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## Genetic differentiation among European whitefish ecotypes based on microsatellite data

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The amount of genetic differentiation at DNA microsatellite loci in European whitefish (*Coregonus lavaretus*) was assessed among ecotypes, populations and run-timing types. The magnitude of genetic changes potentially caused by hatchery broodstock rearing were also compared with those observed in corresponding natural populations. A total of 35 populations were studied, including 33 *Coregonus lavaretus* populations and two samples of *Coregonus peled*. Five of the six whitefish ecotypes in Finland were represented within *C. lavaretus* populations. Genetic diversity among *C. lavaretus* populations proved to be high compared to two *C. peled* populations. The genetic  $D_A$  distance between these two species was as high as 0.86. The genetic differentiation among ecotypes was generally low and thus gives support for the hypothesis of one native European whitefish species in Scandinavia. Among the ecotypes the northern, large sparsely-rakered, bottom-dwelling whitefish was most unique. Thus, observed genetic differences in quantitative traits have either developed independently of phylogenetic lineages, or have mixed and later changed according to environments and selection pressures. Overall genetic distances between the anadromous whitefish populations along the Finnish coast, especially in the Bothnian Bay area, were small. Populations of this area have been heavily influenced by human activities, and they also have the highest probability of mixing by natural means. In two cases, the Rivers Iijoki and Tornionjoki, statistically significant genetic differences could be observed between summer- and autumn-run spawning-time types. Wild populations had slightly higher allelic diversity than hatchery-reared populations of corresponding rivers. Although some reduction in genetic diversity during hatchery rearing is possible, it is an important aid in maintaining endangered populations.

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The European whitefish (*Coregonus lavaretus*), a member of the Coregonid group, is widely distributed across the Northern Hemisphere. It is one of the most economically important fish species in Finnish commercial inland and coastal fisheries (TURUNEN et al. 1998). The value of the commercial catch of whitefish from the Baltic Sea was about 2 million euros in 2005, and the catch totalled 765 000 kilos. According to the present view, all native Scandinavian whitefishes belong to same species, *Coregonus lavaretus* sensu lato (NIKOLSKY and RESHETNIKOV 1970; RESHETNIKOV 1980; HIMBERG and LEHTONEN 1995), rather than the five-species complex based on morphological variation previously proposed by SVÄRDSON (1957, 1979, 1998), although very little genetic research has been carried out on this topic. The only other native Coregonid species in Finland is the vendace (*Coregonus albula*). In addition, a third *Coregonus* species has also been introduced to Finland from the Russian Lake Endyr, which is located in Western Siberia, in the Ob River

valley. This species is known as *Coregonus peled* (NIKOLSKY and RESHETNIKOV 1970), and according to previous genetic studies the genetic distance from *Coregonus lavaretus* supports the consensus that they are separate species (KOLJONEN et al. 1988; VUORINEN 1988).

The Scandinavian whitefish, *Coregonus lavaretus*, is well known for its morphological and ecological diversity. Previously, different species (SVÄRDSON 1979) and later several ecological forms, or ecotypes, have been distinguished for practical management purposes (KALLIO-NYBERG and KOLJONEN 1988). Classification of these ecotypes has usually been based on the gill raker number, which is known to be an inherited quantitative trait with high heritability (SVÄRDSON 1970). The estimated heritability of gill rake number for *Gasterosteus aculeatus* varied from 0.20 to 0.36 (HERMIDA et al. 2002), and was thus less sensitive to environmental effects than traits with lower heritability such as growth rate. When gill

raker number (varying from 18 to 56) and spawning habitat (river versus lake or sea) are used as the main classifying criteria, six ecotypes can be defined to describe the variation of whitefish in Finland (KALLIO-NYBERG and KOLJONEN 1988; KAUKORANTA et al. 2000; Table 1). These forms also differ in their geographical distribution, migratory behaviour, growth rate, morphological traits and diet. However, crossings between all these ecotypes are possible and the offspring is fertile. In contrast, in a crossing experiment between *C. peled* and *C. lavaretus pallasii* (previously *C. pallasii*) the mortality of the progeny was 10% higher than among the parental types when sires were *C. peled*, but more than 90% of the offspring died if the sires were *C. lavaretus* and the dams *C. peled* (KOLJONEN et al. 1988). The scientific names for these ecotypes have been formed by modifying and using the previous species name given to these types of whitefish by SVÄRDSON (1979).

Three of these six ecotypes, the large sparsely-rakered whitefish (*Coregonus lavaretus fera*), migratory whitefish (*Coregonus lavaretus lavaretus*) and northern densely-rakered whitefish (*Coregonus lavaretus pallasii*), undergo a clear feeding migration and spawn in rivers. The lesser sparsely-rakered whitefish (*Coregonus lavaretus widegreni*), blue whitefish (*Coregonus lavaretus wartmanni*) and southern densely-rakered whitefish (*Coregonus lavaretus pallasii*) undergo shorter migrations and spawn in shoals or streams in the sea area or in lakes.

In addition to the known ecotype variation, genetic diversity also occurs among populations of the same ecotype, for example among populations of anadro-

mous whitefish (migratory whitefish) from different rivers. The accuracy of homing behaviour of anadromous whitefish is not as well known as that of other salmonids. Within some anadromous river populations, a substructure based on seasonal variation in run-time and spawning season still occurs. These forms are known as summer and autumn whitefish, and it is unknown whether these forms have a genetic background, and whether the seasonal forms in separate rivers are more similar to each other than the two forms within the same river. The spawning time is generally known to be a genetically-determined trait (SAKAMOTO et al. 1999).

Genetic variation in whitefish populations is also affected by human activities. In 2004, 17.8 million whitefish juveniles were released into Finnish waters (FINNISH GAME and FISHERIES RESEARCH INSTITUTE 2006). Many whitefish populations have declined drastically in numbers during the past century, and are managed by hatchery rearing, by producing offspring for release to support the wild stocks, or for stockings for fishing. One of the reasons behind the decline is high fishing pressure and effective fishing techniques. Another problem especially affecting the reproduction of the anadromous whitefish, as well as many other anadromous salmonids, is the construction of power plants that prevent spawning individuals from reaching their spawning grounds. In addition to this, eutrophication reduces the quality of freshwater lakes and rivers and hence might have a negative impact on whitefish populations (BNINSKA 2000).

Although stocking and supportive releases are crucial for maintaining viable populations of

Table 1. Definition of ecotypes of whitefish in Finland based on the number of gill rakers and spawning habitat. Names and criteria modified from SVÄRDSON (1979). Forms are presented in order of increasing gill raker number (KALLIO-NYBERG and KOLJONEN 1988).

