

# 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.

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## 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<sub>2</sub>, 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 disequilibrium, 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	Antens	2004	39	Äntu, Estonia	Late maturing
	Laxodling AB					
Norway	NA	commercial strain	2004 <sup>a</sup>	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 <sup>a</sup>	39	Rutki, Poland	Unselected control group for family selection program
		Olesnica	2004 <sup>a</sup>	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 <sup>a</sup>	39	Mt. Shasta, USA	NA
Canada	Dean river	Dean	2005 <sup>a</sup>	39	Dean river, Canada, BC	Wild population
	Tatlatui lake	Tatlatui	2005 <sup>a</sup>	39	Tatlatui lake, Canada, BC	Wild population

NA — information not available.

<sup>a</sup> Year of sampling.

incorporated in ARLEQUIN v. 2.00 (Schneider et al., 2000) was used to partition genetic variance hierarchically between countries of origin, between strains within countries and among individuals within the strains. GENECLASS v. 2.0 program (Piry et al., 2004) was applied for assigning individuals to their strain of origin based on the likelihood of multilocus genotypes by using Bayesian approach of the assignment test. Genetic distances between the strains were estimated by the  $D_A$  distance of Nei et al. (1983) and a population tree was constructed with the neighbour joining (NJ) algorithm using DISPAN software (Ota, 1993). Bootstrapping 1000 times over loci assessed the strength of the support for each node in the tree.

### 3. Results

A total of 149 alleles were observed in all studied samples across the ten microsatellite loci, ranging from three alleles at *Ssa197* to 27 alleles at *Ssa85* (Table 2). In domesticated strains, the total number of detected alleles was 128. Further partition of farmed strains according to the geographical region of rearing or origin yielded the allele numbers of 115 for the northern European (Finland, Sweden, Denmark, Norway) strains, 95 for the North American Shasta and Donaldson strains and only 49 for the eastern European strains from Poland. Also, relatively low number of alleles (67) was characteristic for the Canadian wild populations. These differences in variability were observed

also in allelic richness which is a measure of the number of alleles independent of sample size: 95.9, 91.1, 48.9 and 66.2 alleles for northern European, American, Polish and Canadian strains, respectively. The strains shared 111 (74%) alleles over all loci and 38 (26%) alleles were private, appearing in specific strains only (Table 3). The highest number of private alleles was observed in Canadian wild populations with some of them occurring at very high frequency, e.g. alleles 130 and 138 at locus *OMM1315* occurred with a frequency 0.95 and 0.76 in lake Tatlatui and Dean river populations, respectively, while the allele 219 at locus *OMM1019* occurred with a frequency 0.41 only in lake Tatlatui population. Both Canadian populations shared alleles 113, 115 and 117 at locus *OC18* which were not observed in any other strain. The frequency of private alleles in farmed strains was generally much lower (less than 0.05) with an exception of alleles 144 and 156 at locus *OMM1039* in Danish Cofradex strain which occurred with a frequency 0.10 each.

Significant deviations from Hardy–Weinberg equilibrium ( $P < 0.01$ ) at least in one locus were observed in thirteen strains (Table 3) which are most probably due to the use of limited number of breeders and/or unequal sex ratio. Linkage disequilibrium was negligible for most samples: one to two pairs of loci out of 45 tests per population were in linkage disequilibrium after applying Bonferroni correction for multiple tests. Significant linkage disequilibrium for multiple loci (four pairs, not the same loci involved) was detected only in the Polish

Table 2

Characteristics of the studied microsatellite loci ( $A$  — number of observed alleles,  $H_O$  — average observed heterozygosity,  $H_E$  — average expected heterozygosity)

