Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2010) **77**, 2048–2071
doi:10.1111/j.1095-8649.2010.02784.x, available online at wileyonlinelibrary.com

High level of population genetic structuring in lake-run brown trout, *Salmo trutta*, of the Inari Basin, northern Finland

A. Swatdipong*†, A. Vasemägi*, T. Niva‡, M.-L. Koljonen§ and C. R. Primmer*‖

*Department of Biology, University of Turku, Turku, Finland, ‡Finnish Game and Fisheries Research Institute, Oulu, Finland and §Finnish Game and Fisheries Research Institute, Helsinki, Finland

(Received 6 October 2009, Accepted 16 August 2010)

Rivers draining into (Lake) Inarijärvi, northern Finland, sustain a number of lake-run brown trout, *Salmo trutta*, populations but, as with most lake-run *S. trutta* systems, the level of population genetic structuring among populations is unknown. To address this and to assist fish stock management in the region, the population genetic structure of *S. trutta* collected from 28 sampling sites in rivers flowing into Inarijärvi was studied using 13 microsatellite loci. Populations were clustered into three separate groups, largely corresponding to geographic regions, with between-region F_{ST} values ranging from 0·11 to 0·16. The significant differentiation observed between most populations within each region also implies that individual populations should be recognized as separate management units and actions to improve, and subsequently maintain, conditions for natural spawning should be prioritized. The results of this study further indicate that the trout from each of these regions may have different biological characteristics, such as local-lake feeding behaviour among the western populations and strong isolation among the northern stocks. As a consequence, further research is warranted to better understand the level of ecological uniqueness of lake-run *S. trutta* populations.

Key words: Inarijärvi; lake-run brown trout; management unit; microsatellite; population structure; *Salmo trutta*.

INTRODUCTION

Brown trout *Salmo trutta* L. is a salmonid fish native to Europe, North Africa and western Asia (Elliott, 1989), that can be also found far beyond its natural distribution as an introduced species due to its high economic value for both professional and recreational fisheries (Youngson *et al.*, 2003; Nylander, 2004). *Salmo trutta* has a more flexible life history than its closest relative, Atlantic salmon *Salmo salar* L. as both stream resident and migratory stocks are common and both forms can live

‖Author to whom correspondence should be addressed. Tel.: +358 2 333 5571; fax: +358 2 333 6680; email: craig.primmer@utu.fi
†Current address: Department of Genetics, Kasetsart University, Bangkok, Thailand

in sympatry (Elliott, 1994; Klemetsen *et al*., 2003). Migratory trout usually spawn in freshwater streams and rivers, with juveniles typically staying 2–6 years in their natal river before smoltification (Fahy, 1978; L'Abee-Lund *et al*., 1989). Smolts then migrate to a lake or sea where they feed until reaching maturity, returning to their natal spawning grounds to breed. As a result of accurate homing behaviour, the level of gene flow among *S. trutta* populations is rather limited (Ryman, 1983). In addition, gene flow among populations may be restricted due to impassable geographic barriers.

The genetic structure of *S. trutta* populations has been extensively studied during recent decades and, as has been observed in riverine fishes (Hänfling & Weetman, 2006; Raeymaekers *et al*., 2008; Takacs *et al*., 2008; Barson *et al*., 2009), a high level of genetic differentiation among *S. trutta* populations occupying adjacent rivers and even tributaries within the same river has often been observed (Carlsson & Nilsson, 2000; Hansen *et al*., 2002; Fraser *et al*., 2007; Apostolidis *et al*., 2008). Studies have tended to focus, however, on the within-river level of either resident or sea-run populations (Carlsson *et al*., 1999; Carlsson & Nilsson, 2000; Hansen *et al*., 2002; Charles *et al*., 2005; Antunes *et al*., 2006; Hovgaard *et al*., 2006; Apostolidis *et al*., 2008; Lehtonen *et al*., 2009) or over very broad geographical regions (Bernatchez, 2001; Presa *et al*., 2002; Schreiber & Diefenbach, 2005). Thus, detailed studies on lake-run systems, the scale of which falls between within river and between regions, are rare (Jensen *et al*., 2005). Such studies could provide more general information regarding the level of genetic structuring in lake-run *S. trutta*. This information would also be valuable for the development of appropriate management plans for endangered and exploited populations as many lake-run *S. trutta* populations are negatively influenced by various anthropogenic activities. The main threats to Finnish lake-run *S. trutta* populations include, for example, overfishing, pollution and changes in land use (Kallio-Nyberg *et al*., 2001). As a consequence, the majority (71%) of Finnish lake-run *S. trutta* populations have been classified as declining, endangered or highly endangered (Kallio-Nyberg *et al*., 2001).

In order to compensate for the decline or extinction of natural populations, large-scale supportive breeding and release programmes, also known as stock enhancement, have been initiated in many countries (Kitada *et al*., 2009). Supportive breeding programmes commonly use a fraction of the wild spawners for artificial reproduction, and their offspring are subsequently released into the natural habitats occupied by wild conspecifics. A key conservation aim of such supportive release programmes is usually to increase the size of the spawning stock in the wild and simultaneously improve fisheries while avoiding the introduction of foreign genes into the native population. For example, hatchery-born juveniles produced using native spawners are stocked to the sites where their parents were caught and hatchery broodstocks are renewed routinely using wild-caught spawners. There are, however, several potential drawbacks to the use of supportive releases as a conservation measure. For example, a decrease in genetic variability or effective population size as well as the replacement of wild populations by hatchery-born fish have been theoretically predicted (Ryman & Laikre, 1991) and also empirically observed (Taniguchi *et al*., 1983; Koskinen *et al*., 2002; Vasemägi *et al*., 2005; Kitada & Kishino, 2006; Kitada *et al*., 2009). In addition, even when local broodstocks are used for enhancement, unintentional genetic changes can occur in local populations if there is undetected structuring within a system (Koskinen *et al*., 2002).

The lake-run *S. trutta* populations of the Inari Basin in northernmost Finland represent a classic example displaying many of the features described above. As a result of the negative effects of water level regulation on the *S. trutta* populations in (Lake) Inarijärvi and damming of the (River) Paatsjoki, large numbers of *S. trutta* have been stocked annually to compensate for damaged wild stocks since the early 1980s. Simultaneously, wild *S. trutta* still reproduce in at least 10 rivers and dozens of tributaries; and both wild and stocked *S. trutta* use Inarijärvi as their feeding ground. *Salmo trutta* are the target of intensive recreational and commercial mixed stock fisheries in Inarijärvi with a combined annual catch of 40–50 metric tons (Salonen *et al*., 2009). Recent tagging results show that *c*. 50% of the *S. trutta* catch consists of stocked–released fish (T. Niva *et al*., unpubl. data). Until now, however, the number of distinct indigenous *S. trutta* populations occurring in the area and the level of genetic differentiation among these populations is unknown.

The aims of this study were: (1) to characterize the population genetic structure of the majority of wild lake-run *S. trutta* populations in rivers and tributaries draining into Inarijärvi and (2) to compare the level of genetic structuring in the Inari system to that observed in other *S. trutta* and *S. salar* populations. This information is vital for future estimation of the river or tributary-specific proportion of *S. trutta* feeding in Inarijärvi, for the assessment of potential genetic effects of supportive stocking in the Inari system and for understanding the level of genetic uniqueness of natural lake-run *S. trutta* populations.

MATERIALS AND METHODS

THE STUDY SYSTEM

The Inari Basin is a large lake system in northern Finland. Its largest lake, Inarijärvi (69° N; 28° W), has a surface area of 1040 km^2, >3000 islands and 3308 km of shoreline, providing extensive feeding grounds for salmonids, including lake-run *S. trutta*. Small-scale supplementation of *S. trutta* has been conducted in the Inari Basin from the early 1950s to the late 1970s from the Inari hatchery situated close to the Juutuanjoki river outlet. In the early 1980s, another hatchery was built at Lake Sarmijärvi, and thereafter the total number of stocked *S. trutta* increased dramatically to *c*. 100 000 smolts annually. Hatchery broodstocks have been established from wild spawners caught from the Juutuanjoki, Ivalojoki and Siuttajoki. The corresponding hatchery broodstocks have been renewed from the wild at time intervals of *c*. 3 years. During the early years, most stocked fish were newly hatched larvae, but from the early 1980s the age of the stocked *S. trutta* has varied from 1 to 4 years, with 3 year olds being the most frequently used age for stocking.

FISH SAMPLES

Salmo trutta were sampled using electrofishing from 28 locations along tributaries and rivers draining into Inarijärvi (Fig. 1 and Table I). Fin clips or tissue samples from parr or spawning adults were collected during 2002–2008 and stored in 95% ethanol. Scales collected from Muddusjärvi and Juutuanjoki during 1949–1979 have been stored in paper envelopes at the Finnish Game and Fisheries Research Institute.

