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Sturgeon genetics and cytogenetics: a review related to ploidy levels and interspecific hybridization

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Abstract. Sturgeons (Chondrostei: Acipenseriformes) display markedly disjunction distributions with a wide distribution in the northern hemisphere. Their unique benthic specializations and conserved morphology, evolutionary age, the variation in their basic diadromous life history, and the large public interest due to their near extinction or critically endangered status make sturgeons and paddlefishes interesting groups for molecular and cytogenetic studies. From altogether 27 acipenseriform species, seventeen species are supposed to be critically endangered, two species are classified as endangered, four species are vulnerable and other species are near threatened or in low-risk (IUCN Red list 2010). Sturgeons are characteristic by a relatively high number of chromosomes in cell nuclei and differences in ploidy levels. Sturgeons displayed a strong tendency for inter-specific and inter-generic hybridization under altered environmental conditions as well as under conditions of artificial propagation. Almost 20 inter-specific sturgeon hybrids were described. The decrease of natural populations and tendencies leading to restocking may result in uncontrolled restocking, production of hybrid specimens (even with non-native species) and decrease of natural genetic diversity of species in their original distribution area. Identification of parental species of natural hybrids by modern methods of molecular biology is still not easy. Here, we attempt to briefly summarize the major aspects of sturgeon genetics and cytogenetics related to ploidy levels and interspecific hybridization.

Key words: *Acipenser*, polyploidy, conservation, molecular markers

Introduction

Sturgeons are of interest genetically and evolutionarily for a variety of reasons. Firstly, sturgeon fishes are supposed to have evolved about 200 million years ago during the Jurassic period (Bemis et al. 1997). Furthermore, the "living fossils" status of the group makes them important for understanding vertebrate evolution in general and the threatened or endangered status of many of these species indicates that there may be a limited time left to study these organisms. In addition, natural populations of almost all sturgeon species have been seriously affected by overexploitation in combination with a substantial loss and degradation of habitat during the 20th century. These significant changes have invariably initiated conservation efforts and stocking activities. Stocking activities (introductions or reintroductions) with fishes grown in captivity has become a common practice in many countries with the primary aim of getting an increment of angling as well as for the rehabilitation of natural populations. It has been shown that restocking programmes can result in deleterious effects on the natural fish populations that in many cases are the same as those caused by the introduction of exotic species. All this might cause a displacement of the local populations, or in extreme cases, their extinction. Additionally, the natural genetic diversity of species is drastically reduced by uncontrolled

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restocking, especially because of the fast decline in the number of wild individuals remaining and another dangerous practice like the production of hybrids with non-native species (Fontana et al. 2001).

Approaches employed in conservation genetics can help to prioritize populations for active management, to identify suitable donor and recipient populations for stocking (Welsh & May 2006).

Knowledge of sturgeons' phylogenetic and taxonomic relationships is still limited because of their high morphological variability and a peculiar ability to yield fully fertile hybrids in the wild between taxonomically distant species, sometimes even assigned to different genera (Berg 1962). Standard taxonomical studies and their findings based on morphological characteristics were influenced by availability of genetic and molecular data. For example, two species of the genus *Huso* (*Huso huso* and *H. dauricus*) are genetically more distant between themselves than they are from those of the genus *Acipenser* (Birstein & DeSalle 1998). As a last but not least, sturgeon genetics is important from the viewpoint of any economic issues involving these valuable fishes. These tasks include species identification by genetic tools for needs of trade and identification of caviar.

Here we attempt to briefly summarize the major aspects of sturgeon genetics and cytogenetics related to ploidy levels and interspecific hybridization. Firstly we review these topics and then discuss the present and future of pattern and process discovery in sturgeon genetics.

Sturgeon karyology and ploidy levels

It is clear that, within fishes polyploidy orders are phylogenetically diverse, suggesting that polyploidy has evolved on more than one occasion. Within taxa in which polyploidy is known to occur, it may have evolved independently on more than one occasion. The polyploidy may be a significant phenomenon in the evolution of fishes (Le Comber & Smith 2004).