Scientific and local names	Spawning habitat and number of gill rakers	Range
<i>Coregonus lavaretus fera</i> Large sparsely-rakered whitefish Bottom whitefish, (pohjasiika in Finnish)	river-spawning 18–22	In some northern lakes draining into Barents Sea
<i>Coregonus lavaretus widegreni</i> Lesser sparsely-rakered whitefish, (karisiika, hietasiika in Finnish)	sea-spawning 25–31 lake-spawning 23–24	In the coastal area and in some lakes in eastern and northern Finland
<i>Coregonus lavaretus lavaretus</i> River whitefish, migratory whitefish, anadromous whitefish, (vaellussiika in Finnish)	river-spawning 27–31	In the coastal area and in rivers discharging into the Baltic sea. Also in the Oulujoki and Vuoksi watershed
<i>Coregonus lavaretus wartmanni</i> Blue whitefish, (tuppisiika in Finnish)	lake-spawning 29–37	In fresh waters all over the country
<i>Coregonus lavaretus nilssonii</i> Southern densely-rakered whitefish, lake whitefish, (järvisiika in Finnish)	lake-spawning 40–45	In fresh waters in southern and central Finland
<i>Coregonus lavaretus pallasii</i> Northern densely-rakered whitefish, plankton whitefish, (planktonsiika in Finnish)	river-spawning 50–56	In fresh waters in central and eastern Finland

whitefish, they might also have negative effects on the few natural breeding populations that still remain in the wild. Studies have indicated that hatchery-produced individuals may have a major impact on natural breeding populations by, for example, the introduction of diseases, elevated levels of competition, and negative effects on genetic diversity (BOHLIN et al. 2002; HEGGENES et al. 2002). Hatchery rearing also includes the risk of losing genetic diversity. Hatchery-reared populations have been observed in some cases to have lower allelic diversity compared to natural breeding populations (NORRIS et al. 1999; PANTE et al. 2001; KOLJONEN et al. 2002; SÄISÄ et al. 2003). In addition, introgression of individuals from hatchery to wild populations may cause increased genetic similarity, and thus lower the overall genetic diversity among populations. The lower allelic diversity in hatchery-reared populations compared to wild populations is related to the smaller effective population sizes that can generally be maintained in hatcheries. In some cases, a low number of founder individuals is used to create broodstocks, which in turn are used to produce thousands of progeny to be released into the wild. The high level of relatedness among individuals in broodstocks leads to high level of inbreeding and low level of genetic diversity among the released fish. Supportive releases of such fish may in some cases cause a decrease in the genetic diversity of the wild population (RYMAN and LAIKRE 1991; WAPLES and DO 1994; RYMAN et al. 1995; TESSIER et al. 1997). Such a result was reported by KOSKINEN et al. (2002) in the case of the European grayling (*Thymallus thymallus*), in which they observed a significant decrease in genetic diversity between wild and introduced populations since the commencement of stocking in 1986.

Stocking activities might change the original differentiation pattern of the native whitefish populations. This needs to be taken into account as a potential difficulty in describing the population structure, as stockings might have blurred it. To preserve genetic diversity as efficiently as possible and to be able to adopt sustainable management for the whitefish resource, it is important to know the level of genetic diversity and differentiation among the populations. However, despite the economic importance of whitefish and its large-scale stocking programmes in Finland, there is lack of knowledge concerning the genetic status of whitefish populations.

The aim of this work was to assess the amount of genetic differentiation at DNA microsatellite loci in European whitefish among a range of hierarchical levels or substructures. The estimates were assessed on the following four levels: 1) the species level, 2) the ecotype level, 3) among populations and 4) among

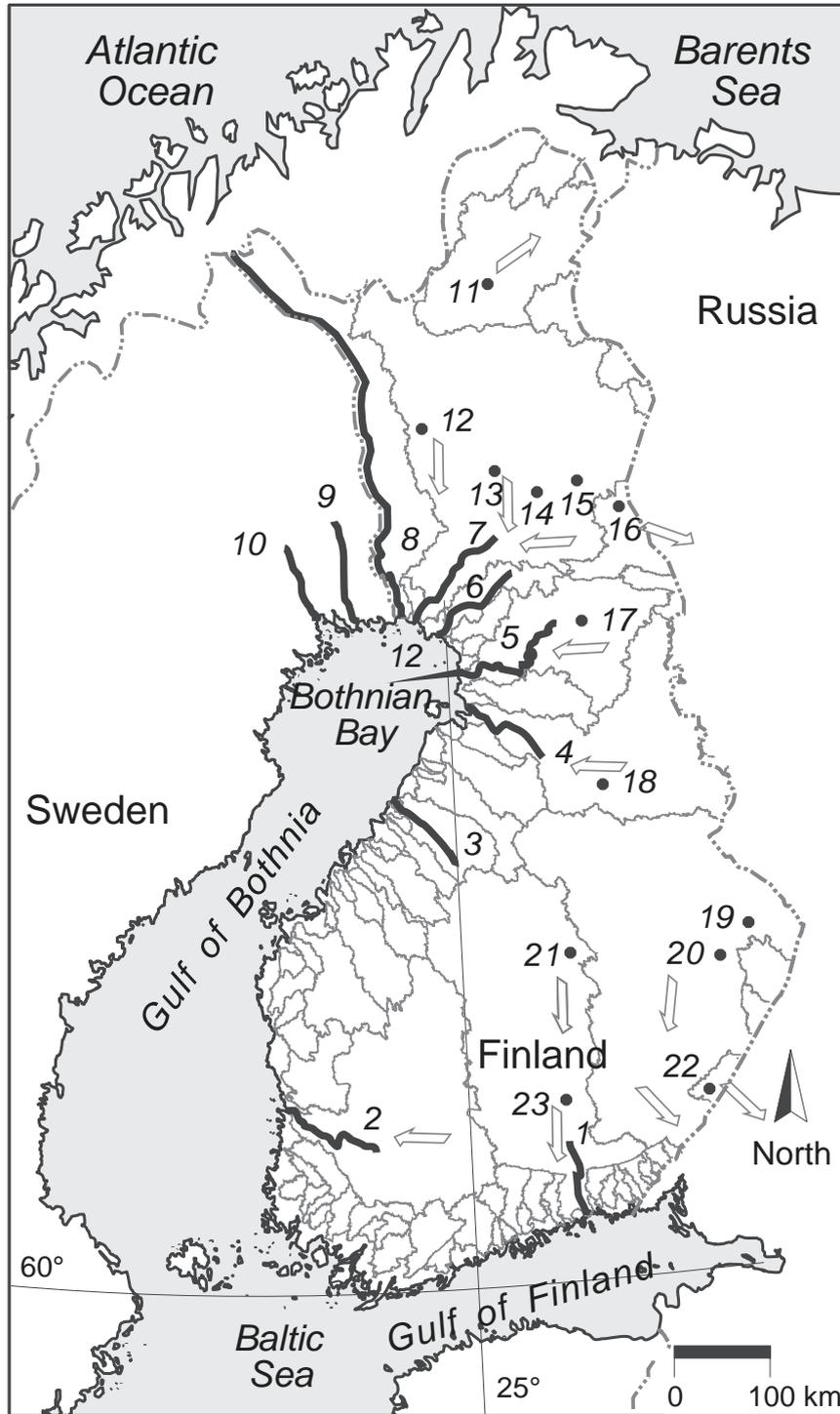
spawning-time forms. The species level was represented by the difference between *C. lavaretus* and *C. peled*. Differentiation between the previously-defined morphologically different ecotypes named as six species by SVÄRDSON (1979), as well as among populations of different watersheds within ecotypes was assessed. Spawning-time types were represented by summer and autumn spawning subpopulations within each river system. In addition, the magnitude of genetic changes potentially caused by hatchery broodstock rearing was measured by comparing them with those observed in natural populations, in cases when both wild and hatchery samples were available.