Locus	Repeat motif	Sequence of the forward (F) and reverse (R) primers	Size range (bp)	$A$	$H_O$	$H_E$
<i>Ssa197</i> <sup>a</sup>	GTGA	F-GGGTTGAGTAGGGAGGCTTG R-TGGCAGGGATTTGACATAAC	106–114	3	0.43	0.42
<i>Ssa85</i> <sup>a</sup>	GT	F-AGGTGGTCTCCAAGCTAC R-ACCCGCTCCTCACTTAATC	96–164	27	0.54	0.56
<i>Ocl8</i> <sup>b</sup>	GT	F-TAGTGTTCCGTGTTCCGCTG R-CACCTTCCATCTCTCATTCCAC	103–153	22	0.78	0.73
<i>OMM1019</i> <sup>c</sup>	AG	F-CCAGCAGTAAACCTTAGGTTG <sup>d</sup> R-GTCAAAGGAGACGTAGAGCTT	197–223	13	0.76	0.72
<i>OMM1020</i> <sup>c</sup>	AG	F-CCTGTGAGTGTTAATTCGACCTGT <sup>d</sup> R-GGTCTTACCTCAACATCGGTGA	177–201	10	0.60	0.64
<i>OMM1036</i> <sup>c</sup>	TATC	F-TGTAGCAGGTGAGAATACCCA <sup>d</sup> R-CACCATCTCCATCCTAGGC	225–321	22	0.75	0.76
<i>OMM1039</i> <sup>c</sup>	GA	F-GGGGTAGGAGTAGACTAGACA <sup>d</sup> R-ATCTTCCCTCCTTGCAC	132–180	17	0.68	0.67
<i>OMM1046</i> <sup>c</sup>	TCTA	F-CAGGCACTATAATGGCAC <sup>d</sup> R-GCCCACGAGTTACAAGA	116–196	20	0.74	0.73
<i>OMM1307</i> <sup>c</sup>	CTAT	F-GCACAACACTCGAAACCCAA <sup>d</sup> R-TGCCAGCTCTGCTATGACATT	186–206	6	0.61	0.55
<i>OMM1315</i> <sup>c</sup>	CATC	F-TACAGGGCTTGGCTCTATCTC <sup>d</sup> R-GCCAAATACTTTCGCAAGG	118–158	9	0.62	0.61
Average across 15 strains/populations				14.9	0.65	0.64

<sup>a</sup> O'Reilly et al. (1996).

<sup>b</sup> Condrey and Bentzen (1998).

<sup>c</sup> Rexroad et al. (2002).

<sup>d</sup> Modified by adding the M13 'tail' (CACGACGTTGTAACGAC) to the 5' end of the forward primer.

<sup>e</sup> Palti et al. (2002).

Table 3

Genetic variability and relatedness within the studied rainbow trout strains: sample size ( $n$ ), average number of alleles per locus ( $A$ ), mean allelic richness per locus ( $A_R$ ), number of private alleles ( $A_{pr}$ ), expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity, significance of the probability test for deviation from expected Hardy–Weinberg proportions ( $P_{HW}$ ), inbreeding coefficient ( $F_{IS}$ ), average kinship ( $F$ ) and relationship ( $r$ ) coefficients

