

SHORT COMMUNICATION

Identification of the origin of an Atlantic salmon (*Salmo salar* L.) population in a recently recolonized river in the Baltic Sea

A. VASEMÄGI,*† R. GROSS,‡ T. PAAVER,‡ M. KANGUR,§ J. NILSSON* and L.-O. ERIKSSON*

*Department of Aquaculture, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden, †Institute of Zoology and Hydrobiology, University of Tartu, 46 Vanemuise, 51014 Tartu, Estonia, ‡Department of Fish Farming, Institute of Animal Science, Estonian Agricultural University, 1 Kreutzwaldi, 51014 Tartu, Estonia, §Estonian Marine Institute, University of Tartu, Viljandi mnt. 18B, 11216 Tallinn, Estonia

Abstract

The founder event in a recently recolonized salmon population in the Baltic Sea (Gulf of Finland) was investigated. To identify the origin of the founders, four wild populations and two hatchery stocks were analysed using six microsatellite loci. The results of assignment tests and factorial correspondence analysis suggest that the initial recolonizers of the river Selja originated from the geographically nearest (7 km) wild population (river Kunda) but as the result of stocking activities, interbreeding between recolonizers and hatchery individuals has occurred in subsequent years. Although the hatchery releases are outnumbering the wild salmon recruitment in the Baltic Sea at present, our results suggest that the native populations may still have an important role in colonization processes of the former salmon rivers.

Keywords: assignment test, microsatellite DNA, multilocus genotypes, natural recolonization, *Salmo salar*

Received 9 May 2001; revision received 23 August 2001; accepted 23 August 2001

Introduction

Understanding the role of gene flow during the evolutionary dynamics of populations is important in conservation programmes both of wild and captive populations. High genetic differentiation among Atlantic salmon populations, described in many studies using different genetic markers (Tessier *et al.* 1997; Koljonen *et al.* 1999; Nilsson *et al.* 2001), indicates restricted gene flow between rivers or even between tributaries or different stretches of a river (Verspoor *et al.* 1991; Beacham & Dempson 1998; Garant *et al.* 2000). As a consequence of accurate homing behaviour in salmon, the founding of new populations or reoccupation of areas where former populations have been driven to extinction may be limited

(Young 1999). However, several studies have shown that some release practices, e.g. delayed release (smolts are kept in cages in the sea for some period before they are released), might increase the straying of hatchery fish (reviewed by Quinn 1993). Considering that hatcheries are releasing approximately nine times more salmon smolts to the Baltic Sea than are estimated to come from wild populations (ICES 1998) the reared individuals may have an important effect on the genetic composition of salmon populations in the Baltic Sea.

A suitable model by which to study gene flow and the formation of genetic structure as a result of the founder effect and enhancement practices occurred in the river Selja salmon population in the Gulf of Finland in the Baltic Sea (Fig. 1) where the native stock was driven to extinction in the beginning of the 1970s as a result of pollution (Loigu *et al.* 2001). When conditions improved during the 1990s, spontaneous recolonization of this river took place. In addition, restocking of the river with hatchery fish was started in 1997.

Correspondence: Anti Vasemägi. *Present address: Department of Aquaculture, Swedish University of Agricultural Sciences, S-901 83 Umeå, Sweden. Fax: +46 90 123729; E-mail: Anti.Vasemagi@vabr.slu.se, vasemagi@eau.ee



Fig. 1 Map of the Baltic Sea showing sampling locations of salmon populations.

