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Genetic characterization of broodstock brown trout from Bled fish-farm, Slovenia

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Abstract

Background and Purpose: *Due to environmental and economic concerns, Bled fish-farm is interested in establishing broodstocks of native brown trout (*Salmo trutta L.*). Progeny would be reared and released into rivers managed by the Fishing Club Bled. In this study was performed genetic characterization of broodstock from Bled fish-farm in order to assess hybridization of native brown trout of Danubian phylogeographic lineage with trout of the allochtonous Atlantic lineage.*

Material and Methods: *DNA was isolated from fin clips of 20 males and 20 females from broodstock. PCR-RFLP technique was used for distinguishing between Atlantic and Danubian lineages on the basis of control region of the mitochondrial DNA (CR mtDNA) and lactate dehydrogenase gene (LDH).*

Results: *Results show a high percentage of allochthonous genetic markers especially among females and confirm hybridization between native and introduced brown trout.*

Conclusions: *Because of low percentage of native trout markers, Bled fish-farm decided to continue with collecting and genotyping fish to establish a broodstock with higher percentage of genetic markers characteristic for native trout of Danubian lineage.*

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INTRODUCTION

Brown trout (*Salmo trutta* L.) has been domesticated and adapted to breeding demands on fish farms. The domesticated strain has been subjected to powerful selective pressure and bottleneck resulting in low genetic variability (*1*). While these trout thrive in hatchery conditions their adaptive success in nature is severely reduced. Investigations have shown that stocking waters with domesticated brown trout is often ineffective, even harmful: the majority of stocked fish fail to adapt and reproduce (*2, 3*), whereas those that manage to spawn decrease genetic diversity of the autochthonous population and may cause extinction of local trout phenotype (*4, 5*). Despite the negative effects, the widely available domesticated strain of brown trout is still used for stocking. In most cases the purpose of stocking is to increase population sizes to the benefit of fisheries, mainly angling (*6*).

A study of the mtDNA of various brown trout populations in Europe, North Africa and parts of Asia revealed five main phylogeographic lineages: Danubian, Atlantic, Adriatic, Mediterranean and *marmoratus* (*7*). In addition, sequence polymorphism of the nuclear gene coding for lactate dehydrogenase (LDH) is also used for distinguishing

trout of Atlantic and Danubian lineages (*8*). It was established that in Slovenia, brown trout of Danubian lineage is native to the Danubian drainage (*9*). Atlantic lineage is allochthonous in Slovenian waters, with stocking history dating back to 1920 (*10*). Recent data suggest that trout of Atlantic lineage is widespread in Slovenian rivers and in fish-farms that rear fish for stocking. In addition, a majority of autochthonous populations hybridized with domesticated trout, especially those in the main waters. In the Danubian drainage only a few streams were found with no sign of hybridization with domesticated trout (*9*).

The Angling Club of Tolmin (Slovenia) faced a similar problem of hybridization of native marble trout with allochthonous Atlantic brown trout in the Adriatic drainage. Fish stocks from headwaters in the uppermost reaches of rivers that had no contact with the domesticated Atlantic brown trout were used for broodstock. Progeny was used to restore downstream populations of native marble trout (*11, 12*). The Angling Club was motivated to use native trout populations for restocking because of environmental concern (improving genetic diversity, avoiding introduction of allochthonous organisms) and economic reasons (marketing trout phenotype of their waters for angling and higher adaptive success of native trout – less fish need to be stocked). Following their example, the Bled Fishing Club established broodstock of presumably native trout of Danubian lineage in order to rear trout for release in the rivers they manage.

The object of this study was to determine the genetic composition of broodstock from Bled fish-farm on the basis of Atlantic lineage specific genetic markers of the control region of mtDNA and LDH gene, using the Restriction Fragment Length Polymorphism (RFLP) analysis. Fish with genetic markers characteristic of Atlantic lineage were excluded from the broodstock.

MATERIAL AND METHODS

Samples and DNA isolation

In 2005 was collected a sample of 40 brown trout (20 ♂ and 20 ♀) from broodstock, maintained in a hatchery of the Bled Fishing Club , Slovenia. Total DNA was isolated from fin clips using the Wizard Genomic DNA Purification (Promega).

