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Short communication

MtDNA and microsatellite variation in Baltic Atlantic salmon

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Variation in mtDNA and DNA microsatellites was examined in Atlantic salmon (*Salmo salar*) from three rivers that drain into the Baltic Sea in order to evaluate the usefulness of DNA markers for genetic characterization of Baltic salmon populations. Three microsatellite loci and variation in the mitochondrial ND1 gene were studied. Polymorphisms were found for all genetic markers studied. Three different haplotypes were found after restriction enzyme digestion of the ND1 gene. The distribution of these haplotypes varied significantly among the salmon populations. The microsatellites included one highly polymorphic locus with eight alleles, one with an intermediate level of variation and one locus with little variation. Two of the microsatellites showed significant differentiation among populations. The study demonstrates that mtDNA and microsatellites provide a large amount of variation useful in genetic studies of Baltic salmon.

Key words: Atlantic salmon, Baltic Sea populations, genetic variation, microsatellites, mtDNA, *Salmo salar*.

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Introduction

Surveys of genetic variation, based on protein electrophoresis, have indicated low levels of variation with few polymorphic loci and low heterozygosity in Baltic Atlantic salmon (*Salmo salar*) (Ståhl, 1987; Koljonen, 1989; Kazakov and Titov, 1991, 1993). Genetic distances between samples from different rivers are often small and attempts to separate stocks in mixed fisheries by means of genetic stock identification have met with limited success (Koljonen, 1995; Koljonen and McKinnell, 1996). During the 20th century, Baltic salmon populations have been damaged by the damming and regulation of rivers, by pollution, by overfishing and by the transfer of fish between rivers. It may be that genetic differences between salmon populations are now small because of such anthropogenic activities. Also, naturally-occurring migration of salmon between rivers may have counteracted genetic differentiation. On the other hand, the few polymorphic genetic markers studied so far may have given a poor description of differentiation among Baltic salmon populations. Considering the importance of salmon in the commercial and recreational fisheries and the efforts that are being made to protect the few wild populations left in the Baltic Sea area, it is obvious that the genetic structure of salmon populations needs to be clarified. This will require the use of new techniques to reveal possible genetic differences between salmon populations. In this study mitochondrial DNA and DNA microsatellites were chosen as potentially useful sources of variability in Baltic salmon, with the aim of investigating if such DNA can increase the amount of variation available for genetic surveys.

Materials and methods

Samples of salmon were collected from two hatchery strains, representing populations of the River Skellefteälven and Ångermanälven, and from a wild population from the River Vindelälven. All three rivers run through northern Sweden and drain into the northern part of the Baltic Sea. Skellefteälv and Ångermanälv salmon were chosen because allozyme studies have failed to detect any genetic differences between them (Koljonen and McKinnell, 1996). Skellefteälven and Vindelälven tissue samples were taken from ascending spawners in June, July, and August 1995. Ångermanälven samples were taken from 1+ year old fish at Forsmo

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hatchery in August 1996. DNA was prepared from small fin-clips in accordance with the method of Laird *et al.* (1991).

mtDNA variation

Variation in the mitochondrial ND1 gene was detected after polymerase chain reaction (PCR) and subsequent fragmentation with restriction enzymes. The PCR was performed in 20 µl volume and contained 1–5 ng total DNA, 0.2 µM of each primer, 100 µM dNTP, 2.3 mM $MgCl_2$, 0.3 u Taq polymerase, and 1 × reaction buffer. The primers used were: 5′CCCGCCTGTTTACCAAA AAC 3′ (forward) and 5′GGTATGAGCCCGAAAGC 3′ (reverse). These primers are modifications of those used by Cronin *et al.* (1993).

After denaturation for 4 min at 95°C, samples were processed through 34 cycles of 1 min at 95°C, 1 min at 55°C, and 2 min at 72°C. The last elongation step was lengthened to 10 min.

Enzyme digestions were performed with 5 µl PCR product. After enzyme digestion the fragments were separated on a 1.5% agarose gel.

