Microsatellite analysis of hatchery stocks and natural populations of Arctic charr, *Salvelinus alpinus*, from the Nordic region: implications for conservation

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Semi-automated fluorescent genotyping of eight polymorphic microsatellite loci was used to assess the level of genetic diversity and population differentiation in Nordic stocks of non-anadromous Arctic charr, *Salvelinus alpinus*, of both wild and hatchery origin. Highly significant genetic heterogeneity was detected globally across wild and or hatchery populations and even between populations from lakes separated by as little as six kilometres. The overall level of genetic differentiation among wild populations ($F_{ST} = 0.360$) was substantially higher than that observed between populations of anadromous Arctic charr in Canada using microsatellite data. Cavalli-Sforza and Edward's chord distance was used to construct a neighbour-joining tree and three population clusters were supported with relatively high bootstrap values which included the populations from north-west, north-east and southern Finland respectively. Use of Paetkau et al's individual assignment test further supported the strong differentiation of most populations as well as their classification into the three predicted geographical areas. No significant difference in average allele number or heterozygosity was observed between populations of wild and hatchery origin. However, the effects of hatchery rearing were revealed by a strong increase in the number of deviations from Hardy-Weinberg equilibrium as well as of linkage disequilibrium events in the hatchery stocks compared to natural populations. The population from Lake Saimaa, in south-east Finland, is completely reliant upon aquaculture assistance for its survival. The broodstocks of this population exhibited particularly low levels of genetic variability. Although the hatchery stocks of this population suffer from increased egg and alevin mortality and disease susceptibility, it remains to be determined if this is due directly to a lack of genetic variation as some abundant unstocked natural populations possessed similarly low levels of microsatellite variability.

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Fish species of the family Salmonidae are highly valued due to their importance as a food source and also for sports fishing. With such an importance to humans, it is not surprising that the exploitation of this natural resource has been to the detriment of many wild salmonid populations (e.g., MAITLAND 1995; RYMAN et al. 1995; ALLENDORF and WAPLES 1996). Such heavy human exploitation poses a unique challenge for those interested in the conservation of salmonid, and indeed other, fish species: how can intraspecific genetic diversity be maintained in intensely harvested populations? In addition, salmonids exhibit a complex and diverse range of life histories at the inter- and intraspecific level (e.g., BALON 1980; TAYLOR 1991). Such ecological diversity further complicates the development of a general set of guidelines for salmonid conservation and virtually necessitates population by population evaluation.

The Arctic charr, *Salvelinus alpinus*, exemplifies the level of intraspecific diversity observed in salmonid fishes (JOHNSON 1980). Not only do anadromous and non-anadromous forms occur, but also various 'morphotypes' (morphs), which can be distinguished according to size and growth rate (JOHNSON 1980), or according to food niche segregation (SKULASON et al. 1993). The sympatric occurrence of several morphs is not uncommon, with up to four different morphs known to occur in a single lake (SANDLUND et al. 1987; SKULASON et al. 1993). As with other salmonid species, populations of Arctic charr have been signficantly affected by human activities with nine of the sixteen countries possessing indigenous Arctic charr stocks reporting the extinction of at least some of these populations (MAITLAND 1995).

Previous genetic studies of Arctic charr populations have produced mixed results. Earlier studies using allozymes (e.g., FERGUSON 1981; OSINOV and PAVLOV 1998), mtDNA RFLPs (DANZMANN et al. 1991; BRUNNER et al. 1998) mtDNA sequencing (VOLPE and FERGUSON 1996; WILSON et al. 1996)

and minisatellite fingerprinting (VOLPE and FERGUSON 1996) revealed little variation and therefore differentiation between populations and or morphs was difficult to detect. Recently however, several studies have revealed high levels of microsatellite variation in populations of Arctic charr from Canada and the Alpine region in central Europe (BERNATCHEZ et al. 1998; BRUNNER et al. 1998) which have enabled the identification of genetically distinct populations situated as little as 10 km apart (BERNATCHEZ et al. 1998).

Countries of the Nordic region contain about 84 % of the world's indigenous Arctic charr stocks (MAITLAND 1995). However, despite extensive surveys, very little information is available as to the genetic structuring of populations in this region due to the limits of detecting genetic variation with more traditional molecular genetic markers mentioned above (ANDERSSON et al. 1983; HINDAR et al. 1986). Detailed information about the charr stocks of specific countries are often difficult to come by. Finland however, has compiled a register of the main Arctic charr stocks in the country (KALLIO-NYBERG and KOLJONEN 1991). This register lists only 40 % of all stocks as being safe from the threat of extinction. The status of populations of the large, fast-growing morph, most prized by anglers, was particularly alarming with three of the six stocks listed as endangered. For example, the Lake Saimaa stock of large morph Arctic charr in south-east Finland is completely reliant on aquaculture for its existence (MAKKONEN 1997). In response to these population declines, the Finnish Game and Fisheries Research Institute (FGFRI) has initiated broodstock rearing programs and hatchery propagation of endangered Arctic charr stocks (PIIRONEN and HEINIMAA 1998). The main aim of these programs is to maintain the original fish stocks and their biodiversity by means of broodstock cultivation and stocking. For successful hatchery management, it is extremely important to monitor the genetic variability of these stocks and how this variation is maintained during hatchery rearing.

Given the vulnerable state of many of these populations, microsatellite analysis would appear to be the marker of choice for genetic analyses not only due to the increased levels of polymorphism detected compared to other marker types, but also because samples can be taken without harming the fish. In this study we present results from the analysis of 320 individuals, originating from 11 non-anadromous populations in Finland, Norway and Sweden, with eight microsatellite loci. The aims were 1—development of a multicolour fluorescent based genotyping system enabling rapid assessment of the level of genetic diversity in hatchery broodstocks used for supporting endangered natural populations in Finland, 2—comparison of the diversity levels observed in hatchery and natural populations and 3—identification of populations which could be considered as distinct units suitable for practical conservation management.

MATERIALS AND METHODS

Samples

Samples were collected during 1997–1998 from hatcheries or from the wild (Table 1; Fig. 1). Eight stocks were classified as being of true hatchery origin on the basis that they were at least F1 generation hatchery stocks (Table 1). Four populations, originating from presumed unstocked lakes (Table 1), were classed as 'wild'. Following comparisons between 'hatchery' and 'wild' populations are therefore based on these stocks and populations. For the majority of samples, DNA was chelex extracted from approx. 1 mm^3 of adipose/dorsal fin according to the method of ESTOUP et al. (1996). For a small number of samples, DNA was chelex extracted from liver or muscle tissue which had been stored at $-20°C$ for up to five years.