DNA EXTRACTION AND MICROSATELLITE DATA

Total genomic DNA was extracted from muscle or scale samples using one of two methods: (1) DNeasy 96 Blood & Tissue Kit method (Qiagen; www.qiagen.com) and (2), salt-based

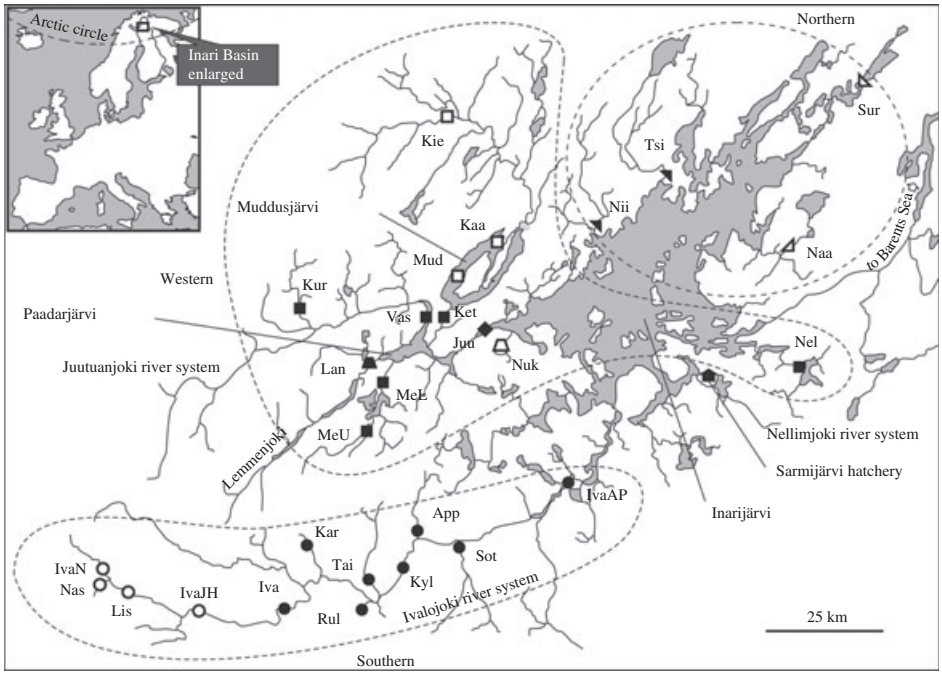

FIG. 1. Map indicating sampling locations. Three population groups indicated by principal component analysis (PCA) are outlined by dotted/broken lines. Ten different symbols are used according to population clustering by Baps. Sample abbreviations are given in Table I.

protocol (Aljanabi & Martinez, 1997) combined with a vacuum-based (Elphinstone *et al*., 2003) extraction method (details available on request).

Samples were genotyped initially for a total of 15 microsatellite loci: BS131, OneU9, Ssa171, Ssa197, Ssa407, Ssa85, Ssosl311, Ssosl417, Ssosl438, Str15INRA, Str543INRA, Str60INRA, Str73INRA, Str85INRA and Strutta58 (see references in Table S1). Data for two of these loci were subsequently excluded either due to poor amplification rates in a large number of samples (Ssa171) or highly significant heterozygote deficiency ($P < 0{\cdot}0001$) in several populations, probably indicating a high frequency of null alleles (Ssa197). The Muddusjärvi population was genotyped at nine loci due to the low-quality DNA template (dried scales from 1949).

The first set of samples, analysed at the University of Helsinki, included a sub-set of individuals collected from seven populations, and these were genotyped at the 15 loci as listed above. The loci were amplified individually and then amplicons were pooled for electrophoresis (details available from M.-L.K. on request). The remainder of the samples (*c*. 650) were analysed at the University of Turku using the same set of microsatellite loci, but multiplex PCR, whereby all 15 loci were amplified in a single PCR reaction, was used instead of individual locus amplification. Conditions for the multiplex PCR reaction and amplification were as in Swatdipong *et al*. (2009), except that the PCR volume was reduced to 5 μl and a 2720 Thermal cycler (Applied Biosystems; www.appliedbiosystems.com) or a Piko thermal cycler (Finnzymes Instruments; www.finnzymes.com) were sometimes used. Primer concentrations and fluorescent dye colours are listed in Table S1. Electrophoresis was performed on an ABI3130xl (Applied Biosystems) as outlined in Swatdipong *et al*. (2009). A sub-sample (80 individuals; 20%) of the first sample set genotyped in Helsinki were re-genotyped using the multiplex protocol to enable allele-size calibration between two data sets.

Old scale samples collected from two sampling sites (Juutuanjoki and Muddusjärvi; Fig. 1) were genotyped using slightly modified protocol consisting of two multiplex PCRs of three

TABLE I. Sample information, microsatellite diversity estimates of Inari *Salmo trutta* populations

Location									
Sampling site (ordered by PCA group)	Code	Co-ordinates		Sampling year	Fish sampled	Sample size	Diversity[a]		H-W[b]
							A_r	H_e	
Surnujoki	Sur	69° 16′ 43·48″ N	28° 45′ 39·98″ E	2005	Parr	15	4·42	0·61	<0·01
Siuttajoki (Tsiutta)	Tsi	69° 12′ 35·29″ N	27° 46′ 2·09″ E	2002–2005	Parr	53	3·74	0·54	>0·05
Niipijoki	Nii	69° 6′ 4·37″ N	27° 32′ 30·39″ E	2008	Parr	15	3·22	0·54	<0·05
Naamajoki	Naa	69° 2′ 32·23″ N	28° 31′ 54·11″ E	2008	Parr	18	3·06	0·45	>0·05
Kielajoki	Kie	69° 17′ 9·83″ N	26° 36′ 2·75″ E	2008	Parr	21	4·26	0·64	>0·05
Kaamajoki	Kaa	69° 3′ 59·59″ N	27° 4′ 45·79″ E	2008	Parr	22	5·10	0·69	>0·05
Muddusjärvi	Mud49	69° 0′ 0·72″ N	26° 48′ 42·72″ E	1949	Parr	29	—	0·58	<0·05
Nellimjoki	Nel	68° 49′ 26·16″ N	28° 36′ 41·00″ E	2008	Parr	15	4·12	0·61	>0·05
Juutuanjoki–Kettukoski	Ket	68° 55′ 8·14″ N	26° 44′ 48·69″ E	2008	Parr	23	4·43	0·58	>0·05
Juutuanjoki–Vaskojoki	Vas	68° 54′ 54·65″ N	26° 39′ 41·39″ E	2006	Parr	43	4·42	0·62	>0·05
Juutuanjoki–Kurtojoki	Kur	68° 59′ 4·83″ N	25° 56′ 38·31″ E	2008	Parr	11	4·00	0·63	>0·05
Juutuanjoki–Ala Menesjoki	MeL	68° 47′ 45·66″ N	26° 25′ 29·38″ E	2007	Parr	16	5·08	0·63	>0·05
Juutuanjoki–Ylä Menesjoki	MeU	68° 39′ 34·48″ N	26° 18′ 31·99″ E	2008	Parr	21	4·66	0·64	>0·05
Juutuanjoki–Lankojoki	Lan	68° 50′ 15·39″ N	26° 21′ 27·75″ E	2008	Parr	28	4·20	0·55	>0·05
Juutuanjoki	Juu76	68° 54′ 23·74″ N	26° 59′ 55·37″ E	1968–1979	Parr	28	3·63	0·56	>0·05
Juutuanjoki	Juu04L			2004	Spawners	82	3·99	0·57	>0·05
Nukkumajoki	Nuk	68° 52′ 50·56″ N	27° 4′ 34·37″ E	2008	Parr	19	4·67	0·66	>0·05
Ivalojoki–Alakoski and Pajakoski	IvaAP	68° 35′ 9·65″ N	27° 20′ 43·09″ E	2008	Parr	24	5·34	0·69	>0·05
Ivalojoki–Sotajoki	Sot	68° 30′ 25·83″ N	26° 50′ 11·99″ E	2008	Parr	21	4·51	0·61	<0·05

TABLE I. Continued

Location								
Sampling site (ordered by PCA group)	Code	Co-ordinates	Sampling year	Fish sampled	Sample size	Diversity[a]		
						A_r	H_e	H-W[b]
Ivalojoki–Appisjoki	App	68° 31′ 22·96″ N 26° 36′ 34·54″ E	2007	Parr	29	4·30	0·62	>0·05
Ivalojoki–Kyläjoki	Kyl	68° 26′ 23·06″ N 26° 33′ 24·99″ E	2008	Parr	33	4·41	0·63	>0·05
Ivalojoki–Taimenjoki	Tai	68° 25′ 41·66″ N 26° 21′ 47·18″ E	2007	Parr	29	4·92	0·67	>0·05
Ivalojoki–Pikku Rullajoki	Rul	68° 22′ 58·01″ N 26° 23′ 21·84″ E	2008	Parr	30	4·77	0·66	>0·05
Ivalojoki–Karvajoki	Kar	68° 27′ 49·07″ N 26° 5′ 18·62″ E	2007	Parr	31	4·34	0·61	>0·05
Ivalojoki	Iva04L	68° 22′ 18·61″ N 25° 55′ 32·19″ E	2004	Spawners	48	4·71	0·64	<0·05
Ivalojoki	Iva06L		2006	Spawners	50	4·50	0·61	>0·05
Ivalojoki	Iva04O		2004 and 2008	Parr	66	4·40	0·62	>0·05
Ivalojoki–Joupinniva and Helkikoski	IvaJH	68° 20′ 13·55″ N 25° 42′ 53·46″ E	2008	Parr	25	4·04	0·60	>0·05
Ivalojoki–Lismajoki	Lis	68° 21′ 15·56″ N 25° 30′ 52·75″ E	2008	Parr	32	3·34	0·55	<0·01
Ivalojoki–Naskamajoki	Nas	68° 20′ 30·60″ N 25° 26′ 9·50″ E	2008	Parr	22	3·21	0·52	<0·01
Ivalojoki, upper Naskamajoki	IvaN	68° 20′ 41·94″ N 25° 25′ 43·25″ E	2008	Parr	18	3·52	0·52	<0·01

[a] A_r, allelic richness (based on re-sampling 11 diploid individuals); H_e, expected heterozygosity; A_r not estimated for Mud49 as only nine of 13 loci genotyped.
[b] P-value for Hardy–Weinberg equilibrium test.

and six loci and single locus PCR for four loci as outlined in Table S1. To evaluate genotyping errors in old specimens due to the degraded DNA, 36 individuals (26 and 10 from Lake Muddusjärvi and Juutuanjoki, respectively) were re-extracted and re-amplified separately. For Lake Muddusjärvi specimens collected in 1949, genotype mismatches were detected for 9·2% of genotypes. The estimated genotyping error was much lower (2·4%) for Juutuanjoki specimens collected in 1979. For both historical samples, genotyping inconsistencies were primarily caused by large allele dropouts. Thus, the majority of genotyping errors probably originated from DNA degradation causing inconsistent amplification in historical samples. In the case of an inconsistent allele call, heterozygote genotypes were retained in most of the cases for further analyses.

