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APPLICATION DNA FINGERPRINT ANALYSIS FOR IDENTIFICATION OF MIXED GROUPS OF SIBERIAN STURGEON (*ACIPENSER BAERI* BRANDT)*

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A b s t r a c t

DNA finferprinting analysis based on microsatellites was applied for separation of mixed gynogenetic offspring of Siberian sturgeon (*Acipenser baeri*) and individuals from commercial production. Variation at 11 microsatellite DNA loci was surveyed for parent of gynogenetic offspring. Thus microsatellite DNA profiles in studied loci were known and this key-point was aplied in segregation analysis of mixed fish. In results 108 individuals of 281 studied were verified as gynogenetic offspring. The present survey of microsatellite variation demonstrated a reliable tool for separation of mixed group of fish.

ZASTOSOWANIE ANALIZY GENETYCZNEGO ODCISKU PALCA DO SEPARACJI MIESZANYCH GRUP JESIOTRA SYBERYJSKIEGO (*ACIPENSER BAERI* BRANDT)

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S ł o w a k l u c z o w e: genetyczny odcisk palca, jesiotr syberyjski, mikrosatelitarny DNA, potomstwo gynogenetyczne.

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Abstrakt

W opracowaniu zastosowano analizę genetycznego odcisku palca (DNA fingerprinting) do odseparowania gynogenetycznego potomstwa jesiotra syberyjskiego (*Acipenser baeri* Brandt) oraz ryb tego samego gatunku pochodzących z komercyjnej produkcji. W badaniach zastosowano 11 par starterów mikrosatelitarnego DNA do identyfikacji profili genetycznych matki gynogenetycznego potomstwa oraz dawcy nasienia. W wyniku przeprowadzonych analiz molekularnych 108 osobników jesiotra syberyjskiego (spośród grupy liczącej 281 osobników) zidentyfikowano jako gynogenetyczne potomstwo posiadające genotyp odziedziczony wyłącznie po matce. Badania ukazują możliwość identyfikacji poszczególnych osobników w mieszanych stadach ryb za pomocą analizy polimorfizmu mikrosatelitarnego DNA.

Introduction

The Siberian sturgeon, *Acipenser baeri* (Brandt), has been domesticated in Europe and Asia (AKIMOVA 1985, WILLIOT et al. 1991, CHEBANOV and BILLARD 2001). The Siberian sturgeon is one of the most common and most important sturgeon species cultured in Poland. The economic importance of sturgeons together with the fact that they occupy a critical position in understanding vertebrate genome evolution has resulted in many genetic studies in those fishes. Genetic studies of sturgeons are further complicated by the reported tetraploid, octaploid and polyploid nature of those species (BLACKLIDGE and BIDWELL 1993).

DNA fingerprinting has become an important tool for genetic identification in fish breeding as well as wild life management and conservation (O'REILLY and WRIGHT 1995, BEACHAM et al. 2000). In the area of broodstock monitoring and conservation, DNA fingerprinting techniques are used to link individuals found in separate areas, determine migration patterns, estimate gene flow among individuals, profile the genetic diversity and manage captive breeding programs.

In planning for DNA fingerprinting one of the important decisions is the selection of the appropriate marker system and the techniques. Various systems and their related techniques are currently in use and those based on the Polymerase Chain Reaction (PCR). A powerful technique for DNA fingerprinting is based on PCR amplification of tandem repeated sequences, which have long been known to be polymorphic and widespread in plant, animal and human genomes referred to as Simple Sequence Repeat (SSR) or microsatellite DNA (O'REILLY and WRIGHT 1995). Microsatellites occur in many places throughout the genome, but in almost all cases they are in non-coding regions of the DNA (SCHLÖTTERER and TAUTZ 1992). Eukaryotic genomes contain large numbers of microsatellite genetic loci that are widely dispersed along and among chromosomes with known DNA sequence and consist of many tandem

repeats (SCHLÖTTERER 2000). Analysis of these sequences has yielded very high levels of polymorphism. This is due to tandem repeats, presumably resulting from unequal mitotic or meiotic exchanges or by DNA slippage during replication (SCHLÖTTERER 2000). Due to the high variability and abundance of microsatellites throughout the genome, their use as markers for DNA fingerprinting has been found to be a powerful tool for population genetic studies (O'REILLY and WRIGHT 1995, FOPP-BAYAT 2008, FOPP-BAYAT and WOZNICKI 2008) and pedigree reconstruction, specially for communally reared populations (HERBINGER et al. 1995, MOAZAMI-GOUDARZI 1997). These fragments are inherited in a Mendelian fashion and provide a technique suitable for genetic variation studies, forensic and ecological studies, breeding programs, and population genetics (TAUTZ 1989).

