GENETIC DIVERSITY AND DIFFERENTIATION OF IMPORTED INTO ESTONIA RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) STRAINS BASED ON MICROSATELLITE DNA VARIATION

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ABSTRACT. Genetic diversity and differentiation of imported into Estonia rainbow trout (*Oncorhynchus mykiss*) strains based on microsatellite dna variation. *The variation of 10 microsatellite loci was analyzed in eight rainbow trout strains (the North American Donaldson strain, two Finnish strains, four Danish strains and a Norwegian strain). The most variable locus was Ssa85 (total of 24 alleles), whereas the least variable was Ssa197 (total of 2 alleles). The strains of Finnish origin were on an average more variable than the strains of Danish origin (allelic richness 5.9 and 4.9, respectively; expected heterozygosity 0.73 and 0.65, respectively), while the variability of Donaldson and Norwegian strains was similar to the Finnish strains. The average level of differentiation (F_{ST}) among the strains was 0.10 and ranged from 0.03 (between the Norwegian and Finnish Arvo-Kala strain) to 0.18 (between the Danish Cofradex and Ollerupgård strains). Based on genetic distances, the strains grouped into three clusters but the clustering was not always consistent with the country of origin, e.g. the Finnish Joutsa strain clustered together with the Danish Cofradex strain and the Finnish Arvo-Kala strain clustered together with the Donaldson strain, while only three Danish strains (Hansen, Ollerupgård and Sangild) formed a single cluster. Based on the likelihood of multilocus genotypes, 86.8% of individuals could be correctly classified into their strain of origin, which demonstrates a good potential of microsatellite loci for genetic tagging of rainbow trout strains*.

Keywords: *rainbow trout, microsatellites, genetic diversity.*

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. It is believed that most of the rainbow trout cultured around the world originate from the McCloud River hatchery in California (Gall, 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, quality etc. (Gjedrem, 2000).

In 1896, rainbow trout was also brought into Estonia and it is nowadays the most important farmed fish species here. During the Soviet period, different strains of rainbow trout were introduced into Estonia from Russia, Germany, Czech Republic, Finland, Sweden, Denmark, Japan and the USA. However, their propagation and crossbreeding was quite stochastic that resulted in loss of strain specific characters and decline of performance, e.g. in reduced survival and growth rate. In recent years, multiple new strains have been imported mainly from Finland and Denmark and they have replaced most of the earlier introduced strains. It is necessary to estimate and monitor the levels of variability and differentiation of introduced strains and to tag them genetically in order to avoid further mixing. Genetic tagging of the strains is very important also in comparative performance testing experiments, enabling communal rearing of the test groups and thus, reducing the number of test tanks and the influence of inter-tank environmental differences. In 1980s, the Estonian rainbow trout strains were genetically characterized based on allozyme markers (Paaver, Lilleorg, 1994; Paaver, 1986, 1988). However, due to the low level of allozyme variation and limited number of polymorphic loci no significant differences were revealed, except the specific genetic profile of the Donaldson strain. Today the situation has changed and it is necessary to characterize the newly introduced strains by applying more variable DNA markers. The aim of the present study was to estimate the levels of variability and differentiation among the introduced into Estonia rainbow trout strains by using hypervariable microsatellite DNA markers and to reveal the genetic relationships among the strains. Also, the potential of the microsatellite loci for genetic tagging of the strains was estimated.

Material and methods

Fish samples, DNA isolation and microsatellite analysis

The variation of 10 microsatellite loci (Table 1) was studied in total of 326 individuals from eight strains of rainbow trout (four strains of Danish origin, two strains of Finnish origin and a strain of Norwegian and USA

origin, respectively) (Table 2). Samples were obtained from the Estonian fish farms Härjanurme, Vohnja, Aravuse and Roosna-Alliku with the exception of the sample of Norwegian trout, which was purchased from a processing factory. Unfortunately, there is no background information available about the origin and previous management of the strains, e.g. about the number of parents or intensity of selection.