STATISTICAL ANALYSES

Microsatellite diversity, Hardy–Weinberg and genotypic linkage equilibrium

GenePop 4.0.7 (Rousset, 2008) was initially used to screen for deviations from Hardy–Weinberg (H–W) equilibrium using default settings, and the Ssa197 locus was excluded as described above. Thus, the final data set consisted of 13 loci. As almost all fish specimens collected were parr, some of which could belong to full-sib families (Hansen *et al*., 1997), which could bias genetic estimates, Colony 1.2 (Wang, 2004) was used to identify related individuals. To cope with simulation bias (Sefc & Koblmuller, 2009) and to precisely exclude full-sib individuals, Colony 1.2 was set to have a typing error rate in the data set = 0 (no error) and 0·0001, for comparative purposes. The results assuming different error rates, however, were very similar. When the family consisted of more than three full-sibs, only three full-sib individuals were retained for further analyses. The threshold of three was chosen based on the study of Hansen & Jensen (2005) that utilized a similar approach in *S. trutta* and observed that occasionally full-sib groups of up to three or four individuals can be erroneously identified among unrelated individuals. In total, 86 individuals from 25 full-sib families (one to six individuals per family, with the exception of 12 and 14 in two families from the Naamajoki and Lismajoki, respectively) were excluded from the data set.

As sampling sites do not necessarily represent biological populations (Waples & Gaggiotti, 2006), Structure 2.3 (Pritchard *et al*., 2000) and BAPS 5.2 (Corander *et al*., 2004) were used to identify genetically distinct populations based on multilocus genotype information. In BAPS, this was done by using the 'trained clustering' function (Corander *et al*., 2006), whereby all samples collected from the same location were pooled and used as a 'prior data' reference. In total, 55 individuals (22 revealed by Structure and 33 by BAPS) belonging to seven populations [one to four individuals per population, but seven, 11 and 27 samples from Lankojoki, Juutuanjoki (Juu04L) and Vaskojoki populations, respectively] were identified as putative non-native individuals clustering to a different population compared to their original sampling location. Inclusion of these individuals to the *a priori* defined population samples resulted in highly significant deviations from H-W equilibrium (data not shown). There are several possible explanations for this observation. First, these individuals could reflect natural dispersal of parr from neighbouring populations. Or alternatively, this can be caused by small-scale stocking activities or sample mislabelling. As it was not possible to determine with certainty whether these individuals reflect a natural dispersal process or represent a consequence of stocking activities, these putative non-natives were excluded from further analyses. The final data set excluding these individuals consisted of 917 individuals from 28 populations (two and three sampling times at Juutuajoki and Ivalojoki, respectively; Table I). If the putative non-native individuals were included to the subsequent analyses, however, the main findings of the study were unchanged (data not shown).

Expected heterozygosity and allelic richness were estimated for each population–locus combination using FSTAT 2.9.3.2 (Goudet, 1995) and subsequently averaged across loci for each population. Deviations from Hardy–Weinberg equilibrium and genotypic linkage equilibrium were calculated using Genepop 4.0.7 (Rousset, 2008). Fisher's procedure (Mosteller & Fisher, 1948) was applied for the combination of separate tests across loci (H-W test) or

locus pairs (linkage disequilibrium test). The sequential Bonferroni correction (Holm, 1979) was used to correct for multiple testing.

Genetic differentiation and relationships between populations

The significance of allele frequencies between sampling sites was tested using the genetic differentiation test in Genepop 4.0.7 (Rousset, 2008), and the extent of divergence was quantified using the multilocus F_{ST} estimator (Weir & Cockerham, 1984) as estimated in FSTAT 2.9.3.2 (Goudet, 1995). The genetic relationships among populations were examined using principal component analysis (PCA) implemented in PCAGEN 1.2.1 (Goudet, 2005). Population clustering was performed with a Bayesian approach using BAPS 5.2 (Corander *et al*., 2003, 2008) with the function 'clustering of groups of individuals' (*i.e.* pre-defined group analysis; Corander *et al*., 2006; Gonzalez-Suarez *et al*., 2009). The upper boundary to the number of clusters (*i.e.* K) was determined using multiple (>10) different values and the simulations were run three times with different K series. The best clustering solution was chosen based on the largest value of the log of marginal likelihood from all simulations. To analyse the genetic characteristics among different population groups identified by BAPS , genetic divergence, allelic richness and expected heterozygosity were compared between the groups with a one-sided test using FSTAT 2.9.3.2 (Goudet, 1995). Old scale samples from Juutuanjoki and recent samples from Nellimjoki (where the sampling sites are close to hatcheries) were compared with other samples to evaluate the potential effects of previous stocking activities by estimating interpopulation genetic divergence and genetic differentiation as well as using the BAPS clustering and PCA.

Analysis of molecular variance

To estimate the amount of molecular variation residing within alternative population groups, an analysis of molecular variance (AMOVA) was performed using Arlequin 3.11 (Excoffier *et al*., 2005). Two alternatives were tested: in version 1, three genetic groups of populations were set (north, west and south) corresponding largely to geographical regions (Fig. 1); in version 2, samples of Pikku Rullajoki (Rul), lower reaches of Ivalojoki (IvaAP) and Taimenjoki (Tai) were grouped together with the western group to resemble the PCA results (see below) while other populations were as in version 1.

Self-assignment

The level of population differentiation and population structure was also evaluated through the self-assignment of individuals to their population of origin using the Bayesian assignment method (Rannala & Mountain, 1997) implemented in GeneClass2 (Piry *et al*., 2004) with an assignment threshold score set to 0·05. The population from Muddusjärvi (Mud49) was not included in the analysis as the population was genotyped at only nine of 13 loci.

Isolation by distance

Determining the geographic scale at which genetic structuring occurs is an important issue for designing conservation and management programmes (Primmer *et al*., 2006). Therefore, the relationship between the riverine (geographic) distance and the genetic divergence was evaluated using the Mantel test implemented in the GenAlEx 6.2 package (Peakall & Smouse, 2006). The riverine distances between populations were obtained using the Finnish Citizen's MapSite (http://kansalaisen.karttapaikka.fi). For the Mantel test, $F_{ST}(1 - F_{ST})^{-1}$ was used as a measure of genetic divergence while riverine distance was used as raw and ln-transformed matrix (Rousset, 1997). The statistical significance of the parameter estimates was assessed by 9999 permutations. Samples of Iva04O and Juu06L were chosen to represent Ivalojoki and Juutuanjoki populations, respectively, as the most recent samples collected from these sites. In addition, isolation by distance (IBD) slopes from previously published studies from *S. trutta* and *S. salar* were used for comparative purposes.

In order to assess whether upstream trout populations exhibit reduced level of genetic diversity as a result of smaller population sizes or decreased levels of migration, compared with downstream populations (Primmer *et al*., 2006), the association between genetic

diversities (allelic richness and expected heterozygosity) and the distance from the river mouth at Inarijärvi was evaluated using a linear regression analysis implemented in PopTools 3.0 (Hood, 2008).

Mapping genetic differences to riverine sections

In addition to IBD analysis, Kalinowski *et al.* (2008) recently developed a method specifically aimed at mapping genetic differences among populations of freshwater fish to the sections of the river that connect them. The programme StreamTree (Kalinowski *et al.*, 2008) was used to partition genetic divergence (F_{ST}) along waterway sections. The only geographic information used by the StreamTree algorithm is the topology of the catchment, and hence, this approach is expected to be useful for describing the spatial distribution of genetic differentiation along the riverine sections irrespective of other underlying factors. As a result, the StreamTree approach can reflect genetic discontinuities related to both landscape features or historical processes unrelated to geography. When reconstructing the topological map, the Nellimjoki population was excluded because it was likely to be affected by releases of non-native fish from the nearby hatchery (see below). Similar to IBD analyses, samples of Iva04O and Juu06L were chosen to represent Ivalojoki and Juutuanjoki populations, respectively, as the most recent samples collected from these sites.

RESULTS

MICROSATELLITE DIVERSITY, HARDY–WEINBERG AND GENOTYPIC LINKAGE EQUILIBRIUM

The average allelic richness varied from 3·06 in Naamajoki (Naa) to 5·34 in the lower reaches of Ivalojoki (IvaAP). The expected heterozygosity ranged from 0·45 (Naa) to 0·69 (IvaAP; Table I). A linkage disequilibrium test indicated non-random association of alleles in 257 locus pairs (10·8% from the total of 2376 pairs; $P < 0{\cdot}05$). No locus pairs, however, were significantly linked in a large number of populations (<11, from total of 31), and six of 13 loci used in the study that have been genetically mapped are located on different linkage groups (Gharbi *et al.*, 2006). Thus, the studied loci were probably not physically linked. In total, eight sampling sites exhibited significant deviations from Hardy–Weinberg equilibrium across loci ($P < 0{\cdot}05$) but none of them remained significant after the sequential Bonferroni correction ($k = 31$; cut-off $P = 0{\cdot}0016$). Two loci (Ssa407 and Ssosl311) deviated from the Hardy–Weinberg equilibrium across populations after the sequential Bonferroni correction ($k = 13$; cut-off $P = 0{\cdot}0042$), however, each of them showing significant heterozygote deficit in different populations.