Country/strain	$n$	$A$	$A_R$	$A_{pr}$	$H_O$	$H_E$	$P_{HW}$	$F_{IS}$	$F$	$r$
<i>Finland</i>										
Arvo–Kala	16	6.1	6.0	1	0.80	0.76	n.s.	–0.05	0.04	0.06
Jalo 3	39	6.0	5.0	–	0.77	0.71	**	–0.08	0.08	0.18
Joutsa 99	39	7.5	6.0	1	0.77	0.71	**	–0.07	0.04	0.14
Joutsa 01	39	6.0	5.1	–	0.68	0.66	***	–0.02	0.04	0.23
average		6.4	5.5 <sup>a</sup>		0.74 <sup>a</sup>	0.70 <sup>a</sup>			0.05	0.15
<i>Sweden</i>										
Antens	39	5.4	4.7	–	0.60	0.61	***	0.02	0.08	0.32
<i>Norway</i>										
commercial strain	39	7.0	5.9	4	0.62	0.69	***	0.10	0.05	0.16
<i>Denmark</i>										
Cofradex	78	7.2	5.7	6	0.68	0.69	***	–0.01	0.06	0.20
Hansen	29	5.9	5.0	–	0.66	0.63	n.s.	–0.06	0.04	0.29
Ollerupgard	39	5.7	4.8	–	0.73	0.64	***	–0.15	0.11	0.33
Sangild 03	39	5.9	4.9	–	0.69	0.67	**	–0.03	0.05	0.21
Sangild 04	39	4.9	4.2	2	0.69	0.61	*	–0.15	0.09	0.37
average		5.9	4.9 <sup>a</sup>		0.69 <sup>a</sup>	0.65 <sup>a</sup>			0.07	0.28
<i>Poland</i>										
Jastarnia	39	4.2	3.9	–	0.56	0.62	***	0.11	0.13	0.31
Olesnica	39	3.2	2.8	–	0.38	0.42	***	0.10	0.15	0.59
average		3.7	3.3 <sup>b</sup>		0.47 <sup>b</sup>	0.52 <sup>b</sup>			0.14	0.45
<i>USA</i>										
Donaldson 99	30	6.5	5.7	–	0.77	0.74	**	–0.05	0.07	0.14
Donaldson 00	25	6.4	6.0	3	0.74	0.75	n.s.	0.01	0.07	0.09
Shasta	39	5.6	4.9	4	0.63	0.65	***	0.04	0.16	0.29
average		6.2	5.5 <sup>a</sup>		0.70 <sup>a</sup>	0.70 <sup>a</sup>			0.10	0.17
<i>Canada</i>										
Dean	39	5.1	4.1	10	0.49	0.49	n.s.	–0.01	0.37	0.57
Tatlatui	38	3.8	3.2	7	0.41	0.39	n.s.	–0.03	0.53	0.69
Average		4.5	3.6 <sup>b</sup>		0.45 <sup>b</sup>	0.44 <sup>b</sup>			0.45	0.63

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , n.s. — not significant.

<sup>a, b</sup> average values of  $A_R$ ,  $H_E$  and  $H_O$  for groups of strains with different letters in superscript are significantly different ( $P < 0.05$ ).

Olesnica and Danish Cofradex strains. Physical linkage is not probable for the studied loci because at least eight loci have been mapped on distinct linkage groups (Nichols et al., 2003). However, significant linkage disequilibrium between loci can be caused also by selection on certain multilocus genotypes, presence of subgroups within some samples and/or sampling the siblings (Ohta, 1982).

The genetic variability (as estimated by the allelic richness,  $A_R$  and the observed heterozygosity,  $H_O$ ) of northern European strains that are farmed in Finland, Sweden, Denmark, Norway and Estonia was similar and did not differ significantly from the strains of USA origin (Shasta and Donaldson):  $A_R$  ranged from 4.2 (Sangild 04) to 6.0 (Arvo–Kala and Joutsa 99,

Donaldson 00) and  $H_O$  ranged from 0.60 (Antens) to 0.80 (Arvo–Kala) (Table 3). In contrast, the Polish strains and wild Canadian populations had significantly lower estimates of variability (average  $A_R = 3.3$  and 3.6, respectively; average  $H_O = 0.47$  and 0.45, respectively; Table 3). Inbreeding coefficient ( $F_{IS}$ ) was significantly positive only in Polish and Norwegian strains (Table 3). The Polish strains had also relatively high average kinship ( $F$ ) and relationship ( $r$ ) coefficients but the highest average  $F$  and  $r$  values were observed in wild Canadian populations (Table 3).

Results of the hierarchical gene diversity analysis revealed that only 0.9% of the total genetic variation among the farmed strains (wild Canadian populations excluded) was accounted

Table 4  
Assignment accuracy of rainbow trout individuals to their strain of origin

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total no.	Correctly assigned, %
1 Arvo–Kala	10	1	1		4											16	62.5
2 Jalo3		36	3													39	92.3
3 Joutsa	4	1	72									1				78	92.3
4 Antens		1		34	1	1	1	1								39	87.2
5 Norwegian	1		1	1	31		2		1			2				39	79.5
6 Cofradex			1			76		1								78	97.4
7 Hansen		1			3		24						1			29	82.8
8 Ollerupgard								39								39	100.0
9 Sangild						1		4	73							78	93.6
10 Jastarnia				1	1					37						39	94.5
11 Olesnica							1			1	37					39	94.5
12 Donaldson	4		3				3						45			55	81.8
13 Shasta			1										38			39	97.4
14 Dean														39		39	100.0
15 Tatlatui															38	38	100.0
<i>Total</i>																684	92.0