Semi-automated fluorescent microsatellite analysis

A total of 56 pairs of primers, isolated from seven salmonid species, were tested for their ability to amplify a homologous product in Arctic charr. Eight loci were chosen for analysis of the entire material (Table 2). Four of these, *Sfo8*, *23*; *Cocl3*; *MST85*, had been used in previous studies of Arctic charr (BERNATCHEZ et al. 1998; BRUNNER et al. 1998), three had been identified as polymorphic in Arctic charr (*Ssa197*; *μ60*; *SSOSL85*, (BRUNNER et al. 1998) but not investigated further, plus an additional marker, *Ssa14*, which we identified to be polymorphic. The general protocol for 10 μl PCR reactions was as follows: 1 % of the chelex extracted DNA solution (see above), 3–6 pmol of each primer, 200 μM dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.25/0.5 U AmpliTaq/AmpliTaq Gold DNA polymerase (Perkin Elmer). All reactions were carried out on either PTC100, PTC200 (MJ Research) or Mastercycler gradient (Eppendorf) thermal cyclers. The general PCR profile for most loci was as follows: 94°C for 3 min, followed by 30–35 cycles of 94°C for 60 s, x°C (see Table 2) for 60 s, 72°C for 60 s, with a final 72°C extension of 5 min. For several markers, a touchdown PCR protocol was used which consisted of 94°C for 10 min, x°C (Table 2) for 60 s, 72°C for 60 s, followed by 20 cycles of 94°C for 30 s, x°C for 30 s, 72°C for 30 s, with the

annealing temperature decreasing 0.5°C per cycle followed by 15 cycles of 94°C for 30 s, (x-10)°C for 30 s and 72°C for 30 s and a final 72°C extension of 5 min. All loci were PCR amplified separately.

Based on a preliminary evaluation of genetic diversity, the eight markers were divided into two groups and end-labelled fluorescent primers (FAM, HEX or NED) ordered so as to enable co-migration of each marker 'panel' in a single gel lane i.e., within each panel, loci labelled with the same dye colour had non-overlapping size ranges (Table 2). In order to produce signals of relatively even intensity in each panel, varying volumes of each locus PCR product were pooled (Table 2) along with a loading buffer/size standard mix (400HD; Perkin Elmer). Samples were then electrophoresed using an ABI377 sequencer, according to the recommended guidelines (http://www2.perkin-elmer.com/ab/about/dna/377/377str.html) using filter set D. Genotypes were scored with the aid of the Genotyper 2.0 program (Perkin Elmer) using locus specific macros made by CRP, followed by manual corrections and then exported into a spreadsheet program for further statistical analyses (see below).

Genetic diversity analyses

Genetic diversity indices, including number of observed alleles (A), observed number of heterozygotes (H_O) and expected level of gene diversity (H_E), were calculated using GENEPOP3.1b (RAYMOND and ROUSSET 1995). Differences in mean genetic diversity indices between hatchery and wild populations were assessed using a nested ANOVA with logarithmic transformation of the data. For the test on allele number, we adjusted to a common sample size (that of the largest population analysed) using the formula of Ewens (1972). GENEPOP3.1b was used to conduct exact tests for deviations from Hardy-Weinberg (H-W) equilibrium and for genotypic linkage. Corrections for multiple significance tests were performed using Fisher's method as computed in GENEPOP3.1b and applying a sequential Bonferroni type correction (RICE 1989).

Table 1. *Details of stocks and populations examined in this study*

Location	Abbreviation	Latitude	Longitude	Description
Pihtsosjärvi	PIH	69°14′	21°17′	Wild caught fish maintained in the hatchery for 4 years. 30 % mortality during this period
Somasjärvi	SOM	69°17′	21°33′	Wild caught fish maintained in the hatchery for 5 years. 73 % mortality during this period
Toskaljärvi	TOS	69°11′	21°28′	Wild caught fish maintained in the hatchery for 4 years. 61 % mortality during this period
Hornavan	HOR	66°13′	17°46′	At least 3rd generation hatchery stock. No information on number of founders available.
Haukejavri	HAU	69°57′	29°14′	Wild caught fish from a presumed unstocked lake.
Buevattnet	BUE	70°37′	30°05′	Wild caught fish from a presumed unstocked lake.
Lisma	LIS	70°08′	28°04′	Wild caught fish from a presumed unstocked lake.
Inari	INA-L	69°00′	28°00′	Large morph. Descendants of several broodstocks initiated in the late 1960's with occasional addition of wild individuals.
	INA-W			Large morph. Wild caught fish from the lake stocked with fish produced by INA-L.
	INA-S			Small morph: 1st generation hatchery stock. Founders taken from several different spawning sites.
Saimaa	SAI-85	61°15′	27°35′	Large morph: 1st generation hatchery stock produced from wild caught fish. Includes offspring from 2 broodstocks
	SAI-91			Large morph: 1st generation hatchery stock produced from wild caught fish and SAI-85 offspring.
	SAI-92			Large morph: 1st generation hatchery stock produced from wild caught fish and SAI-85 offspring.
	SAI-93			Large morph: 1st generation hatchery stock produced from wild caught fish.
	SAI-94			Large morph: 1st generation hatchery stock produced from wild caught fish and SAI-85 offspring.
	SAI-W			Large morph. Wild caught fish from the lake stocked with fish produced by SAI-85-94[1].
Riasten	RIA	62°51′	11°46′	Wild caught fish from a presumed unstocked lake.
Germany, Königssee	GER	47°30′	12°57′	History unknown. From the Danube drainage system.

[1] Taking into account the generation time of arctic charr, these wild fish are most likely of natural origin.

Genetic differentiation among populations

Tests for significant genic differentiation among population pairs or larger groups of populations were made using exact tests for genetic heterogeneity in GENEPOP 3.1b. Corrections for multiple significance tests were performed using Fisher's method as computed in GENEPOP 3.1b and applying a sequential Bonferroni type correction (RICE 1989). For those cases in which significant genic differentiation was observed, Wright's F-statistics were estimated in order to measure the extent of genetic differentiation, using the classical estimator Θ of WEIR and COCKERHAM (1984) or by taking into account the difference between allelic size using the estimator ρ_{ST} of ROUSSET (1996). The relationships and differences between these statistics are reviewed in ROUSSET (1996) and ESTOUP and ANGERS (1998). These values were estimated globally over all populations and also for the groups of populations defined as natural and of hatchery origin (see above). Tests for multilocus differences between F_{ST} and ρ_{ST} for the same population groups were assessed using a Wilcoxon signed rank test.

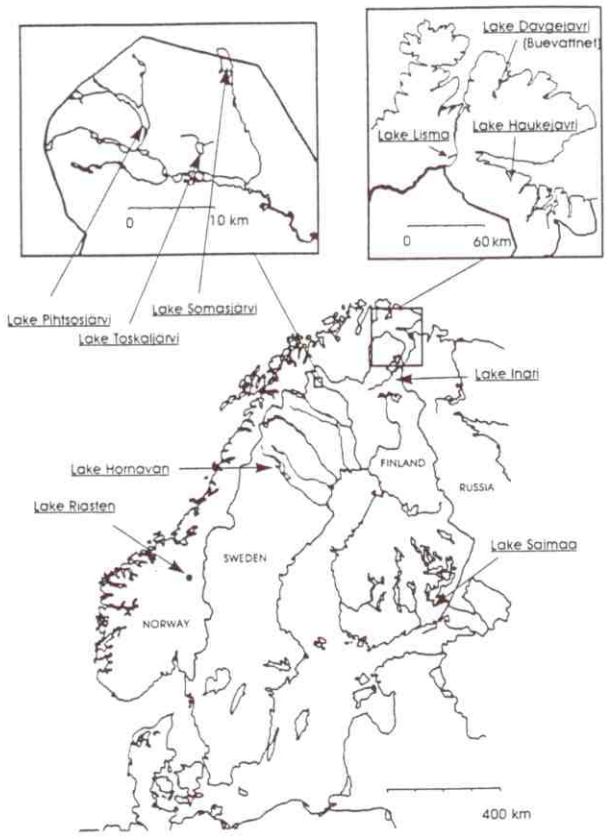

Fig. 1. Map showing the locations of Arctic charr populations from the Nordic region included in this study. All hatchery stocks are kept in facilities within 150 km of their origin except for the Hornaven (HOR) stock which is maintained in the Taivalkoski hatchery in central Finland.