The main objective of the present study was genetic separation of two mixed groups of Siberian sturgeon (gynogenetic group and commercial group) based on microsatellite DNA analysis.

Material and Methods

The fin clips were sampled from 281 specimens reared in experimental fish farm Dgal in Pieczarki, Inland Fisheries Institute in Olsztyn, Poland. One part of fish were gynogenetic offspring of Siberian sturgeon breeded in Wasosze Fish farm near Konin, Poland in 2006 year, and the second group were reprsented by individuals of Siberian sturgeon from commercial aquaculture production, breeded in the same farm and at the same time. The procedure of obtaining the gynogenetic offspring of Siberian sturgeon was described by FOPP-BAYAT (2007). The fin clips were also sampled from female used in gynogenesis in 2006 and male – sperm donor. The two groups of fish were mixed and there are not morphological distinctive characters for separate its. Genomic DNA for amplification of eleven microsatellite loci [*Afu-39, Afu-68, AfuB-68,* (MAY et al. 1997), *Spl-104, Spl-105, Spl-113, Spl-163, Spl-168* (MCQUOWN et al. 2000) *Aox-45* (KING et al. 2001) and *AfuG-9*, *Afu-G122* (WELSH and MAY 2006)] was extracted using Chelex 100 (WALSH et al. 1991) – Table 1. All microsatellite loci were amplified for parent of gynogenetic group of fish and three primers pair (*Spl-104*, *Spl-113*, *Spl-168*) were clasified as reliable tool for identification gynogenetic offspring. From the three loci (*Spl-104*, *Spl-113*, *Spl-168*) one *Spl-168* was selected for gynogenetic offspring identification. Reaction mixes for amplification microsatellites were prepared in a total volume of 25 μl with 40 ng DNA template, 1x PCR reaction buffer (50 mM KCl, pH 8.5; Triton X-100), 0.4 mM of each primer, 0.25 mM) of each deoxynucleotide triphosphate (dNTP), 3.3 mM $MgCl_2$ and 0.6 unit Go Taq

Flexi DNA Polymerase (Promega, Madison, WI, USA). Re-distilled water was used to bring the reaction mixture to the desired final volume. Amplification was conducted with a Mastercycler gradient thermocycler (Eppendorf, Germany), with initial denaturation at 94°C for 5 min, followed by 35 amplification cycles (94°C, 1 min; 52–57°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 visualized by the silver staining method (TEGELSTRÖM 1986). Electrophoresis was conducted on a Bio-Rad SequiGen Sequencing Cell-system, and the gel size was 38 x 30 cm. Amplified fragments were sized by comparing migration with two DNA standards: ϕX 174 DNA/*Hinf* I DNA Step Ladder (Promega, Madison, WI, USA) and 25bp DNA Step Ladder (Promega, Madison, WI, USA). Every gel analyzing samples included two lanes containing the appropriate parental microsatellite PCR amplification products. Specific microsatellite profiles for parents were noted and compared to those from analyzed specimens.

