Microsatellite DNA polymorphism in sturgeon species and their hybrids reared in Polish aquaculture farms

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ABSTRACT

Highly variable microsatellite DNA loci show high levels of variation per locus and provide molecular markers for species and for populations of small effective size. In this study we applied microsatellite markers to identify specimens belonging to several sturgeon species and their interspecific hybrids. Nine microsatellite loci (*Afu-19, Afu-22, Afu-34, Afu-39, Afu-54, Afu-57, Afu-58, Afu-68, Afu-69*) were analysed for five species (beluga, Siberian sturgeon, Russian sturgeon, sterlet and paddlefish) and for their three interspecific hybrids: Siberian sturgeon x Russian sturgeon, beluga x sterlet (called bester) and for beluga x bester. Certain alleles at five loci: *Afu-22, Afu-39, Afu-54, Afu-57* and *Afu-68* were diagnostic for the examined sturgeon species and their interspecific hybrids.

INTRODUCTION

The family Acipenseridae consists of 25 extant sturgeon species (Birstein 1993; Birstein and Bemis 1997). Together, with the family Polyodontidae (two paddlefish species), it composes the order Acipenseriformes, which inhabits the Northern Hemisphere. The present bio-geographic distribution of this group reflects ancient relationships among fish faunas of Europe, Asia, and North America (Birstein and DeSalle 1998).

Almost all Acipenseriformes species are threatened and some species are in danger of extinction (Birstein et al. 1997a; Pikitch et al. 2005). The causes of the decline in sturgeon populations include environmental pollution, the destruction of spawning habitats, human intervention preventing the migration of fish to their spawning grounds, and overfishing (Birstein 1993). Although increased stocking of waters with farm reared fish may increase the abundance of fish, it may reduce genetic variation of the species and destroy gene pool integrity, particularly in those cases where interspecific hybridization occurs (or when hybrid fish reared in aquaculture conditions escape to natural waters) (Tave 1986).

In the beginning of the 1990s, fertilized eggs of sturgeons were imported into Poland from sturgeon breeding centres in Konakovo near Moscow, from Krasnodarskij Yar near Krasnodar, and from Gorachij Kluch near Rostov na Donu (all in Russia). At first the eggs were transported to the Fisheries Experimental Station Dgal (Inland Fisheries Institute in Olsztyn, Poland) and to the Fish Farm Wasosze near Konin, Poland. Then, sturgeons were introduced to several fish farms throughout Poland. Since then many different sturgeon species and their interspecific hybrids have occurred in Poland (such as Siberian sturgeon, Russian sturgeon, sterlet, bester, and others) (Table 1).

Due to the late maturation of sturgeons, breeding under controlled conditions began in Poland only several years ago. Moreover, the numbers of spawners of different species are small, resulting in a substantial risk of losing genetic variability of the breeding stocks and of their offspring.

Hybridization between species imposes difficulties in offspring identification based on morphological features (Birstein and Bemis 1997; Birstein et al. 1997b). Due to cryptic morphology it is necessary to apply genetic markers in order to identify species or to prove the hybrid origin of examined specimens. Also, the application of molecular markers enables estimation of the kind and amount of genetic variation existing within each breeding stock (Allendorf et al. 2001; Tranah et al. 2004; Wirgin et al. 2002).

Microsatellite DNA polymorphism analysis is often applied in fish population studies (Estoup 1998). Microsatellite loci assayed with the help of polymerase chain reaction (PCR; Saiki et al. 1988) allow for the use of nonlethal tissue sampling (fin, blood, etc.), which is of extreme importance in studies of endangered and threatened species.

Microsatellites show high levels of variation and have become important genetic markers for studying parentage, linkage, and intraspecific population structure in many organisms (Schug et al. 1998), including sturgeons (May et al. 1997; Tranah et al. 2004), salmon (McConnel et al. 1994; Nielsen, 1996; O'Reilly and Wright 1995; Slettan et al. 1997), trout

Table 1. Examined sturgeon species and hybrids, location of sturgeon stocks and number of sampled individuals.