DNA amplification and Restriction fragment length polymorphism – RFLP

PCR amplification was performed of the entire control region (CR mtDNA) (1088 bp) using primers 28RIBa (*13*) and HN20 (*14*). Partial LDH gene was amplified (428 bp) using primers Ldhxon3F and Ldhxon4F (*8*). The PCR conditions for CR mtDNA were: initial denaturation (95°C, 3 min) followed by 30 cycles of strand denaturation (94°C, 45 s), primer annealing (52°C, 45 s) and DNA extension (72°C, 2 min). The program for LDH differed from the mtDNA program only in primer annealing (62°C, 1 min) and DNA extension (72°C, 1 min) steps. All PCR amplifications were performed in a programmable thermocycler GeneAmp® PCR System 9700 (AB Applied Biosystems). The total PCR volume of 30 μL contained 1 μM of each primer, 0.2 μM dNTP, 1.5 μM $MgCl_2$, 1×PCR buffer, 1 U *Taq* polymerase (PE Applied Biosystems) and 100 ng of genomic DNA. Amplified DNA fragments were checked on 1.5% agarose gel.

Polymorphisms of the amplified fragments were detected using *SatI* endonuclease for CR mtDNA and *BseLI* for LDH. Restriction reaction contained: PCR product (5 μL), digestion buffer (2 μL), restriction enzyme (5 U) and autoclaved distilled water (12.5 μL). The samples were digested for 3h at the appropriate incubation temperature (37°C for *SatI* and 55°C for *BseLI*). The total restriction reaction was loaded onto 1.5% agarose gel with 0.5×TBE electrophoresis buffer, stained with ethidium bromide. After 15 min on 120 V the gel was observed and photographically documented under UV light (302 nm).

RESULTS

Endonuclease *SatI* generated a single cut on haplotypes of Atlantic lineages at the polymorphic site C_{434} of the amplified CR mtDNA fragment. Endonuclease *BseLI* generated a single cut on LDH-C1*90 allele, specific for Atlantic lineage at polymorphic site G_{353} of the amplified fragment. Analysis of the CR mtDNA and LDH gene revealed that broodstock was a mixture of both autochthonous Danubian and allochtonous Atlantic lineage of brown trout (Figures 1, 2).

Analysis of the CR mtDNA showed that 3 out of 20 males (15%) and 14 out of 20 females (70%) from the broodstock sample were of Atlantic lineage. In total, 42.5% of broodstock fish had mtDNA of allochthonous Atlantic lineage (Table 1). The high frequency (42.5%) of Atlantic lineage in the broodstock recorded, using the mtDNA as a marker, came from the dominance (70%) of that lineage in females. Since mtDNA is inherited exclusively maternally we used the nuclear LDH gene to assess the introgression of allochthonous trout. There were 80% of individuals with the allochthonous LDH-C1*90 allele in the broodstock (genotypes LDH-C1*90/90 and LDH-C1*90/100). The frequency of the autochthonous Danubian genotype (LDH-C1*100/100) in females of the broodstock was 0%, whereas in males it was 40%. As in the case of the mtDNA marker, higher frequency of occurrence (52.5%) of allochthonous LDH-C1*90 allele

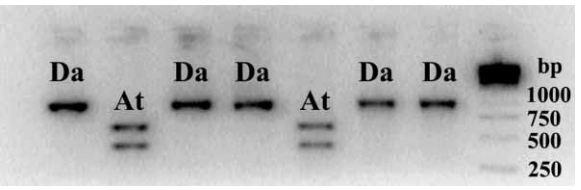

Figure 1. *Restriction of mtDNA control region (1088 bp) with* SatI *enzyme. Control region of Danubian (Da) lineage remains uncut while Atlantic (At) lineage is cut into two fragments (654 and 434 bp). The marker used is a 1kb.*