Table 1
Primer sequences, annealing temperature and references of studied micxrosatellite loci in Siberian sturgeon (*Acipenser baeri*) specimens

Microsatellite locus	Primer sequence	T °C	References
Afu-39	F..TCCTGAAGTTCACACATTG R..ATGGAGCATTATTGGAAGG	57	MAY et al. 1997
Afu-68	F..TTATTGCATGGTGTAGCTAAAC R..AGCCCAACACAGACAATATC	55	MAY et al. 1997
AfuB-68	F..AACAATATGCAACTCAGCATAA R..AGCCCAACACAGACAATATC	55	WELSH and MAY 2006
Spl-104	F..TTATATGGGTGGGGTGGATG R..TCCTCTTTGGCATTTGTTCC	57	MCQUOWN et al. 2000
Spl-105	F..GCGATTTGATTGGCTCTTGT R..GGCACTGAATAAATGGACCG	57	MCQUOWN et al. 2000
Spl-113	F..TCCCACATGGCTTGTATTGA R..ACCACACCATGCGTCATAAG	57	MCQUOWN et al. 2000
Spl-163	F..TGCTTGTAAACTGCCCCACT R..CCACATGCAGTTTGAGCTGC	57	MCQUOWN et al. 2000
Spl-168	F..CACTGATTCGCTACAACCGT R..AGAAGGACTTGCAGTCCGAA	57	MCQUOWN et al. 2000
Aox-45	F..TTGTCCAATAGTTTCCAACGC R..TGTGCTCCTGCTTTTACTGTC	53	MCQUOWN et al. 2001
AfuG-9	F..CATAATGTAAAGCAAAAGT R..ACCTGAAATGTATGTTATG	52	WELSH and MAY 2006
AfuG-122	F..AACACGACAACAAACTTATTCA R..TGTGTTTCTATGTCTGTCTGTCTA	52	WELSH and MAY 2006

Results and Discussion

In results 108 specimens were verified as gynogenetic offspring while 173 were identified as fish from commercial production using microsatellite locus *Spl-168*. Alleles *212* and *224* bp were observed at locus *Spl-168* in female of Siberian sturgeon (mother of gynogenetic offspring) while allele *184* bp was characteristic for male – sperm donor at the same locus. All verified gynogenetic offspring possesed alleles identical to female used for meiotic gynogenesis. In this group of fish allele *212* and *224* base pairs (bp) were observed. In the second verified group of fish eleven alleles was identified: *248*, *244*, *224*, *220*, *216*, *212*, *208*, *204*, *200*, *112* and *94* bp at studied microsatellite *Spl-168* locus (Figure 1). The alleles identified in gynogenetic group of fish was also observed in the commercial breeding group because the same female was also used to commercial reproduction.

Genetic structure of broodstock analysis of commercially important fish species is essential for optimizing management strategy or stock improvement program. Genetic monitoring is necessary for an effective management strategy because a population can suffer severe genetic erosion for example: bottleneck, genetic drift, inbreeding, founder effect etc. DNA fingerprinting technology with special emphasis on microsatellites is useful in application to fisheries and aquaculture for example in selection and breeding programmes for aquaculture broodstock.

Many applications of microsatellite DNA analysis have been used to investigate stock structure and stock contributions to mixed-stock fisheries. McConnel et al. (1995) were able to discriminate clearly between Canadian and European *Salmo salar*, Ruzzante et al. (1996) differentiated inshore and offshore Atlantic cod, Taylor (1995) described genetic variation among North Pacific populations of steelhead and rainbow trout. Microsatellite markers were also applied for stock identification to management of coho salmon in British Columbia (Beacham et al. 2001).

In sturgeon fishes microsatellite DNA analysis was applied for example in:
- analysis of variation in Atlantic sturgeon (*Acipenser oxyrhinchus oxyrhinchus*) and cross-species amplification in the Acipenseridae (King et al. 2001),
- analysis of genetic variation in the Chinese sturgeon *Acipenser sinensis*; estimating the contribution of artificially produced larvae in a wild population (Zhu et al. 2002),
- genetic identification of black caviar (Jenneckens et al. 2001, Fopp-Bayat 2007, Ludwig 2008),
- estimation of parentage and relatedness in white sturgeon *Acipenser transmontanus* (Rodzen et al. 2004),
- study of genetic population structure of lake sturgeon *Acipenser fluvescens* (DeHaan et al. 2006),