Species or hybrid	Symbol	Number of fish sampled from stocks in Poland		
		Wasosze	Acipol	Dgal
Siberian sturgeon, *Acipenser baeri*	SB	27	50	4
Russian sturgeon, *Acipenser gueldenstaedti*	R	15		2
A. baeri x A. gueldenstaedti	SBxR	19	20	2
beluga x bester, *Huso huso x (H. huso x A. ruthenus)*	BxBS	none	none	4
Sterlet, *Acipenser ruthenus*	S	none	18	none
beluga x sterlet, *Huso huso x A. ruthenus*	BxS	none	6	2
Paddlefish, *Polyodon spathula*	W	11	none	none
Beluga, *Huso huso**	B	none	none	none

* tissue samples of 7 beluga specimens were obtained from the River Volga (Russia)

(Estoup et al. 1993; Hansen et al. 2000; Nielsen and Sage 2001), tilapia (Kocher et al. 1998; Streelman and Kocher 2002), and many others. Microsatellite loci were applied in several genetic studies in Acipenseridae, for example in:

- studies of the inheritance of microsatellite loci (Pyatskowit et al. 2001; Rodzen and May 2002),
- studies of genome duplication events and functional reduction of ploidy levels (Ludwig et al. 2001),
- characterization of the sturgeon population structure and gene flow (Smith et al. 2002; Wirgin et al. 2002),
- genetic identification of hybridization events (Tranah et al. 2004).

In this study nine microsatellite loci (*Afu-19, Afu-22, Afu-34, Afu-39, Afu-54, Afu-57, Afu-58, Afu-68, Afu-69*), described by May et al. (1997) for American sturgeons, were analysed for five sturgeon species (Siberian sturgeon, Russian sturgeon, sterlet, beluga and paddlefish) and for three interspecific hybrids: Siberian sturgeon x Russian sturgeon, beluga x sterlet (the hybrid is called bester) and beluga x bester (Table 1). All the examined fish, except beluga, were reared on Polish aquaculture farms. Certain alleles at five diagnostic loci: *Afu-22, Afu-39, Afu-54, Afu-57* and *Afu-68*, allowed for the identification of sturgeon species and their hybrids.

MATERIAL AND METHODS

Fins were clipped or scutes were taken from fish on the fish farms. Pieces of fin were stored in 96% ethanol and scutes were dried and stored in envelopes at room temperature. The DNA was isolated using Wizard genomic Purification Kit (Promega, Madison, WI, USA). The research was conducted on 4 sturgeon species and 3 sturgeon hybrids obtained from three farms in Poland, whereas the belugas were caught from the River Volga, Russia (Table 1).

Nine loci were analysed: *Afu-19*, *Afu-22, Afu-34, Afu-39, Afu-54, Afu-57, Afu-58, Afu-68, Afu-69* (May et al. 1997). Reaction mixes were prepared in a total volume of 15 μl with 0.5 μl DNA template, 1.5 μl PCR reaction buffer (50 mM KCl, pH 8.5; Triton X-100), 0.5 μl of each primer, 1μl (500 μM) of each deoxynucleotide triphosphate (dNTP), 0.8 μl MgCl2 and 1 μl Schark Max DNA polymerase (DNA-Gdansk). Re-distilled water was used to bring the reaction mixture to the desired final volume. Amplification was conducted with a Perkin Elmer thermocycler Gene Amp-System 9600 (PE-Applied Biosystem, California, USA), with initial denaturation at 94°C for 5 min, followed by 30 amplification cycles (94°C, 1 min; 53-57°C, 30s; 72°C-30s) and final elongation at 72°C for 5 min. Aliquots containing PCR products and reaction buffer were electrophoresed using 6% polyacrylamide gel, and DNA bands were then visualized by the silver staining method (Tegelström 1986). Electrophoresis was conducted on Bio-Rad SequiGen Sequencing Cell-system, and gel size was 38x30cm. Amplified fragments were sized by comparison with a DNA standard ϕX 174 DNA/*Hinf* I DNA Step Ladder (Promega).

Haplotype frequencies estimation were analysed with the use of a computer program Arlequin 2.0 (Schneider et al. 2000). The "Heterozygosity test" as implemented in the software Bottleneck (Cornuet and Luikart 1996) was applied. This test is based on the principle that populations with a recent reduction in effective population size typically exhibit a reduction in both allele number and heterozygosity, but with a faster reduction of allele number as compared to genetic diversity (Maruyama and Fuerst 1985). If a recent population bottleneck has occurred, the observed gene diversity (H_o) will be larger than the expected gene diversity (H_e) based on the number of haplotypes present in the population. We tested for recent population bottlenecks assuming a Stepwise Mutation Model (SMM) and Infinite Allele Model (IAM) for sterlet, beluga, bester, beluga x bester, Russian sturgeon, Siberian sturgeon, hybrid of Siberian sturgeon and Russian sturgeon and paddlefish microsatellite evolution.

