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Genetic identification of black caviar based on microsatellite DNA analysis

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ABSTRACT

Sturgeons (Acipenseridae) are producers of black caviar, which is sold world-wide. Black caviar differs widely in quality, availability, price and taste and for these reasons it is often the subject of commercial fraud. Identification of sturgeon species is frequently based on molecular methods such as analysis of nuclear DNA and mitochondrial DNA. In this study two fragments of microsatellite DNA: *Afu-39* and *Afu-68* were analyzed. In caviar samples, four alleles were observed in locus *Afu-39*, and six alleles in locus *Afu-68*. This paper describes the use of microsatellite DNA markers for species identification of black caviar.

INTRODUCTION

Sturgeons (Acipenseridae) are the source of the raw material for producing black caviar, sold world-wide. Habitat degradation and overfishing threaten the survival of many sturgeon species (Birstein 1993), and almost all natural populations of the 27 species of this group are endangered.

Traditionally, three sturgeon species have been used for commercial caviar production: *Huso huso* L., *Acipenser gueldenstaedti* Brand, and *Acipenser stellatus* Pallas. Different processing techniques, from egg to caviar, have their effect; but the source of the eggs has the main impact on taste, and supply the main impact on the final value of the caviar. This determines the nature of the resulting fraud, mainly mis-labeling, and indicates the need for an easy method of product-source identification to frustrate it (Gessner et al. 2002).

Caviar dealers have usually relied on crude indicators, such as the size and the general appearance of the eggs, and their smell, texture and colour. But this method is often unreliable in identifying the species origin of black caviar. Several molecular methods have been developed to identify species for both forensic and conservation purposes, mainly as a result of the increasing commercial demand for sturgeon products (caviar and meat). Most of these methods were based on mtDNA analysis (Birstein et al. 1998; Ludwig 2006; Ludwig et al. 2002). However, the main drawback of mitochondrial markers is their limited usefulness in monitoring interspecific hybridization events. Thus, a method for species identification of caviar is needed in order to help the enforcement of current fisheries regulations and to ensure the conservation of the fish. The method of caviar brand identification should be simple and quick if it is to help in monitoring the production and distribution of black caviar around the world.

Analysis of microsatellite DNA seems to be accurate enough for correct species identification (Estoup 1998; Fopp-Bayat 2004). Only very small tissue samples, a few eggs of the fish, are needed when microsatellite loci are assayed with the help of polymerase chain reaction (PCR). This makes the method suitable in identifying caviar obtained from endangered and threatened species.

This paper describes the use of microsatellite DNA markers for the identification of black caviar and is focused on two loci: *Afu-39* and *Afu-68*.

MATERIAL AND METHODS

Research was conducted on five samples of black caviar: two of them (caviar A and B) were taken from two jars of caviar produced in Astrakhan (Russia), two others (C and D) were produced in the Wasosze Fish Farm near Konin, Poland. Caviar "C" was produced from Russian sturgeon eggs and caviar "D" from a hybrid of the first generation between Russian sturgeon and Siberian sturgeon. Eggs for caviar "C" and "D" samples were obtained after artificial reproduction of the sturgeon species. The fifth sample (E) was an artificial caviar, a substitute produced in Russia, that consisted of "jelly granules" of an unknown composition.

DNA isolation was conducted on four eggs (or granules) from each sample: A, B, C, D, and E. Three methods for DNA isolation were applied: 1-DNeasy Tissue Kit (QIAGEN, GmbH, Hilden), 2-Wizard Genomic Purification Kit (Promega, Madison, WI, USA), and 3-phenol-chloroform method (Sambrook et al. 1989) modified with proteinase K digestion (1mg·ml^{-1} final concentration) for 1 hour at 60°C.