Species of genera *Acipenser*, *Huso*, *Scaphirynchus* and *Polyodon* are separable into the different classes of chromosome numbers: (A) species with ~ 120 chromosomes including all taxa with between 110 and 130 chromosomes; (B) species with ~ 250 chromosomes including all taxa with between 220 and 276; and the last class (C) is represented only by *A. brevirostrum* having ~ 360 chromosomes (Ludwig et al. 2001). This species has the highest chromosome number and highest amount of DNA among all acipenseriform species. Number of chromosomes for different acipenseriform species are summarized in Table 1.

The first data on sturgeon karyology reported chromosome number 2n = 60 inferred from metaphase plates from blastomeres and cells of branchial mucosa of *H. huso* and *A. ruthenus* which were obtained in the early 1960's (Serebryakova 1972). Other data were published by Ohno et al. (1969), who constructed metaphase plates from squashes of tissue fragments of *Scaphirhynchus platorhynus*. These preparations revealed a diploid number of 112 chromosomes where 48 chromosomes were distinguished as microchromosomes. The high number of chromosomes led the authors to the hypothesis that *S. platorhynus* was tetraploid. Observation of preparations of several tissues of *Polyodon spathula* reported chromosome number 2n = 120 where 72 were microchromosomes (Dingerkus & Howell 1976). These authors arranged chromosomes in groups of fours suggesting tetraploidy of these species.

Another method of ploidy level determination is observation of nuclear organizer regions (NORs). A study of a number of stained active NORs in four different sturgeon species suggested tetraploidy of species with ~ 120 chromosomes and octaploidy of species with ~ 250 chromosomes (Birstein & Vasil'ev 1987). This was particularly discussed by Fontana (1994) who localized NORs in *A. ruthenus* on the telomeric regions of two morphologically different pairs of chromosomes. These NORs are localized on eight chromosomes arranged in two quadruplets in *A. baeri*, *A. transmontanus* and *A. naccarii*. Based on these findings he considered the species with 120 chromosomes to be diploid and species with 240-250 chromosomes to be tetraploid. Varying results concerning the ploidy level of acipenseriform species were also found among studies focused on expressed gene products. Birstein et al. (1997) observed duplicated protein loci in species with ~ 120 chromosomes which they attributed to tetraploidy. If the 120 chromosome species were to be considered tetraploid, then the ones later described with 240 chromosomes should be considered octaploid. On the other hand, a densitometric study of serum albumins performed by Kuzmin (1996) assigned diploidy to species with ~ 120 chromosomes. Other authors hypothesized that all species having ~ 120 chromosomes were tetraploids (e.g. Ohno et al. 1969, Birstein & Vasil'ev 1987, Birstein & DeSalle 1998, Flajšhans & Vajcová 2000).

The theory of genome duplication and subsequent reduction in functional ploidy is frequently applied, even in the evolution of diploid vertebrates; genome quadruplication is discussed (Spring 1997). Piferrer et al. (2009) reviewed that polyploidy has been involved in the speciation of both animals and plants

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Table 1. *Chromosome numbers of different acipenseriform species.*

Species	Chromosome number	Reference
Scaphirhynchus platorynchus	112	Ohno et al. (1969)
A. nudiventris	116 ± 4	Nowruzfashkhami et al. (2006)
	118 ± 2	Sokolov & Vasil'ev (1989)
Huso huso	116 ± 4	Fontana & Colombo (1974)
	118 ± 2	Fontana et al. (1998)
A. sturio	116 ± 4	Fontana & Colombo (1974)
	121 ± 3	Tagliavini et al. (1999)
A. ruthenus	118 ± 2	Fontana et al. (1975)
	118 ± 4	Ráb (1986)
	118 ± 2	Birstein & Vasil'ev (1987)
A. stellatus	118 ± 2	Birstein & Vasil'ev (1987)
	146 ± 6	Chicca et al. (2002)
Polyodon spathula	120	Dingerkus & Howell (1976)
A. oxyrinchus	121 ± 3	Fontana et al. (2008b)
A. baerii	229 – 240	Fopp-Bayat et al. (2006)
	246 ± 8	Fontana (1994)
	246 ± 10	Fontana et al. (1997)
	249 ± 5	Vasil'ev et al. (1980)
A. naccarii	239 ± 7	Fontana & Colombo (1974)
	246 ± 8	Fontana (1994)
	248 ± 4	Fontana et al. (1999)
A. transmontanus	246 ± 10	Fontana et al. (1997)
	248 ± 8	Fontana (1994)
	256 ± 6	Wang et al. (2003)
	271 ± 2	Van Eenennaam et al. (1998)
A. mikadoi	247 ± 33	Vishnyakova et al. (2008)
	262 ± 4	Vasil'ev et al. (2009)
A. gueldenstaedtii	249 ± 2	Arefjev & Nikolaev (1991)
	250 ± 8	Birstein & Vasil'ev (1987)
	258 ± 4	Fontana et al. (1996)
A. medirostris	249 ± 8	Van Eenennaam et al. (1999a)
A. persicus	258 ± 4	Nowruzfashkhami et al. (2000)
A. fulvescens	262 ± 6	Fontana et al. (2004)
A. sinensis	264	Yu et al. (1987)
	264	Zhou et al. (2008)
Huso dauricus	268 ± 4	Vasil'ev et al. (2009)
A. brevirostrum	372	Kim et al. (2005)
	372 ± 6	Fontana et al. (2008)