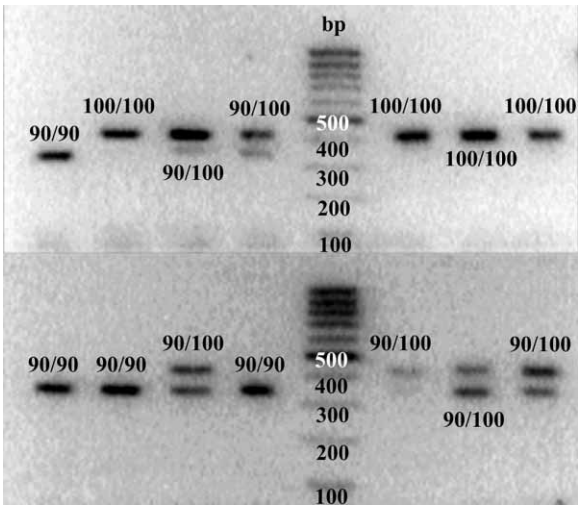

Figure 2. *Restriction of partial LDH gene (428 bp) with* BseLI *enzyme. LDH-C1*100 of Danubian lineage remains uncut while LDH-C1*90 gene of Atlantic lineage is cut into two fragments (353 and 75 bp; fragments of 75 bp were too small to be visible on gels). The marker used is a 100 bp.*

came from the dominance (72.5%) of that allele in females. Seven males (Danubian mtDNA and LDH-C1*100/100) had no genetic markers characteristic of domesticated trout and eight females were 100% domesticated trout (Atlantic mtDNA and LDH-C1*90/90).

Opposing results were observed in some fish. Thus, we found in one male and female the Danubian mtDNA marker, whereas the same individuals were both homozygotes for the LDH-C1*90 allele (Atlantic). Likewise, one male was of Danubian lineage at nuclear DNA (LDH-C1*100/100) and had mtDNA of Atlantic lineage.

DISCUSSION

The results suggest the remarkable occurrence of allochthonous Atlantic lineage in the broodstock of brown trout in the Bled fish-farm.

Both mtDNA and LDH markers revealed greater contamination of females in the broodstock by the allochthonous material, probably due to the greater number of females reared for artificial spawning. The male part of the broodstock was also contaminated, though notably less than in females, probably due to less need for males than for females in the hatcheries, which resulted in a relatively small number of allochthonous males used for stocking.

The crossing between individuals of Atlantic and Danubian phylogenetic lineages can result in F2 individuals that hold maternally inherited mtDNA from one lineage and a major part of nuclear genetic markers from another phylogenetic lineage. Individuals, which were LDH-C1*90/90 homozygotes with the mtDNA of Danubian lineage, as well as those which were LDH-C1*100/100 homozygotes with the mtDNA of Atlantic lineage can appear after only two generations. The intensity of hybridization between Atlantic and Danubian lineage could be assessed more accurately if more nuclear markers were used. However, even with just mtDNA and one nuclear marker we can conclude that broodstock from the Bled fish-farm contains a high proportion of genetic markers characteristic of allochthonous trout.

Since fish stocking is considered the main conservation activity in the restoration of populations that are in immediate danger of extinction due to demographic factors (*6, 15, 16, 17)*, special care should be taken to ensure aboriginality of the broodstock. In the case of the Bled fish-farm, one possibility is to improve existing broodstock through selection of individuals with a higher proportion of autochthonous genetic markers. Due to the danger of reducing genetic diversity, particularly with regard to the low proportion of autochthonous genetic markers found, this option was not used.

Instead, the Bled fish-farm has decided to continue collecting fish from remote and presumably unstocked locations. New fish will be tested for allochthonous genetic markers and a new broodstock will be formed from a sufficient number of autochthonous fish found.

TABLE 1

Frequencies of mtDNA haplotypes, LDH alleles and LDH genotypes in the broodstock sample of brown trout.

genetic markers	♂		♀		♂ + ♀	
At mtDNA	3/20	(15 %)	14/20	(70 %)	17/40	(42.5 %)
Da mtDNA	17/20	(85 %)	6/20	(30 %)	23/40	(57.5 %)
LDH-C1*90	13/40	(32.5 %)	29/40	(72.5 %)	42/80	(52.5 %)
LDH-C1*100	27/40	(67.5 %)	11/40	(27.5 %)	38/80	(47.5 %)
genotypes						
LDH-C1* 90/90	1/20	(5 %)	9/20	(45 %)	10/40	(25 %)
LDH-C1* 90/100	11/20	(55 %)	11/20	(55 %)	22/40	(55 %)
LDH-C1*100/100	8/20	(40 %)	0/20	(0 %)	8/40	(20 %)