On the basis of my earlier results (Fopp 2003), two microsatellite loci were analyzed: *Afu-39* and *Afu-68* (May et al. 1997). Reaction mixes were prepared in a total volume of 15μl with a 0.5μl DNA template, 1.5μl PCR reaction buffer (50mM KCl, pH 8.5; Triton X-100), 0.5μl of each primer, 1μl (500μM) of each deoxynucleotide triphosphate (dNTP), 0.8μl $MgCl_2$ and 1μl Shark MAX DNA polymerase (DNA-Gdansk, Poland). 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 minutes, followed by 30 amplification cycles (94°C, 1min; 53-57°C, 30s; 72°C, 30s) and final elongation at 72°C for 5 minutes. Aliquots containing PCR products and reaction buffer were electrophoresed using 6% polyacrylamide gel, and DNA bands were then visualized by the silver staining method. Electrophoresis was conducted on Bio-Rad SequiGen Sequencing Cell-system, and gel size was 38x30cm. Amplified fragments were sized by comparison with the DNA standard ΦX 174 DNA/Hinf I DNA Step Ladder (Promega).

RESULTS

Of the three different DNA isolation methods applied in this study, only the isolation with DNeasy Tissue Kit (QIAGEN, GmbH, Hilden) was successful because amplification of microsatellite DNA loci was possible only with this method.

Amplification of two fragments of microsatellite DNA: *Afu-39* and *Afu-68*, produced fragments of microsatellite DNA characteristic for the studied caviar samples (Figure 1 and Figure 2). Locus *Afu-39* was characterized by four alleles: *114, 117, 123* and *126* base pairs (bp) long (Figure 1). Two alleles (*114* and *117* bp) were observed in the sample of caviar "A". Allele *117* was also found in caviar "B". But A and B samples were differentiated by other alleles (Figure 1). Locus *Afu-39* for caviar D was characterized by the two alleles: *123* and *126* bp, that were distinctive for Russian sturgeon and Siberian sturgeon (Figure 1). Whereas two alleles: *114*, and *117* indicated the Russian sturgeon in caviar C.

Locus *Afu-68* was represented by seven alleles: *128, 136, 140, 148, 156, 160*, and *232* bp (Figure 2). In sample A four of them: *128, 136, 140* and *148* were found while caviar B sample showed three alleles: *156, 160,* and *232*. DNA amplification of locus *Afu-68* was unsuccessful for caviar C, D, and E (Figure 2).

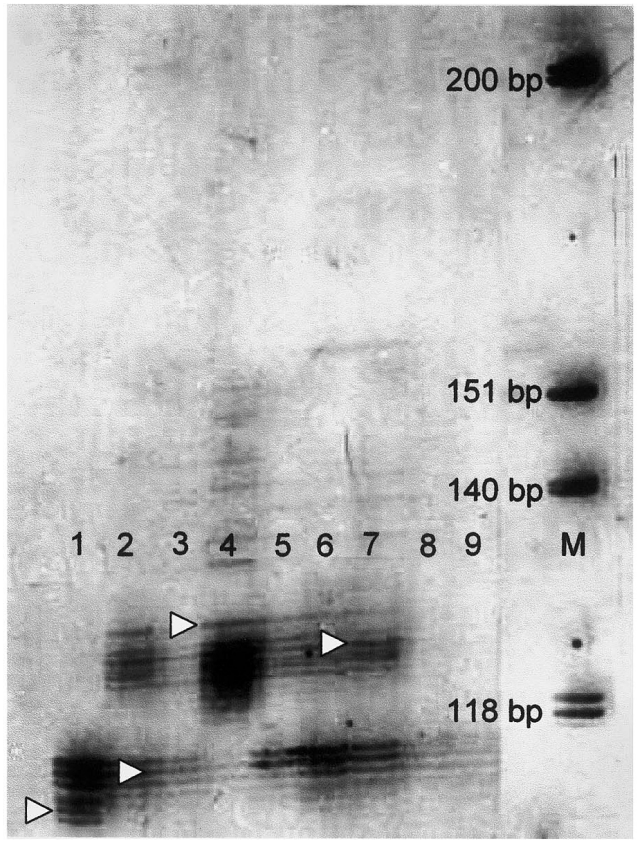

Figure 1. Polymorphism at locus *Afu-39* observed in samples of black caviar. Lane 1 – caviar A, lane 2 and lanes from 6 to 9 – caviar B, lane 3 – caviar C, lane 4 – caviar D, lane 5 – caviar E, and lane M – marker ΦX 174. Arrowheads point marker alleles (from left to right): *114, 117, 126,* and *123*.