Genetic affinities among populations and individual fish

Neighbor-Joining (NJ) trees relating the natural and domestic samples were constructed using the chord distance of CAVALLI-SFORZA and EDWARDS (1967) and the D_A distance of NEI et al. (1983). These two distances have been shown to have a higher probability of producing trees with a correct topology than many other distances, regardless of the mutational model of the markers (TAKEZAKI and NEI 1996). As it is suspected that the mutation pattern of microsatellite sequences nearly follows a stepwise mutation model (SMM, OHTA and KIMURA 1973), a NJ tree was also constructed from microsatellite data using the genetic distance based on allele size differences of GOLDSTEIN et al. (1995). Bootstrap values were computed by resampling loci and are given as percentages over 2000 replications (HEDGES 1992). In order to study the genetic affinities among individuals, we used an assignment method of individual fish to the set of sampled populations based on individual genotypes and population allele frequencies (PAETKAU et al. 1995). This method assigns an individual to the population in which its multilocus genotype has the highest probability of occurring, assuming Hardy-Weinberg equilibrium and linkage equilibrium in all locus-population combinations. All NJ trees and assignment computations were processed using personal programs.

RESULTS

Cross species amplification and genetic diversity

Of the 56 salmonid microsatellites tested, 35 amplified a specific product in Arctic charr. Eight loci, which were polymorphic in at least some of the populations tested, could be co-electrophoresed in two 'panels' of markers on an ABI377 semi-automated sequencer (Table 2). The total number of alleles per locus varied from 4 to 56, and the average heterozygosity level across all sampled populations and broodstocks was between 0.05 and 0.73 (Table 2; Appendix A). Genotype frequencies at a ninth highly polymorphic locus, *Ssa289*, appeared to deviate significantly from H-W equilibrium in a number of populations, all such cases being due to heterozygote deficiency. A situation whereby heterozygote deficiency at a microsatellite locus is observed across a number of populations may suggest the presence of a null allele (PEMBERTON et al. 1995, BROOKFIELD 1996). Using formula (4) from BROOKFIELD (1996), the potential null allele frequency at *Ssa289* in the four natural populations was calculated to range from 0.06–0.29. This locus was excluded from further

analyses. Genetic diversity indices for the remaining eight markers in each population are given in Appendix A. Details of allele frequencies in each population are available on request from the corresponding author.

H-W and linkage disequilibrium in natural populations

In the four natural populations included in the study, three deviations from H-W equilibrium, significant at the 5% level, were observed in 25 locus/population comparisons (Table 3). Each of these results occurred in a different population and with a different marker (Appendix A) and none were significant after using a Bonferroni-type correction for multiple tests. Multiple probability tests at each locus and over populations (Fisher's method) revealed one locus (*Sfo23*) and one population (Buevattnet) which deviated from H-W equilibrium across populations/loci at the 5% level (Appendix A) however neither were significant after correcting for multiple tests. No significant genetic disequilibrium events were observed in any locus/population combinations ($P > 0.07$) nor for any multiple probability tests across populations (Fisher's method, $P > 0.19$).

Hatchery vs. wild population genetic diversity

When considering natural populations compared to hatchery stocks as a whole, no significant difference in average allele number (corrected for sample size) was observed (nested ANOVA, $F_{1,10} = 1.4$ $P = 0.24$), nor were there any differences in average observed heterozygosity ($F_{1,10} = 0.58$ $P = 0.45$). In contrast to the natural populations however, a number of significant deviations from H-W equilibrium were observed in the hatchery stocks (Table 3, Appendix A). In addition, of 51 possible locus/hatchery stock exact tests, 17 deviations from H-W equilibrium, significant at the 5% level, were observed (only 2.5 are expected due to type I error). After correction for multiple tests, five remained significant. Multiple probability tests across loci for each population indicated that there were significant deviations from H-W equilibrium at five of the eight hatchery stocks (three after

Table 2. *Summary of the salmonid microsatellite loci used in this study and their analysis conditions. N—number of individuals successfully analysed. Total A—total number of alleles observed at the locus and Ave H_O—average observed heterozygosity level across populations/broodstocks*

Locus[a,b]	Species of origin	Fluoro. Label	Primer amount (pmol)	Anneal. Temp. (°C)	Marker 'panel'	Loading volume (µl)	Size Range (bp)	N	Total A	Ave. H_o
SSOSL85[1]	*Salmo salar*	FAM	4	55	1	0.250	171[c], 224–280	334	19	0.63
Cocl3[2]	*Coregonus clupeaformis*	HEX	6	50	1	0.375	223–281	325	27	0.69
Ssa14[3]	*Salmo salar*	HEX	6	58	1	0.250	148–166	336	7	0.18
Ssa197[4]	*Salmo salar*	HEX	3	55	1	0.125	121–129	327	4	0.05
MST85[5]	*Salmo trutta*	NED	3	55	1	0.500	183–275	327	33	0.68
Sfo8[6]	*Salvelinus fontinalis*	FAM	3	60	2	0.375	263–315	329	26	0.68
Sfo23[6]	*Salvelinus fontinalis*	HEX	6	55[d]	2	0.500	139–269	333	56	0.73
MST60[7]	*Salmo trutta*	NED	6	60[d]	2	0.375	161–233	331	24	0.65
Ssa289[3,e]	*Salmo salar*	FAM	3	53	2	0.250	176–230	328	24	0.50

[a] Microsatellite references: 1—SLETTAN et al. 1995, 2—BERNATCHEZ 1996, 3—MCCONNELL et al. 1995, 4—O'REILLY et al. 1996, 5—Presa and Guyomard 1996, 6—ANGERS et al. 1995, 7—ESTOUP et al. 1993.

[b] In order to enhance 3' adenylation (BROWNSTEIN et al. 1996), a GTTT 'PIGtail' sequence was added to the 5' end of the non-labelled primer for all primer pairs except *MST85* and *Sfo23*.

[c] Primers co-amplify a monomorphic fragment 171bp in length in addition to the polymorphic locus.

[d] Touchdown PCR protocol (see Methods).

[e] Due to the possible presence of a null allele in some populations, not used in further analyses.

