ORIGIN AND GENETIC STRUCTURE OF SEVERAL HUNGARIAN WILD AND DOMESTICATED BROWN TROUT POPULATIONS BASED ON PCR-RFLP AND MICROSATELLITE MARKERS

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POREKLO I GENETIČKA STRUKTURA NEKOLIKO MAĐARSKIH DIVLJIH I DOMESTIFIKOVANIH POPULACIJA POTOČNE PASTRMKE NA OSNOVU PCR-RFLP I MIKROSATELITSKIH MARKERA

Apstrakt

U Evropi je, na osnovu studija mitohondrijalne DNK, identifikovano pet evolutivnih linija potočne pastrmke *(Salmo trutta m. fario* L. 1758): Atlanska, Dunavska, Mediteranska, Jadranska i Mramorna. Mađarske linije bi teorijski trebalo da pripadaju Dunavskoj liniji na osnovu hidrogeografije zemlje, ipak, ovo nije potvrđeno genetičkim studijama. Korišćeni su molekularni markeri da bi se ispitalo genetička pozadina populacije potočne pastrmke u Mađarskoj. Istraživanja su uključila jedini matični nasad potočne pastrmke u Mađarskoj, kao i po jednu populaciju u planinskim vencima Bükk, Aggtelek, Börzsöny i Visegrádi, po jedna populacija u svakom. Genetička analiza je do sada sprovedena na 533 individue, isečak peraja je uzorkovan sa svake ribe i PCR-RFLP (kontrolni region mitohondrijalne DNK, laktat dehidrogenaza i somatolaktin geni), kao i analiza mikrosatelitnih markeri (BFRO002, OMM1064, Ssa408uos, SsoSL417, SsoSL438) su bili korišćeni da bi se razlikovale Dunavska i Atlanska linija potočne pastrmke. Na osnovu genetičke analize mitohondrijalne DNK divljih populacija, udeo Dunavskog haplotipa je nizak (< 10 %), sa izuzetkom potoka Apátkúti u planinama Visegrádi, gde je nađena relativno visok udeo Dunavskog haplotipa (34%). 401 analizirani primerak matičnog jata farme je skoro u potpunosti Atlanski haplotip, što ukazuje

na efekat osnivača. Iako su kasnije prikupljeni primerci iz obližnjeg potoka i pridodati matičnom jatu, njihov broj je bio ograničen jer su uglavnom svi bili mužjaci. Budući da je jedino matično jato u Mađarskoj, ribe sa ove farme se koriste za poribljavanje od strane ribolovaca, što može dovesti do značajnog uticaja na prirodne populacije. Na osnovu analize nuklearnih markera sve populacije su veoma heterogene. Veliki udeo (60-80%) Atlanskih alela primećen za ove markere na svim lokacijama gde je obavljano uzorkovanje ukazuje na efekat intenzivnog poribljavanja mađarskih salmonidnih regiona. Analize mikrosatelitskih markera su ukazale na visoku heterozigotnost i Hardy-Weinberg-ovu ravnotežu svih populacija.

Ključne reči: potočna pastrmka, PCR-RFLP, mikrosateliti, populaciona genetika, Mađarska

Keywords: Brown trout, PCR-RFLP, microsatellite, population genetics, Hungary

INTRODUCTION

Brown trout (*Salmo trutta m. fario*) is a salmonid species native to the freshwater streams of Eurasia. Analyses of variation in the mitochondrial DNA revealed the existence of five major evolutionary lineages in the native range of the species: Atlantic, Danubian, Mediterranean, Adriatic and *marmoratus* (Bernatchez, 2001). The species is cultured primarily for stock enhancement of natural waters and those managed by anglers. As most hatchery-maintained broodstocks originate from the Atlantic lineage (Jug et al, 2005), several European populations have been affected by hybridization and introgression with stocked trout of this lineage (Snoj et al, 2002, Marić et al 2010).

Hungary is a landlocked country with all its streams and rivers belonging to the Black Sea drainage through the river Danube, therefore, it is expected that local brown trout would also belong to the Danubian lineage. Moreover, due to the relative scarcity of typical salmonid waters in the country, currently there is only one hatchery that has a constant brown trout broodstock. Unless fish are imported from other countries, all brown trout stocked in the country theoretically originates from this broodstock.

The objective of this work was to elucidate the origin and genetic composition of the only Hungarian brown trout broodstock and that of natural populations in some streams using mitochondrial and nuclear (PCR-RLFP and microsatellite) markers.