RESULTS

Six (*Afu-19, Afu-22, Afu-39, Afu-54, Afu-57, Afu-68*) of the nine microsatellite primer pairs produced electrophoretically resolvable products when tested on sturgeon species and hybrids. The number of alleles per locus ranged from 7 (*Afu-19*) to 29 (*Afu-68*), and the allele size ranged from 114 to 324 base pairs (bp). Unique diagnostic alleles were found at loci: *Afu-22* for sterlet, Siberian sturgeon and Russian sturgeon, *Afu-39* for beluga and Russian sturgeon, *Afu-54* for beluga, Siberian sturgeon, Russian sturgeon and paddlefish, *Afu-57* for sterlet and Siberian sturgeon, and *Afu-68* for sterlet, Siberian sturgeon, Russian sturgeon and paddlefish. Polymorphism was observed at all the examined loci in all 5 sturgeon species and in their three interspecific hybrids.

Seven alleles were observed at the locus *Afu-19*. Amplification of the locus *Afu-19* for beluga and paddlefish was not successful; at this locus no diagnostic alleles for studied sturgeon species were observed.

Locus *Afu-22* was characterized by 23 alleles. Amplification of locus *Afu-22* in beluga was not successful. The allele of 186 bp was characteristic for sterlet (Table 2). For Siberian sturgeon 6 private alleles were found, but their frequencies were very low; Russian sturgeon was characterized by 2 private alleles: *144, 210* (Table 2).

Table 2. Allele frequencies at locus *Afu-22* in examined sturgeon species and hybrids. Certain alleles of this locus were diagnostic for sterlet, Siberian sturgeon, Russian sturgeon and paddlefish.

Allele	Examined fish						
(bp)	**S**	**BS**	**BBS**	**SB**	**R**	**SBxR**	**W**
n	18	8	4	54	16	30	11
144	-	-	-	-	0.03	-	-
147	-	-	-	0.26	-	0.08	1.00
150	-	0.06	-	-	-	-	-
153	-	-	-	0.01	0.06	0.05	-
156	-	0.06	0.25	-	-	-	-
159	-	0.38	-	0.03	-	-	-
162	-	-	-	0.05	-	0.02	-
165	-	-	-	0.19	-	0.02	-
168	0.28	-	-	0.03	0.03	-	-
171	-	-	-	0.06	-	-	-
174	-	0.06	-	-	-	-	-
177	0.25	-	-	0.25	0.31	0.32	-
180	-	-	-	0.01	-	-	-
183	-	-	-	0.05	-	0.02	-
186	0.47	-	0.25	-	-	-	-
189	-	0.13	-	-	-	0.05	-
192	-	0.06	-	0.01	-	-	-
195	-	0.19	-	-	-	0.10	-
198	-	-	*0.50	-	0.16	-	-
201	-	0.06	-	0.02	-	-	-
204	-	-	-	0.01	-	0.15	-
207	-	-	-	0.03	0.06	0.02	-
210	-	-	-	-	0.35	0.18	-

"-" – 0.00

n – number of examined fish, bp - allele size in base pairs

S – sterlet, BS – bester (hybrid of beluga and sterlet), BBS - hybrid of beluga and bester, R – Russian sturgeon, SB – Siberian sturgeon, SBxR – hybrid of Siberian sturgeon and Russian sturgeon, W – paddlefish

* – fishes morphologically identified as BBS, whereas allele frequencies preclude this indication

Table 3. Allele frequencies at locus *Afu-39* in examined sturgeon species and hybrids. Certain alleles at this locus were diagnostic for beluga and Russian sturgeon.