## MATERIAL AND METHODS

### *Sampled fish populations*

A total of 29 whitefish population belonging to the species *Coregonus lavaretus* were sampled from Finnish waters, and two samples from the northern Swedish rivers Kalixälven and Råne were additionally included (Fig. 1, Table 2). Five of the six whitefish ecotypes in Finland were represented in the samples. Most of the samples (23 out of 35) were from anadromous whitefish populations (*Coregonus lavaretus lavaretus*) in the Bothnian Bay area, and four of them were from landlocked, river-spawning populations of this same type. Four samples were from northern densely-rakered, planktivorous, river-spawning freshwater whitefish (*C. l. pallasi*), two from large, sparsely-rakered, bottom-dwelling, river-spawning freshwater whitefish (*C. l. fera*), and two were from southern densely-rakered, lake-spawning freshwater whitefish, (*C. l. nilssonii*) populations. In addition, two samples were taken from sea catches of the sea-spawning whitefish populations (*C. l. widegreni*), one from the Bothnian Bay and one from the Gulf of Finland (Table 2). Three of the large anadromous populations (the Rivers Kemijoki, Iijoki and Tornionjoki) were represented by two samples spawning in different seasons: summer- and autumn-run populations (Table 2). Hatchery broodstocks of all the main whitefish populations were analysed and, if possible, also compared with the respective naturally-reproducing stocks. Moreover, a broodstock and a stocked, naturally reproducing population of peled whitefish (*C. peled*) were included in the study. Samples 11, 16, 19, 20 and 22 in Fig. 1 are taken from watersheds draining eastwards into the Russia.

The anadromous whitefish (*C. l. lavaretus*) occurs in Baltic Sea rivers and also as a freshwater form in some other watersheds. This fast-growing whitefish originally spawned in at least 30 Finnish rivers draining into



- |                |              |                  |                 |                 |
|----------------|--------------|------------------|-----------------|-----------------|
| 1 Kymijoki     | 6 Simojoki   | 11 Ivalojoiki    | 16 Kuusinkijoki | 21 Rautalampi   |
| 2 Kokemäenjoki | 7 Kemijoki   | 12 Ounasjoki     | 17 Livojoki     | 22 Simpelejärvi |
| 3 Kalajoki     | 8 Torniojoki | 13 Luirojoiki    | 18 Sotkamo      | 23 Vuohijärvi   |
| 4 Oulujoki     | 9 Kalixälven | 14 Kemijärvi     | 19 Koitajoki    |                 |
| 5 Iijoki       | 10 Råne      | 15 Kallunkijärvi | 20 Pielisjoki   |                 |

**Fig. 1.** Sampling locations of the studied whitefish populations. Boundaries of main watersheds in Finland and their flowing directions are shown.

Table 2. Population origin, ecotype, latin name of the whitefish (*Coregonus lavaretus* and *Coregonus peled*) forms, and the type of propagation of the sampled population.

No	Population	Ecotype	Species/form	Propagation
1	Kymijoki	anadromous	<i>C. l. lavaretus</i>	wild
2	Kymijoki	anadromous	<i>C. l. lavaretus</i>	hatchery
3	Kokemäenjoki	anadromous	<i>C. l. lavaretus</i>	hatchery
4	Kalajoki	anadromous	<i>C. l. lavaretus</i>	wild
5	Kalajoki	anadromous	<i>C. l. lavaretus</i>	hatchery
6	Oulujoki, autumn	anadromous	<i>C. l. lavaretus</i>	wild
7	Oulujoki	anadromous	<i>C. l. lavaretus</i>	hatchery
8	Iijoki, summer	anadromous	<i>C. l. lavaretus</i>	wild
9	Iijoki, autumn	anadromous	<i>C. l. lavaretus</i>	wild
10	Iijoki, hatchery	anadromous	<i>C. l. lavaretus</i>	hatchery
11	Simojoki	anadromous	<i>C. l. lavaretus</i>	wild
12	Kemijoki, summer	anadromous	<i>C. l. lavaretus</i>	wild
13	Kemijoki, autumn	anadromous	<i>C. l. lavaretus</i>	wild
14	Kemijoki, upper part	anadromous	<i>C. l. lavaretus</i>	wild
15	Tornionjoki, summer	anadromous	<i>C. l. lavaretus</i>	wild
16	Tornionjoki, autumn	anadromous	<i>C. l. lavaretus</i>	wild
17	Kalixälven	anadromous	<i>C. l. lavaretus</i>	wild
18	Råne	anadromous	<i>C. l. lavaretus</i>	wild
19	Bothnian Bay	sea-spawning	<i>C. l. widegreni</i>	–
20	Gulf of Finland	sea-spawning	<i>C. l. widegreni</i>	–
21	Ivalojoki	bottom-dwelling	<i>C. l. fera</i>	hatchery
22	Ounasjoki	river-spawning	<i>C. l. lavaretus</i>	wild
23	Luirojoki	river-spawning	<i>C. l. lavaretus</i>	wild
24	Kemijärvi	river-spawning	<i>C. l. lavaretus</i>	wild
25	Kallunkijärvi	bottom-dwelling	<i>C. l. fera</i>	wild
26	Kuusinkijoki	river-spawning	<i>C. l. lavaretus</i>	hatchery
27	Livojoki	river-spawning	<i>C. l. lavaretus</i>	wild
28	Sotkamo	planktivorous	<i>C. l. pallasi</i>	hatchery
29	Koitaajoki	planktivorous	<i>C. l. pallasi</i>	wild
30	Pielisjoki	planktivorous	<i>C. l. pallasi</i>	wild
31	Rautalampi	planktivorous	<i>C. l. pallasi</i>	hatchery
32	Simpeleenjärvi	lake-spawning	<i>C. l. nilssoni</i>	wild
33	Vuohijärvi	lake-spawning	<i>C. l. nilssoni</i>	wild
34	Endyr, Lokka	lake-spawning	<i>C. peled</i>	wild
35	Endyr (Russia)	lake-spawning	<i>C. peled</i>	hatchery

the Baltic Sea. Many of the populations have been lost due to the damming of rivers and as a consequence of the drastic decline of water quality in rivers. Some of the populations have, however, survived as result of active breeding programmes. Anadromous whitefish populations in coastal, dammed rivers are largely dependent on supportive releases. The anadromous whitefish is the most intensively reared form of whitefish in Finland.

Indigenous populations of the northern densely-rakered whitefish (*C. l. pallasi*) occur in lakes and rivers in central and northern Finland, and in the Vuoksi, Kymijoki and Oulujoki watersheds. Being the only plankton-feeding whitefish, it is most commonly known as the plankton whitefish, and it also has the highest number of gill rakers (50–56).

The large sparsely-rakered bottom-dwelling whitefish (*C. l. fera*) is indigenous only in some of the lakes

draining into the Barents Sea in northern Lapland and in the Kuusamo area of southern Lapland. This is the most northern whitefish ecotype.

The southern densely-rakered whitefish (*C. l. nilssoni*) is distributed in lakes and rivers in southern and central Finland, but indigenous non-mixed stocks are currently only found in a few watersheds. This whitefish ecotype has mixed with other ecotypes of introduced whitefish in many lakes.

Indigenous populations of lesser sparsely-rakered sea- or lake-spawning whitefish (*C. l. widegreni*) live in the coastal areas of the Baltic Sea and in a few lakes in eastern and northern Finland.