In a preliminary study, restriction enzyme digestions were made using enzymes *Alu*I, *Hae*III, *Hind*III, *Hinf* I and *Taq*I with a small set of samples. Of the tested enzymes *Hae*III and *Hinf* I showed variation and were chosen for the study.

Microsatellites

Salmon microsatellites studied were SSOSL85 and SSOSL417 (Slettan *et al.*, 1995) and F43 (Sanchez *et al.*, 1996). Amplification of microsatellites was made in 15 µl volumes with 5–50 ng DNA, 0.2 µM of each primer, 100 µM dNTP, 1.5 mM $MgCl_2$, 0.3 u Taq polymerase and 1 × reaction buffer. The PCR programme consisted of initial denaturation for 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C, and ending with 10 min at 72°C. The amplification products were mixed with one volume of formamide loading buffer and heated to 95°C for 3 min before separation on sequencing gels (5 M urea, 6% acrylamide). After electrophoresis the gels were stained with silver using the method developed by Bassam *et al.* (1991). Size determinations of alleles was made from comparisons with a sequencing reaction.

Exact tests of microsatellite variation for Hardy–Weinberg equilibria and population differentiation were performed using Markov chain methods and the GENEPOP 1.2 software package (Raymond and Rousset, 1995). Nei's genetic distance (Nei, 1972) was estimated using the PHYLIP package (Felsenstein, 1993).

Table 1. mtDNA ND1 gene haplotype frequencies in three Baltic salmon populations.

Haplotype	Ångermanälv	Vindelälv	Skellefteälv
n	62	55	50
I	0.69	0.25	0.68
II	0.16	0.04	0.32
III	0.15	0.71	0.00
h	0.48	0.43	0.44

n = sample size, h = nucleon diversity.

Results

mtDNA variation

Variation from digestion of the ND1 mitochondrial gene with *Hae*III was caused by the alternate cutting of a 560 base pair (bp) fragment into a 60 bp and a 500 bp fragment. With *Hinf* I, a 520 bp fragment was cut into a 430 bp and a 90 bp fragment in some individuals. Three composite haplotypes were detected among the salmon studied. The distribution of the mtDNA variation is presented in Table 1. Salmon from Vindelälven had haplotype frequencies which were strikingly different from the other two populations. For example, the most common haplotype in Vindelälven (haplotype III) was not present among salmon from Skellefteälven and was present at a low frequency in Ångermanälven. A chi-square test of haplotype distribution among the populations indicated a significant heterogeneity ($\chi^2 = 78.0$; d.f. = 4; $p < 0.001$).

Microsatellites

All three microsatellites studied were polymorphic (Table 2). Least variation was detected with F43, which was only marginally variable in Vindelälven salmon and showed low levels of variation in the other two populations (Table 2). The SSOSL85 microsatellite was polymorphic in the three populations with two common and several rare alleles. Most variation was obtained from SSOSL417, with a total of eight alleles. For each population exact tests were made for conformation with Hardy–Weinberg equilibrium. None of the loci showed any case of significant deviation from equilibrium frequencies. Tests for population differentiation showed that variation at the SSOSL417 locus was significantly different among populations ($p < 0.001$; s.e. < 0.0001). Among population differentiation was also detected for the F43 locus ($p = 0.030$; s.e. $= 0.003$) but not for locus SSOSL85.

Since allozyme studies have failed to find any significant differences between Ångermanälven and Skellefteälven salmon, it was of interest to perform

Table 2. Microsatellite variation in three Baltic salmon populations.