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. Differences between allele frequencies across all loci were highly significant for all population pairs ( $P < 0.001$ ). The level of differentiation among the farmed rainbow trout strains was moderate (average  $F_{ST} = 0.14$ ) and ranged from 0.04 (between Finnish Arvo–Kala and Norwegian commercial strain) to 0.36 (between the two Polish strains). The Danish strains were on an average more diverged than the Finnish strains (average  $F_{ST} = 0.10$  and 0.05, respectively). The individuals of rainbow trout could be assigned to their strain of origin with an

accuracy that ranged from 63 to 100% (on an average 92.0%; Table 4). All individuals were correctly assigned in Canadian wild populations and in Danish Ollerupgard strain, while the lowest accuracy of assignment (62.5%) was observed in Finnish Arvo–Kala strain (Table 4).

The NJ dendrogram of the  $D_A$  distances revealed only clear separation of Canadian wild populations from the farmed rainbow trout strains and a highly supported cluster of two Danish strains (Fig. 1). The other farmed strains did not form any strongly supported clusters. However, it is noteworthy that all farmed strains clustered together with the Shasta strain and not with the wild Canadian populations.

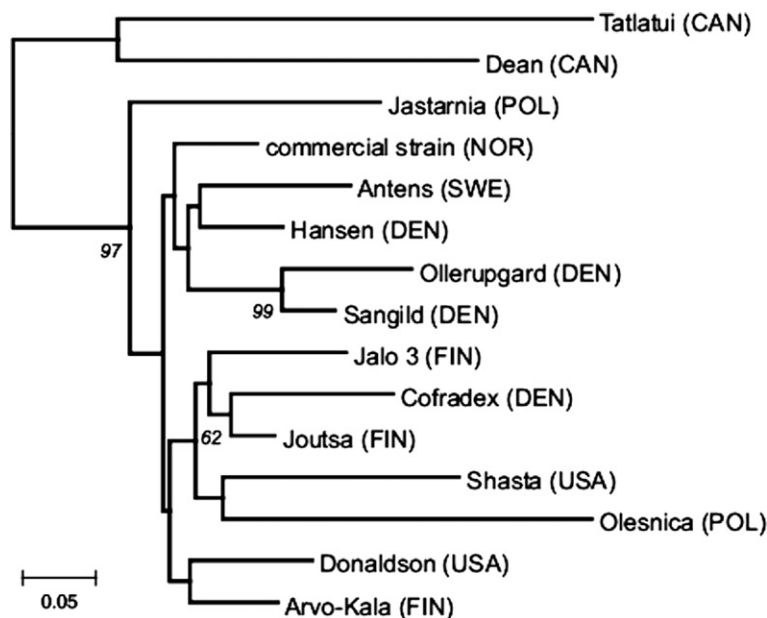


Fig. 1. NJ tree of rainbow trout strains based on Nei's  $D_A$  distance.

#### 4. Discussion

The level of genetic variation in the domesticated rainbow trout strains that are farmed in northern European countries (Finland, Sweden, Denmark, Norway, Estonia) did not differ significantly neither within nor between the countries and was comparable with that of the North American Shasta and Donaldson strains. In contrast, significantly lower variation was detected in the Polish strains and also in the wild populations from Canada. The two Polish strains are being used as an unselected control groups in a family selection program and may have lost the variation due to the low breeding population size and/or past population size bottlenecks. This can be inferred also from their significantly positive inbreeding coefficients and the relatively high average relatedness and relationship coefficients. Low variability and high relatedness in the studied Canadian wild populations is more difficult to explain as we have no background information about these populations. Again, low effective population size or historical population size bottleneck might explain the reduced variability of these populations. Unfortunately we had no access to Californian wild populations of rainbow trout or steelhead and thus, direct comparison of variability in domesticated strains and in their wild progenitors was not possible. Collating our results with published data is also somewhat complicated because different sets of microsatellite loci have been used by different authors. Nielsen (1999) used 11 microsatellite loci to study variation in six steelhead populations along the US Pacific Coast and found that for all populations combined, the number of alleles per locus averaged 16 and the average heterozygosity was 0.68. Narum et al. (2004) used six microsatellite loci for studying variation in sympatric resident and anadromous forms of *Oncorhynchus mykiss* in the Walla Walla River in south-eastern Washington and found on an average 14.5 alleles per locus for anadromous collections, 13.7 for the resident rainbow population and 13.8 for the collections of mixed life-history forms. Average  $H_O$  for the three collection types was 0.78 for anadromous, 0.70 for resident rainbow trout and 0.67 for mixed collections. Average  $H_E$  was 0.80 for anadromous and 0.78 for resident rainbow trout, and 0.79 for mixed collections. All of these indicators revealed higher genetic variation in steelhead than resident rainbow trout. Silverstein et al. (2004) assessed variation among three domesticated strains of rainbow trout in USA at nine microsatellite loci and found an average of 14 alleles at a locus and an average heterozygosity of 0.72. If we compare these results with our data for domesticated strains (average