Table 3. *Number of effective founders (N_{ef}) and average genetic diversity indices at eight microsatellite loci for hatchery broodstocks and natural populations with no known stocking history*

	Hatchery broodstocks								Wild populations			
	SAI-85	SAI-91	SAI-92	SAI-93	SAI-94	INA-L	INA-S	HOR	RIA	HAU	BUE	LIS
N_{ef}^{a}	3.4	5.1	4.8	6.9	≈ 12	≈ 30	48	?	–	–	–	–
Mean A	3.5	3.0	2.9	2.4	2.6	6.3	8.1	5.6	4.3	3.1	10.0	4.4
Mean H_e	0.72	0.50	0.50	0.42	0.52	0.59	0.68	0.57	0.45	0.43	0.62	0.51
Mean GD	0.50	0.42	0.42	0.36	0.45	0.60	0.68	0.55	0.43	0.40	0.71	0.51
H-W deviations[b]	6 6[d]	1 6	0 6	2 5	3 6	2 7	1 7	2 8	1 6	1 5	1/8	0/6
L D[c]	9 20[d]	2 15	2 15	1 14	1 15	2 21	0 20	2 21	0 14	0 15	0/21	0 14

[a] The maximum number of effective founders, based on hatchery records. The actual number of founders is likely to be lower due to variation in reproductive success between individuals.
[b] Single locus deviations from Hardy-Weinberg equilibrium.
[c] Pairwise linkage disequilibrium.
[d] Number of linkage disequilibrium events possibly elevated due to the combination of two broodstocks.

a Bonferroni-type correction). Similarly in contrast to the natural populations, many genotypic disequilibrium events were observed in the hatchery stocks i.e., 21 in 148 pairwise locus tests. Two of these were significant following a Bonferroni-type correction. As has been noted and discussed earlier (ESTOUP et al. 1998), significant disequilibria most often involved markers with the highest polymorphism levels, especially the highly polymorphic *Sfo23* which was involved in 10 of the 21 significant events.

Level of differentiation and genetic affinities among populations

Highly significant genetic heterogeneity was detected across all populations and also when considering the wild and hatchery populations as separate groups (Fisher's test $P < 1 \times 10^{-5}$). The global level of differentiation, as calculated by F_{ST} and ρ_{ST}, was similarly high using both measures for wild and hatchery populations as well as over all populations (Table 4). There were no significant differences between the two differentiation estimators for any group of populations (Wilcoxon signed rank test, all P values > 0.72). Pairwise D_{CE} distances are given in Appendix B. The distance of GOLDSTEIN et al. (1995), which takes into account allele size differences, produced a meaningless NJ tree topology with only two of 15 nodes with bootstrap values greater than 30 % (tree not shown) and is therefore not discussed further. A more robust topology was obtained using the classical distance of CAVALLI-SFORZA and EDWARDS (1967) (Fig. 2). Although some internal nodes are associated with low bootstrap values, three main clusters were supported by relatively high (53 %) to high (99 and 100 %) bootstrap values: north-east Finland populations (Inari-L, -S and-W), north-west Finland populations (Pihtsosjärvi, Somasjärvi, Toskaljärvi) and the hatchery and wild stocks from south-east Finland (Saimaa-85, -91-94, -W). Similar branching pattern and bootstrap values with respect to these three clusters were produced using Nei's D_A distance (tree not shown). It is interesting to note that the Saimaa hatchery and wild stocks from south-east Finland appear to show more genetic similarities with the Riasten population from southern Norway (bootstrap value of 82 %) than with any of the northern populations. In both cases where we were able to obtain samples of wild caught fish from lakes with stocking programs (Inari-W and Saimaa-W), the wild fish population clustered with high bootstrap values with the hatchery stocks from the same lake and morphotype, i.e. Inari-W with Inari-L and Saimaa-W with Saimaa-85-94.

Genetic affinities among individuals

Assigning individuals to populations based on the probability of an individual's multilocus genotype arising from a population with known allele frequencies has proved to have a wide range of applications not only in population genetics, but also forensics, conservation genetics and stock management (reviewed in WASER and STROBECK (1998)). Individual assignment can also give an indication of the level of population differentiation and, to a lesser extent, of their genetic relationships (PAETKAU et al. 1995; NIELSEN et al. 1997; ESTOUP et al. 1998). Over 80 % (257 out of 320) of individuals were assigned to their correct population using the individual assignment test developed by PAETKAU et al. (1995). 100 % assignment success was achieved for seven population groups (Saimaa-W, Germany, Haukejavri, Lisma, Pihtsosjärvi, Riasten, Toskaljärvi) with 80 % of individuals correctly assigned for an additional six populations (Saimaa-85, -91, -93, Hornavan, Inari-W,

Table 4. *Population differentiation estimates based on global F_{ST} and ρ_{ST}*

Locus	F_{ST}			ρ_{ST}		
	Wild	Hatchery	All	Wild	Hatchery	All
SSOSL85	0.472	0.296	0.317	0.599	0.361	0.375
Cocl3	0.385	0.286	0.336	0.275	0.354	0.400
Ssa14	0.627	0.739	0.742	0.803	0.493	0.591
Ssa197	0.022[a]	0.033	0.105	0.022[a]	0.042	0.125
MST85	0.364	0.340	0.348	0.057	0.577	0.621
Sfo8	0.393	0.275	0.309	0.271	0.197	0.218
Sfo23	0.100	0.235	0.284	0.384	0.270	0.653
MST60	0.274	0.178	0.305	0.255	0.131	0.155
Over all loci	0.360	0.317	0.361	0.336	0.385	0.531

[a] No significant genic differentiation detected between these populations with this locus.

Somasjarvi). The worst assignment success was for the small and large morphs from lake Inari (Inari-L and Inari-S; 27 % and 36 % respectively). In both these cases, the vast majority of incorrect assignments were due to individuals being classified in Inari-W, the group of wild caught large-morph fish from the same lake. Individual classification was in good agreement with a larger scale grouping of populations into the three distinct clades observed in NJ trees with 313 320 individuals (98 %) correctly assigned to a population from one of these clades.

DISCUSSION

Population differentiation at different geographic scales and implications for stock management

Neighbour-joining trees constructed using the D_{CE} genetic distance revealed three population clusters with high bootstrap support that are in general agreement with the geographic distribution of the populations: north-west Finland, north-east Finland and south Finland/Norway. Previous studies of anadromous Arctic charr microsatellite diversity have revealed significant genic differentiation between populations separated by as little as 10 km (BERNATCHEZ et al. 1998). Similarly in this study, there was significant genic differentiation between the three populations from north-east Finland (Pihtsosjärvi, Somasjärvi, Toskaljärvi; $P < 1 \times 10^{-5}$). The maximum distance between these lakes is 11 km (Pihtsosjärvi-Somasjärvi) and the minimum six (Somasjärvi-Toskaljärvi). The level of differentiation between these populations is further evidenced by the assignment of individual multilocus genotypes to populations (PAETKAU et al. 1995). All individuals but one (98 %) from north-west Finland were indeed assigned to the correct population. On a broader geographical scale, the level of genetic differentiation observed between the non-anadromous natural populations examined in this study (global $F_{ST} = 0.360$) was considerably greater than that observed for anadromous Arctic charr populations from Labrador and Newfoundland, Canada (global $F_{ST} = 0.059$; BERNATCHEZ et al. 1998). This may reflect a general difference in the level of genetic differentiation between anadromous and non-anadromous fishes (WARD et al. 1994).