GENETIC RELATIONSHIPS AND DIFFERENTIATION BETWEEN POPULATIONS

Both Bayesian clustering analysis [Fig. 2(a)] and PCA [Fig. 2(b)] revealed that populations in the Inari Basin were structured into three main groups. The population structure largely corresponded to geographic areas: north, west and south. In the first PCA axis (explaining 35·59% of variation), the southern population group was separated from the others. The northern group was isolated from the western group by the second PCA axis (explaining 15·28% of the variation). The northern group comprised four populations that also exhibited relatively high genetic divergence

among each other. The southern group comprised 12 samples collected from the Ivalojoki system, and this group was the most homogeneous showing relatively low differentiation among samples within the group. The western group clustered 11 populations from the western side of the lake, as well as the population from Nellimjoki (Nel). The inclusion of the Nellimjoki population in this group was unexpected as it is located on the opposite side of the lake. Both old scale (1968–1979) and recent (2004) samples collected from Juutuanjoki clustered together within the western group using PCA. Bayesian clustering analysis also indicated a finer level of structuring within each sub-group [Fig. 2(a)]. AMOVA confirmed the presence of three groups initially suggested by PCA and BAPS and supported the clustering of Pikku Rullajoki (Rul), lower reaches of Ivalojoki (IvaAP) and Taimenjoki (Tai) with the southern group ($F_{CT} = 0{\cdot}0702$, $F_{ST} = 0{\cdot}1240$; both associated $P < 0{\cdot}001$) rather than with the western group ($F_{CT} = 0{\cdot}0609$, $F_{ST} = 0{\cdot}1202$; both associated $P < 0{\cdot}001$).

The global F_{ST} across all 28 populations (Juutuanjoki and Ivalojoki sections were sampled two and three times, respectively) was 0·101, indicating a considerable level of genetic differentiation among *S. trutta* populations in the Inari Basin. Within each of the three population clusters, the estimated global F_{ST} was 0·118, 0·078 and 0·044 for the northern, western and southern groups, respectively. The F_{ST} among groups was 0·16, 0·15 and 0·106 for north–west, north–south and west–south comparisons, respectively [Fig. 2(b)]. The F_{ST} in the northern group was significantly higher than in the southern group (one-sided test, $P < 0{\cdot}05$), while other F_{ST} comparisons were not significantly different from each other (north *v*. west and south *v*. west; both one-sided tests, $P > 0{\cdot}05$). Allelic richness and expected heterozygosity were as high as 4·45 and 0·61 in the western group, 4·26 and 0·61 in the southern and significantly lower (both one-sided tests, $P < 0{\cdot}05$) in the northern group (3·61 and 0·54, respectively). Genetic differentiation was significant for all

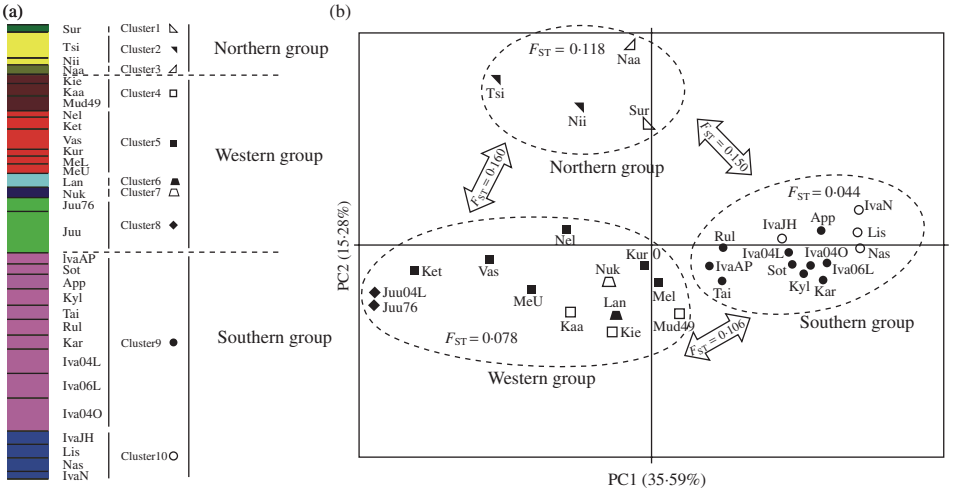

FIG. 2. Population structure of Inari basin *Salmo trutta* populations based on: (a) Bayesian clustering analysis (BAPS); and (b) principal component analysis (PCA). F_{ST} values measured within and among groups are also indicated. The 10 population clusters identified by BAPS are indicated with different symbols. Sample abbreviations are given in Table I.

but one population pair and two samples with temporal replicates: Kie-Mud49 and Iva04L-Iva06L (associated $P > 0{\cdot}05$; Appendix 1).

SELF-ASSIGNMENT

Individual assignment success to the population of origin varied considerably among population groups. In the northern group, the assignment success ranged from 80 (Surnujoki) to 100% (Naamajoki). The success rate was more variable in the western and southern groups ranging from 24% in lower reaches of Ivalojoki sample (IvaJH) to 89·3% in Lankojoki (Lan). Most misassignments, however, were between populations within the same group as indicated by the high level of assignment success to group of origin (93–98%; Appendix 2).

ISOLATION BY DISTANCE AND WITHIN-RIVER DIVERSITY PATTERNS

A highly significant isolation-by-distance (IBD) signal was observed when plotting genetic and geographic distances of the 28 populations ($R_{XY} = 0{\cdot}392$, $P < 0{\cdot}001$; Fig. 3). The IBD signal was also highly significant within the southern group ($R_{XY} = 0{\cdot}496$, $P < 0{\cdot}01$). No significant IBD, however, was observed in the western group ($P > 0{\cdot}05$, both with and without Nellimjoki) while in the northern group IBD test was not performed because of the small number of populations. The results also remained similar when the natural logarithm (ln) of geographic distance rather than the raw geographic distance was used (data not shown).

In the southern population group (Ivalojoki system), a highly significant correlation between genetic diversity estimates and the distance from the river mouth was also observed (Pearson's $r = -0{\cdot}85$ and $-0{\cdot}81$, for allelic richness and expected heterozygosity, respectively; $P < 0{\cdot}001$ in both cases; Fig. 4).

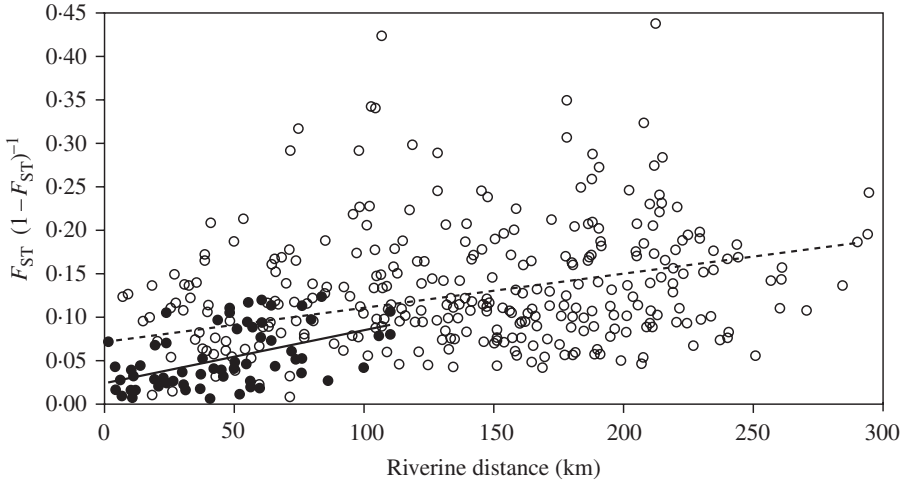

FIG. 3. Isolation by distance (IBD) in Inari Basin *Salmo trutta*. The open dots and broken trend line correspond to the 28 population comparison, while filled dots and solid trend line indicate the within southern group comparison (Ivalojoki river system).

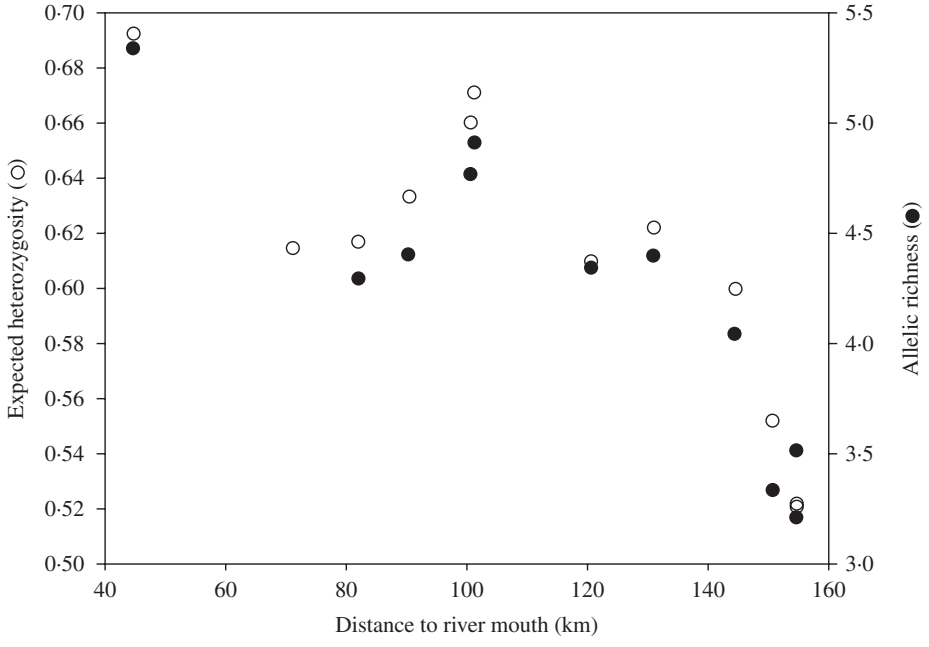

FIG. 4. Correlation between *Salmo trutta* genetic diversity estimates and the waterway distance of the Ivalojoki river system.