Allele	Examined fish						
(bp)	S	BS	BBS	B	R	SB	SBxR
n	18	7	4	5	39	65	31
114	-	-	-	0.20	-	-	-
117	-	-	*0.87	-	-	-	-
120	-	-	0.13	0.10	0.03	0.01	-
123	0.17	-	-	-	0.28	0.42	0.50
126	0.19	0.50	-	0.50	0.04	0.49	0.40
129	0.50	0.36	-	0.10	0.01	0.08	0.10
132	0.14	-	-	-	0.13	-	-
135	-	0.14	-	0.10	0.48	-	-
150	-	-	-	-	0.01	-	-
156	-	-	-	-	0.01	-	-
159	-	-	-	-	0.01	-	-

" – " – 0.00

n – number of examined fish, bp – allele size in base pairs

S – sterlet, BS – bester (hybrid of beluga and sterlet), BBS – hybrid of beluga and bester, B – beluga, R – Russian sturgeon, SB – Siberian sturgeon, SBxR – hybrid of Siberian sturgeon and Russian sturgeon

* – fishes morphologically identified as BBS, whereas allele frequencies preclude this indication

Table 4. Allele frequencies at locus *Afu-54* in examined sturgeon species and hybrids. Certain alleles at this locus were diagnostic for sterlet, Siberian sturgeon, Russian sturgeon and paddlefish.

Allele	Examined fish						
(bp)	S	BS	B	R	SB	SBxR	W
n	18	6	6	38	64	30	11
164	-	-	-	-	-	-	0.50
172	1.00	0.08	-	-	-	-	0.50
176	-	-	-	-	-	0.02	-
184	-	-	-	-	0.01	0.10	-
188	-	-	-	0.03	-	-	-
192	-	-	-	0.15	0.02	0.05	-
196	-	-	-	0.29	0.36	0.25	-
200	-	-	-	0.07	0.01	0.03	-
204	-	-	-	0.16	0.29	0.10	-
208	-	-	-	0.13	-	0.12	-
212	-	-	-	0.08	0.22	0.28	-
216	-	-	-	0.09	0.05	0.05	-
224	-	-	0.08	-	0.01	-	-
228	-	-	-	0.01	-	-	-
232	-	-	0.17	-	0.01	-	-
236	-	-	-	-	0.01	-	-
240	-	-	-	-	0.01	-	-
248	-	0.42	0.58	-	-	-	-
252	-	-	0.08	-	-	-	-
272	-	-	0.08	-	-	-	-
324	-	*0.50	-	-	-	-	-

" – " – 0.00

n – number of examined fish, bp – allele size in base pairs

S – sterlet, BS – bester (hybrid of beluga and sterlet), B – beluga, R – Russian sturgeon, SB – Siberian sturgeon, SBxR – hybrid of Siberian sturgeon and Russian sturgeon, W – paddlefish

* – fishes morphologically identified as BS, whereas allele frequencies preclude this indication

Table 5. Allele frequencies at locus *Afu-57* in examined sturgeon species and hybrids. Two allles at this locus were diagnostic for sterlet.

Allele	Examined fish					
(bp)	S	BS	R	SB	SBxR	W
n	18	6	31	62	28	11
126	-	-	-	-	0.02	-
138	-	-	-	0.01	-	-
141	-	0.08	0.01	0.11	0.09	-
144	-	-	0.18	0.15	0.14	-
147	-	0.08	0.26	0.31	0.30	-
150	-	-	0.23	0.28	0.30	0.50
153	-	-	0.13	0.02	-	0.50
156	-	-	0.10	0.02	-	-
159	-	-	0.03	0.02	-	-
162	-	-	0.01	0.01	-	-
165	-	-	-	0.01	0.06	-
168	-	-	-	0.03	0.02	-
174	-	-	0.03	0.02	0.07	-
177	-	0.08	-	0.01	-	-
183	0.08	-	-	-	-	-
186	0.75	0.08	0.01	-	-	-
189	0.11	0.34	-	-	-	-
192	0.06	0.34	-	-	-	-

" – " – 0.00

n – number of examined fish, bp – allele size in base pairs

S – sterlet, BS – bester (hybrid of beluga and sterlet), R – Russian sturgeon, SB – Siberian sturgeon, SBxR – hybrid of Siberian sturgeon and Russian sturgeon, W – paddlefish

In total we observed 11 alleles at the locus *Afu-39*. For paddlefish, amplification of this locus was not successful. Four alleles were observed at the *Afu-39* locus for sterlet, and four alleles for Siberian sturgeon. Private alleles for Russian sturgeon were: *150, 156*, and *159* (Table 3).

At the locus *Afu-54* there were 21 alleles. There was one allele 172 bp for sterlet, and three diagnostic alleles for beluga (Table 4). This locus appeared useful for beluga and sterlet identification. For Siberian sturgeon 11 alleles were observed, whereas Russian sturgeon was characterized by 9 alleles. Two alleles were observed for paddlefish: *164* and *172* bp (Table 4).