One of the primary aims of stocking programs in Finland has been to maintain the genetic integrity of the respective populations by only stocking fish produced from lake specific broodstocks (PIIRONEN and

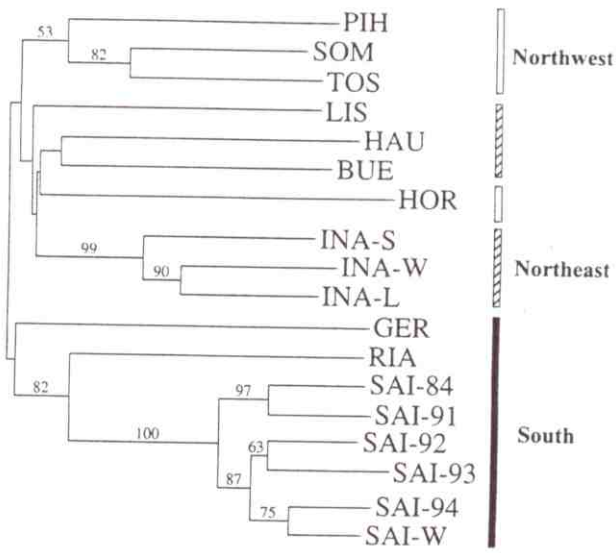

Fig. 2. Neighbour-joining phenogram, based on Cavalli-Sforza and Edward's chord distance, showing the genic relationships among populations and stocks of Arctic charr populations from the Nordic region and one population from Germany. Values along branches represent bootstrap values in percent. Nodes lacking values were supported by less than 50 % of bootstrap replicates. Black, hatched and white bars indicate the approximate geographic location of the populations.

HEINIMAA 1998). Several lines of evidence would suggest that this practice has succeeded. Firstly, the clear differentiation between hatchery broodstocks (e.g., Saimaa, Inari-L and HOR; Fig. 2) indicates that little or no population introgression has occurred, as opposed to the levels observed in alpine charr populations where stock transfer is known to have taken place (BRUNNER et al. 1998). Secondly, in the two lakes with stocking programs from which we were able to obtain wild caught samples (Inari-W and Saimaa-W), both 'populations' grouped most closely (and with high bootstrap support) with the hatchery broodstock of the same lake. It could be argued that the high level of differentiation of the Saimaa stocks from other populations may have arisen simply due to the low number of founding individuals in hatchery increasing the effect of genetic drift. However broodstocks from several years have been independently produced using different wild-caught individuals (all released fish are marked and can therefore be identified if they return to spawn) and all year classes cluster together with 100% bootstrap support, therefore suggesting 'true' differentiation of this population from the others in this study. Interestingly, the Saimaa population appears to show a closer genetic affinity to a population from southern Norway (Riasten) than to any of the other populations in this study. Although further investigations are required, north-south clinal gradients have been observed in a number of other species in the Nordic region (e.g., MERILÄ et al. 1996) and may also be occurring in Arctic charr.

Effects of hatchery stock composition and management on genetic patterns

Previous comparisons of genetic diversity between hatchery stocks and wild populations have revealed significant differences in genetic diversity and equilibrium indices such as allele number (TESSIER et al. 1997), allele frequencies (NYMAN and RING 1989; TESSIER et al. 1997; CLIFFORD et al. 1998) and heterozygosity (NYMAN and RING 1989; CLIFFORD et al. 1998). When comparing the overall level of diversity in hatchery stocks as a whole to that in the natural populations examined here, no significant differences in the average number of alleles per locus nor average H_O were observed. This was partly due to the high level of variation observed in some hatchery stocks with higher numbers of founding individuals (e.g., Inari-S; Table 3). The effects of hatchery production were however revealed in other ways when compared to non-stocked natural populations in the form of an increased occurrence of H-W and linkage disequilibrium events in hatchery stocks than in natural populations (17 vs. 3 and 21 vs. 0, respectively).

Taking into account the history of hatchery stocks proved to be very useful for explaining the observed levels of genetic diversity and the frequency of disequilibrium events in several of the hatchery broodstocks. For example, the highest average number of alleles observed in the hatchery stocks was in Inari-S, the broodstock with the highest number of effective founders (N_{ef}) and also known to have been initiated using fish from several different spawning sites which may have represented different gene pools. In addition, over one third of the H-W and genetic disequilibrium events occurred in a single broodstock (Saimaa-85). This can perhaps be explained by the fact that this stock actually comprises a combination of two broodstocks produced independently in 1985 and 1986, each with a low N_{ef}. Therefore, the increased number of disequilibrium events compared to other hatchery stocks may be the result of a Wahlund-type effect (HARTL and CLARK 1989).

Sustainable management of exploited populations: Lake Saimaa charr as an example

Aquaculture plays an important role in the maintenance of several Arctic charr stocks analysed in this study (e.g., MAKKONEN 1997). The value of aquaculture and stock supplementation as a tool for the conservation of aquatic species is currently debated (RYMAN and LAIKRE 1991; THORNHILL 1993, WAPLES and DO 1994; ALLENDORF and WAPLES 1996). In some cases however, when a population is fished to near extinction with little or no reproduction in the wild, aquaculture supplementation is perhaps the only means to maintain a viable population. An obvious alternative would be to prohibit fishing until the population in question recovered. This is however a politically sensitive issue. An example of the former scenario is the Arctic charr population of Lake Saimaa in south-east Finland where very few wild spawners could be caught for producing annual broodstocks (MAKKONEN and NURMIO 1997). Such a situation creates quite a dilemma: should the effective number of founders be increased by using fish from other lakes, and therefore risk the loss of habitat/population specific gene combinations? Or should the genetic integrity of the population be maintained, but risk the loss of genetic variation? In the case of the Saimaa population, the decision was made to risk the loss of genetic variation in order to maintain genetic integrity. In contrast to this procedure, broodstock production of two morphotypes from the same lake in north-east Finland was initiated while the respec-