MAPPING GENETIC DIFFERENCES AGAINST RIVERINE SECTIONS

The correlation (R^2) between F_{ST} fitted onto riverine sections and the observed F_{ST} among populations was 0·705, indicating that the StreamTree model of genetic structure explained a reasonable proportion of genetic differentiation among *S. trutta* populations of the Inari Basin (Fig. 5). As suggested by PCA analysis, the four riverine sections connecting northern populations to Inarijärvi had higher levels of F_{ST} compared to other riverine sections on the map. A high level of F_{ST} (>0·05) was also present in the waterway section connecting the Lankojoki river population to the Inari system.

DISCUSSION

HIERARCHICAL POPULATION STRUCTURE OF INARI *SALMO TRUTTA*

Three genetically distinct population clusters, broadly corresponding to separate geographical regions, were identified in lake-run *S. trutta* of the Inari Basin. The northern population group had the highest level of genetic differentiation and the lowest level of genetic diversity compared to the other groups, and strong differentiation was also observed among populations within the northern group (Fig. 5 and Appendix 1). This was also reflected by the high self-assignment success (80–100%) within the northern group. Although small sample sizes of several of the

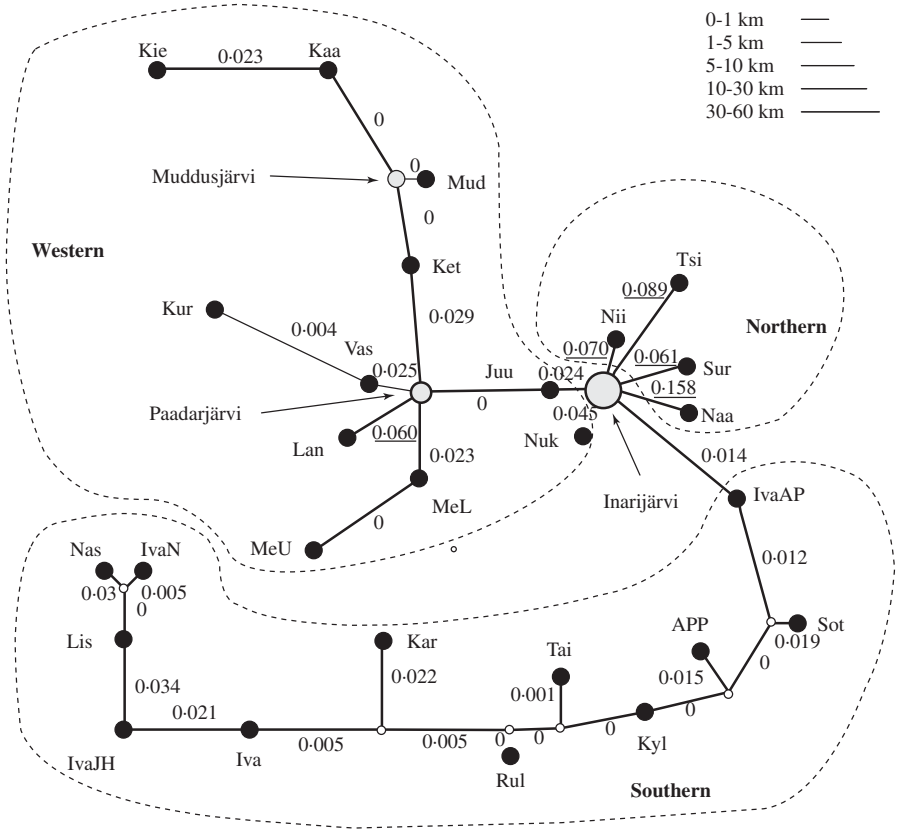

FIG. 5. Stream Tree diagram fitting *Salmo trutta* F_{ST} values onto riverine sections. F_{ST} values higher than 0·05 are underlined. River sections of different length categories are indicated with lines of different length. Sample abbreviations are given in Table I.

northern populations could have potentially inflated the genetic divergence estimates, it is unlikely that the limited number of individuals alone explains the elevated differentiation in northern samples. Indeed, pair-wise genetic differentiation estimates were highly similar even if the sample sizes of all populations were reduced to 20 (data not shown). Instead, several factors could contribute to this, including the relatively large geographical distance between populations in the north group compared to populations in the other groups. Alternatively, the catchment area and the corresponding water flow and spawning grounds of the northern rivers are considerably smaller than in the main river systems (T. Niva *et al*., unpubl. data), Juutuanjoki and Ivalojoki, which together account about two-thirds of the total flow of the Paatsjoki that is the outlet of the Inari Basin to the Arctic Ocean. The northern population group also exhibited significantly lower allelic richness and expected heterozygosity than the other two population clusters which can be a consequence of smaller effective population sizes of the northern populations. Thus, random genetic drift potentially plays a larger role in the northern populations, and therefore, these populations may be particularly vulnerable to population decline. As a result, additional monitoring and assessment of the temporal genetic stability are warranted.

Populations in the southern group, within the Ivalojoki river system, were substructured into a series of interconnected populations showing a significant IBD pattern. In addition, a negative association between genetic diversity and the geographical distance from the river mouth was observed (Table I and Figs 1 and 4) with a strong break in genetic diversity between populations in the upper and lower reaches. These features show similarities to those observed in other within-river system studies of both *S. salar* (Primmer *et al*., 2006) and sea-run *S. trutta* (Lehtonen *et al*., 2009). The trend of decreasing genetic diversity in the upper reaches of Ivalojoki is likely to be caused by two factors: an increasing role of genetic drift, as population sizes tend to decrease towards upper reaches, and the decreasing effect of gene flow, as the number of migrants decreases towards the upper reaches of the river system. The low level of genetic diversity of upstream populations has been observed not only in migratory fishes but also in sedentary ones (*i.e. Cottus gobio* L., Hänfling & Weetman, 2006; *Thymallus thymallus* L., Meldgaard *et al*., 2003; *Poecilia reticulata* Peters, Barson *et al*., 2009; *Gasterosteus aculeatus* L., Raeymaekers *et al*., 2008) since gene flow often occurs in a downstream direction while upstream gene flow can be more costly and be subject to obstruction. As a result, upstream populations are probably more affected by random genetic drift and inbreeding that increases the risk of population extinction (Speirs & Gurney, 2001; Morita & Yamamoto, 2002; Morita & Yokota, 2002). Therefore, similar to the northern populations in the Inari Basin, routine genetic and ecological monitoring of the far upstream populations would be valuable for conservation management and planning.

The population substructuring was more complex in the western group possibly because of the additional lakes that fish can utilize as feeding grounds, instead of completely relying on Inarijärvi. For example, the Bayesian clustering identified a western sub-group comprising Muddusjärvi and Kaamajoki and Kiellajoki populations. It is possible that *S. trutta* from these populations co-utilize Muddusjärvi to reach maturity which at least partly explains the high genetic similarity among them. Similarly, it is possible that Lake Paadarjärvi is an important feeding ground for fish from Kurtojoki, Vaskojoki, upper and lower Menesjoki and Kettukoski. These results are consistent with the recent tagging studies, where *S. trutta* from Vaskojoki have been mostly found in Lake Paadarjärvi (T. Niva, unpubl. data). Based on the relatively high F_{ST} fitted onto the Lankojoki river section (fitted $F_{ST} = 0{\cdot}06$; Fig. 5), it is also possible that the Lankojoki river population forages on the riverine expansions in the lower reaches of Lemmenjoki (Fig. 1) rather than in the western lakes and Inarijärvi.

HIGH LEVEL OF GENETIC STRUCTURING IN *S. TRUTTA*

The homing ability of salmonid fishes is very well known as they accurately return to natal spawning grounds from lacustrine or marine feeding areas for reproduction (Youngson *et al*., 2003). To compare the level of genetic differentiation of *S. trutta* in the Inari Basin with other regions, the relationship between genetic and geographic waterway distances observed in lake-run *S. trutta* of the present study was compared to other life-history types; resident *S. trutta* (Carlsson & Nilsson, 2000) and sea-run *S. trutta* (Lehtonen *et al*., 2009; Samuiloviene *et al*., 2009) and anadromous *S. salar* (Primmer *et al*., 2006; Vähä *et al*., 2008). The comparison of linear IBD slopes showed that lake-run *S. trutta* exhibit a similar level of structuring to that of sea-run

trout, but clearly lower than resident *S. trutta*. The correlation between genetic and riverine distances in all *S. trutta* forms, however, was much steeper than that of *S. salar*, indicating a high level of structuring in *S. trutta* (Fig. 6). This finding contradicts an earlier suggestion that the homing accuracy of *S. trutta* is roughly equal to that of *S. salar* or even less precise (Banks, 1969). Among *S. trutta*, the lake-run and sea-run forms had similar levels of genetic structuring (Fig. 6), while the level of structuring was highest in resident trout, most likely because they spend their entire life cycle in their natal stream or river.