Locus *Afu-57* was not scoreable for beluga and for the hybrid of beluga and bester. For sterlet there were three diagnostic alleles: *183, 189* and *192* bp, and for Siberian sturgeon three diagnostic alleles were observed (*138, 165* and *168* bp) (Table 5).

There were 29 alleles at the locus *Afu-68*. Siberian sturgeon was characterized by 15 alleles of a length ranging from *132* to *248* bp. Allele *204* bp was diagnostic for Siberian sturgeon. Alleles *124, 128, 136, 244* and *252* bp were diagnostic for Russian sturgeon (Table 6).

All the studied loci, except locus *Afu-54* for sterlet and *Afu-22* for paddlefish, were polymorphic.

All the studied groups of fish were characterized by various gene diversity in analyzed microsatellite loci. The heterozygosity excess test revealed that the paddlefish population had a higher observed gene diversity than expected under the SMM and IAM in three studied microsatellite loci (Table 7). The observed gene diversity in beluga at locus *Afu-54*, Siberian sturgeon at locus *Afu-22* and *Afu-54*, and Russian sturgeon at locus *Afu-39*, deviated strongly from the expected gene diversity under SMM and had a significantly lower gene diversity whereas the observed gene diversity in Siberian sturgeon and Russian sturgeon at locus *Afu-68* and Russian sturgeon at locus *Afu-54* was significantly lower than the diversity expected under IAM.

DISCUSSION

In spite of the economical importance of sturgeons, knowledge of their phylogenetic and taxonomic relationships is still limited, mainly because of their morphological variability, ontogenic allometry, and peculiar ability to yield fully fertile hybrids in the wild between taxonomically distant species (Berg 1962). Taxo-

Table 6. Allele frequencies at locus *Afu-68* in examined sturgeon species and hybrids. Certain alleles at this locus were diagnostic for sterlet, Siberian sturgeon, Russian sturgeon and paddlefish.

Allele	Examined fish							
(bp)	S	BS	BBS	B	R	SB	SBxR	W
n	18	6	4	6	44	81	26	11
116	-	-	-	-	-	-	0.03	-
124	-	-	-	-	0.07	-	0.01	-
128	-	-	-	-	0.14	-	0.05	-
132	-	-	-	0.08	0.02	0.02	0.12	-
136	-	-	-	-	0.37	-	0.12	-
140	-	-	-	0.42	0.06	0.18	0.06	0.45
144	-	-	-	0.08	0.02	0.06	-	-
148	-	-	-	-	0.12	0.03	0.13	-
152	-	-	-	0.08	0.02	0.01	0.09	-
156	-	-	-	-	-	0.33	0.11	0.1
160	-	-	-	0.25	-	0.01	0.01	-
164	-	0.17	0.75	-	-	-	-	-
168	-	-	-	-	-	-	-	0.45
176	-	-	-	0.08	0.02	0.04	-	-
180	-	-	*0.25	-	-	0.09	0.06	-
184	0.11	-	-	-	0.09	-	-	-
188	-	-	-	-	-	-	-	-
200	-	0.25	-	-	-	0.06	-	-
204	-	-	-	-	-	0.07	0.01	-
208	0.39	-	-	-	-	0.02	-	-
212	0.11	-	-	-	-	-	-	-
216	0.03	0.08	-	-	-	-	-	-
224	-	0.08	-	-	-	-	-	-
228	0.36	-	-	-	-	0.06	0.10	-
232	-	0.17	-	-	-	0.01	-	-
236	-	0.25	-	-	-	-	-	-
244	-	-	-	-	0.01	-	0.03	-
248	-	-	-	-	0.05	0.01	0.04	-
252	-	-	-	-	0.01	-	0.03	-

"-" – 0.00

n – number of examined fish, bp - allele size in base pairs

S – sterlet, BS – bester (hybrid of beluga and sterlet), BBS - hybrid of beluga and bester, R – Russian sturgeon, SB – Siberian sturgeon, SBxR – hybrid of Siberian sturgeon and Russian sturgeon,

W – paddlefish

* – fishes morphologically identified as BBS, whereas allele frequencies preclude this indication

nomical diagnoses based exclusively on morphological characters are often disclaimed by genetic and molecular data: for example, the two species of the genus *Huso* (*H. huso*, and *H. dauricus*) are genetically more distant to each other than they are to those of the genus *Acipenser* (Birstein and DeSalle 1998).