Genomic DNA was isolated from fin clips or muscle tissue according to the simplified method of Laird *et al*. (1991). Each polymerase chain reaction (PCR) was composed of ca 100 ng DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.1 μM dNTPs, 0.2 μM of each primer (forward primers were end-labeled with the florescent dye Cy5) and 0.8 U of Taq DNA polymerase (MBI-Fermentas), in a total volume of 10 μl. We used the following "touch down" PCR profile: initial denaturation at 94 °C for 3 min, 10 cycles of 40 sec at 94 °C, 40 sec at 60 to 50 °C (1 °C decrease per cycle), 1 min at 72 °C and 25 cycles of 40 sec at 94 °C, 40 sec at 50 °C, 1 min at 72 °C and final extension at 72 °C for 10 min. Thermal cycling was performed by Eppendorf Mastercycler Gradient. The length of the microsatellite alleles was determined by an ALFexpress II DNA analyzer (Amersham Pharmacia Biotech) 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.

Table 1. Characterization of the studied microsatellite loci
Tabel 1. *Uuritud mikrosatelliidilookuste kirjeldus*

Locus	Repeat	Primer sequence 5'→3'	T_a (°C)	Size range (bp)	A	H_o	H_e
Ssa197[1]	GTGA	F-GGGTTGAGTAGGGAGGCTTG R-TGGCAGGGATTTGACATAAC	60–50	110–114	2	0.50	0.46
Ssa85[1]	GT	F-AGGTGGGTCCTCCAAGCTAC R-ACCCGCTCCTCACTTAATC	60–50	102–164	24	0.57	0.60
Ocl8[2]	GT	F-TAGTGTTCCGTGTTCGCCTG R-CACCTTCCATCTCTCATTCCAC	60–50	103–153	17	0.86	0.81
OMM1039[3]	GA	F-GGGGTAGGAGTAGACTAGACA* R-ATCTTTCCCTCCTTGCAC	60–50	132–176	16	0.80	0.74
OMM1019[3]	AG	F-CCAGCAGTAAACCTTAGGTTG* R-GTCAAAGGAGACGTAGAGCTT	60–50	201–217	9	0.84	0.78
OMM1020[3]	AG	F-CCTGTGAGTGTTAATTCGACCTGT* R-GGTCTTACCTCAACATCGGTGA	60–50	179–201	8	0.61	0.69
OMM1036[3]	TATC	F-TGTAGCAGGTGAGAATACCCA* R-CACCATCTCCATCCTAGGC	60–50	225–317	15	0.74	0.75
OMM1046[3]	TCTA	F-CAGGCACTATAATGGCAC* R-GCCCACGAGTTACAAGA	60–50	116–176	14	0.80	0.77
OMM1307[4]	CTAT	F-GCACAACTACGAAACCCAA* R-TGCCAGCTCTGCTATGACATT	60–50	186–198	4	0.73	0.64
OMM1315[4]	CATC	F-TACAGGGCTTGGCTCTATCTC* R-GCCAAATACTTTCGCAAGG	60–50	118–158	7	0.70	0.67
Average					11.6	0.71	0.69

* modified by adding M13 sequence (cacgacgttgtaaaacgac) to the 5' end of the forward primer
T_a – annealing temperature, A – number of observed alleles, H_o – average observed heterozygosity, H_e – average expected heterozygosity
[1] O´Reilly *et al*. (1996), [2] Condrey, Bentzen (1998), [3] Rexroad *et al*. (2002), [4] Palti *et al*. (2002)

Data analysis

FSTAT v. 2.9.3.2 program package (Goudet, 2002) was used for calculating allele frequencies and for estimating the expected and observed heterozygosities (H_e, H_o) and the allelic richness (A_R). FSTAT was used also for testing the significance of differences in average values of A_R, H_e and H_o among the groups of strains (1000 permutations, one-side test of the null hypothesis of no difference). The number of private alleles (A_{pr}) was calculated by using GDA v. 1.0 program (Lewis, Zaykin, 2002). GENEPOP v. 3.3 (Raymond, 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, for calculating F_{ST} values and for estimating the significance of genotypic differentiation between stock pairs. All probability tests were based on the Markov chain method (Guo, Thompson, 1992; Raymond, 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.

Analysis of molecular variance (AMOVA) 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 strains of origin by using Bayesian approach of the assignment test. Genetic distances between the strains were estimated according to the D_A distance of Nei et al. (1983) and a phylogenetic 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.