(Mable 2004, Hegarty & Hiscock 2007), and seems to have arisen independently several times during the evolution of fishes with higher incidence in the more primitive groups (Legatt & Iwama 2003). In case of sturgeons, a few theories about polyploidization events were proposed. Ludwig et al. (2001) suggested four polyploidization events in the evolution of sturgeon species. Vasil'eva et al. (2009a) proposed that at least three polyploidization events occurred in acipenserid evolution. Due to functional genome reduction events, some authors (e.g. Fontana 1994, Fontana et al. 1998, Tagliavini et al. 1999, Jenneckens et al. 2000, Ludwig et al. 2001) consider all sturgeons with ~ 120 chromosomes to be modern (functional) diploids. Accordingly, species with ~ 250 chromosomes are then called modern (functional) tetraploids.

Fontana et al. (2007) supposed that the common diploid ancestor of all Acipenseriformes had a karyotype of 60 chromosomes. A genome duplication event must have occurred in this ancestor to produce the acipenserid lineage with species having ~ 120 chromosomes (Birstein et al. 1997). In the first chromosomal duplication the chromosome number probably increased from 2n = 60 to 4n = 120. This event is believed to have occurred at least 200 million years ago since no Acipenseridae species with 60 chromosomes presently survives. Hybridization among the different 120 species resulted in hybrids which, after genome duplication, became allotetraploid or allooctaploid according the ploidy theory. This process may have independently occurred

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several times (Fontana et al. 2007). A high number of chromosomes in cell nuclei and high number of microchromosomes among these chromosomes can be considered the main reason of controversial opinion about ploidy levels of sturgeon species.

The amount of nuclear DNA has also been employed to study the ploidy relationship among groups. Vialli (1957) in *A. sturio* detected an amount of nuclear DNA 3.6 picograms per nucleus (pg/N) and Ohno et al. (1969) found 3.2 pg/N in *S. platyrhynchus*. The amount of nuclear DNA of *A. naccarii* (6.26 pg/N) showed to be twice higher than the amount of nuclear DNA in *H. huso* (3.6 pg/N) and *A. sturio* (3.6 pg/N; Fontana 1976). The amount of DNA higher than 13 pg/N was reported in *A. brevirostrum* by Blacklidge & Bidwell (1993) and in *A. mikadoi* by Birstein et al. (1993). In this case Birstein et al. (1993) assumed 500 chromosomes in *A. mikadoi* by the finding of a two times higher DNA content than in species with ~ 250 chromosomes. This finding was discussed many times. Zhou et al. (2009) observed DNA content of 8.0-9.1 pg/N similarly to *A. transmontanus*, which has karyotype containing 270 chromosomes. Similarly, 262 chromosomes were reported in a karyotypic study of *A. mikadoi* by Vasil'ev et al. (2009). Observation of unexpected chromosome numbers in *H. dauricus* (268 chromosomes) published in this study is also interesting. *H. dauricus* was assumed to be a species with 120 chromosomes (Birstein et al. 1993, Birstein & DeSalle 1998, Fontana et al. 2001, Ludwig et al. 2001). Ploidy level of *A. mikadoi* was also discussed by Vishnyakova et al. (2008).