Most studies on molecular characteristics of sturgeons have been performed on American species and only recently have the studies been extended to Eurasian species. There are still few studies of genetic relationships among sturgeons based on nuclear genome. Due to the variability in genome size (different ploidy levels) among species, and the ease of inter-specific hybridization, this approach appears to be difficult. To date, the identification of sturgeons and of the origin of black caviar were limited to the use of mitochondrial DNA markers (Birstein et al. 1997b; Birstein and DeSalle 1998; Birsteinet al. 1998a,b; DeSalle and Birstein 1996) and microsatellite DNA analysis

Table 7. Comparison of observed (H_o) and expected (H_e) gene diversity in studied sturgeons. Expected gene diversities were calculated assuming a Stepwise Mutation Model (SMM) and Infinite Allele Model (IAM).

Species or hybrid	Gene diversity						
	Observed			Expected			
locus				SMM		IAM	
	n	k_o	H_o	H_e	*P*	H_e	*P*
sterlet							
Afu-19	36	2	0.49	0.28	0.14	0.23	0.10
Afu-22	36	3	0.66	0.50	0.07	0.39	0.04
Afu-39	36	4	0.68	0.62	0.31	0.51	0.11
Afu-57	36	4	0.43	0.62	0.06	0.51	0.31
Afu-68	36	5	0.71	0.71	0.45	0.60	0.19
beluga							
Afu-39	10	5	0.76	0.82	0.17	0.79	0.34
Afu-54	12	5	0.67	0.79	0.04	0.76	0.19
Afu-68	12	2	0.80	0.36	0.00	0.32	0.00
bester (beluga x sterlet)							
Afu-19	10	3	0.60	0.60	0.45	0.56	0.58
Afu-22	16	8	0.84	0.89	0.07	0.87	0.25
Afu-39	14	3	0.65	0.56	0.29	0.50	0.17
Afu-54	12	3	0.62	0.58	0.53	0.53	0.36
Afu-57	12	6	0.82	0.85	0.25	0.83	0.46
Afu-68	12	6	0.88	0.85	0.29	0.82	0.15
beluga x bester							
Afu-22	8	3	0.71	0.64	0.26	0.61	0.19
Afu-39	8	2	0.25	0.41	0.35	0.39	0.44
Afu-68	8	2	0.43	0.41	0.67	0.38	0.53
Siberian sturgeon							
Afu-19	62	5	0.68	0.68	0.41	0.55	0.19
Afu-22	108	14	0.83	0.89	0.02	0.81	0.48
Afu-39	132	4	0.58	0.58	0.39	0.41	0.21
Afu-54	148	11	0.74	0.86	0.01	0.73	0.45
Afu-57	124	13	0.80	0.88	0.08	0.79	0.45
Afu-68	162	15	0.83	0.80	0.38	0.89	0.01
Russian sturgeon							
Afu-19	62	6	0.76	0.74	0.44	0.61	0.08
Afu-22	32	7	0.77	0.81	0.21	0.73	0.38
Afu-39	78	9	0.69	0.83	0.01	0.72	0.30
Afu-54	76	9	0.84	0.83	0.42	0.72	0.04
Afu-57	62	10	0.84	0.85	0.23	0.77	0.19
Afu-68	84	13	0.82	0.81	0.46	0.88	0.01
Siberian sturgeon x Russian sturgeon							
Afu-19	42	5	0.78	0.70	0.11	0.59	0.02
Afu-22	60	11	0.84	0.87	0.09	0.80	0.36
Afu-39	62	3	0.59	0.49	0.20	0.36	0.08
Afu-54	60	9	0.83	0.84	0.36	0.74	0.12
Afu-57	56	8	0.79	0.82	0.23	0.71	0.24
Afu-68	52	16	0.93	0.89	0.04	0.77	0.49
paddlefish							
Afu-54	22	2	0.52	0.31	0.03	0.27	0.03
Afu-57	22	2	0.52	0.31	0.03	0.26	0.02
Afu-68	22	3	0.61	0.53	0.29	0.45	0.16

n – sample size (number of gene copies); k_o – number of alleles; H_o – observed gene diversity (Nei 1978); H_e – expected average gene diversity under mutation drift equilibrium

(Jenneckens et al. 2001). In some cases information obtained from studies of mitochondrial DNA markers should be considered with some caution. For instance, caviar derived from "bester" females (the cross of a *H. huso* female and an *A. ruthenus* male) when identified solely on the mtDNA would be designated as beluga (*H. huso*) caviar.