number of alleles per locus 12.8, average  $H_O=0.67$  and  $H_E=0.76$ ), we can infer that most of the domesticated strains in our study are not significantly less variable than e.g. the resident rainbow trout populations in the Walla Walla River or the domesticated strains in USA. In contrast to rainbow trout, several studies on Atlantic salmon have demonstrated that the farmed fish show less genetic variability than the wild populations (in terms of allelic diversity but not necessarily in terms of overall heterozygosity), a likely result of founder effects and the ongoing selection programmes (Mjølnerod et al., 1997; Norris et al., 1999; Skaala et al., 2005). Reduced genetic variability is characteristic also for domesticated/captive stocks of common carp as compared to wild populations (Kohlmann et al., 2003, 2005; Zhou et al., 2004). This can be explained by founder effects but also by low effective breeding population size as relatively low numbers of breeders are typically used for reproduction of common carp strains due to the very high fecundity of female spawners. While the last factor may explain the difference between common carp and rainbow trout (females of which possess much smaller number of eggs and thus, much higher numbers of breeders are used for reproduction of farmed strains), the difference between rainbow trout and Atlantic salmon is more complicated to explain and direct comparison of farmed strains and their wild progenitors of rainbow trout is warranted to confirm or decline our inferences.

The average level of differentiation among the farmed strains in our study ( $F_{ST}=0.14$ ) is comparable with that of the steelhead populations along the US Pacific Coast ( $F_{ST}=0.17$ ; Nielsen, 1999) but higher than among the three domesticated strains in USA ( $F_{ST}=0.09$ ; Silverstein et al., 2004). The Danish strains were significantly more diverged than the Finnish strains (average  $F_{ST}=0.10$  and 0.05, respectively). This can be explained by the higher isolation of the Danish strains because the fish or egg transfers between hatcheries in Denmark have been limited in order to prevent spread of dangerous viral or bacterial diseases. However, the differentiation between strains is still sufficient for assigning the individuals to their strain of origin with rather high accuracy.

The farmed rainbow trout strains in this study were genetically rather similar and did not form any clear clusters on the dendrogram. However, clear separation of Canadian wild populations from all domesticated strains and genetic similarity of northern and eastern European strains with the Shasta strain provide support for the hypothesis that most rainbow trout strains in Europe have their origin in California.

## 5. Conclusions

The level of genetic diversity in northern European rainbow trout strains is comparable with that of the North American domesticated strains and wild populations, indicating that the northern European rainbow trout strains have not significantly lost their variability due to breeding practices in contrast to the eastern European Polish strains.

The northern and eastern European rainbow trout strains are moderately differentiated and have significantly different allele frequencies at microsatellite loci. High individual assignment accuracy indicates a good potential for genetic tagging of the strains.

The Danish strains were more divergent than the Finnish strains that can be explained by their higher degree of isolation as transfers of strains between hatcheries in Denmark have been limited to avoid transmission of infectious diseases.

The country of origin has only minor impact on the level of differentiation and relatedness of the strains, indicating that the hatcheries in different countries have created the strains by mixing different stocks and may exchange breeding material even nowadays.

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