 9 out of 254 primer pairs (3.5%) developed for lake sturgeon, a species with presumably the same ploidy level as Persian sturgeon (Ludwig *et al*., 2001), exhibited diploid inheritance. In the only previous study that compared microsatellite variations in sturgeons which included the Persian sturgeon, Ludwig *et al*. (2001) found that none of the five micro-satellite loci tested were disomic, four showed tetraploid banding patterns and at least one seemed to exhibit octosomy. Taken together, the process of functional genome reduction seems to be still active in species with ~250 chromo-somes including the Persian sturgeon (Ludwig *et al*., 2001).

In the present study, Persian sturgeon fin clips were collected from the Iranian sturgeon fish landings and preserved in 95% ethanol. Genomic DNA was extracted using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA) and stored at –20°C. The microsatellite primer sequences tested were as reported in McQuown *et al*. (2000). The screening of the microsatellite loci using a single ^{32}P-labeled primer

followed Schrey *et al*. (2007). Briefly, one of the two primers was labeled with γ-^{32}P using polynucleotide kinase. We amplified genomic DNA from 24 individuals in an Eppendorf master cycler gradient thermocycler with annealing temperature ranging from 54° to 66°. We used an initial 5 minute denaturing step at 95° followed by 40 cycles of 95° 30 sec., 54-66° for 30 sec, and 72° for 30 sec. Finally the products were allowed to extend for five minutes at 72°. Stop solution (5µl 95% formamide, 0.05% xylene cyanol, 0.05% bromophenol blue, and 10mM NaOH) was added and the products were denatured at 95° for 2 minutes prior to loading onto a 42 cm long 6% denaturing polyacrylamide gel. After 1-3 hours of electrophoresis at 1500X volts/cm the gels were dried and visualized via auto-radiography.

We interpreted a locus as being polymorphic if multiple bands of the appropriate predicted sizes and appearance were present in most individuals. If bands were present in less than one half of all the individuals tested we performed a second PCR reaction using a single annealing temperature chosen to optimize amplification. We determined whether the bands were of the appropriate sizes based on the allele sizes reported by McQuown *et al*. (2000) and their migrations relative to the dye in the stop solution. Most of the loci that we scored as being polymorphic exhibited stutter bands characteristic of microsatellite banding patterns on poly-acrylamide gels. Loci were scored as mono-morphic if a single band was present in all individuals, diploid if one or two bands were present in every individual, tetraploid if 3-4 bands were present in some individuals, and octoploid if 5-8 bands were present in some individuals. Loci that produced multiple bands but lacked stutter bands or were otherwise difficult to interpret were scored as "ambiguous".

Of the 56 sets of primer pairs we tested, 50 (89%) produced bands. We scored one locus as being monomorphic (Spl 40), 27 as tetraploid (Spl 1, Spl 2, Spl 4, Spl 7, Spl 10, Spl 11, Spl 12, Spl 13, Spl 14, Spl 16, Spl 23, Spl 30, Spl 35, Spl 36, Spl 37, Spl 38, Spl 44, Spl 45, Spl 46, Spl 47, Spl 49, Spl 50, Spl 52, Spl 53, Spl 54, Spl 55 and Spl 56), four as octoploid (Spl 22, Spl 31, Spl 43 and Spl 60), and 18 as ambiguous (Spl 3, Spl 5, Spl 6, Spl 8, Spl 9, Spl 15, Spl 18, Spl 19, Spl 20, Spl 21, Spl 24, Spl 25, Spl 32, Spl 33, Spl 39, Spl 51, Spl 58 and Spl 59).

None of the loci exhibited diploid inheritance. Hence, it is possible to use many of the interspecific microsatellite primer pairs which we tested to detect tetraploid and octoploid loci to be used as dominant genetic markers to characterize the various geographical populations of the Persian sturgeon from the Caspian Sea. We are also in the process of trying to develop diploid microsatellite loci for the Persian sturgeon. It is expected that microsatellite primer pairs developed for the Persian sturgeon

could also be used to characterize genetically species of the closely related Russian sturgeon complex (i.e., *A. gueldenstaedtii*, *A. naccarii* and *A. baerii*) (Birstein *et al*., 2005).

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