Results and discussion

Genetic diversity

A total of 116 alleles were observed across the ten microsatellite loci with an average of 11.6 alleles per locus, ranging from two alleles at Ssa197 to 24 alleles at Ssa85 (Table 1). Significant deviations from Hardy-Weinberg equilibrium (P<0.01) at least in one locus were observed in five strains (Table 2). The deviations are most probably due to the use of limited number of breeders and/or unequal sex ratio. However, in strains from which two age classes were sampled (Finnish Joutsa and Donaldson), the deviations may also have resulted from a Wahlund effect caused by genetic heterogeneity between cohorts.

Table 2. Microsatellite diversity indices of rainbow trout strains in Estonia
Tabel 2. *Eestisse imporditud vikerforelliliinide geneetiline varieeruvus*

Country of origin / strain	*n*	*A*	A_R*	A_{pr}	H_e*	H_o*	P_{HW}
Finland							
Joutsa	78	8.4	5.8	1	0.705	0.722	***
Arvo-Kala	14	6.0	6.0	1	0.757	0.793	n.s.
Average, Finland		7.2	5.9[a]	–	0.731[a]	0.757[a]	–
Denmark							
Hansen	29	5.9	4.9	–	0.626	0.660	n.s.
Cofradex	39	6.2	5.3	4	0.658	0.672	***
Sangild	39	5.9	4.9	–	0.671	0.688	**
Ollergupgård	39	5.7	4.7	1	0.642	0.734	***
Average, Denmark		5.9	4.9[b]	–	0.649[b]	0.688[a]	–
USA/Donaldson	55	7.6	5.9	4	0.744	0.758	**
Norway/market fish	33	7.0	5.9	5	0.699	0.642	**
Total average		6.6	5.4	2.7	0.688	0.708	–

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, results of the probability test for deviation from expected Hardy-Weinberg proportions (P_{HW}): ** P<0.01, *** P<0.001, n.s. – not significant.
* Average values of A_R, H_e and H_o for Finnish and Danish strains with different superscript letters are significantly different (P<0.05)

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 (4 pairs; not the same loci involved) was detected only in the Donaldson and Danish Hansen 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).

Among the studied strains, the total number of alleles varied from 57 (Danish Ollerupgård) to 84 (Finnish Joutsa). The strains shared 100 (86.2%) alleles over all loci, whereas a total of 2, 5, 4 and 5 private alleles were observed among the strains of Finnish, Danish, North American and Norwegian origin, respectively. The strains of Finnish origin were on an average more variable than the strains of Danish origin (allelic richness 5.9 and 4.9, respectively; expected heterozygosity 0.731 and 0.649, respectively), while the variability of Donaldson and Norwegian strains was similar to the Finnish strains (Table 2). The level of genetic variation in the imported to Estonia rainbow trout strains was comparable with three North American domesticated strains from the study of Silverstein *et al*. (2004) where five microsatellite loci coincided with our set of loci (Table 3).

Table 3. Comparison of genetic variability at five microsatellite loci in Estonian and North American samples of domesticated rainbow trout
Tabel 3. *Eesti ja Põhja-Ameerika kodustatud vikerforelliliinide geneetilise varieeruvuse võrdlus viie mikrosatelliidi-lookuse põhjal*

Locus	Estonia[1]		North America[2]	
	A	H_o	*A*	H_o
OMM1039	16	0.80	12	0.81
OMM1019	9	0.84	12	0.66
OMM1020	8	0.61	9	0.66
OMM1036	15	0.74	15	0.80
OMM1046	14	0.80	14	0.80

[1] 8 strains; our study
[2] 3 strains; Silverstein *et al*. (2004)

Hierarchical diversity analysis and differentiation among the strains

Results of the hierarchical gene diversity analysis revealed that only 2.9% of the total genetical variation was accounted for by differences between the countries of origin, 8.4% was due to differentiation among the strains within the countries of origin and 88.7% was due to variation within strains. Differences between allele frequencies across all loci were highly significant for all population pairs ($P<0.001$). The overall level of differentiation (as estimated by F_{ST}) for the studied rainbow trout strains in Estonia was 0.10 and ranged from 0.03 (between the Finnish Arvo-Kala and Norwegian strain) to 0.18 (between the Danish strains Cofradex and Ollerupgård) (Table 4).