POTENTIAL EFFECTS OF SUPPLEMENTARY STOCKING

Small-scale supplementary stocking using hatchery-reared fish has been conducted in the Inari Basin from the early 1950s to the late 1970s in order to sustain *S. trutta* populations for professional and recreational fishing in the region. Large-scale stocking started in the 1980s and in the new Sarmijärvi hatchery surplus egg production of *S. trutta* was considerable, due to oversized broodstocks. The records from the Sarmijärvi hatchery show that *c*. 7 million surplus eyed eggs and newly hatched larvae and *c*. 100 000 parr (age of 0+ to 4+) have been released into Nellimjoki since 1989 (T. Niva, unpubl. data). These fish originated from wild spawners collected from Juutuanjoki (1989–2003) and later from Ivalojoki (2004 to today). Based on the present study, the stocking activities have affected the genetic composition of the Nellimjoki population as genetic analyses of contemporary samples indicated that the Nellimjoki population grouped together with the populations from Juutuanjoki river system, despite a large geographical distance between them. Genetic analyses of Nellimjoki samples collected in 2008, however, did not reveal any effect of more recent stocking from the Ivalojoki. Analyses of old scales (mainly collected in 1976) of parr (3+ to 5+) from the population occupying Juutuanjoki river mouth showed no significant differences between the historical and recent gene pools, indicating that the genetic composition of the Juutuanjoki river population has been stable over

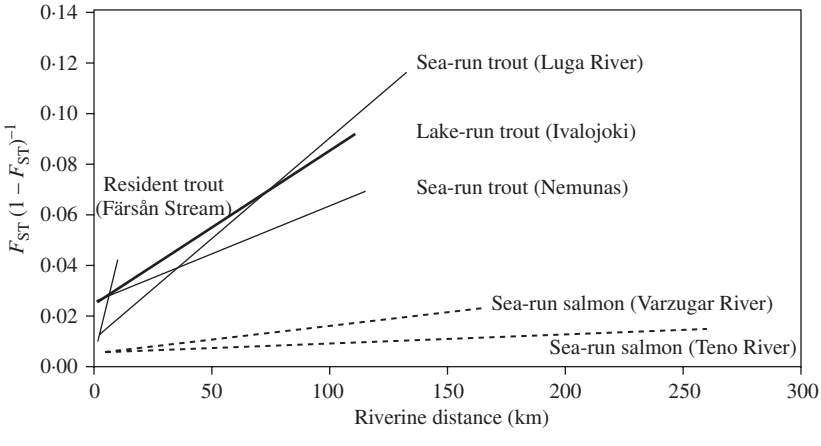

FIG. 6. Isolation-by-distance (IBD) trend lines in two *Salmo* species. Solid lines correspond to different life-history types of *Salmo trutta* while dashed lines correspond to anadromous Atlantic salmon *Salmo salar*. The IBD trend line for *S. trutta* in Ivalojoki is marked in bold.

20 years (Appendix 1), even with a hatchery working at the Juutuanjoki river mouth since 1950s.

MANAGEMENT UNITS IN THE INARI BASIN

Salmo trutta populations collected from the Inari water Basin were significantly differentiated from each other even when comparing geographically close samples collected from the main stem or tributaries within the same river. Therefore, it is clear that most of the populations could potentially be recognized as separate or genetically independent management units. As a result of the large number of genetically distinct units, artificial maintenance or appropriate supplementation of all separate populations is clearly not feasible. Thus, the best management plan for sustaining these trout populations, as well as for long-term sustainable fisheries, in the Inari Basin would be to ensure actions to improve, and subsequently maintain, as many areas suitable for natural spawning as possible. Northern populations and populations upstream of Ivalojoki river would also be conservation targets due to their low genetic diversity and small population sizes. In addition, further research to understand the level of ecological separation and boundaries of these populations is warranted. If supportive stocking is considered necessary for short-term management purposes, as is currently the case for a number of water systems in Finland (Salonen *et al*., 2009), at least three separate stocks representing the northern, southern and western regions of the Inari Basin should be maintained and only stocked locally. It is also important to recognize that the results of this study indicate that the *S. trutta* from each of these regions may have different biological characteristics, such as the local-lake feeding behaviour of the western populations and strong isolation among the northern stocks. As a consequence, further research to better understand the level of ecological uniqueness of different lake-run *S. trutta* populations is clearly necessary.

We acknowledge E. E. Nielsen, J. Carlsson, J. Aspi, B. Hänfling and two anonymous reviewers for valuable comments that improved the final manuscript. We thank the electrofishing group led by A. Savikko from Inari Fisheries Research and Aquaculture station of FGRI for supplying fish specimens. We also thank A. J. Vallunen for his technical advice in the laboratory. This study was supported by a Royal Thai Scholarship (to A.S.), the Academy of Finland (Postdoctoral Fellowship to A.V. and Centre of Excellence in Evolutionary Genetics and Physiology to C.R.P.) and the Estonian Science Foundation (to A.V., grant no. 6802).

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics and PCR conditions for the microsatellite loci used in the study.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

Aljanabi, S. M. & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Research* **25,** 4692–4693.

Antunes, A., Faria, R., Johnson, W. E., Guyomard, R. & Alexandrino, P. (2006). Life on the edge: the long-term persistence and contrasting spatial genetic structure of distinct brown trout life histories at their ecological limits. *Journal of Heredity* **97,** 193–205.

Apostolidis, A. P., Madeira, M. J., Hansen, M. M. & Machordom, A. (2008). Genetic structure and demographic history of brown trout (*Salmo trutta*) populations from the southern Balkans. *Freshwater Biology* **53,** 1555–1566.

Banks, J. W. (1969). A review of literature on upstream migration of adult salmonids. *Journal of Fish Biology* **1,** 85–136.

Barson, N. J., Cable, J. & van Oosterhout, C. (2009). Population genetic analysis of microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source-sink metapopulation structure, founder events and population bottlenecks. *Journal of Evolutionary Biology* **22,** 485–497.

Bernatchez, L. (2001). The evolutionary history of brown trout (*Salmo trutta* L.) inferred from phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA variation. *Evolution* **55,** 351–379.

Carlsson, J. & Nilsson, J. (2000). Population genetic structure of brown trout (*Salmo trutta* L.) within a northern boreal forest stream. *Hereditas* **132,** 173–181.

Carlsson, J., Olsen, K. H., Nilsson, J., Overli, O. & Stabell, O. B. (1999). Microsatellites reveal fine-scale genetic structure in stream-living brown trout. *Journal of Fish Biology* **55,** 1290–1303.

Charles, K., Guyomard, R., Hoyheim, B., Ombredane, D. & Bagliniere, J. L. (2005). Lack of genetic differentiation between anadromous and resident sympatric brown trout (*Salmo trutta*) in a Normandy population. *Aquatic Living Resources* **18,** 65–69.

Corander, J., Waldmann, P. & Sillanpaa, M. J. (2003). Bayesian analysis of genetic differentiation between populations. *Genetics* **163,** 367–374.

Corander, J., Waldmann, P., Marttinen, P. & Sillanpaa, M. J. (2004). BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics* **20,** 2363–2369.

Corander, J., Marttinen, P. & Mantyniemi, S. (2006). A Bayesian method for identification of stock mixtures from molecular marker data. *Fishery Bulletin* **104,** 550–558.

Corander, J., Marttinen, P., Siren, J. & Tang, J. (2008). Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. *BMC Bioinformatics* **9,** 539.

Elliott, J. M. (1989). Wild brown trout *Salmo trutta*: an important national and international resource. *Freshwater Biology* **21,** 1–5.

Elliott, J. M. (1994). *Quantitative Ecology and the Brown Trout.* Oxford: Oxford University Press.

Elphinstone, M. S., Hinten, G. N., Anderson, M. J. & Nock, C. J. (2003). An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. *Molecular Ecology Notes* **3,** 317–320.

Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J. M., Adriamanga, M. & Guyomard, R. (1998). Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology* **7,** 339–353.

Excoffier, L., Laval, G. & Schneide, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1,** 47–50.

Fahy, E. (1978). Variation in some biological characteristics of British sea trout, *Salmo trutta* L. *Journal of Fish Biology* **13,** 123–138.

Fraser, D. J., Hansen, M. M., Ostergaard, S., Tessier, N., Legault, M. & Bernatchez, L. (2007). Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Molecular Ecology* **16,** 3866–3889.

Gharbi, K., Gautier, A., Danzmann, R. G., Gharbi, S., Sakamoto, T., Hoyheim, B., Taggart, J. B., Cairney, M., Powell, R., Krieg, F., Okamoto, N., Ferguson, M. M., Holm, L. E. & Guyomard, R. (2006). A linkage map for brown trout (*Salmo trutta*): chromosome homeologies and comparative genome organization with other salmonid fish. *Genetics* **172,** 2405–2419.

Gonzalez-Suarez, M., Flatz, R., Aurioles-Gamboa, D., Hedrick, P. W. & Gerber, L. R. (2009). Isolation by distance among California sea lion populations in Mexico: redefining management stocks. *Molecular Ecology* **18,** 1088–1099.

Goudet, J. (1995). FSTAT (Version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86,** 485–486.

Hänfling, B. & Weetman, D. (2006). Concordant genetic estimators of migration reveal anthropogenically enhanced source-sink population structure in the River Sculpin, *Cottus gobio*. *Genetics* **173,** 1487–1501.

Hansen, M. M. & Jensen, L. F. (2005). Sibship within samples of brown trout (*Salmo trutta*) and implications for supportive breeding. *Conservation Genetics* **6,** 297–305.

Hansen, M. M., Nielsen, E. E. & Mensberg, K. L. D. (1997). The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. *Molecular Ecology* **6,** 469–474.

Hansen, M. M., Ruzzante, D. E., Nielsen, E. E., Bekkevold, D. & Mensberg, K. L. D. (2002). Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology* **11,** 2523–2535.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics* **6,** 65–70.

Hovgaard, K., Skaala, O. & Naevdal, G. (2006). Genetic differentiation among sea trout, *Salmo trutta* L., populations from western Norway. *Journal of Applied Ichthyology* **22,** 57–61.

Jensen, L. F., Hansen, M. M., Carlsson, J., Loeschcke, V. & Mensberg, K. L. D. (2005). Spatial and temporal genetic differentiation and effective population size of brown trout (*Salmo trutta*, L.) in small Danish rivers. *Conservation Genetics* **6,** 615–621.