The main aim of this study was to find the microsatellite loci that could be applied in genetic identification of various sturgeon species and their interspecific hybrids in Polish aquaculture conditions. Six alleles diagnostic for Siberian sturgeons were found in locus *Afu-22* (*162, 165, 171, 180, 183* and *204* bp; Table 2), three alleles in locus *Afu-54* (*184, 236* and *240* bp; Table 4), three alleles in locus *Afu-57* (*138, 165, 168*; Table 5), and one allele in locus *Afu-68* (*204* bp; Table 6), whereas for Russian sturgeons we showed two diagnostic alleles in locus *Afu-22* (*144* bp and *210* bp; Table 2), three in locus *Afu-39* (*150, 156* and *159* bp; Table 3), three alleles in locus *Afu-54* (*188, 208, 228* bp; Table 4), and five alleles in locus *Afu-68* (*124, 128, 136, 244* and *252* bp; Table 6).

For sterlet the diagnostic alleles were observed in locus *Afu-22* (186 bp; Table 2), in locus *Afu-57* (*183, 189* and *192* bp) and in locus *Afu-68* (*212* and *216* bp; Table 6).

Alleles diagnostic for beluga were in locus *Afu-39* (*114* bp) and in locus *Afu-54* (*248, 252, 272* bp; Table 4).

All the loci and alleles listed above can be applied in sturgeon identification under the conditions of Polish aquaculture farms.

F_1 hybrids: Siberian sturgeon x Russian sturgeon, beluga x sterlet and beluga x bester, were characterized by alleles belonging to both respective parental species. Some hybrids did not have alleles characteristic for their parental species (Table: 2, 3, 4 and 6). This was possibly due to the mistaken morphological identification of fish by the farmers as the observed allele frequencies precluded such morphological indication.

Unambiguous species identification has become important for two main reasons: to expose commercial frauds due to intentional species substitution, and to help protect endangered species (for instance by genetic identification of caviar). In sturgeons, most efforts applied to species identification concern commercial caviar and mainly involve the analysis of mitochondrial DNA markers. However, mtDNA does not allow for recognition of interspecific hybrids. As sturgeon hybrids are increasingly being employed in aquaculture projects (Birstein et al. 1997b) and sport fishing, the ability to identify hybrids becomes important.

The use of genetic markers provides valuable information on population genetic structure in captive breeding fish. Under aquaculture conditions the broodstock sometimes have the gene pool of reduced diversity. Studies involving fish show that small populations quickly lose the rarest alleles (Allendorf 1986). Careful genetic management of the founder population can allay these losses (Lacy 1989), and multi-locus molecular markers (e.g. microsatellite DNAs) can be used to monitor the retention of diversity. The breeding system (e.g. small broodstock, biased sex-ratio, harem formation, inbreeding, high reproductive variance among individuals, etc.) and fluctuations in population size over time (the bottleneck effect) can reduce effective population size (*N*e) and result in the loss of allelic diversity in stocks. From a breeding perspective detection of recent dramatic changes in population size is an important aspect of any population monitoring programme. Signs of population bottleneck can be detected by using, for example, analyses of microsatellite DNA markers; in a recently bottlenecked population, the observed heterozygosity is higher than the expected equilibrium heterozygosity when calculated from the observed number of alleles (Luikart and Cornuet 1998).

In this study all examined groups of fish were characterized by various gene diversity in analyzed microsatellite loci. These results show that Polish breeding centres apply the proper breeding system for sturgeons, including, for example, artificial fertilization of eggs coming from one female by the mixture of sperm obtained from (usually) two males.

We have detected signs of a recent bottleneck in a population of paddlefish. This confirms the supposition that paddlefish, before their importation to Poland, probably suffered a bottleneck effect (for instance due to the small number of parental fish from which fertilized eggs we obtained). In this context, direct intervention (import of additional individuals of paddlefish or cryopreserved sperm) would be required to increase genetic diversity to the appropriate level. Also, regular monitoring of breeding populations, recognition of the normal population size fluctuations and estimation of the level of genetic diversity, as even if the number of founder specimens of the broodstock was genetically appropriate, wrong management of the stock can lead to inbreeding and a rapid decline in gene diversity in successive generations of fish.

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