Table 4. Pair wise F_{ST} estimates between rainbow trout strains
Tabel 4. *Vikerforelli liinide vahelised F_{ST} väärtused*

Strain,	Finland		Denmark				USA	Norway
(country of origin)	Joutsa	Arvo-Kala	Hansen	Cofradex	Sangild	Ollerupg.	Donaldson	
Joutsa (FIN)	0							
Arvo-Kala (FIN)	0.044	0						
Hansen (DEN)	0.093	0.075	0					
Cofradex (DEN)	0.066	0.108	0.135	0				
Sangild (DEN)	0.102	0.087	0.065	0.132	0			
Ollerupgård (DEN)	0.126	0.110	0.128	0.179	0.084	0		
Donaldson (USA)	0.077	0.044	0.106	0.105	0.101	0.136	0	
NOR	0.047	0.030	0.047	0.114	0.073	0.107	0.067	0

Based on the likelihood of multilocus genotypes, 86.8% of individuals could be correctly classified to their strain of origin, which demonstrates a good potential of microsatellite loci for genetic tagging of rainbow trout strains (Table 5).

Genetic relationships among the strains

Pair-wise genetic distances (D_A) were calculated between all trout samples to investigate evolutionary relationships in allele frequencies. The greatest genetic distance (0.33) was observed between the Danish Cofradex and Ollerupgård strains, and the smallest (0.12) was observed between the Norwegian and Finnish Arvo-Kala strains. The unrooted NJ dendrogram depicting the underlying structure of the D_A distance matrix revealed that the clustering of strains was not always consistent with the country of origin: the Finnish Joutsa strain clustered together with the Danish Cofradex strain and the Finnish Arvo-Kala strain clustered together with the Donaldson strain, while only Danish Hansen, Ollerupgård and Sangild strains formed a single cluster (Fig. 1). This indicates, that in the Northern Europe the rainbow trout strains are developed from different mixtures of several stocks and exchange of breeding material between the countries has taken place.

Table 5. Accuracy of self-assignment of rainbow trout individuals to their strain of origin based on the likelihood of multilocus microsatellite genotypes (Bayesian method)
Tabel 5. *Vikerforelli indiviidide tõulise päritolu määramise täpsus mikrosatelliidilookuste genotüüpide põhjal (Bayesi meetod)*

Strain, (country of origin)	FIN			DEN			USA	NOR	Sample size	No. correctly classified	% correctly classified
	1	2	3	4	5	6	7	8			
1 Joutsa (FIN)	71	4	–	–	1	–	2	–	78	71	91.0
2 Arvo-Kala (FIN)	2	8	–	–	–	–	–	4	14	8	57.1
3 Hansen (DEN)	1	–	24	–	–	–	1	3	29	24	82.8
4 Cofradex (DEN)	–	–	–	38	–	1	–	–	39	38	97.4
5 Sangild (DEN)	–	–	–	1	34	4	–	–	39	34	87.2
6 Ollerupgård (DEN)	–	–	–	–	1	38	–	–	39	38	97.4
7 Donaldson (USA)	3	4	3	–	1	–	44	–	55	44	80.0
8 NOR	1	2	2	–	–	–	2	26	33	26	78.8
Total									326	283	86.8