Kalinowski, S. T., Meeuwig, M. H., Narum, S. R. & Taper, M. L. (2008). Stream trees: a statistical method for mapping genetic differences between populations of freshwater organisms to the sections of streams that connect them. *Canadian Journal of Fisheries and Aquatic Sciences* **65,** 2752–2760.

Kallio-Nyberg, I., Koljonen, M.-L. & Jutila, E. (2001). *Taimenatlas.* Helsinki: Finnish Game and Fisheries Research Institute.

Kitada, S. & Kishino, H. (2006). Lessons learned from Japanese marine finfish stock enhancement programs. *Fisheries Research* **80,** 101–112.

Kitada, S., Shishidou, H., Sugaya, T., Kitakado, T., Hamasaki, K. & Kishino, H. (2009). Genetic effects of long-term stock enhancement programmes. *Aquaculture* **290,** 69–79.

Klemetsen, A., Amundsen, P. A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F. & Mortensen, E. (2003). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish* **12,** 1–59.

Koskinen, M. T., Sundell, P., Piironen, J. & Primmer, C. R. (2002). Genetic assessment of spatiotemporal evolutionary relationships and stocking effects in grayling (*Thymallus thymallus*, Salmonidae). *Ecology Letters* **5,** 193–205.

L'Abee-Lund, J. H., Jonsson, B., Jensen, A. J., Saettem, L. M., Heggberget, T. G., Johnsen, B. O. & Naesje, T. F. (1989). Latitudinal variation in life-history characteristics of sea-run migrant brown trout *Salmo trutta*. *Journal of Animal Ecology* **58,** 525–542.

Lehtonen, P. K., Tonteri, A., Sendek, D., Titov, S. & Primmer, C. R. (2009). Spatio-temporal genetic structuring of brown trout (*Salmo trutta* L.) populations within the River Luga, northwest Russia. *Conservation Genetics* **10,** 281–289.

Meldgaard, T., Nielsen, E. E. & Loeschcke, V. (2003). Fragmentation by weirs in a riverine system: a study of genetic variation in time and space among populations of European grayling (*Thymallus thymallus*) in a Danish river system. *Conservation Genetics* **4,** 735–747.

Morita, K. & Yamamoto, S. (2002). Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. *Conservation Biology* **16,** 1318–1323.

Morita, K. & Yokota, A. (2002). Population viability of stream-resident salmonids after habitat fragmentation: a case study with white-spotted charr (*Salvelinus leucomaenis*) by an individual based model. *Ecological Modelling* **155,** 85–94.

Mosteller, F. & Fisher, R. A. (1948). Questions and answers. *American Statistician* **2,** 30–31.

Nylander, E. (2004). *Kalatalous tilastoina 2004: Finnish fisheries statistics.* Helsinki: Finnish Game and Fisheries Research Institute.

Peakall, R. & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6,** 288–295.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. & Estoup, A. (2004). GeneClass2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95,** 536–539.

Presa, P., Pardo, B. G., Martinez, P. & Bernatchez, L. (2002). Phylogeographic congruence between mtDNA and rDNA ITS markers in brown trout. *Molecular Biology and Evolution* **19,** 2161–2175.

Primmer, C. R., Veselov, A. J., Zubchenko, A., Poututkin, A., Bakhmet, I. & Koskinen, M. T. (2006). Isolation by distance within a river system: genetic population structuring of Atlantic salmon, *Salmo salar*, in tributaries of the Varzuga River in northwest Russia. *Molecular Ecology* **15,** 653–666.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155,** 945–959.

Raeymaekers, J. A. M., Maes, G. E., Geldof, S., Hontis, I., Nackaerts, K. & Volckaert, F. A. M. (2008). Modeling genetic connectivity in sticklebacks as a guideline for river restoration. *Evolutionary Applications* **1,** 475–488.

Rannala, B. & Mountain, J. L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* **94,** 9197–9201.

Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145,** 1219–1228.

Rousset, F. (2008). Genepop′007: a complete re-implementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* **8,** 103–106.

Ryman, N. (1983). Patterns of distribution of biochemical genetic variation in salmonids: differences between species. *Aquaculture* **33,** 1–21.

Ryman, N. & Laikre, L. (1991). Effects of supportive breeding on the genetically effective population size. *Conservation Biology* **5,** 325–329.

Salonen, E., Niva, T., Raineva, S., Pukkila, H., Savikko, A., Aikio, E., Leinonen, K. & Jutila, H. (2009). *Inarijärven ja sen sivuvesistöjen kalataloudellinen velvoitetarkkailu 2008.* Helsinki: Riista-ja kalatalouden tutkimuslaitos.

Samuiloviene, A., Kontautas, A. & Gross, R. (2009). Genetic diversity and differentiation of sea trout (*Salmo trutta*) populations in Lithuanian rivers assessed by microsatellite DNA variation. *Fish Physiology and Biochemistry* **35,** 649–659.

Schreiber, A. & Diefenbach, G. (2005). Population genetics of the European trout (*Salmo trutta* L.) migration system in the River Rhine: recolonisation by sea trout. *Ecology of Freshwater Fish* **14,** 1–13.

Sefc, K. M. & Koblmuller, S. (2009). Assessing parent numbers from offspring genotypes: the importance of marker polymorphism. *Journal of Heredity* **100,** 197–205.

Slettan, A., Olsaker, I. & Lie, O. (1996). Polymorphic Atlantic salmon, *Salmo salar* L., microsatellites at the SSOSL438, SSOSL439 and SSOSL444 loci. *Animal Genetics* **27,** 57–58.

Speirs, D. C. & Gurney, W. S. C. (2001). Population persistence in rivers and estuaries. *Ecology* **82,** 1219–1237.

Swatdipong, A., Vasemägi, A., Koskinen, M., Piironen, J. & Primmer, C. (2009). Unanticipated population structure of European grayling in its northern distribution: implications for conservation prioritization. *Frontiers in Zoology* **6,** 6.

Takacs, P., Csoma, E., Eros, T. & Sandor, N. A. (2008). Distribution patterns and genetic variability of three stream-dwelling fish species. *Acta Zoologica Academiae Scientiarum Hungaricae* **54,** 289–303.

Taniguchi, N., Sumantadinata, K. & Iyama, S. (1983). Genetic change in the 1st and 2nd generations of hatchery stock of black seabream. *Aquaculture* **35,** 309–320.

Vähä, J. P., Erkinaro, J., Niemela, E. & Primmer, C. R. (2008). Temporally stable genetic structure and low migration in an Atlantic salmon population complex: implications for conservation and management. *Evolutionary Applications* **1,** 137–154.

Vasemägi, A., Gross, R., Paaver, T., Koljonen, M. L. & Nilsson, J. (2005). Extensive immigration from compensatory hatchery releases into wild Atlantic salmon population in the Baltic Sea: spatio-temporal analysis over 18 years. *Heredity* **95,** 76–83.

Wang, J. L. (2004). Sibship reconstruction from genetic data with typing errors. *Genetics* **166,** 1963–1979.

Waples, R. S. & Gaggiotti, O. (2006). What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* **15,** 1419–1439.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38,** 1358–1370.

Youngson, A. F., Jordan, W. C., Verspoor, E., McGinnity, P., Cross, T. & Ferguson, A. (2003). Management of salmonid fisheries in the British Isles: towards a practical approach based on population genetics. *Fisheries Research* **62,** 193–209.

Electronic References

Goudet, J. (2005). PCAGEN 1.2. Lausanne: Uniersity of Lausanne, Department of Ecology & Evolution. Available at www2.unil.ch/popgen/softwares/pcagen.htm

Hood, G. M. (2008). *PopTools Version 3.0.6.* Canberra: Commonwealth Scientific & Industrial Research Organization. Available at http://www.cse.csiro.au/poptools/

APPENDIX I. Genetic distance measured as F_{ST} (above diagonal) and geographic distance measured as riverine distance (km, below diagonal) among Inari *Salmo trutta* populations. Population pairs where genetic differentiation test was non-significant ($P > 0{\cdot}05$, before sequential Bonferroni correction) are underlined. Three population groups are shaded with grey. Sample site abbreviations are given in Table 1