MATERIALS AND METHODS

Brown trout broodstock was maintained at the fish farm of Hoitsy & Rieger Kft in Lillafüred, Hungary. Fish were sampled between June and November of 2011. In total, 401 fish of the broodstock were samples. Samples from wild populations were collected at the Bán stream in the Bükk mountains (25 fish), the Jósva stream in the Aggtelek mountains (33 fish, both on April 26th, 2012), the Kemence stream in the Börzsöny mountains (24 fish on October 25th, 2012) and finally the Apátkúti stream in the Visegrád mountains (50 fish on February 13th, 2013). On all locations, fish were anesthetized in a 0.04% solution of 2-phenoxyethanol, then laid on a wet towel. Fin clips of approximately 1 cm^2 were collected from each fish and stored in a 1,5-mL microcentrifuge tube in 96% ethanol. A photo was taken of each fish. Collected samples were shipped to the laboratory of the Department of Aquaculture of Szent István University, Gödöllő,

Hungary where DNA isolation and a part of the genotyping work was conducted. A part of the genotyping work of broodstock samples was performed at the Biotechnical Faculty of the University of Ljubljana in Domžale, Slovenia. Results received at the two laboratories were compared and verified using test samples. Samples collected from natural streams were processed entirely at the Department of Aquaculture of Szent István University.

Whole DNA isolate was made from the tissues according to Kovacs et al. (2001).The quality and concentration of DNA were assessed by a photometer (IMPLEN). The DNA concentration were adjusted to 50 ng/µL before PCR amplification.

Primers were synthesized to the flanking regions of five microsatellites (BFRO002, Sušnik et al, 1997, OMM1064, Rexroad et. al., 2002, Ssa408uos: Cairney et al, 2000, SsoSL417, Slettan et al, 1995, SsoSL438, Slettan et al, 1996), two genomic PCR-RFLP (lactate dehydrogenase: LDH-C1; McMeel et al, 2001 and somatolactin: SL Ford, 1998), and one mitochondrial PCR-RFLP (CRmtDNA; Bernatchez and Danzmann, 1993).

During the microsatellite analyses in Slovenia, fluorescently labelled primers were used, while tailed primers (Shimizu et al, 2002) were employed in the investigations in Hungary. In this latter method, a 17-bp tail (5'-ATTACCGCGGCTGCTGG-3') was attached to the 5' end of one of the forward primers. A third FAM, NED, PET or VIC dye labelled primer corresponding to the sequence of the tail was used in the PCR reactions.

Amplification of microsatellite alleles were performed according to Bogataj (2010) with tailed primer pair (250 nM) and an additional tail specific labeled (50 nM) primer.

For separation of microsatellite alleles, labeled PCR products of individuals were separated on the ABI 3130 sequencer with 38cm long capillaries, POP7 polymer, GeneScan™-500 LIZ®. size standard, The length of PCR products was determined by Genotyper software (Applied Biosystems). Allele sizes read during the fragment analyses were reduced by the length of the tail (17 bp). Results received with the two methods were compared and verified using test samples.

The PCR-RFLP analyses of CRmtDNA and LDH-C1were performed according to Sušnik et.al (2008), while PCR-RFLP Somatolactin were made according to Marić at al. (2010).

The digested PCR products were separated on 3% agarose gels, containing 0.5 µg/ml ethidium bromide. Band patterns were photographed under UV light.

Statistical analyses of allele frequencies, genetic diversity, Hardy-Weinberg equilibrium per locus were calculated using the free software GenoDive version 20b22 for Mac OS X (Patrick G Meirmans, the Netherlands).

RESULTS AND DISCUSSION

To date altogether 533 brown trout individuals were genotyped. Antlantic and Danubian alleles were distinguished according to Bogataj (2010). The proportion of the mitochondrial haplotype characteristic of the Danubian lineage was low (< 10 %), with the exception of the Apátkúti stream with a relatively high percentage of Danubian haplotype (34 %). The 401 analysed individuals of the broodstock almost uniformly displayed the Atlantic haplotype. Analyses of nuclear markers showed a varying proportion of alleles characteristic of the Atlantic or Danubian lineages although those representing Atlantic origin dominated in every population (Table 1).

Table 1. Proportions of alleles characteristic of the Danubian or Atlantic lineages of brown trout in the analyzed populations at 3 tested PCR-RFLP loci (mtDNA, SL and LDH-C1)

Locus	mtDNA		SL		LDH	
Population	Danubian	Atlantic	Danubian	Atlantic	Danubian	Atlantic
Broodstock	0,002	0,998	0,221	0,779	0,370	0,630
Bán	0,080	0,840	0,300	0,580	0,300	0,660
Jósva	0,091	0,909	0,167	0,803	0,106	0,894
Kemence	0,083	0,917	0,417	0,583	0,292	0,708
Apátkút	0,340	0,660	0,180	0,820	0,190	0,810

Results indicate that the original broodstock that was introduced to the farm following its construction in 1933 was of the Atlantic lineage female. The low proportion of Danubian genes observed originated probably from male individuals collected from local streams. In addition, stocking of natural waters with fish of non-native origin was conducted in the past, as revealed by the results.

Overall 99 alleles were found across the five microsatellite loci and the effective number of alleles varied greatly at all locations. Microsatellite OMM 1064 was found to be the most polymorphic whereas, BFRO002 displayed the lowest PIC values. On population level, the populations are effectively in Hardy-Weinberg equilibrium for both PCR-RFLP and microsatellite markers.

CONCLUSIONS

Overall we found a remarkably high proportion of allochthonous Atlantic alleles in the Hungarian brown trout populations as a clear indicator of the import and stocking of non-native populations. According to these findings, further stocking of brown trout in Hungary should be conducted in more controlled conditions.

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