#### Microsatellite analysis

DNA was extracted from ethanol-stored fin samples according to the Chelex method of ESTOUP et al. (1996). Five microsatellite loci, *Bwf1*, and *Bwf2*

(PATTON et al. 1997), *C2-157* (TURGEON et al. 1999), *Cocl-23* (BERNATCHEZ 1996), and *SSBgIIIM.26* (L. Bernatchez, Univ. Laval, Quebec, Canada, pers. comm.), were used for the analysis of genetic variation. All 10 ml PCR reactions were performed in a buffer concentration of 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 1.5 mM MgCl<sub>2</sub>, 25 mM dNTPs, 0.5 U *Ampli Taq* polymerase, 0.3-0.6 mM of each primer (one of each pair being labelled with the fluorescent label, either FAM, HEX or NED) and 1 ml of the Chelex-extracted DNA. Following PCR, semi-automated microsatellite analysis was carried out by analysing all loci in an ABI377 sequencer (Perkin Elmer).

From each population, 50 individuals were randomly sampled. The numbers of alleles in samples were compared by an allelic richness measure ( $A_r$ ; EL MOUSADIK and PETIT 1996, PETIT et al. 1998) that was calculated by the rarefaction approach with FSTAT software ver. 2.9.3. (GOUDET 1995, 2001). The program calculates allelic richness for the smallest number of individuals typed for any locus. Due to missing information for locus *C2-157*, the wild *C. peled* sample was excluded from the analysis.

The populations were tested for a recent reduction in their effective population size by the Wilcoxon sign-rank test, as implemented in the BOTTLENECK program ver. 1.2.02 (CORNUET and LUIKART 1996), assuming the two-phase model of mutation (with 5% multi-step changes and variance of 12) for microsatellite loci as recommended by PIRY et al. (1999). Reduction in population size was also tested according the method of GARZA and WILLIAMSON (2001), which compares the mean ratio of the number of alleles to the range in allele sizes, named M. This method has been especially developed for microsatellite data.

Exact tests for the Hardy-Weinberg (H-W) equilibrium (GUO and THOMPSON 1992) and population differentiation were analysed with the GENEPOP 3.2 program package (RAYMOND and ROUSSET 1995) with Markov chain parameters, 300 batches and 3000 iterations. Probabilities of H-W equilibrium tests for populations were adjusted over loci using the sequential Bonferroni procedure for multiple tests (RICE 1989). In addition, the observed and expected heterozygosity level in each population was calculated using GENEPOP ver. 3.2 (RAYMOND and ROUSSET 1995).

Analysis of the differences between populations was based on allele frequency differences, using pairwise  $F_{ST}$  values (WEIR and COCKERHAM 1984), which were estimated with FSTAT ver. 2.9.3. (GOUDET 2001).

Standard deviation and confidence intervals were estimated through bootstrapping.

Genetic distances between populations were calculated using Nei's  $D_A$  distances (NEI et al. 1983). A phylogenetic tree was constructed with DISPAN software (OTA 1993) using a neighbour joining (NJ) algorithm (SAITOU and NEI 1987). Bootstrapping with 1000 replicates was used to test the statistical strength of the branches.

To provide estimates of the number of founders in the broodstocks, effective population size estimates ( $N_e$ ) were calculated using the maximum-likelihood method (WANG 2001, WANG and WHITLOCK 2002) in the MLNE program (WANG and WHITLOCK 2002). The method assumes the factors causing temporal allele frequency changes to be genetic drift and, unlike earlier temporal methods where this was neglected, migration. The method assumes mutation and direct selection of the markers not to be significant.

## RESULTS

### *General genetic diversity*

A total of 182 alleles were observed across the five microsatellite loci with an average of 36.4 alleles per locus ranging from 24 at *Bwf2* to 53 alleles at *Cocl-23* (Table 3). *C. lavaretus* populations were clearly more polymorphic in all loci than the two *C. peled* populations. The naturally-reproducing *C. peled* population was even fixed for the allele \*197 at the locus *Bwf1*. The highest number of alleles (30) was observed at locus *Cocl-23* in the large population of the River Kalixälven. This population also had the highest observed total number of alleles (93). The lowest total number (29) of alleles among *C. lavaretus* populations was found in the wild bottom-dwelling (*C. l. fera*) population in Lake Kallunkijärvi.

Almost 25% of all alleles (45 of 182) were private alleles that are unique to a single population. The River Kalixälven population had eight private alleles, which was the highest observed value for any single population, although the mixed sea sample from the Gulf of Finland included nine private alleles.

Deviations from H-W equilibrium with a probability smaller than or equal to 0.001 after Bonferroni correction were observed in eight out of 35 samples, including following: Rautalampi (hatchery), *C. peled* from Lake Endyr (wild), *C. peled* (hatchery), Iijoki (autumn sample), Iijoki (hatchery), Kemijoki (wild summer sample), Kalixälven and Råne. These deviations were all due to deficiency of heterozygotes. This might suggest that these populations are not at mutation-drift equilibrium, but have perhaps a

Table 3. Genetic diversity for all populations. Abbreviations in column 2: *A* = anadromous, *P* = planktivorous, *B* = bottom-dwelling, *S* = sea-spawning, *L* = lake-spawning. *SD* = standard deviation, *A<sub>r</sub>* = allelic richness, *M* = *M*-value (GARZA and WILLIAMSON 2001), *H<sub>e</sub>* = expected heterozygosity.