tive natural populations were still relatively abundant (Inari-L and Inari-S). It has previously been noted that Saimaa charr appeared to have a lower level of genetic diversity than many alpine populations of Arctic charr (BRUNNER et al. 1998), a feature supported by the results of this study. This was particularly evident when considering those loci with the highest overall level of variation such as *Sfo23*, with only two allelic variants commonly observed across Saimaa broodstocks, compared to up to 14 and 23 observed alleles in other hatchery and wild populations, respectively (Appendix A). In addition, hatchery records reveal a number of life history features which raise concerns about the viability of the Saimaa broodstocks. These include low fertilisation success, a high level of egg and alevin mortality and vulnerability to some bacterial infections and eye disease in adult fish (e.g., PYLKKÖ et al. 1996). Reduced fitness is commonly stated as a potential result of lack of genetic variation (e.g., FRANKHAM 1995) however concluding that reduced fitness in Saimaa charr is directly due to loss of genetic variation would be premature for several reasons. Firstly, it appears that the level of genetic variation can vary markedly between unstocked populations. This is evidenced most dramatically when considering the Haukejavri and Buevattnet populations from the north-west Norway Tana Fjord region (Appendix A). Although situated less than 80 km apart, the average number of alleles per locus was over three times higher in Buevattnet (3.1 vs. 10) and average gene diversities also differed markedly (0.40 vs. 0.71). Secondly, environmental factors specific to the Saimaa region, such as temperature, should also be considered before making conclusions about the effect of low genetic variability. Egg mortality has been shown to increase significantly if the water temperature is increased by just two or three degrees (GILLET 1991). In addition, the mortality of adult fish at the Saimaa hatchery is several times higher in the warmest month of the year compared to the coldest (JP unpub. data). Thirdly, a much lower level of egg and alevin mortality was observed in eggs of the Saimaa-93 broodstock which were transferred to a hatchery 600 km north of Lake Saimaa (and therefore a lower water temperature than the Saimaa hatchery).

Although it remains to be determined if a low level of genetic variation has been detrimental to the Lake Saimaa charr population, it is clear that the low N_{ef} in all broodstock year classes has affected their genetic structure, with the majority of deviations from H-W and linkage equilibrium occurring in these stocks (Table 3). This would suggest that a significant increase in the N_{ef} of future broodstocks is warranted. Due to the poor status of the natural Saimaa population, this would necessitate the inclusion of individuals from other lake systems. As has been discussed earlier, the line between inbreeding and outbreeding depression is very unclear in salmonid populations (ALLENDORF and WAPLES 1996). Of utmost priority is therefore the identification of an appropriate 'sister population' as genetically similar as possible to the Saimaa population and from a lake with similar ecological characteristics. Clearly, none of the other populations in this study fulfil these requirements and additional populations need to be studied. Perhaps the most promising areas to explore in this respect are the Arctic charr populations from lakes in Russian Karelia such as Lakes Ladoga and Onega. These, and surrounding smaller lakes, are thought to have been connected with Lake Saimaa for a period after the last ice age (DONNER 1995) and still contain a number of abundant Arctic charr populations (Alexei Veselov, Karelia Research Centre, pers. comm.).

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REFERENCES

Allendorf FW and Waples RS, (1996). Conservation and genetics of salmonid fishes. In: Conservation genetics: case histories from nature (eds Avise and Hamric), Chapman & Hall, NY, pp. 238–280.

Andersson L, Ryman N and Ståhl G, (1983). Protein loci in the Arctic charr, Salvelinus alpinus L.: electrophoretic expression and genetic variability patterns. J. Fish Biol. 23: 75–94.

Angers B, Bernatchez L, Angers A and Desgrosseillers L, (1995). Specific microsatellite DNA loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish Biol. (Suppl. A) 47: 177–185.

Balon EK, (1980). Charrs: Salmonid fishes of the genus Salvelinus. Dr. W. Junk, The Hague.

Bernatchez L, (1996). Résau de suivi environnemental du complex la grande. Caractérisation génétique des formes naines et normales de grande corégone du réservoir Caniapiscau et du lac Sérigny à l'aide de marqueurs microsatellites. Rapport présenté par l'universite Laval à la vice-présidence Environnement et Collectivites d'Hydro-Québec.

Bernatchez L, Dempson JB and Martin S, (1998). Microsatellite gene diversity analysis in anadromous arctic

char, Salvelinus alpinus, from Labrador, Canada. Can. J. Fish. Aquat. Sci. 55: 1264–1272.

Brookfield JFY, (1996). A simple new method for estimating null allele frequency from heterozygote deficiency. Mol. Ecol. 5: 453–455.

Brownstein MJ, Carpten JD and Smith JR, (1996). Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. BioTechniques 20: 1004–1010.

Brunner PC, Douglas MR and Bernatchez L, (1998). Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr Salvelinus alpinus (Teleostei: Salmonidae) from central alpine lakes. Mol. Ecol. 7: 209–223.

Cavalli-Sforza LL and Edwards AWF, (1967). Phylogenetic analysis: models and estimation procedures. Am. J. Hum. Genet. 19: 233–257.

Clifford SL, McGinnity P and Ferguson A, (1998). Genetic changes in an Atlantic salmon population resulting from escaped juvenile farm salmon. J. Fish Biol. 52: 118–127.

Danzmann RG, Ferguson MM, Skulason S, Snorrason SS and Noakes DLG, (1991). Mitochondrial DNA diversity among four sympatric morphs of Arctic charr, Salvelinus alpinus, L., from Thingvallavatn, Iceland. J. of Fish Biol. 39: 649–659.

Donner J, (1995). The quarternary history of Scandinavia. Cambridge University Press, Cambridge.

Estoup A and Angers B, (1998). Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations. In: Advances in Molecular Ecology, (ed. G. Carvalho), IOS Press, pp. 55–86.

Estoup A, Presa P, Krieg F, Vaiman D and Guyomard R, (1993). $(CT)_n$ and $(GT)_n$ microsatellites: a new class of genetic markers for Salmo trutta L. (brown trout). Heredity 71: 488–496.

Estoup A, Largiader CR, Perrot E and Chourrout D, (1996). Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol. Biotech. 5: 295–298.

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

Ewens WJ, (1972). The sampling theory of selectively neutral alleles. Theor. Pop. Biol. 3: 87–112.

Ferguson A, (1981). Systematics of Irish charr as indicated by electrophoretic analysis of tissue proteins. Biochem. Syst. Ecol. 9: 225–232.

Frankham R, (1995). Conservation genetics. Annu. Rev. Genetics 29: 305–327.

Gillet C, (1991). Egg production in Arctic charr (Salvelinus alpinus L.) brood stock: effects of temperature on the timing of spawning and the quality of eggs. Aquat. Living Resour. 4: 109–116.

Goldstein DB, Linares AR, Feldman MW and Cavalli-Sforza LL, (1995). Genetic absolute dating based on microsatellites and the origin of modern humans. Proc. Nat. Acad. Sci. USA 92: 6723–6727.

Hartl DL and Clark AG, (1989) Principles of population genetics, second edition. Sinaur Associates, Sunderland.

Hedges SB, (1992). The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. Mol. Biol. Evol. 9: 366–369.

Hindar K, Ryman N and Ståhl G, (1986). Genetic differentiation among local populations and morphotypes of Arctic charr, Salvelinus alpinus. Biol. J. Linn. Soc. 27: 269–285.

Johnson L, (1980). The Arctic charr, Salvelinus alpinus. In: Charrs: Salmonid fishes of the genus Salvelinus (ed EK Balon) Dr. W. Junk, The Hague, pp. 1–98.

Kallio-Nyberg I and Koljonen M-L, (1991). The Finnish char (Salvelinus alpinus) stock register. Finnish Fish. Res. 12: 77–82.

Maitland PS, (1995). World status and conservation of the Arctic charr Salvelinus alpinus (L.). Nordic J. Freshw. Res. 71: 113–127.