Blacklidge & Bidwell (1993) observed a genome size of 13.08 pg/N in *A. brevirostrum* and suggested dodecaploidy in this species. This was partly supported by Kim et al. (2005) who found 372 chromosomes in *A. brevirostrum*, but they could not discriminate whether this species was hexaploid or dodecaploid. Hexaploidy of *A. brevirostrum* was suggested by Fontana et al. (2008a) by observation of hybridization signal with 5S rDNA probe. They detected six florescent signals in all analyzed metaphases and from previous observation of two florescent signals in 120 chromosome species, four fluorescent signals in 250 chromosome species they made the conclusion that *A. brevirostrum* is hexaploid. In agreement with Kim et al. (2005) they also found 372 chromosomes in *A. brevirostrum* karyotype.

New insight on ploidy levels in Acipenseridae was enabled by methods of molecular biology. The *in vitro* amplification of polymorphic nuclear markers, such as microsatellite loci, permits a direct although partial view of the genome (Ludwig et al. 2001). A pattern of microsatellite alleles' inheritance is more important for studies based on microsatellite genotyping. The study of Ludwig et al. (2001) reported disomic allelic patterns in species with ~ 120 chromosomes and tetrasomic allelic patterns in species with ~ 250 chromosomes. One group of species (*A. brevirostrum*, *A. fulvescens*, *A. gueldenstaedtii*, *A. medirostris*, *A. mikadoi* and *A. naccarii*) was characteristic by microsatellite patterns indicating octosomic or greater allelic band patterns at a minimum of one locus and allelic band patterns in another group of species (*A. baerii*, *A. persicus*, *A. sinensis* and *A. transmontanus*) showed evidence of possible octosomy at a minimum of one locus. Allelic band patterns indicating higher than octosomic inheritance pattern were observed in *A. mikadoi* at two loci (Ludwig et al. 2001). The tetrasomic, double disomic or even octasomic inheritance of the different microsatellite loci was described in various acipenserid species. Pyatskowit et al. (2001) examined twelve microsatellite loci in *A. fulvescens*. They observed five polymorphic loci, LS-19, LS-34 and LS-54 showed two alleles, LS-39 three alleles and LS-68 four alleles. They also suggested tetrasomic inheritance for loci LS-19, LS-34, LS-39, and LS-54. In another study, one of these loci, LS-39, showed no more than two alleles in 501 fishes from six sturgeon species with ~ 120 chromosomes (*A. stellatus*, *H. huso*, *A. nudiventris*, *A. ruthenus*, *A. oxyrinchus* and *A. sturio*) and four alleles in 265 samples from four species with ~ 250 chromosomes (*A. gueldenstaedtii*, *A. baerii*, *A. naccarii* and *A. persicus*) (Jenneckens et al. 2001).

Nine microsatellite loci were developed by Rodzen & May (2002) for *A. transmontanus*. They reported inheritance patterns with a range from disomic system to tetrasomy and octasomy, with some null alleles. Fopp-Bayat (2008) screened microsatellite loci in haploid progeny of *A. baerii*. She suggested that three studied loci segregate disomically (Afu-68, Spl 163 and Spl 168), three tetrasomically (Aox-45, Afu 19 and Afu 39) and one octasomically (Spl-104).

We can conclude that all studies based on microsatellite loci and inherritance of microsatellite alleles supported theory of di- and tetraploidy rather than tetra- and octaploidy in species with ~ 120 and ~ 250 chromosomes, respectively.

Sturgeon sex determination

Generally, fish sex determination is one of the most changeable and diverse among all vertebrates (Vasil'eva et al. 2009b). This problem is more