No.	Population	Observed number of alleles								<i>A<sub>r</sub></i>		Heterozygosity		
		<i>Bwfl</i>	<i>Bwf2</i>	<i>C2-157</i>	<i>Cocl-23</i>	<i>SSBgIIIM.26</i>	Total	Mean	SD	Total	Mean	<i>M</i>	<i>H<sub>e</sub></i>	SD
1	A Kymijoki, wild	11	10	21	18	16	76	15.2	4.66	55.4	11.1	0.71	0.88	0.07
2	A Kymijoki, hatchery	12	9	14	20	14	69	13.8	4.03	50.5	10.1	0.58	0.85	0.07
3	A Kokemäenjoki, hatchery	7	5	9	8	6	35	7.0	1.58	29.3	5.9	0.62	0.70	0.18
4	A Kalajoki, wild	11	9	18	20	17	75	15.0	4.74	52.0	10.4	0.59	0.86	0.05
5	A Kalajoki, hatchery	13	8	18	13	13	65	13.0	3.54	50.1	10.0	0.71	0.83	0.13
6	A Oulujoki Autumn, wild	13	9	15	15	18	70	14.0	3.32	51.7	10.3	0.60	0.86	0.06
7	A Oulujoki, hatchery	13	11	18	16	12	70	14.0	2.92	52.1	10.4	0.73	0.86	0.05
8	A Iijoki Summer, wild	10	11	19	17	18	75	15.0	4.18	53.7	10.7	0.69	0.86	0.09
9	A Iijoki Autumn, wild	12	10	19	23	21	85	17.0	5.70	57.7	11.5	0.69	0.88	0.06
10	A Iijoki, hatchery	12	10	20	20	14	76	15.2	4.60	53.2	10.6	0.72	0.87	0.04
11	A Simojoki, wild	12	8	16	19	20	75	15.0	5.00	51.6	10.3	0.63	0.85	0.06
12	A Kemijoki Summer, wild	10	7	20	21	22	80	16.0	6.96	54.4	10.9	0.72	0.85	0.08
13	A Kemijoki Autumn, wild	10	10	19	22	15	76	15.2	5.36	51.0	10.2	0.71	0.85	0.05
14	A Kemijoki (upper part) wild	10	9	17	21	14	71	14.2	4.97	49.8	10.0	0.72	0.85	0.05
15	A Tornionjoki, Summer, wild	12	9	17	20	19	77	15.4	4.72	54.1	10.8	0.72	0.86	0.07
16	A Tornionjoki, Autumn, wild	9	9	18	13	12	61	12.2	3.70	49.8	10.0	0.61	0.86	0.06
17	A Kalixälven, wild	15	11	15	30	22	93	18.6	7.50	65.1	13.0	0.65	0.88	0.09
18	A Råne, wild	15	14	13	15	16	73	14.6	1.14	53.2	10.6	0.64	0.87	0.03
19	S Bothnian Bay	12	8	15	26	17	78	15.6	6.73	52.9	10.6	0.56	0.83	0.14
20	S Gulf of Finland	14	11	19	25	23	92	18.4	5.90	54.6	10.9	0.54	0.87	0.07
21	B Ivalojoiki, hatchery	7	6	11	14	16	54	10.8	4.32	40.5	8.1	0.63	0.81	0.09
22	A Ounasjoki, wild	9	7	12	17	11	56	11.2	3.77	46.5	9.3	0.68	0.83	0.08
23	A Lurojoki, wild	11	7	15	20	10	63	12.6	5.03	46.0	9.2	0.64	0.81	0.10
24	A Kemijärvi, wild	13	8	15	17	15	68	13.6	3.44	48.7	9.7	0.66	0.82	0.13
25	B Kallunkijärvi, wild	4	7	4	7	7	29	5.8	1.64	24.0	4.8	0.78	0.68	0.11
26	A Kuusinkijoki, hatchery	11	10	15	19	13	68	13.6	3.58	47.6	9.5	0.60	0.84	0.08
27	A Livojoki, wild	7	5	8	10	13	43	8.6	3.05	34.2	6.8	0.60	0.75	0.13
28	P Sotkamo, hatchery	6	6	11	10	14	47	9.4	3.44	36.4	7.3	0.65	0.77	0.09
29	P Koitajoki, wild	8	8	9	12	12	49	9.8	2.05	37.2	7.4	0.71	0.79	0.06
30	P Pielisjoki, wild	8	8	15	17	11	59	11.8	4.09	42.7	8.5	0.69	0.79	0.11
31	P Rautalampi, hatchery	7	6	7	12	11	43	8.6	2.70	36.5	7.3	0.54	0.81	0.07
32	L Simpeleenjärvi, wild	12	10	17	14	16	69	13.8	2.86	51.0	10.2	0.74	0.87	0.03
33	L Vuohijärvi, wild	9	9	11	21	11	61	12.2	5.02	49.2	9.8	0.69	0.86	0.05
34	Lake Endyr, Lokka, wild	1	3		3	4	11	2.2	1.64				0.16	0.23
35	Lake Endyr, hatchery	4	5	11	2	3	25	5.0	3.54	14.8	3.0	0.78	0.15	0.10
	Total	27	24	31	53	47	182	36.4		58.9	11.8		0.88	0.07

sub-population structure, or have experienced an expansion in population size.

A possible indication of a recent bottleneck was only observed in a sample of the hatchery stock of Rautalampi plankton whitefish *C. l. pallasii* (one-tailed Wilcoxon sign-rank test for heterozygosity excess;  $P < 0.05$ ). This Rautalampi hatchery stock also had a low  $M$  value (0.54; Table 3;  $M$  = mean ratio of the number of alleles to the range in allele sizes; GARZA and WILLIAMSON 2001), indicating a possible reduction in population size. The highest  $M$  value of this study was observed in the wild stock of Kallunkijärvi (0.78), and the lowest in the wild *C. peled* sample (0.41). The overall average of  $M$  was 0.64.  $M$  values below average were observed in following stocks: Kalajoki, wild (0.59), Ivalojoiki hatchery (0.63), Kuusinkijoki hatchery (0.60), Kokemäenjoki (0.62), Kymijoki hatchery (0.58), Livojoki wild (0.60), Simojoki wild (0.63), Tornionjoki Autumn wild (0.61), Oulujoki Autumn wild (0.60) and also the sea samples of the Bothnian Bay and the Gulf of Finland (0.56 and 0.54 respectively).

The average mean heterozygosity ( $H_e$ ) for all populations was high, varying from 0.68 (Lake Kallunkijärvi) to 0.88 (Iijoki autumn wild, Kymijoki wild and Kalixälven wild; Table 3). Only the two *C. peled* whitefish samples were an exception to this, with their extremely low mean heterozygosity (0.16), which was only 19% of that for an average *Coregonus lavaretus* population. Mean heterozygosity for different ecotypes varied from 0.82 for the bottom whitefish (*C. l. fera*) to 0.89 for the plankton whitefish (*C. l. nilssoni*). The mean heterozygosity for wild populations, excluding *C. peled* samples, was on average 0.84, which was slightly greater than that of the hatchery populations, for which the average was 0.81.

The sample-size-corrected mean number of alleles per locus (allelic richness, mean  $A_r$ ) varied from 13.0 in the wild anadromous population of the River Kalixälven to 4.8 in a bottom whitefish (*C. l. fera*) population of Lake Kallunkijärvi. When compared to *C. lavaretus* populations, *C. peled* populations had an

extremely low number of alleles; the mean  $A_r$  for the hatchery population was only 2.96. On average, wild populations had somewhat more alleles than the hatchery-reared populations (mean  $A_r$  9.81 and 8.80 respectively, when *C. peled* populations were excluded). In the mixed sea samples, both mean heterozygosity and mean allelic richness was as high as 0.85 and 10.8, respectively.

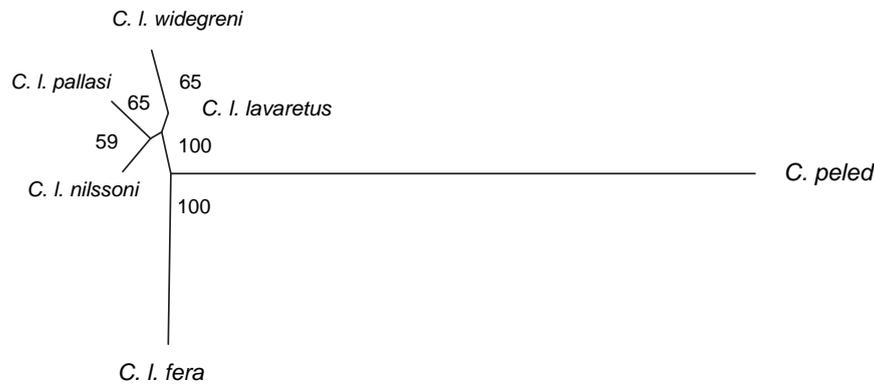
The average genetic distances ( $D_A$ ) between the ecotypes of *C. lavaretus* and the species *C. peled* was as high as 0.86, varying between 0.82 and 0.90 among *C. lavaretus* ecotypes (Table 4, Fig. 2, 3). For a single population the maximum distance to *C. peled* was 0.98 (Kallunkijärvi). The genetic identity (NEI 1972) between *C. peled* and *C. lavaretus* ecotypes varied from 0.02 (*C. l. pallasii*) to 0.12 (*C. l. widegreni*). Several individual population pairs of *C. lavaretus* and *C. peled* had a genetic identity of less than 0.01 (Kokemäenjoki, Koitajoki, Kallunkijärvi and Livojoki). The  $F_{ST}$  value between the species *C. peled* and *C. lavaretus* was on average 0.50.