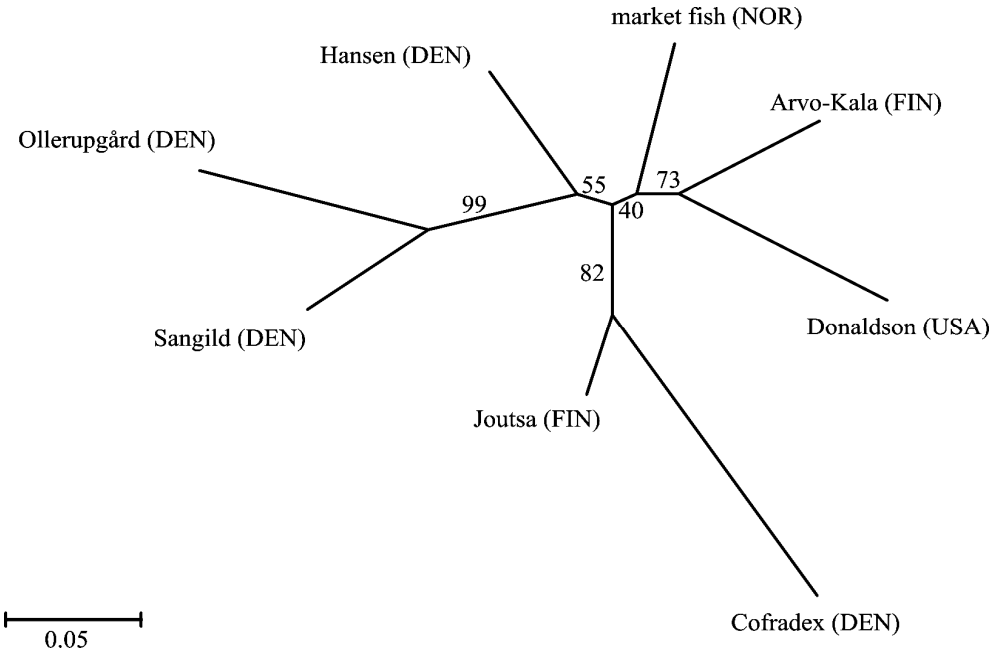

Figure 1. Unrooted NJ dendrogram of the studied rainbow trout strains, based on Nei's D_A distances. Numbers indicate bootstrap support for the nodes
Joonis 1. *Eestisse imporditud vikerforellliliinide geneetilise sarnasuse dendrogramm Nei D_A distantsi põhjal*

Conclusions

The level of genetic diversity in imported to Estonia rainbow trout strains, is comparable with the North American domesticated strains, indicating that the Northern European rainbow trout strains have not significantly lost their variability due to breeding practices. The strains of Finnish origin are on an average genetically more variable than the strains of Danish origin. The strains are moderately differentiated and have significantly different allele frequencies at microsatellite loci, offering a good potential for genetic tagging of the strains. However, 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 quite stochastically and maybe exchange breeding material even nowadays.

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Eestisse imporditud vikerforelli (*Oncorhynchus mykiss*) liinide geneetiline mitmekesisus ja diferentseerumisaste mikrosatelliitse DNA varieeruvuse põhjal

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Kokkuvõte

Vikerforell on üks maailma tähtsamaid kalakasvatuse objekte, kelle aastane toodang ületab 500 tuhat tonni. Arvatakse, et enamus kasvatatavaid vikerforelli liine on saanud alguse McCloudi jõe haudemajast

Californias, kust seda levitati nii Põhja-Ameerika kui Euroopa kalakasvandustesse 19. sajandi lõpul (Gall, Crandell, 1992). Ka Eestisse toodi vikerforell juba 1896. aastal ja on siin tänapäeval kõige olulisemaks kalakasvatuse objektiks. Vikerforelli erinevaid liine on Eestisse toodud mitmel korral Venemaalt, Saksamaalt, Tšehhist, Soomest, Rootsist, Taanist, Jaapanist ja USA-st. Et nõukogude perioodil puudus Eestis vikerforelli aretust ja paljundamist koordineeriv keskus, siis toimus liinide paljundamine ja ristamine ilma asjatundliku juhendamiseta, mistõttu ilmnesid inbriidingu nähud ja liinide kontrollimatu segunemise tõttu läksid kaduma tõuspetsiifilised omadused. Nende liinide pidamine ja paljundamine on lõpetatud ja lootuses omandada uuemat ja kvaliteetsemat tõumaterjali on kalakasvatajad viimasel aastakümnel toonud Eestisse arvukalt uusi liine ja tõuge Soomest, Taanist ja Rootsist. Nende geneetilistest erinevustest on teada väga vähe ja seetõttu on vajalik kõigi Eestisse toodud vikerforelliliinide geneetiline iseloomustamine, mis võimaldaks neid geneetiliselt märgistada ja seetõttu edaspidi ka nende puhtust kontrollida. Geneetiline märgistamine võimaldab ka liine kasvatada koos ühises tiigis/basseinis, et võrrelda identsetes keskkonnatingimustes nende produktiivomadusi ja välja selgitada parimate omadustega tõud. Aretustöö jaoks oluliseks aspektiks on ka geneetiliselt võimalikult erinevate tõugude ja liinide väljaselgitamine, mis hõlbustab heteroosiefekti saavutamiseks tehtavate ristamiskatsete planeerimist. Tõugude ja liinide geneetilise muutlikkuse hindamine lubab ka teha järeldusi inbriidingu ja juhusliku geenitriivi taseme kohta. 1980-ndatel aastatel Eestis kasvatatud vikerforelliliinide geneetilist muutlikkust iseloomustati tollal standardiks olnud polümorfsete valkude ja ensüümide (allosüümide) põhjal (Paaver, Lilleorg, 1994; Paaver, 1986, 1988), kuid lookuste vähese arvu ja madala muutlikkuse tõttu saadi välja tuua vaid kõige silmatorkavamaid erinevusi nagu näiteks Donaldsoni forelli vähenenud muutlikkuse tase. Eestis kasvatatavate tõugude väljavahetamine täiesti uute vastu nõuab nende geneetilise mitmekesisuse uurimist DNA markerite abil. Käesoleva uurimuse eesmärgiks oli hinnata Eestisse imporditud vikerforelliliinide geneetilist muutlikkust, diferentseerumis- ja sugulusastet hüpervarieeruvate mikrosatelliitse DNA markerite abil. Samuti hinnati mikrosatelliidilookuste geneetiliste märgistena kasutamise potentsiaali.