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complicated in acipenseriform species due to high number of polyploidization events in their evolution (Vasil'ev et al. 2009). Sex in vertebrates is generally determined by environmental and genetic factors. To the best of our knowledge, no evidence about environmental effects on sex determination in sturgeons has been found yet. There is no evident view on the sturgeon sex determination system up to now. It is still very difficult to distinguish sex chromosomes in sturgeons' karyotypes because of the large number of microchromosomes (Vasil'eva et al. 2009b). Cytogenetical studies have not revealed any presence of heteromorphic chromosomes in sturgeon species (Van Eenennaam et al. 1999b). In order to reveal sturgeon sex determination in absence of the evidence of heteromorphic chromosomes, genetic approaches can be used based on artificial genome manipulation techniques such as gynogenesis (Arai 2001). This is also of great importance for aquaculture, as potentially monosexual, all-female populations should increase economical feasibility in caviar production. Gynogenesis will produce all-female progeny only in case of sex determination, where the homogamety will be carried by females (XX female/XY male). In the acipenseriform species it has been demonstrated for North American paddlefish (*P. spathula*; Mims et al. 1997). A gynogenetic, all-female population of *P. spathula* was produced by activating eggs with ultraviolet-irradiated shovelnose sturgeon (*S. platorynchus*) spermatozoa and heat shocking. On the other hand the evidence of female heterogamety (WZ female/ZZ male), where gynogenesis did not produce all-female population, was reported several times. Van Eenennaam et al. (1999b) used gynogenesis for the investigation of the sex determination system in *A. transmontanus* and they observed both sexes in 24 month old progeny. This supported the hypothesis of a female heterogametic sex determination system. The female heterogametic sex determination system was also supposed for bester (♀ *H. huso* × ♂ *A. ruthenus*) by Omoto et al. (2005). Flynn et al. (2006) used gynogenesis to study sex determination in *A. brevirostrum*. They received 35 % of males and 65 % of females and based on this observation they suggested female heterogamety in this species. Flow cytometry and microsatellite DNA analysis were used for the verification of gynogenesis induction. Microsatellite DNA analysis was also used by Fopp-Bayat (2010) to verify induction of gynogenesis in *A. baerii*. Histological analysis of gonads of all gynogenetic progeny showed 81 % of females and 19 % of males. This sex ratio provides supposition that *A. baerii* has no female homogametic sex determination system (Fopp-Bayat 2010). The problem of sturgeon sex determination was deeply reviewed by Keyvanshokooh & Gharaei (2010).

An unexpected sex ratio observed in gynogenetic progeny of *A. stellatus* Vasil'eva et al. (2009b) produced hypothesis that sex determination system in *A. stellatus* should be more complicated and connected with the balance of sex chromosomes and autosomes on the one hand or hypothesis that WW homozygotes superfemale should be lethal. Vasil'eva et al. (2009b) mentioned the possibility that *A. stellatus* has ZO female heterogametic sex determination system.

The utilization of molecular markers for sturgeon sex determination would be very profitable but there is no evidence about sex specific markers up to now. Wuertz et al. (2006) did not detect sex specific markers for *A. naccarii*, *A. baerii* and *A. ruthenus* using of RADP techniques. They also used AFLP and ISSR, but no sex specific marker was found using these techniques similarly to RAPD.

RAPD technique used by McCormick et al. (2008) also failed to find sex specific markers in *A. fulvescens* and Keyvanshokooh et al. (2007) obtained similar results by screening the genome of *H. huso*. Wuertz et al. (2006) supposed that gene expression profiling could be used as an alternative method to failed DNA-based techniques. This was partly doubted by Keyvanshokooh et al. (2009). They analyzed protein expression in gonads of mature males and females of *A. persicus*, but they did not find differences in proteins directly linked to the sex determining genes.

Problems of sturgeon diversity inferred from hybridization events

Sturgeon hybrids are being increasingly utilized in aquaculture projects and sport fishing, therefore the ability to detect the accidental introduction of hybrids in the wild becomes extremely valuable. In general, natural interspecific hybridization happens more frequently between closely related fish species. Interspecific hybridization between taxonomically distant verterbrate species differing in their chromosome numbers is a rare event due to genetic incompatibility of parental genomes (Arnold 1997). Even if such interspecific hybrids survive, they are usually sterile because their chromosomes cannot correctly pair during the zygotene stage of prophase I and such impairment interferes with gonadal development and gametogenesis (Piferrer et al. 2009). Due to the unusual genetic structure of acipenserids (all are polyploid); they hybridize more easily than other