#### Genetic differences between ecotypes

The genetic distances ( $D_A$ ) among the five analysed ecotypes of *C. lavaretus* was in general clearly less than among the two species, being on average 0.15, with a range of 0.06–0.26 (Table 4, Fig. 2). It was only about 17% of that between the species *C. peled* and *C. lavaretus*. However, the genetic distances between *C. l. fera* and other ecotypes was about twice as great (0.22, 0.19–0.26) as among the other four ecotypes (0.10, 0.06–0.14, for *C. l. lavaretus*, *C. l. widegreni*, *C. l. pallasii* and *C. l. nilssoni*), corresponding to 26% and 12% of the species level differentiation. Inter-ecotype differentiation varied from 0.10 to 0.31 among the four more similar ecotypes, which was more than the variation between the ecotypes, also indicating other strong differentiation patterns in addition to ecotypes. In *C. l. fera* the inter-ecotype differences between populations was as high as 0.50, which was more than the distance of this type from any other *C. lavaretus* ecotype.

Table 4. Pair-wise  $F_{ST}$  estimates (above the diagonal) and genetic distances ( $D_A$ , NEI et al. 1983) (below the diagonal) between different ecotypes of *C. lavaretus*, and *C. peled*, and average distances within groups (in italics along the diagonal).

Ecotype	<i>C. l. lavaretus</i>	<i>C. l. pallasii</i>	<i>C. l. nilssoni</i>	<i>C. l. widegreni</i>	<i>C. l. fera</i>	<i>C. peled</i>
<i>C. l. lavaretus</i>	0.20	0.02	0.01	0.01	0.04	0.35
<i>C. l. pallasii</i>	0.09	0.31	0.02	0.03	0.05	0.42
<i>C. l. nilssoni</i>	0.07	0.10	0.25	0.03	0.05	0.44
<i>C. l. widegreni</i>	0.06	0.14	0.13	0.10	0.06	0.43
<i>C. l. fera</i>	0.19	0.20	0.23	0.26	0.50	0.47
<i>C. peled</i>	0.82	0.90	0.85	0.82	0.89	0.06



**Fig. 2.** Neighbour-joining dendrogram of the studied whitefish ecotypes based on  $D_A$  distances. Percent support from 1000 bootstrap replications is given above each branch.

### Genetic differences among populations

Genetic differentiation among populations was not always strong, and in many cases no statistically significant differences could be observed at all. For example, the following pairs of populations from the Bothnian Bay area had no statistically significant differences in their allele frequencies for the studied gene loci:

wild Tornionjoki autumn	- Kemijoki autumn
wild Kalajoki	- Kemijoki autumn
Kemijoki autumn	- Kemijoki summer
wild Tornionjoki autumn	- Kemijoki summer
wild Kalajoki	- Kemijoki summer
Kemijoki autumn	- wild Simojoki.

In addition, strong similarity was observed for the sea sample from Bothnian Bay and two river populations (Tornionjoki summer and Iijoki summer).

Overall genetic distances (Nei  $D_A$  distances) among all *C. lavaretus* whitefish populations were small ( $D_A=0.24$ ) and varied from 0.07 (wild Kemijoki autumn to wild Simojoki) to 0.55 (Rautalampi to Livojoki). The  $F_{ST}$  (WEIR and COCKERHAM 1984) estimate for all *C. lavaretus* populations was 0.05, and varied from  $<0.01$  (several population pairs within Bothnian Bay area) to 0.21 (Kallunkijärvi to Sotkamo). In a dendrogram based on  $D_A$  distances, populations from the Bothnian Bay area mainly group on the same branch, with a few exceptions (Fig. 3). Populations from the same ecotype did not always cluster together.

Comparison of sub-populations differing in run timing from the Rivers Kemijoki, Iijoki and Tornionjoki revealed no statistically significant differences in genetic diversity, the number of observed alleles or allelic richness at the individual population level. Virtually no differences could be observed in mean genetic diversity (0.86 for both summer-run and in

autumn-run samples) or mean allelic richness ( $A_i$ ; summer-run 10.8 and autumn-run 10.6). However, the number of observed alleles was on average slightly higher for the summer-run type than for the autumn-run type (77 compared to 74).

### Effective population size

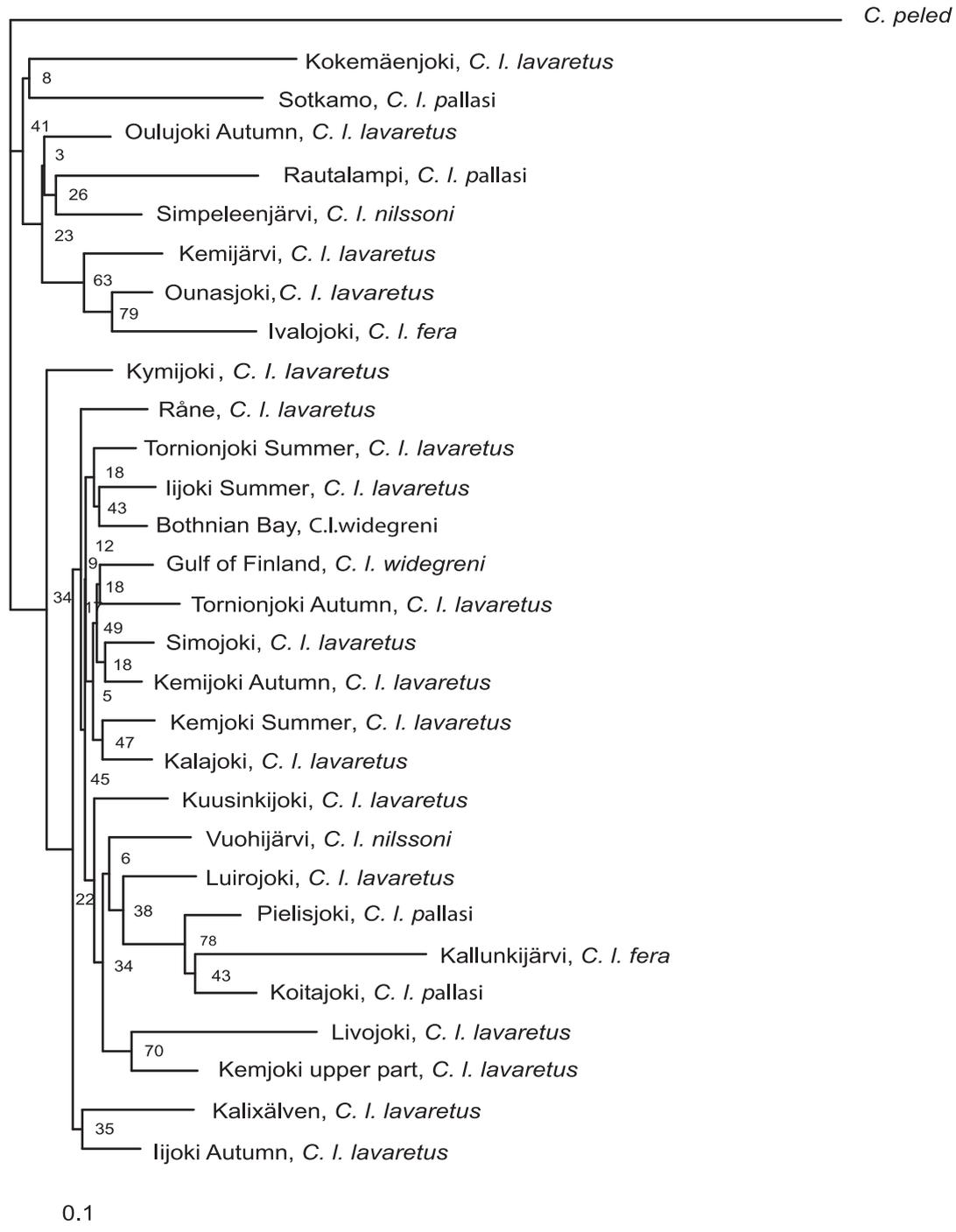
Effective population size estimates,  $N_e$ , for hatchery populations of Oulujoki, Kymijoki and Kalajoki were less than 50 fish (33 to 46 fishes; Table 5). The estimate of  $N_e$  for Iijoki hatchery population was the largest, 122 fishes. The 95% confidence intervals were also greater in Iijoki compared to other cases.