Locus	Allele	Ångermanälv	Vindelälv	Skellefteälv
SSOSL417				
n		45	38	49
	190	0.189	0.000	0.061
	188	0.067	0.013	0.276
	186	0.056	0.158	0.112
	184	0.178	0.105	0.102
	182	0.100	0.237	0.041
	178	0.100	0.000	0.071
	176	0.111	0.237	0.112
	174	0.200	0.250	0.224
h		0.853	0.789	0.831
SSOSL85				
n		49	62	50
	200	0.020	0.008	0.030
	198	0.704	0.718	0.730
	196	0.010	0.000	0.000
	190	0.041	0.040	0.000
	180	0.225	0.234	0.240
h		0.452	0.428	0.409
F43				
n		42	34	41
	124	0.083	0.015	0.134
	122	0.012	0.000	0.012
	120	0.905	0.985	0.854
h		0.174	0.030	0.253

n=sample size, h=expected heterozygosity.

pairwise tests between these populations. These tests revealed significant differences for the haplotype distribution ($\chi^2=9.6$; d.f.=2; $p<0.01$) and for the SSOSL417 allele distribution ($p<0.001$; s.e.<0.0001). The SSOSL85 and F43 loci were not significantly different between Ångermanälven and Skellefteälven salmon.

Nei's genetic distance, estimated from three microsatellite loci, was 0.026 between Ångermanälven and Skellefteälven. The other two distances, Ångermanälven–Vindelälven and Skellefteälven–Vindelälven, were 0.031 and 0.053, respectively.

Discussion

This study shows that mtDNA and DNA-microsatellite markers provide a large amount of variation that can be used to study population genetic structure in Baltic salmon. The data presented here suggest that mtDNA, in particular, reveals more pronounced differences between salmon populations compared to nuclear genes. Nielsen *et al.* (1996) recently described variation in the ND1 mtDNA gene among European populations of Atlantic salmon and demonstrated significant genetic differentiation among populations. The present study shows that such variation is also present among Baltic salmon populations and that geographically close populations can be strongly differentiated.

The stronger differentiation for mtDNA is expected since mtDNA has a population size of only a quarter that of the nuclear genes and, accordingly, genetic drift occurs at a faster rate. Nevertheless, there are drawbacks with mtDNA. The maternal inheritance of mitochondria can result in misleading results if migration between rivers differs between male and female salmon. If gene flow among rivers is caused mainly by males spawning in non-native rivers, then mitochondrial differentiation may persist despite little differentiation in nuclear genes. A full description of the genetic relationships between populations should for this reason also make use of nuclear genetic markers. As such microsatellites appear to be suitable. The amount of variation obtained in this study with a few microsatellites clearly exceeds that which can be obtained by applying all known polymorphic allozymes. Average heterozygosity ranged between 0.40 and 0.50 for microsatellites. In Baltic salmon the average heterozygosity estimated from allozymes did not exceed 0.03 (Ståhl, 1987). This difference between microsatellite and allozyme variation is of the same order as described by Brooker *et al.* (1994) in cod (*Gadus morhua*).

Significant differences in allele and haplotype frequencies were obtained between two populations which show no differences with allozymes. This suggests that the DNA markers may be useful for characterizing and identifying salmon populations. In a study of Irish and Spanish salmon populations with allozymes and microsatellites, Sanchez *et al.* (1996) also found that microsatellite loci gave larger genetic distances compared with allozymes. Estimates by Estoup *et al.* (1993) suggest that more than 100 000 microsatellite loci are present in the salmonid genome. Accordingly, microsatellites provide practically unlimited amounts of variation to be used in population genetic studies of Baltic salmon.

The study of the population genetics of Baltic salmon will be especially important for monitoring the combined effects of mortality caused by the M74 syndrome and the heavy fishing in the Baltic Sea. Swedish hatchery-produced Baltic salmon fry are now routinely treated with thiamine, which eliminates the mortality from M74. Consequently, since approximately 90% of smolts entering the Baltic Sea come from hatcheries a high fishing pressure may persist to the detriment of the wild stocks. The first year class of salmon which has experienced a high level of M74 mortality will return as spawners in 1997. A reduction in population size in wild populations accompanied by loss of genetic variation is a realistic expectation for the coming years. The mtDNA and microsatellite markers will be useful tools for detecting such changes.

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