DISCUSSION

Unambiguous species identification has become important for two main reasons: to expose commercial fraud (by species substitution) and to help to protect endangered species. Molecular markers are useful tools in solving forensic problems of identification of sturgeon and their commercial products, such as caviar and meat (Estoup 1998, Ludwig 2006).

The use of mitochondrial genes for identifying sturgeon species has been described elsewhere (Birstein et al. 1998; DeSalle and Birstein 1996). However, the results based on mtDNA are not always conclusive, since only maternal mtDNA is present in the cells of the progeny. Hybridizations between different sturgeon species are known both from natural waters and aquaculture conditions (Birstein et al. 1997), and hybrids between species with the same chromosome number are fertile. For instance, caviar derived from females of the

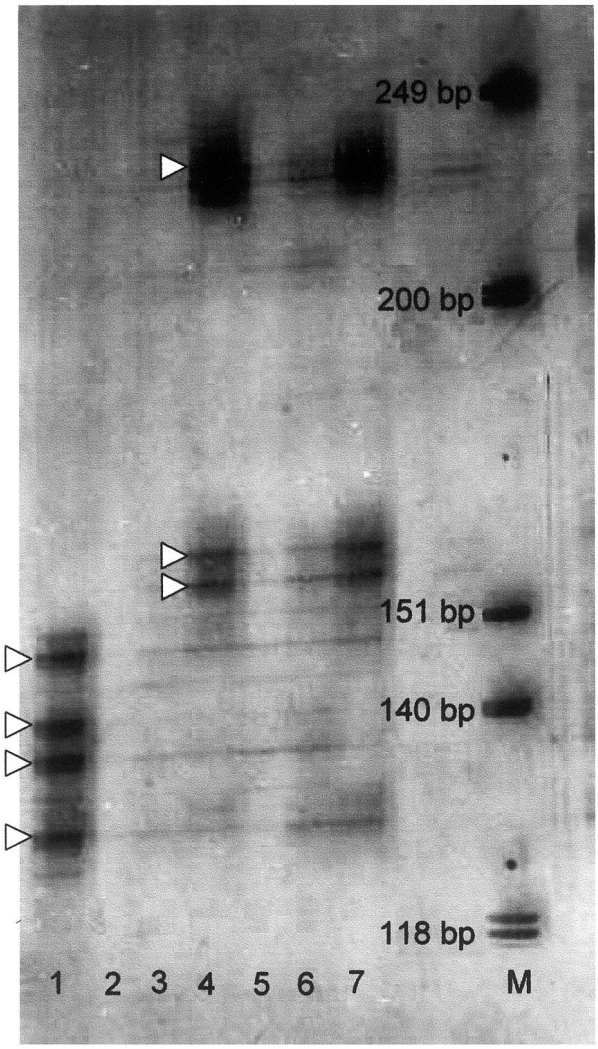

Figure 2. Polymorphism at locus *Afu-68* observed in samples of black caviar. Lane 1 – caviar A, lanes 4, 6, 7 – caviar B, lane 2 – caviar C, lane 3 – caviar D, lane 5 – caviar E, and lane M – marker ΦX 174. Arrowheads point marker alleles: lane 1 (top to bottom) – *148, 140, 136, 128*; lane 4 (top to bottom) – *232, 160, 156*.

hybrid called "bester", which is produced by crossing a *Huso huso* (beluga) female and an *Acipenser ruthenus* male, would be identified as derived from beluga if based only on mitochondrial DNA markers. Thus, a reliable identification of the black caviar species origin must include additional genetic markers, for example microsatellite DNA (Pyatskowit et al. 2001). A microsatellite locus *Afu-39* (May et al. 1997) was applied (*Afu-39* was earlier named as locus *LS-39* by Jenneckens et al. 2001) for genetic identification of black caviar derived from *Acipenser stellatus* and a unique allele was found that is missing in all other sturgeon species. Microsatellite DNA analysis is applicable only for species which don't have null alleles (Ludwig 2006). A null allele is an allele which is not amplified by a specific pair of primers. Null alleles are often detectable if primers developed for one species are used for another species (Ludwig 2006).