## DISCUSSION

When compared to earlier (PATTON et al. 1997, DOUGLAS et al. 1999, LU and BERNATCHEZ 1999, TURGEON et al. 1999, LU et al. 2001, ØSTBYE et al. 2006) whitefish studies including the same microsatellite loci, somewhat more alleles were observed in the present study. However, the observed allele sizes were generally consistent with those of the earlier studies. Only one locus, *Cocl-23*, had a wider allele range with smaller alleles in the earlier studies of LU and BERNATCHEZ (1999), LU et al. (2001) and ØSTBYE et al. (2006) than observed in this study.

### Species-level genetic diversity and differentiation between *C. peled* and *C. lavaretus*

The number of alleles and level of heterozygosity proved to be high among all stocks included in this study, except for the *C. peled* stocks, for which the diversity was extremely low. The *C. peled* whitefish was imported as fertilised eggs from Russia and the progeny from that batch has subsequently been used to found new broodstocks, which in turn have produced offspring to be released into Lake Lokka in Finnish Lapland. The number of founders of the



**Fig. 3.** Neighbour-joining dendrogram (SAITOU and NEI 1987) of the studied whitefish populations based on  $D_A$  distances. Percent support from 1000 bootstrap replications is given above each branch.

original broodstock is unknown, but was most likely low, contributing to the poor genetic diversity of the stocks seen today.

In both *C. peled* populations studied, departures from the Hardy-Weinberg equilibrium and positive  $F_{IS}$  values were detected at each locus, which may be a result of inbreeding. The wild *C. peled* population also

had a low  $M$ -value estimate, less than 0.68, which according to GARZA and WILLIAMSON (2001) indicates a recent reduction in population size if at least seven loci have been studied. The genetic distance measured here between *C. peled* and *C. lavaretus* was 0.86. In the earlier study of KOLJONEN et al. (1988) based on 24 allozyme loci the standard genetic

Table 5. Observed change in allelic richness, heterozygosity and temporal effective population size ( $N_e$ ) (WANG and WHITLOCK 2002) estimates in hatchery breeding of Finnish whitefish stocks studied.  $t$  = number of generations,  $w$  = wild,  $h$  = hatchery,  $\text{Chance}^{\circ}/t$  = per cent of chance per generation, CI = confidence intervals.

Population	Allelic richness					Heterozygosity				$N_e$	
	$t$	$w$	$h$	Chance/ $t$	Chance $^{\circ}/t$	$w$	$h$	Chance/ $t$	Chance $^{\circ}/t$	$N_e$	95% CI
Kymijoki, hatchery	1	55.4	50.5	4.9	8.84	0.88	0.85	0.03	3.41	46	31–81
Kalajoki, hatchery	1	52.0	50.1	1.9	3.65	0.86	0.83	0.03	3.49	33	25–48
Oulujoki, hatchery	1	51.7	52.1	−0.4	−0.77	0.86	0.86	0	0	34	26–50
Iijoki, hatchery	1	55.7	53.2	2.5	4.49	0.87	0.87	0	0	122	69–427

distance (NEI 1978) between *C. peled* and *C. pallasii* was 0.158, and genetic identity (I; NEI 1972) was 0.854, which was considered to indicate a small but clear species-level difference. In our study the genetic distance between *C. peled* and *C. L. pallasii* groups was as high as 0.90 (Table 4), and the genetic identity estimate only 0.02. The result clearly supports the species-level difference between these two whitefishes. This is, however, not true for the studied ecotypes.

#### Genetic differentiation of ecotypes of *C. lavaretus*

Whitefish species have a wide range of variation in phenotypic and ecological traits, and different classifications and a multitude of unjustified taxonomic nomenclatures have been formed on the basis of these traits, one of the latest by KOTTELAT (1997). It is important to understand the taxonomic and ecological distinctiveness of populations in order to identify and form biological units for conservation purposes, however, the species concept need to be kept as clear as possible. Here, the ecotypes are named according to the SVÄRDSON (1979), and the classification is performed according to the gill raker number and spawning habitat. This classification has also previously been presented by KALLIO-NYBERG and KOLJONEN (1988).

In this study the pair-wise  $F_{ST}$  estimates of the ecotypes were small, varying from 0.01 to 0.06. The genetic distances among different ecotypes were generally lower than those between populations within ecotypes (Table 4), the only exception being ecotype *C. l. widegreni* (sea-spawning lesser sparsely-rakered whitefish). The shortest observed genetic distance was between geographically close ecotypes *C. l. widegreni* (sea-spawning lesser sparsely-rakered whitefish) and *C. l. lavaretus* (migratory, anadromous whitefish) ( $D_A$  0.06), which also had the lowest pair-wise  $F_{ST}$  estimates (0.01). The most distinctive ecotype was the bottom-dwelling whitefish (or northern large sparsely-rakered whitefish, *C. l. fera*), with the largest genetic distance to all other groups. This ecotype, also had the largest within-group distance ( $D_A$  0.50; Table 4,

Fig. 2). The pair-wise  $F_{ST}$  estimate between the two bottom-dwelling whitefish populations from Ivalojoiki (hatchery) and Kallunkijärvi (wild) was as high as 0.19. The Kallunkijärvi population also had the lowest number of alleles and heterozygosity of all *C. lavaretus* populations, which is clearly a result of genetic drift in this particular population. However, the data support the idea that the bottom-dwelling whitefish populations generally differ more from the other four *C. lavaretus* ecotypes (*C. l. widegreni*, *C. l. lavaretus*, *C. l. nilssoni* and *C. l. pallasii*) than the other four from each other. No separate phylogenetic background could be assumed for the four similar types on basis of our data from the neutral DNA-markers used in this study. For the case of *C. l. fera* the case remains open and needs more research and comprehensive sampling to be solved.

The genetic differentiation among ecotypes remained far beyond the species-level differentiation observed between *C. peled* and *C. lavaretus*, and thus the result clearly supports the existence of only one native European whitefish species in Scandinavia. In some cases the ecotypes might, however, reflect phylogeographic lineages within the species, especially in the case of the bottom-dwelling whitefish, *C. lavaretus fera*, although further research is needed to verify this. Genetic differences in quantitative traits such as gill-raker number, spawning or run timing, migration behaviour and spawning habitat preference do exist, and have resulted from natural selection. However, the quantitative traits seem not in general to have a direct correlation with phylogenetic lineages, and have either developed independently of them or have mixed and changed later, according to the actual environments and their selection pressures.