Makkonen J, 91997). Saimaan nieriän emokalasto ja istutukset. Kalatutkimuksia 133: 1–129 (in Finnish).

Makkonen J and Nurmio T, (1997). Kuolimon nieränkalastus ja nykytila. Kalatutkimuksia 133: 10–43, (in Finnish).

McConnell SK, O'Reilly P, Hamilton L, Wright JM and Bentzen P, (1995). Polymorphic microsatellite loci from Atlantic salmon (Salmo salar): genetic differentiation of North American and European populations. Can. J. Fish. Aquat. Sci. 52: 1863–1872.

Merilä J, Björklund M and Baker AJ, (1996). Genetic population structure and gradual northward decline of genetic variability in the Greenfinch (Carduelis chloris). Evolution 50: 2548–2557.

Nei M, Tajima F and Tateno Y, (1983). Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19: 153–170.

Nielsen EE, Hansen MM and Loeschcke V, (1997). Analysis of microsatellite DNA from old scale samples of Atlantic salmon Salmo salar: a comparison of genetic composition over 60 years. Mol. Ecol. 6: 487–492.

Nyman L and Ring O, (1989). Affects of hatchery environment on three polymorphic loci in arctic char (Salvelinus alpinus species complex). Nordic J. Freshw. Res. 65: 34–43.

Ohta T and Kimura M, (1973). A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Gen. Res. 22: 201–204.

O'Reilly PT, Hamilton LC, McConnell SK and Wright JM, (1996). Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. 53: 2292–2298.

Osinov AG and Pavlov SD, (1998). Allozyme variation and genetic divergence between populations of arctic char and dolly varden (Salvelinus alpinus-Salvelinus malma complex). J. Ichthyol. 38: 42–55.

Paetkau D, Calvert W, Stirling I and Strobeck C, (1995). Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. 4: 347–354.

Pemberton JM, Slate J, Bancroft DR and Barrett JA, (1995). Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol. Ecol. 4: 249–252.

Piironen J and Heinimaa P, (1998). Preservation programs for endangered fish stocks in Finland. In: Action before extinction: an international conference on conservation of fish genetic diversity (eds. B Harvey, C Ross, Greer D and J Carosfeld), World Fisheries Trust, p. 105–114.

Presa P and Guyomard R, (1996). Conservation of microsatellites in three species of salmonids. J. Fish Biol. 49: 1326–1329.

Pylkkö P, Pohjanvirta T and Pursiainen M. (1996). Nieriän (Salvelinus alpinus) silmäsamentumat. Kalatutkimuksia 109: 1–21. (in Finnish).

Raymond M and Rousset F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity 86: 248–249.

Rice WR. (1989). Analysing tables of statistical tests. Evolution 43: 223–225.

Rousset F. (1996). Equilibrium values of measures of population subdivision for stepwise mutation processes. Genetics 142: 1357–1362.

Ryman N and Laikre L. (1991). Effects of supportive breeding on the genetically effective population size. Conserv. Biol. 5: 325–329.

Ryman N, Utter F and Laikre L. (1995). Protection of intraspecific biodiversity of exploited fishes. Rev. Fish Biol. Fisher. 5: 417–446.

Sandlund OT, Jonsson B, Malmquist HJ, Gydemo R, Lindem T, Skulason S, Snorrason SS and Jonasson PM. (1987). Habitat use by Arctic charr Salvelinus alpinus in Thingvallavatn, Iceland. Environ. Biol. Fish. 20: 263–274.

Skulason S, Snorrason SS, Ota D and Noakes LG. (1993). Genetically based differences in foraging behaviour among sympatric morphs of Arctic charr (Pisces: Salmonidae). Anim. Behav. 45: 1179–1192.

Slettan A, Olsaker I and Lie O. (1995). Atlantic salmon, Salmo salar, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. Animal Genet. 26: 277–285.

Takezaki N and Nei M. (1996). Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144: 389–399.

Taylor EB. (1991). A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture 98: 185–207.

Tessier N, Bernatchez L and Wright JM. (1997). Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analyses in land-locked Atlantic salmon Salmo salar L. Mol. Ecol. 6: 735–750.

Thornhill NM. (1993). The natural history of inbreeding and outbreeding: theoretical and empirical perspectives. Univ. Chicago Press, Chicago.

Volpe JP and Ferguson MM. (1996). Molecular genetic examination of the polymorphic Arctic charr Salvelinus alpinus of Thingvallavatn, Iceland. Mol. Ecol. 5: 763–772.

Waples RS and Do C. (1994). Genetic risk associated with supplementation of Pacific salmonids: captive broodstock programs. Can. J. Fish. Aquat. Sci. 51 (Suppl. 1): 310–329.

Ward RD, Woodstock M and Skibinski DOF. (1994). A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J. Fish Biol. 44: 213–232.

Waser PM and Strobeck C. (1998). Genetic signatures of dispersal. Trends Ecol. Evol. 13: 43–44.

Weir BS and Cockerham CC. (1984). Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Wilson CC, Hebert PDN, Reist JD and Dempson JB. (1996). Phylogeography and postglacial dispersal of Arctic charr Salvelinus alpinus in North America. Mol. Ecol. 5: 187–197.

APPENDIX A: *Measures of genetic diversity detected at eight microsatellite loci from 18 populations/hatchery broodstocks. Listed are the number of chromosomes analysed (N), the number of alleles (A), observed heterozygote proportion (H_O), gene diversity (H_E) and significant ($P < 0.05$) exact probability estimates for departure from H-W equilibrium for locus/population combinations. Abbreviations for population names are given in Table 1*