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vertebrates (Birstein et al. 1997) and this concerns species with the same and/or differing ploidy level. The latter do hybridize both in nature (e.g. Birstein et al. 1997; Ludwig et al. 2002, 2009) and in captivity (Nikolyukin 1964, Arefjev 1997, Flajšhans & Vajcová 2000 and others). Morphological description is not enough to prove that a particular individual is hybrid but only a genetic study can provide necessary proof of sturgeon hybridization. Research efforts on nuclear markers should increase the possibility of hybrid detection and consequently the control of admixture at inter-specific but also at intra-specific level. Interspecific hybridization has a negative effect on outbreeding level. Hybrids are often characterized by greater growth performance which leads to replacing native species and often causing their extinction (Ludwig et al. 2009). There was also demonstrated that hybridization is the most rapidly acting genetic threat to endangered populations, with extinction often occurring in less than five generations (Wolf et al. 2001). Hybrids sometimes resulted from intended release programs (Becker et al. 2007), and in other cases from habitat alterations (Freyhof et al. 2005) or from unintended escape of hatchery fish (Birstein & DeSalle 1998). Most of the time backcross with native species resulted in a genetic cleaning of nuclear genotypes so that evidence for ancestral hybridization is often only detectable in mtDNA (Birstein et al. 2005).

Species determination and identification of hybrid specimens based on single locus nuclear markers, suitable for different species is very difficult because of the complexity of sturgeon genome and because of different ploidy conditions. Furthermore a single locus marker would be useless in case of second generation hybrids or backcross hybridization. Multilocus nuclear markers could solve the problem (Fontana et al. 2001). There is a need to establish suitable methods for identification at species level of caviar, for population identification, for determination of source of products from sturgeons and for identification of age of caviar according to Ludwig (2008). Utilization of mtDNA – cytochrome *b* sequences was recommended for species identification Ludwig (2008). Correct species identification based on mtDNA is limited due maternal inheritance of mitochondrial DNA and identification of interspecific hybrids is very limited. This method also showed limitation for differentiation of closely related acipenseriform species complex (e.g. *A. gueldenstaedti*, *A. baerii*, *A. persicus*, *A. naccarii*) due to overlapping mtDNA profiles (Birstein et al. 2000). But mitochondrial DNA can be used for species identification as mentioned above. For example, the mtDNA control region has been employed to distinguish the three species of genus *Scaphirhynchus* (*S. albus*, *S. platorhynchus* and *S. suttkusi*) where the first two species are sympatric (Campton et al. 2000). *S. suttkusi* is easy to identify, while the distinction between the two sympatric species is rather difficult because of interferences due to interspecific hybridization. As previously mentioned, mtDNA does not allow recognition of inter-specific hybrids and this problem is particularly relevant to sturgeons, among which hybridization is a common event (Campton et al. 2000). Some species of sturgeon are preferred in aquaculture because they are easy to handle, fast growing and reproduce quickly under hatchery conditions. The escape of farmed sturgeons is often reported especially during flooding events (Maury-Brachet et al. 2008), which have become increasingly frequent in Europe during a last few decades. A clear example of this is reported by Jenneckens et al. (2000) on contamination of *A. gueldenstaedtii* with *A. baerii*. The analysis of mitochondrial cytochrome *b* gene performed on 34 sturgeons captured in the River Volga and morphologically classified as *A. gueldenstaedtii* showed that eleven of them actually had *A. baerii* haplotype. The possibility of molecular identification of sturgeon hybrids, which is often very difficult distinguish by morphological approach, could be a powerful tool for both conservation biology and quality certification of commercial products (Congiu et al. 2001). Ludwig et al. (2009) described a natural spawning of non-native species *A. baerii* in the River Danube basin and hybridization with native *A. ruthenus* by analysis of mtDNA control region and nine microsatellite loci. This hybridization possesses a serious threat for the survival of this native isolated population of *A. ruthenus* in the Danube drainage. The natural hybridization was also demonstrated by combining nuclear (microsatellites) and mitochondrial (cytochrome *b*) markers (Jenneckens et al. 2000, Ludwig et al. 2001), between *A. gueldenstaedti* and *A. stellatus*, *A. gueldenstaedti* and *A. ruthenus* hybrid, as well as five hybrids between *A. gueldenstaedti* or *A. persicus* and *A. nudiventris*. Using the cytochrome *b* sequences, in all these cases Ludwig et al. (2002) identified *A. gueldenstaedti* or *A. persicus* as the maternal species. In all specimens they observed triploid band patterns at several microsatellite loci. The *A. gueldenstaedti*/*A. stellatus* and the *A. gueldenstaedti*/*A. ruthenus* hybrids came from the River Volga and the *A. nudiventris*-hybrids were caught in the Iranian waters of the Caspian Sea near the mouth of the River Safid Rud (Ludwig et al. 2002). Tranah et al. (2004) used microsatellites to observe hybridization between *S. albus* and *S. platorhynchus* in lower part of