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REFERENCES

1. AURELLE D, CATTANEO-BERREBI G, BERREBI P 2002 Natural and artificial secondary contact in brown trout (Salmo trutta, L) in the French western Pyrenees assessed by allozymes and microsatellites. *Heredity 89*: 171–183
2. MARTÍNEZ P, ARIAS J, CASTRO J, SÁNCHEZ L 1993 Differential stocking incidence in brown trout (*Salmo trutta*) populations from northwestern Spain. *Aquaculture 114*: 203–216
3. GARCIA-MARIN J L, SANZ N R, PLA C 1999. Erosion of the native genetic resources of brown trout in Spain. *Ecol Freshw Fish 8*: 151–158
4. HANSEN M M, LOESCHCKE V 1994 Effects of releasing hatchery-reared brown trout to wild trout populations. *In:* Loeschcke V, Tomiuk J, Jain S K (*ed*). *Conserv Genet.* Birkhäuser Verlag, Basel, p 273–289
5. RYMAN N, JORDE P E, LAIKRE L 1995 Supportive breeding and variance effective population size. *Conserv Biol 9*: 1619–1628
6. LAIKRE L, ANTUNES A, APOSTOLIDIS A, BERREBI P, DUGUID A, FERGUSON A, GARCIA – MARIN J L, GUYOMARD R, HANSEN M M, HINDAR K, KOLJONEN M L, LARGIADER C, MARTINEZ P, NIELSEN E E, PALM S, RUZZANTE D E, RYMAN N, TRIANTHAPHYLLIDIS C 1999 Conservation genetic management of brown trout (*Salmo trutta*) in Europe. Report by the concerted action on identification, management and exploitation of genetic resources in the brown trout (*Salmo trutta*), »TROUT-CONCERT«; EU FAIR CT97–3882. Silkeborg, Danmarks fiskeriundersrgelser, p 91
7. BERNATCHEZ L, GUYOMARD R, BONHOMME F 1992 DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. *Mol Ecol 1*: 161–173
8. McMEEL M O, HOEY M E, FERGUSON A 2001 Partial nucleotide sequences, and routine typing by polymerase chain reaction–restriction fragment length polymorphism, of the brown trout *(Salmo trutta*) lactate dehydrogenase, LDH-C1*90 and *100 alleles. *Mol Ecol 10*: 29–34
9. JUG T, BERREBI P, SNOJ A 2005 Distribution of non-native trout in Slovenia and their introgression with native trout populations as observed through microsatellite DNA analysis. *Biol Conserv 123*: 381–388
10. GRIDELLI E 1936 I pesci d'aqua dolce della Venezia Giulia. *Bollettino della Societa Adriatica di Scienze Naturali in Trieste* 35: 7–140
11. POVŽ M, JESENŠEK D, BERREBI P, CRIVELLI A 1996 The marble trout, Salmo trutta marmoratus, Cuvier 1817, in the Soča River basin, Slovenia. Tour du Valat Publication, p 65
12. BERREBI P, POVŽ M, JESENŠEK D, CATTANEO – BERREBI G, CRIVELLI A J 2000 The genetic diversity of native, stocked, and hybrid populations of marble trout in Soča river, Slovenia. *Heredity 85 (3)*: 277–287
13. SNOJ A, JUG T, MELKIČ E, SUŠNIK S, POHAR J, DOVČ P, BUDIHNA N 2000 Mitochondrial and mirosatellite DNA analysis of marble trout in Slovenia. *Journal of Freshwater Biology (Quaderni ETP) 29*: 5–11
14. BERNATCHEZ L, DANZMANN R G 1993 Congrugence in control – region sequence and restriction – site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill). *Mol Biol Evol 10*: 1002–1014
15. TAYLOR E B 1991 A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture 98*: 185–207
16. LEARY R F, ALLENDORF F W, FORBES S H 1993 Conservation genetics and bull trout in the Columbia and Klamath River drainages. *Conserv Biol 7*: 856–865
16. ALLENDORF F W, WAPLES R S 1996 Conservation and Genetics of Salmonid Fishes. *In*: Avise J C & Hamrick J L (*eds*.) Conservation Genetics. Case Stories from Nature, Chapman & Hall, New York, p 238–280