One of the important problems of microsatellite DNA analyses is slippage of *Taq* polymerase during PCR, resulting in the addition of an extra base at the end of the amplified fragments (Hu 1993). These additional bands are referred to as stutter or shadow bands (such bands are shown in Figures 1 and 2). Scoring of the gel can still be carried out unambiguously by making an assumption that the true band reflecting an actual allele is the most intense one, located close to the neighbouring group of its stutter bands.

This paper reports the suitability of microsatellite DNA markers for sturgeon identification. The results showed that caviar A was most probably produced from beluga or Russian sturgeon, and caviar B from Siberian sturgeon (Figures 1 and 2), sterlet or beluga, because alleles observed in this study had been earlier found in those species (Fopp 2003). On the other hand, in a single sample of caviar alleles were found which were indicative of different sturgeon species, this might result from mixing different kinds of caviar by the producer and selling them as a mis-labeled product, under a false trade name.

This study is only a preliminary research project based on nuclear DNA isolated from black caviar, but the technique seems to be valuable. The method enables species recognition and could help in identifying mis-labeled caviar on the market.

REFERENCES

Birstein, V.J. 1993. Sturgeons and paddlefishes: threatened fishes in the need of conservation. Conservation Biology 7: 773-787.

Birstein, V.J., R. Hanner, R. DeSalle. 1997. Phylogeny of the Acipenseriformes: Cytogenetic and molecular approaches. Environmental Biology of Fishes 48: 127-155.

Birstein, V.J., P. Doukakis, B. Sorkin, R. DeSalle. 1998. Population aggregation analysis of three caviar-producing species of sturgeons and implications for the species identification of black caviar. Conservation Biology 12: 766-775.

DeSalle, R., V.J. Birstein. 1996. PCR identification of black caviar. Nature 381: 197-198.

Estoup, A. 1998. Application of VNTR and especially microsatellite markers to analysis of genetic structure and relationships among populations and individuals. Advances in Molecular Ecology. Course Information and Lecture Summaries, pp. 33–41. Italy, 20–31 March, 1998.

Fopp, D. 2003. Analysis of microsatellite DNA polymorphism for genetic identification of sturgeons. Ph.D. Thesis. 84 p. University of Warmia and Mazury in Olsztyn, Poland (in Polish with English summary).

Fopp-Bayat, D. 2004. Genetic identification of sturgeons, and sturgeon-derived food products: meat and caviar. Archives of Polish Fisheries 12: 231-241.

Gessner, J., M. Wirth, F. Kirschbaum, N. Patriche. 2002. Processing techniques for caviar and their effect on product composition. International Review of Hydrobiology 87: 645-650.

Hu, G. 1993. Polymerase-catalyzed addition of nontemplated extra nucleotides to the 3' end of a DNA fragment. DNA and Cell Biology 12: 763-770.

Jenneckens, I., J.N. Meyer, G. Horstgen-Schwark, B. May, L. Debus, H. Wedekind, A. Ludwig. 2001. A fixed allele at microsatellite locus LS-39 exhibiting species-specificity for the black caviar producer *Acipenser stellatus*. Journal of Applied Ichthyology 17: 39-42.

Ludwig, A. 2006. Identification of Acipenseriformes species in trade. The World Conservation Union and Species Commission. 65 p.

Ludwig, A., L. Debus, I. Jenneckens. 2002. A molecular approach to control the international trade in black caviar. International Review of Hydrobiology 87: 661-674.

May, B., C.C. Krueger, H.L. Kincaid. 1997. Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. Canadian Journal of Fisheries and Aquatic Sciences 54: 1542-1547.

Pyatskowit, J.D., C.C. Krueger, H.L. Kincaid, B. May. 2001. Inheritance of microsatellite loci in the polyploid lake sturgeon (*Acipenser fulvescens*). Genome 44: 185-191.

Sambrook, J., E.F. Fritch, T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.