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the River Mississippi. Using nine microsatellites loci they found evidence of hybridization between these sturgeon species. Congiu et al. (2001) used amplified fragment length polymorphism (AFLP) to separate hybrids (*A. naccarii* × *A. transmontanus*) from their parental species. Ludwig (2008) discussed about AFLP as very useful method for backcross screening (crosses between hybrid and one of its parental species).

Identification of pure species by means of suitable molecular markers is a necessary prerequisite to distinguish hybrids and this concerns also the identification of sturgeon products, either for conservation or forensic purposes. Species specific PCR, which is based on the presence of diagnostic nucleotide differences in mtDNA sequences, was recommended as a very easy, inexpensive and fast method for the identification of two very important caviar producing species *A. stellatus* and *H. huso* (Ludwig 2008). DeSalle & Birstein (1996) proposed the sequencing of parts of cytochrome *b*, 12S and 16S rDNA genes and designing specific primers for diagnostic substitution as a method to identify caviar of three species. Jenneckens et al. (2001) observed one species specific allele on microsatellite locus LS 39 for *A. stellatus*. He recommended microsatellite locus LS 39 to identify caviar origin due to fixed allele of 111 bp, which was absent in the other species. With big probability it is the first genomic marker described for sturgeon with the potential to identify one of the main caviar-producing species and its hybrids.

Many molecular studies have incorporated microsatellite loci in examining genetic variability within and among species. May et al. (1997) demonstrated that primers designed from *A. fulvescens* subgenomic library amplified microsatellites at eight of eleven loci examined in other *Acipenser* species, as well as in *Scaphirhynchus* sturgeon. Furthermore six of the nine loci that amplified in *Scaphirhynchus* species were polymorphic, and *Acipenser* polymorphism ranged from 33 % to 80 %. The dynamics of sturgeon genome appear to mandate using a rapidly evolving marker in lieu of those once used in traditional molecular techniques. Furthermore, the conservation of microsatellite flanking regions among related taxa suggests that the development of microsatellite loci for one species would prove a useful family-wide scale (May et al. 1997). McQuown et al. (2000) developed a large set of microsatellite loci by sequencing of three subgenomic of *S. platorynchus*. Authors designed primers for 113 of sequences and tested against *S. platorynchus*, *S. albus*, *A. transmontanus*, *A. fulvescens* and *A. medirostris*. They observed that 96 % from 113 primer sets amplified on one or more species. Similarly King et al. (2001) described six microsatellite loci isolated from *A. oxyrinchus* and this six microsatellite loci they tested for cross amplification in ten acipenseriform species. Four of six microsatellite primer sets developed for *A. oxyrinchus* produced DNA fragments in all ten of the additional sturgeon taxa (King et al. 2001).

Conclusion

In this review we summarized the main problems on sturgeon polyploidy and interspecific hybridization. A study of sturgeon genetics can show us to what extent the polyploidization events played an important role during the evolution of vertebrates and new results indicate that these events still continue among different sturgeon species. There is still no clear view on the status of sturgeon polyploidy (e.g. paleotetraploidy vs. modern diploidy) even inside the scientific community dealing with this question till now. Polyploidization is closely connected with frequent interspecific hybridization events. At least twelve different types of interspecific hybrids and five intergeneric ones have been described, some of which even fertile, such as the bester (Birstein et al. 1997). Identification of parental species is not easy in natural hybrids. Research efforts on nuclear markers should increase the possibility of detection of hybrids and consequently the control of admixture at interspecific but also intraspecific level. It is evident that this topic is still very complicated and partly unsolved and that it will require further studies in the future.

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