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Genetic variability and differentiation of rainbow trout (*Oncorhynchus mykiss*) strains in northern and Eastern Europe

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Abstract

Variation of 10 microsatellite loci was analyzed in 12 rainbow trout strains reared in northern and Eastern Europe (Finland, Denmark, Sweden, Norway, Estonia and Poland). For comparison, two wild populations from Canada and a farmed strain from USA (Shasta strain from California) were analyzed. In majority of European strains, the level of variability, measured as the average allele richness and observed heterozygosity, was similar to that of Shasta strain with the exception of two Polish strains which exhibited significantly lower variability and elevated level of inbreeding. Only 0.9% of the total genetic variation in farmed strains was accounted for by differences between the countries of origin, 13.7% was due to differentiation among the strains within the countries and 85.5% was due to variation within strains. The farmed strains were moderately differentiated (average $F_{ST}=0.14$) and the individual fish could be assigned to their strain of origin with an average of 90% accuracy. The European strains were genetically more similar to the Shasta strain than to the Canadian wild populations which provide support to their ancestry from rainbow trout populations in California.

Keywords: Rainbow trout; *Oncorhynchus mykiss*; Microsatellites; Genetic diversity

1. Introduction

Rainbow trout, *Oncorhynchus mykiss*, is among the most important cultivated fish species in the world with the total annual production exceeding 500 thousand tons (FAO, 2004). It is believed that most of the rainbow trout strains cultured around the world originate from the McCloud River hatchery in California which was established already in 1879 (Gall and Crandell, 1992). Since then, numerous strains of rainbow trout have been developed by selective breeding and crossbreeding with the goal of improving economically important traits like growth rate, viability, disease resistance, age at maturity, time of spawning, flesh quality etc. (Gjedrem, 2000). However, not much is known about the effect of different breeding practices on genetic diversity and differentiation, especially among the rainbow trout strains in northern and Eastern Europe.

The aim of the present study was to test if there are any differences in the levels of genetic variability and differentiation among the rainbow trout strains in this region and if the strains within the countries are genetically more related to each other than the strains between the countries. To address these questions, we used highly variable microsatellite DNA markers. Additionally, we explored the potential of microsatellite

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markers for 'genetic tagging' of the individuals, i.e. for identification of their strain of origin.

2. Materials and methods

13 farmed strains and two wild populations of rainbow trout (Table 1), total of 684 individuals, were genotyped for 10 microsatellite loci (Table 2). Most of the farmed strains (except the Norwegian, Polish, and Shasta strains) were sampled in Estonia where they were imported for rearing as fertilized eggs during the period from 1999 to 2004 (Table 1). The tissue samples of these strains were collected from fingerlings or 0+fish in Estonian fish farms in the year of introduction. The samples of Norwegian strain were obtained from imported market fish and the samples of Canadian, Polish and Shasta strains were provided by foreign colleagues.

Genomic DNA was isolated from fin clips or muscle tissue according to the simplified method of Laird et al. (1991). PCR was composed of ca 10 ng DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.1 μM dNTPs, 0.2 μM of each primer (Table 2) and 0.8 U of Taq DNA polymerase (MBI-Fermentas), in a total volume of 10 μl. For cycling, the following "touch down" thermal profile was used: initial denaturation at 94 °C for 3 min, 10 cycles of 40 s at 94 °C, 40 s at 60 to 50 °C (1 °C decrease per cycle), 1 min at 72 °C and 25 cycles of 40 s at 94 °C, 40 s at 50 °C, 1 min at 72 °C and final extension at 72 °C for 10 min. The length of the microsatellite alleles was determined by ALFexpress II DNA analyzer and AlleleLinks v. 1.02 software (Amersham Pharmacia Biotech). A reference sample with known genotype was included on each gel and internal standards were included in each lane to ensure consistent scoring of genotypes across all gels.

For data analysis, FSTAT v. 2.9.3.2 program package (Goudet, 2002) was used for calculating allele frequencies and pair-wise F_{ST} values, for estimating the expected and observed heterozygosities (H_E, H_O) and the allelic richness (A_R), and for testing the significance of differences in average values of A_R, H_E and H_O among the groups of strains (1000 permutations, two- and one-side tests of the null hypothesis of no difference). The number of private alleles (A_{pr}) was calculated by using GDA v. 1.0 program (Lewis and Zaykin, 2001). GENEPOP v. 3.3 (Raymond and Rousset, 1995a) was used to test genotypic distributions for conformance to Hardy–Weinberg (HW) expectations and for deficiency or excess of heterozygosity, to test the loci for genotypic disequilibria, and for estimating the significance of genotypic differentiation between strain pairs. All probability tests were based on the Markov chain method (Guo and Thompson, 1992; Raymond and Rousset, 1995b) by using 1000 de-memorization steps, 100 batches and 1000 iterations per batch. The sequential Bonferroni adjustments (Rice, 1989) were applied to correct for the effect of multiple tests. SPAGeDi 1.2 software (Hardy and Vekemans, 2002) was used for estimating the average kinship (F) and relationship (r) coefficients within the strains. Analysis of molecular variance (AMOVA)

Table 1
Origin and characteristics of the studied rainbow trout strains

Country of origin	Fish farm/water-body	Strain/population	Year-class	Sample size	Sampling site	Strain characteristics
Finland	Arvo–Kala	Arvo–Kala	2001	16	Härjanurme, Estonia	All-female
	Tervo	Jalo 3	2003	39	Härjanurme, Estonia	All-female
	Joutsa	Joutsa-99	1999	39	Rutikvere, Estonia	All-female
		Joutsa-01	2001	39	Härjanurme, Estonia	All-female
Sweden	Antens Laxodling AB	Antens	2004	39	Äntu, Estonia	Late maturing
Norway	NA	commercial strain	2004[a]	39	Härjanurme, Estonia	NA
Denmark	Cofradex	Cofradex	2002	78	Härjanurme, Estonia	NA
	Hansen	Hansen	1999	29	Rutikvere, Estonia	All-female
	Ollerupgard	Ollerupgard	2003	39	Härjanurme, Estonia	Late maturing
	Sangild	Sangild-03	2003	39	Härjanurme, Estonia	Late maturing
		Sangild-04	2004	39	Äntu, Estonia	Late maturing
Poland	Rutki	Jastarnia	2004[a]	39	Rutki, Poland	Unselected control group for family selection program
	Rutki	Olesnica	2004[a]	39	Rutki, Poland	Unselected control group for family selection program
USA	University of Washington	Donaldson-99	1999	30	Rutikvere, Estonia	Fast growing
		Donaldson-00	2000	25	Vohnja, Estonia	Fast growing
	Mt. Shasta	Shasta	2005[a]	39	Mt. Shasta, USA	NA
Canada	Dean river	Dean	2005[a]	39	Dean river, Canada, BC	Wild population
	Tatlatui lake	Tatlatui	2005[a]	39	Tatlatui lake, Canada, BC	Wild population

NA — information not available.
[a] Year of sampling.