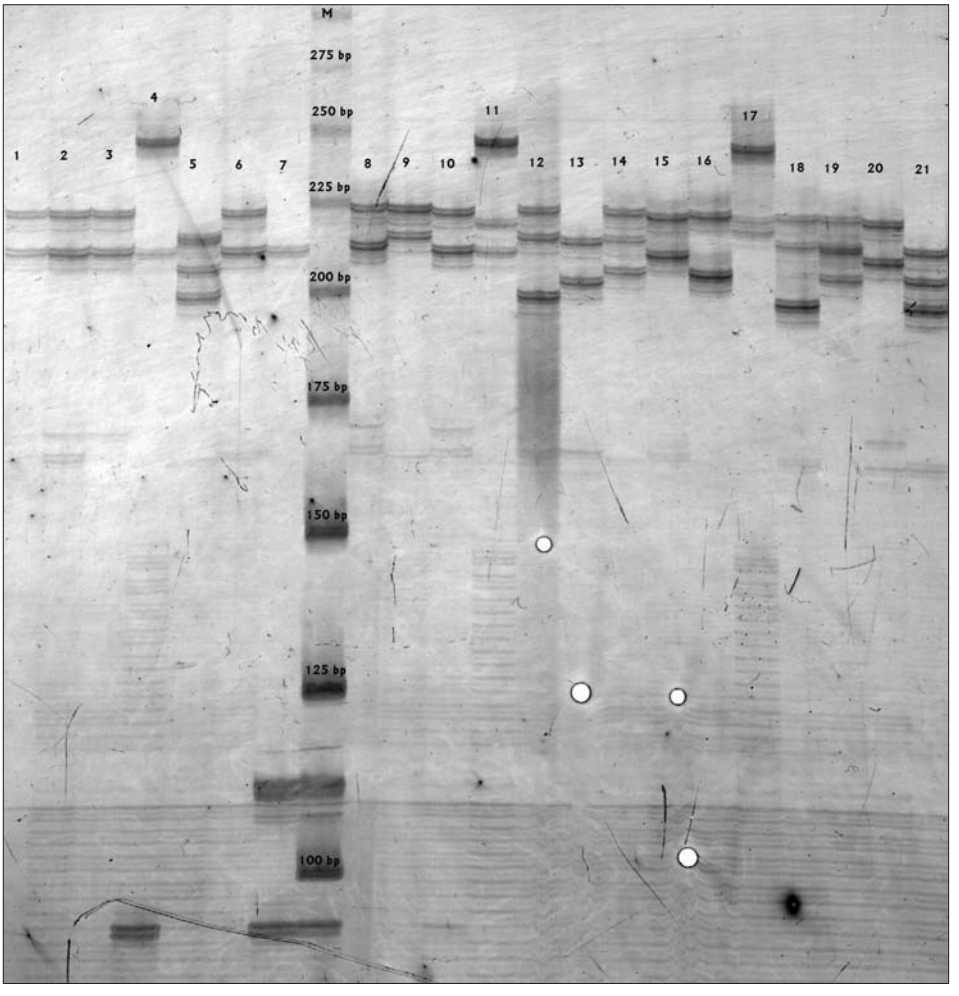

Fig. 1. Allele segregation at microsatellite locus *Spl-168* in Siberian sturgeon (*Acipenser baeri*): M – marker 25 bp (Promega); female of Siberian sturgeon (mother of gynogenetic offspring) – sample 1; gynogenetic offspring – samples: 2, 3, 6, 8, 10, 15, and 20; individuals from comercial production – samples: 4, 5, 7, 9, 11, 12, 13, 14, 16, 17, 18, 19, and 21

– verification of genome manipulation in sterlet *Acipenser ruthenus* and siberian sturgeon *Acipenser baeri* (FOPP-BAYAT et al. 2007, FOPP-BAYAT 2007).

Conclusion

The present study was designed to characterize genetically the progeny of Siberian sturgeon from experimental group mixed with group of fish from commercial production. This method is especially important for genetic monitoring of broodstock condition and it could be applicable in simmilar situations when some unfortunately mixing of fish groups occurred. Moreover identification of polymorphic markers with consistent scorable alleles is a crucial step to generate genetic profile data for spawners.

The present survey reveal enough differentiation between gynogenetic group of fish and group from commercial production. This method could be applied in estimation of broodstock composition in aquaculture or for identification of mixed stock in natural ekosystem.

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