	Sur	Tsi	Nii	Naa	Kie	Kaa	Mud49	Nel	Ket	Vas	Kur	MeL	MeU	Lan	Nuk
Sur	—	0·085	0·083	0·173	0·154	0·102	0·140	0·101	0·136	0·119	0·116	0·092	0·105	0·183	0·112
Tsi	63·8	—	0·054	0·175	0·175	0·131	0·170	0·106	0·119	0·106	0·157	0·148	0·122	0·185	0·143
Nii	60·7	42·0	—	0·157	0·150	0·119	0·141	0·107	0·139	0·104	0·152	0·122	0·111	0·151	0·146
Naa	41·0	53·4	50·0	—	0·259	0·229	0·297	0·226	0·225	0·179	0·197	0·254	0·224	0·253	0·241
Kie	191	173	145	178	—	0·023	0·009	0·092	0·122	0·101	0·104	0·082	0·079	0·089	0·087
Kaa	132	113	85·6	119	59·6	—	0·015	0·058	0·069	0·057	0·065	0·058	0·032	0·087	0·059
Mud49	120	101	73·9	107	71·3	11·7	—	0·098	0·112	0·105	0·075	0·068	0·062	0·076	0·071
Nel	88·5	90·0	65·8	71·1	180	121	109	—	0·094	0·071	0·102	0·089	0·069	0·129	0·086
Ket	111	92·1	64·8	97·9	80·4	20·8	9·10	100	—	0·010	0·076	0·099	0·037	0·130	0·109
Vas	109	89·7	62·4	95·5	98·8	39·2	27·5	97·6	18·4	—	0·052	0·091	0·031	0·120	0·097
Kur	158	139	112	145	148	88·6	76·9	147	67·8	49·4	—	0·103	0·058	0·145	0·108
MeL	116	97·0	69·7	103	106	46·5	34·8	105	25·7	17·2	66·6	—	0·051	0·087	0·060
MeU	141	122	95·0	128	131	71·5	59·8	130	50·7	42·5	91·9	25·3	—	0·103	0·082
Lan	117	98·5	71·2	104	108	48·0	36·3	106	27·2	18·2	67·6	14·8	40·1	—	0·096
Nuk	85·0	65·9	38·8	74·4	113	53·9	42·2	73·9	33·1	30·7	80·1	37·9	63·2	39·5	—
Juu76	84·9	66·0	38·7	71·8	107	46·9	35·2	73·9	26·1	23·7	73·1	31·0	56·3	32·5	6·97
Juu04L	84·9	66·0	38·7	71·8	107	46·9	35·2	73·9	26·1	23·7	73·1	31·0	56·3	32·5	6·97
IvaAP	119	105	80·6	102	184	125	113	95·3	104	102	151	109	134	110	77·1
Sot	146	131	107	129	211	151	140	122	130	128	177	135	161	137	104
App	157	142	118	139	222	162	150	133	141	139	188	146	171	148	114
Kyl	165	151	126	148	230	170	159	141	150	147	197	154	180	156	123
Tai	176	161	137	159	241	181	170	152	160	158	207	165	191	167	134
Rul	175	161	136	158	240	181	169	151	160	157	207	165	190	166	133
Kar	195	181	157	178	260	201	189	171	180	178	227	185	210	186	153
Iva04L	206	191	167	188	271	211	199	182	190	188	237	195	220	197	163
Iva06L	206	191	167	188	271	211	199	182	190	188	237	195	220	197	163
Iva04O	206	191	167	188	271	211	199	182	190	188	237	195	220	197	163
IvaJH	219	205	180	202	284	225	213	195	204	201	251	209	234	210	177
Lis	225	211	186	208	290	231	219	201	210	207	257	215	240	216	183
Nas	229	215	191	212	294	235	223	205	214	212	261	219	244	220	187
IvaN	229	215	191	212	294	235	223	205	214	211	261	219	244	220	187

Juu76	Juu04L	IvaAP	Sot	App	Kyl	Tai	Rul	Kar	Iva04L	Iva06L	Iva04O	IvaJH	Lis	Nas	IvaN
0·174	0·158	0·087	0·103	0·095	0·098	0·092	0·064	0·117	0·081	0·109	0·103	0·113	0·162	0·165	0·160
0·138	0·131	0·128	0·171	0·146	0·160	0·139	0·113	0·170	0·131	0·161	0·157	0·150	0·187	0·221	0·188
0·151	0·141	0·087	0·116	0·105	0·126	0·103	0·091	0·147	0·093	0·122	0·117	0·138	0·172	0·214	0·168
0·260	0·227	0·186	0·197	0·172	0·193	0·183	0·167	0·235	0·182	0·193	0·173	0·198	0·244	0·304	0·215
0·115	0·117	0·078	0·097	0·099	0·089	0·077	0·072	0·100	0·078	0·100	0·097	0·120	0·156	0·195	0·163
0·064	0·068	0·043	0·079	0·086	0·071	0·056	0·053	0·077	0·063	0·077	0·082	0·086	0·132	0·150	0·134
0·104	0·122	0·044	0·077	0·066	0·053	0·051	0·040	0·055	0·049	0·050	0·048	0·093	0·114	0·158	0·130
0·098	0·087	0·072	0·097	0·090	0·096	0·069	0·072	0·115	0·073	0·097	0·100	0·101	0·121	0·172	0·146
0·025	0·015	0·080	0·125	0·143	0·127	0·086	0·085	0·140	0·118	0·140	0·124	0·110	0·187	0·193	0·181
0·035	0·027	0·052	0·088	0·108	0·097	0·058	0·072	0·111	0·086	0·106	0·097	0·092	0·156	0·171	0·148
0·110	0·104	0·043	0·054	0·082	0·080	0·051	0·045	0·064	0·058	0·077	0·069	0·054	0·125	0·136	0·125
0·118	0·121	0·057	0·070	0·067	0·067	0·063	0·047	0·091	0·061	0·077	0·073	0·081	0·127	0·136	0·122
0·050	0·052	0·042	0·072	0·101	0·081	0·054	0·059	0·085	0·066	0·084	0·087	0·092	0·143	0·156	0·144
0·111	0·120	0·102	0·123	0·108	0·097	0·082	0·092	0·142	0·097	0·123	0·094	0·122	0·142	0·186	0·151
0·098	0·110	0·072	0·118	0·099	0·086	0·070	0·060	0·100	0·084	0·105	0·095	0·101	0·122	0·144	0·146
—	0·001	0·100	0·157	0·162	0·148	0·106	0·113	0·167	0·132	0·157	0·146	0·146	0·195	0·227	0·206
0·0	—	0·103	0·151	0·158	0·141	0·100	0·108	0·165	0·132	0·154	0·142	0·146	0·200	0·224	0·205
77·9	77·9	—	0·025	0·033	0·030	0·019	0·026	0·034	0·018	0·023	0·026	0·040	0·073	0·096	0·075
104	104	26·4	—	0·032	0·028	0·022	0·036	0·040	0·015	0·022	0·019	0·048	0·089	0·110	0·073
115	115	37·3	11·1	—	0·016	0·030	0·026	0·038	0·010	0·011	0·012	0·041	0·058	0·101	0·050
123	123	45·6	19·4	11·6	—	0·008	0·017	0·018	0·006	0·012	0·006	0·044	0·072	0·102	0·068
134	134	56·4	30·2	22·5	10·9	—	0·009	0·026	0·007	0·022	0·017	0·046	0·081	0·107	0·085
134	134	55·8	29·6	21·9	10·3	6·56	—	0·018	0·010	0·021	0·016	0·038	0·080	0·106	0·085
154	154	76·0	49·8	42·1	30·5	26·8	21·2	—	0·016	0·013	0·024	0·049	0·088	0·098	0·096
164	164	86·2	60·0	52·3	40·7	37·0	31·4	24·4	—	0·003	0·010	0·039	0·059	0·092	0·061
164	164	86·2	60·0	52·3	40·7	37·0	31·4	24·4	0	—	0·009	0·047	0·071	0·107	0·070
164	164	86·2	60·0	52·3	40·7	37·0	31·4	24·4	0	0	—	0·043	0·063	0·095	0·066
178	178	99·8	73·6	65·9	54·3	50·6	45·0	38·0	13·6	13·6	13·6	—	0·027	0·037	0·014
184	184	106	79·7	71·9	60·3	56·6	51·0	44·0	19·6	19·6	19·6	6·04	—	0·042	0·016
188	188	110	83·7	76·0	64·4	60·7	55·1	48·1	23·7	23·7	23·7	10·1	4·06	—	0·067
188	188	110	83·7	75·9	64·3	60·6	55·1	48·1	23·7	23·7	23·7	10·1	4·02	1·12	—

APPENDIX II. Number of individual *Salmo trutta* assigned to specific populations based on multilocus microsatellite genotypes. Assignment within three population groups is marked with grey. Sample site abbreviations are given in Table 1

	PCA northern group				PCA western group											PCA southern group													
Assigned to	Sur	Tsi	Nii	Naa	Kie	Kaa	Nel	Ket	Vas	Kur	MeL	MeU	Lan	Nuk	Juu04L	IvaAP	Sot	App	Kyl	Tai	Rul	Kar	Iva	IvaJH	Lis	Nas	IvaN	Total no.	Correct assigned (%)
Origin																													
Sur	12	2							1																			15	80·0
Tsi		50	2					1																				53	94·3
Nii		1	13	1																								15	86·7
Naa				18																								18	100
Kie					16	3						1						1										21	76·2
Kaa					4	15										1				2								22	68·2
Nel							10					1			3								1					15	66·7
Ket								9	1	1		1			9						1		1					23	39·1
Vas							2	4	25	1					8	1				1				1				43	58·1
Kur										8					1			1						1				11	72·7
MeL					2						11		1		1				1									16	68·8
MeU					1			1				14			4							1						21	66·7
Lan								1	1				25			1												28	89·3
Nuk						1		1						15	2													19	78·9
Juu04L		1						9	4			4			62	1					1							82	75·6
IvaAP								1	1							11	4		1	1			4		1			24	45·8
Sot																1	12	2	2				4					21	57·1

APPENDIX II. Continued

	PCA northern group				PCA western group										PCA southern group												
Assigned to	Sur	Tsi	Nii	Naa	Kie	Kaa	Nel	Ket	Vas	Kur	MeL	MeU	Lan	Nuk	Juu04L	IvaAP	Sot	App	Kyl	Tai	Rul	Kar	Iva	IvaJH	Lis	Nas	IvaN	Total no.	Correct assigned (%)
Origin																													
App								1	1							1	1	16	4	2			3					29	55·2
Kyl																2	2	5	11	3		2	6	1	1			33	33·3
Tai									2							1	1	2	6	12	2	1	2					29	41·4
Rul								2		1						2	1	3	1	5	9	3	3					30	30·0
Kar																1	1	2	1	1	3	19	3					31	61·3
Iva04O																2	3	10	6	3	5	4	31	1			1	66	47·0
IvaJH										1					1	1	2				3	1	1	6	2	2	5	25	24·0
Lis																		1					1	3	16	4	7	32	50·0
Nas																					1			2	2	15	2	22	68·2
IvaN				1													1	1						2	3	1	9	18	50·0
North group				99											2												0	101	98·0
West group				1											283												17	301	94·0
South group				1											11												348	360	96·7