In their study based on allozyme loci, NÆSJE et al. (2004) observed that genetic differences were associated with gill raker numbers in whitefish ecotypes of Norwegian Lake Femund, and the possible different origins of these forms were discussed.

ØSTBYE et al. (2005, 2006) concluded that the observed phenotypic variation of European whitefish

populations is of rather recent origin and reflects the adaptive radiation and rapid diversification due to gene flow and competition, and that parallel evolution of the same ecotypes has occurred in many places only since the last glaciation. Thus their results did also not support the SVÄRDSON (1957, 1979, 1998) hypothesis of five separate whitefish species in Fennoscandia, which was in concordance with our results, although they had sampled different ecotypes. Due to their findings, they also concluded that traditional phenotype-based naming of whitefish species is somewhat misleading, as the trinomial names have no taxonomic background. Despite the fact that no systematic, phylogenetic differentiation between all ecotypes has not yet been observed, the use of the trinomial names could be defended because of the importance of adaptive genetic differentiation known among ecotypes.

#### *Population-level genetic diversity and differentiation*

Phylogenetic analysis revealed that the allelic frequencies differ very little and overall genetic distances between the anadromous whitefish populations along Finnish coast, especially in the Bothnian Bay area (the Rivers Råne, Kalixälven, Tornionjoki, Kemijoki, Simojoki and Iijoki), are small. This is not surprising, since the populations in this area have been heavily stocked for a long time. Natural reproduction in these rivers has nearly ceased and they are mainly maintained by stocking and supportive release. These also are the populations that have the highest probability of mixing by natural gene flow.

Overall genetic distances among anadromous whitefish are small, while the genetic distances among freshwater forms and between residents and anadromous fish are larger. This could be explained by intense stocking with anadromous forms or the fact that anadromous forms more easily stray to different rivers and hybridise compared to resident stocks. The bootstrap values for the anadromous whitefish populations (*C. l. lavaretus*) were low and no clear conclusions can be made on their genetic structure based on the phylogenetic analysis or on the basis of Nei's  $D_A$  distances or  $F_{ST}$  values. Further studies with more loci are required to obtain more detailed estimates of this structure.

#### *Genetic diversity of different spawning time types within each population*

Sub-populations based on seasonal spawning-time differences can be identified within each river population of anadromous whitefish, and are known as summer and autumn whitefish. The spawning time is generally known to be a genetically determined trait

(SAKAMOTO et al. 1999), although actual genetic differences are unknown. Here, samples of summer- and autumn-spawning whitefish from three rivers in the Bothnian Bay area (the Rivers Iijoki, Kemijoki and Tornionjoki) were compared.

Statistically significant genetic differences could be observed between spawning-time types in two out of three cases: only Kemijoki autumn and summer samples did not differ in their allelic distribution. However, highly significant allelic distribution differences could be observed between the River Iijoki summer and autumn samples ( $p < 0.001$ ) and the River Tornionjoki summer and autumn samples ( $p < 0.01$ ). Part of the observed microsatellite variation was therefore correlated with the spawning-time differentiation, which thus confirms the genetic basis of spawning-time sub-structuring.

On average, summer samples had a higher allelic richness when compared to autumn samples from the corresponding rivers. The 95% confidence intervals of the heterozygosity estimates overlapped in all cases. Deficiency of heterozygosity was observed in Iijoki autumn, Kemijoki summer and Tornionjoki summer samples, which could indicate the seasonal sub-population structure.

#### *Magnitude of genetic changes potentially caused by hatchery breeding*

In this study, wild populations had a slightly higher allelic diversity than hatchery-reared populations, averaging one allele more per locus (mean allelic richness 9.81 and 8.80 respectively; *C. peled* populations excluded). However, the mean heterozygosity between wild and hatchery-reared populations did not differ as markedly ( $H_e$  0.84 and 0.81 respectively; *C. peled* populations excluded). Similar observations have also been reported in earlier studies (NORRIS et al. 1999, KOLJONEN et al. 2002, SÄISÄ et al. 2003). A bottleneck of short duration, as is often the case when founding new stocks in hatcheries, can reduce the number of alleles by eliminating rare alleles without having much effect on the level of heterozygosity (NORRIS et al. 1999). The loss of genetic variation due to a small effective population size could also be even larger if directed selection is adopted when picking out the founder individuals that will later be used as founders. Comparison of the wild and hatchery-reared populations of corresponding rivers revealed highly significant differences in allelic frequencies.

Despite the high level of maintained heterozygosity, the effective population size estimates ( $N_e$ ) of the hatchery broodstocks (33–122) were somewhat low (Table 5). The maximum likelihood-based estimator

used should give more accurate and precise estimates than the former variance-based estimators (WILLIAMSON and SLATKIN 1999, WANG 2001, WANG and WHITLOCK 2002). According to WANG and WHITLOCK (2002), the maximum likelihood (ML) based method is especially suitable for markers with rare alleles (less than 0.04 frequency), such as microsatellite loci. Here, the loci *C2-157* and *Cocl-23* had a large number of such rare alleles. Another advantage of the ML-based temporal method is that it is applicable to a wide range of population structures and migration patterns, since it does not make any assumptions about the mating system or genetic status of the populations studied.

The hatchery stock of Rautalampi plankton whitefish indicated a recent bottleneck. Also some other hatchery stocks have most likely gone through some reduction in population size. The state of the naturally reproductive *C. l. pallasii* population of Rautalampi waterway was evaluated to be endangered or vulnerable at the study of KALLIO-NYBERG and KOLJONEN (1988), and altogether 32 out of 181 registered Finnish whitefish stocks were classified with similar status (KALLIO-NYBERG and KOLJONEN 1988, 1990). Hatchery rearing can be an important aid in maintaining such endangered populations, although it includes risks such as loss of genetic diversity and adaptations to the hatchery environment instead of the wild.

In all, very extensive genetic differentiation was observed between the two *Coregonus* species, Scandinavian *Coregonus lavaretus* and Siberian *Coregonus peled*. The differentiation among the five studied *Coregonus lavaretus* ecotypes was clearly less, and it did not reach the species differentiation level. Especially for the four ecotypes *C. l. widegreni*, *C. l. lavaretus*, *C. l. nilssoni* and *C. l. pallasii* the genetic differentiation was also less than could be assumed for different preglacial phylogenetic lineages. The number of populations studied for each of the four ecotypes was not large, but if marked systematic differences would exist, they would have been observed. For the case of *C. l. fera* (northern, bottom-dwelling, sparsely-rakered whitefish), no conclusions can be drawn as a more comprehensive study on its phylogenetic status is needed. The differentiation among populations within ecotypes was generally low, and in some cases could be explained by bottlenecks or small population sizes. However, the genetic differentiation between spawning-run timing types was statistically significant in two out of three cases, confirming the genetic background of this form of adaptation. Hatchery rearing had in some cases decreased the allelic richness of the populations, but the general diversity levels were about the same. The estimated effective numbers of the

hatchery populations were in some cases somewhat under the recommended minimum values.

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