Locus	PIH	SOM	TOS	HOR	HAU	BUE	LIS	INA-L	INA-W	INA-S	SAI-85	SAI-91	SAI-92	SAI-93	SAI-94	SAI-W	RIA	GER
SSOSL85																		
N	14	38	42	40	38	38	22	44	16	26	52	54	54	54	36	10	30	10
A	2	5	5	6	1	5	5	6	4	4	4	4	4	3	4	4	5	4
H_o	0.43	0.68	0.81	0.90	–	0.47	0.91	0.55	0.88	0.46	0.96	0.48	0.56	0.85	0.33	0.60	0.67	0.80
H_e	0.36	0.72	0.77	0.78	–	0.71	0.81	0.65	0.73	0.55	0.67	0.40	0.56	0.65	0.59	0.71	0.64	0.64
p	ns	ns	ns	ns	–	0.023	ns	ns	ns	ns	<0.0001	ns	ns	ns	0.034	ns	ns	ns
Coe13																		
N	8	28	38	44	40	36	22	44	16	30	52	56	54	48	36	8	32	10
A	4	9	4	3	2	11	5	7	5	13	4	4	4	4	3	3	7	5
H_o	0.75	0.93	0.26	0.27	0.05	0.83	0.73	0.64	0.75	0.93	0.96	0.54	0.82	0.63	0.72	1.0	0.88	0.80
H_e	0.86	0.87	0.25	0.25	0.05	0.84	0.71	0.73	0.70	0.88	0.67	0.60	0.65	0.69	0.64	0.71	0.75	0.87
p	ns	ns	ns	ns	ns	ns	ns	0.044	ns	ns	<0.0001	ns	ns	ns	ns	ns	ns	ns
Ssa14																		
N	14	36	40	44	38	40	22	44	12	30	50	56	52	56	36	10	32	10
A	3	1	1	3	2	2	1	3	3	4	2	1	1	1	1	1	2	2
H_o	0.43	–	–	0.55	0.21	0.3	–	0.46	0.17	0.53	0.04	–	–	–	–	–	0.19	0.40
H_e	0.58	–	–	0.43	0.19	0.51	–	0.38	0.32	0.53	0.04	–	–	–	–	–	0.18	0.53
p	ns	–	–	ns	ns	ns	–	ns	ns	ns	ns	–	–	–	–	–	ns	ns
Ssa197																		
N	14	40	34	44	34	36	22	42	18	22	52	56	54	56	36	10	24	10
A	1	1	1	2	1	2	1	1	1	1	1	1	1	2	1	1	1	2
H_o	–	–	–	0.14	–	0.06	–	–	–	–	–	–	–	0.04	–	–	–	0.60
H_e	–	–	–	0.13	–	0.16	–	–	–	–	–	–	–	0.04	–	–	–	0.47
p	–	–	–	ns	–	ns	–	–	–	–	–	–	–	ns	–	–	–	ns
MST85																		
N	14	38	40	44	40	34	18	44	18	30	46	52	46	56	36	10	32	6
A	3	8	5	4	5	15	6	4	4	10	6	5	5	3	4	4	1	5
H_o	0.29	0.63	0.5	0.32	0.75	0.88	0.78	0.64	0.78	0.73	0.91	0.96	0.96	0.57	0.89	1	–	0.67
H_e	0.54	0.75	0.62	0.32	0.77	0.85	0.83	0.58	0.58	0.77	0.72	0.66	0.69	0.47	0.70	0.78	–	0.93
p	ns	ns	ns	ns	0.032	ns	ns	ns	ns	ns	<0.0001	0.001	ns	0.034	0.007	ns	ns	ns
Sto8																		
N	14	38	42	44	40	38	22	44	16	26	42	48	50	54	36	10	30	10
A	5	9	12	9	5	11	3	9	7	11	4	3	3	1	3	3	4	6
H_o	0.43	1.0	0.71	0.82	0.85	0.74	0.18	0.91	0.63	1.0	1.0	0.71	0.72	–	0.72	0.40	0.53	0.80
H_e	0.76	0.84	0.85	0.8	0.68	0.8	0.26	0.81	0.63	0.93	0.7	0.61	0.52	–	0.66	0.38	0.48	0.84
p	0.008	0.007	ns	0.034	ns	ns	ns	ns	ns	ns	0.001	ns	ns	–	ns	ns	ns	ns
Sto23																		
N	14	40	42	40	36	38	22	44	18	30	52	54	48	52	36	8	32	10
A	9	5	7	12	6	23	10	11	10	14	3	3	2	2	3	2	9	5
H_o	0.57	0.45	0.38	0.85	0.83	0.95	0.82	0.86	0.89	1.0	0.92	0.56	0.38	0.46	0.78	0.75	0.81	0.80
H_e	0.93	0.46	0.38	0.88	0.78	0.96	0.9	0.88	0.92	0.94	0.52	0.52	0.31	0.36	0.52	0.54	0.88	0.82
p	0.012	ns	ns	ns	ns	ns	ns	ns	ns	ns	<0.0001	ns	ns	ns	0.0192	ns	0.013	ns
MST60																		
N	14	40	42	40	36	36	22	40	18	28	52	50	52	54	36	10	32	10
A	2	6	2	6	3	11	4	9	9	8	4	3	3	3	2	2	5	2
H_o	0.14	0.45	0.29	0.75	0.78	0.72	0.64	0.65	1	0.79	0.96	0.72	0.54	0.78	0.72	0.80	0.50	0.40
H_e	0.14	0.43	0.25	0.79	0.68	0.86	0.61	0.76	0.92	0.87	0.69	0.52	0.63	0.67	0.50	0.53	0.55	0.36
p	ns	ns	ns	0.034	ns	ns	ns	0.045	ns	0.016	0.002	ns	ns	0.016	ns	ns	ns	ns
All Loci																		
Mean A	3.6	5.5	4.6	5.6	3.1	10.0	4.4	6.3	5.4	8.1	3.5	3.0	2.9	2.4	2.6	2.5	4.3	3.9
Mean H_o	0.38	0.52	0.37	0.57	0.43	0.62	0.51	0.59	0.64	0.68	0.72	0.50	0.50	0.42	0.52	0.57	0.45	0.66
Mean H_e	0.52	0.51	0.39	0.55	0.4	0.71	0.51	0.60	0.60	0.68	0.50	0.42	0.42	0.36	0.45	0.46	0.43	0.68
p (all loci)	0.014	ns	ns	ns	ns	0.028	ns	0.014	ns	0.042	<0.0001	0.016	0.044	0.006	0.003	ns	ns	ns

APPENDIX B: *Pairwise genic differentiation estimates based on the D_{CE} distance*

	BUE	SAI-85	SAI-91	SAI-92	SAI-93	SAI-94	SAI-W	GER	HAU	HOR	INA-W	LIS	INA-S	PIH	RIA	INA-L	SOM
SAI-85	5.86																
SAI-91	5.86	1.65															
SAI-92	5.82	2.08	2.19														
SAI-93	6.03	2.91	3.00	1.76													
SAI-94	5.87	2.82	2.54	2.05	2.17												
SAI-W	5.91	2.34	2.21	1.68	2.09	1.29											
GER	6.01	5.88	5.93	5.90	6.07	5.92	5.91										
HAU	4.72	6.09	6.09	6.09	6.21	6.11	6.11	5.70									
HOR	5.30	5.93	6.07	6.04	6.10	6.08	5.93	6.29	5.68								
INA-W	5.08	5.65	5.85	5.74	6.02	5.80	5.80	5.69	5.10	5.41							
LIS	4.71	5.84	5.80	5.90	6.02	5.85	5.80	5.38	5.24	5.45	4.89						
INA-S	4.65	5.29	5.46	5.36	5.68	5.60	5.51	5.56	5.22	5.41	3.19	4.82					
PIH	5.29	5.44	5.50	5.22	5.53	5.27	5.34	5.89	5.16	5.84	5.56	5.25	5.31				
RIA	5.85	5.09	4.99	4.82	4.97	4.79	4.83	5.83	5.92	6.13	5.33	6.11	5.14	5.29			
INA-L	4.78	5.52	5.69	5.53	5.85	5.66	5.63	5.69	5.05	5.17	2.44	4.80	2.76	5.62	5.25		
SOM	5.19	5.72	5.81	5.58	5.65	5.67	5.69	5.66	5.06	5.22	4.92	4.65	4.70	4.46	5.55	4.70	
TOS	5.28	5.38	5.38	5.47	5.84	5.51	5.63	5.93	5.23	5.78	5.29	4.72	5.10	4.17	5.58	5.12	3.09