Analüüsiti 10 mikrosatelliidilookuse varieeruvust kaheksal Eestisse imporditud Soome, Taani, USA ja Norra päritolu vikerforelliliinil (tabelid 1 ja 2). Analüüsimeetodina kasutati polümeraasi ahelreaktsiooni (PCR). Mikrosatelliidilookuste genotüübid määrati ALFexpress II DNA analüsaatoriga (Amersham Pharmacia Biotech) ja AlleleLinks v. 1.02 tarkvaraga (Amersham Pharmacia Biotech). Liinide geneetilist varieeruvust iseloomustati alleelide keskmise arvukuse (A_R) ning faktilise (H_o) ja teoreetilise (H_e) heterosügootsuse põhjal, mille leidmiseks kasutati FSTAT v. 2.9.3.2 programmipaketti (Goudet, 2002). Liinide jaoks unikaalsete alleelide (A_{pr}) leidmiseks kasutati programmi GDA v. 1.0 (Lewis, Zaykin, 2002). Kõrvalekallete olulisust Hardy-Weinbergi tasakaalulistest genotüübisagedustest ja liinide geneetilise diferentseerumise astet (F_{ST}) hinnati GENEPOP v. 3.3 (Raymond, Rousset, 1995a) tarkvara abil. Hierarhiline molekulaarne dispersioonanalüüs (AMOVA) teostati ARLEQUIN v.2.0 tarkvaraga (Schneider et al. 2000). Indiviidide tõulise päritolu määramise võimalikkust nende genotüüpide põhjal uuriti GENECLASS v. 2.0 programmiga (Piry et al., 2004). Liinide geneetilist sarnasust hinnati Nei D_A distantsi põhjal ja populatsioonide dendrogramm koostati DISPAN tarkvara (Ota, 1993) abil.

Kõige suurema varieeruvusega mikrosatelliidilookuseks (tabel 1) osutus Ssa85 (24 alleeli) ja kõige väiksema alleelide arvuga oli lookus Ssa197 (2 alleeli). Soome ja Norra päritolu vikerforelliliinid ja Donaldsoni tõug olid keskmiselt varieeruvamad kui Taani liinid (tabel 2). Põhja-Ameerikas kasvatatavate ja Eestisse imporditud vikerforelliliinide geneetilise varieeruvuse tase on üsna sarnane (tabel 3), mis näitab, et Põhja-Euroopas kasvatatavate vikerforellide genofond ei ole aretustöö käigus oluliselt vaesunud. Eesti vikerforelliliinide geneetiline diferentseerumine on mõõdukas (keskmine F_{ST} = 0.10) ja alleelisageduste erinevused liinide vahel on statistiliselt olulised. See võimaldab üsna suure täpsusega (86,8%) määrata indiviidide tõulist päritolu (tabel 5) ja näitab mikrosatelliitide head potentsiaali geneetiliste märgistena kasutamiseks. Uuritud vikerforelliliinid moodustasid geneetilise distantsi põhjal kolm gruppi, mis ei seostunud alati päritolumaaga. Näiteks Soome Joutsa liin oli geneetiliselt sarnane Taanist pärit Cofradexi liiniga, teine Soome liin (Arvo-Kala) aga sarnasem hoopis Donaldsoni forelliga (joonis 1). See näitab, et erinevates riikides on kalakasvatajad forellitõuge luues lähtunud erinevatest forelliliinide segudest ja tõenäoselt vahetanud ka omavahel aretusmaterjali.