Table 1 Studied salmon samples and the number of individuals assigned to each baseline population directly or after simulation (in parenthesis; with the threshold of rejection 0.05) using the Bayesian approach of assignment test (Cornuet *et al.* 1999)

Sample	<i>n</i>	Year of sampling	Age-class	Status	Number of individuals assigned to:						Rejected from all samples
					Neva	Narva	Pärnu	Keila	Kunda	Loobu	
<i>Recolonized population</i>											
Selja	33	1995	0+	Unknown	0(5)	0(5)	0(0)	1(8)	28(24)	4(10)	(9)
Selja	45	1999	1+	Unknown + Hatchery	19(10)	12(8)	0(0)	5(4)	1(1)	8(3)	(26)
<i>Potential donor populations</i>											
Neva	115	1995–97	0+	Hatchery	108(98)	6(13)	0(0)	1(0)	0(0)	0(0)	(17)
Narva	35	1998	Adults	Hatchery	4(8)	28(24)	2(0)	1(0)	0(0)	0(0)	(10)
Pärnu	21	1997	0+	Wild	0(0)	0(2)	19(15)	2(2)	0(0)	0(0)	(6)
Keila	30	1997	0+, 1+	Wild	0(1)	2(10)	1(4)	25(25)	1(3)	1(0)	(3)
Kunda	78	1996–99	0+, 1+	Wild	0(2)	2(26)	1(5)	1(26)	72(66)	2(13)	(9)
Loobu	67	1996, 1999	0+, 1+	Wild	0(5)	0(7)	0(6)	0(40)	2(39)	65(64)	(2)

Highly variable microsatellite loci provide sufficient variation for the application of a growing number of novel statistical methods, which can utilize multilocus genotype information (reviewed by Luikart & England 1999). Assigning individuals to given groups based on their multilocus genotypes (Paetkau *et al.* 1995; Cornuet *et al.* 1999) is one promising approach that has already been applied in different studies on plants (Muir *et al.* 2000; Sefc *et al.* 2000), invertebrates (Bogdanowicz *et al.* 1997; Pálsson 2000), mammals (e.g. Reed *et al.* 1997; Kyle *et al.* 2000) and fishes (e.g. Estoup *et al.* 1998; Roques *et al.* 1999; Hansen *et al.* 2000; Primmer *et al.* 2000). We applied this method to determine the genetic origin of founders of the recently recolonized river Selja salmon population and to detect the genetic effects on this population of the subsequent stocking activities.

Materials and methods

Samples

Juvenile and adult salmon from five rivers and two hatcheries were collected during 1995–99 (Table 1). The samples from the recolonized river Selja population were collected by electrofishing before stocking (1995) and after 3 years of stocking (1999) with hatchery salmon of river Neva origin. Samples were collected from the rivers Kunda, Loobu, Keila and Pärnu to represent the potential wild donor populations (Fig. 1). Additionally, hatchery fish of river Neva origin from the Laukaa hatchery (Finland) and adults from the river Narva with mixed genetic origin (river Neva and river Salatsa; Kazakov & Titov 1993) were sampled. Fin clips or muscle tissue were

preserved in 96% ethanol until used for total DNA extraction according to Laird *et al.* (1991); except the Selja 1995 samples, where DNA was extracted from dried scales of 0+ old parr according to Nielsen *et al.* (1997).

Genetic analysis

Three tetranucleotide (*Ssa202*, *Ssa197*, *Ssa171*; O'Reilly *et al.* 1996) and three dinucleotide (*SSOSL417*, *SSOSL311*, *SSOSL85*; Slettan *et al.* 1995) microsatellite loci were amplified according to the polymerase chain reaction (PCR) conditions recommended by the authors. For the scale samples 40 instead of 33 PCR cycles were used to increase the amount of PCR product. Different electrophoresis and visualization procedures were applied in different laboratories: nondenaturing polyacrylamide gel electrophoresis and silver staining, denaturing polyacrylamide gel electrophoresis followed by autoradiography or silver staining, and fluorescent analysis on a Pharmacia ALFexpress automated sequencer. Known genotypes from three to seven individuals were included in all gel runs to ensure the correct determination of allele sizes. In tetranucleotide loci allelic ladders up to 12 alleles were used for correct allele determination.

Data analysis

The GENETIX 4.0 program (Belkhir *et al.* 2000) was used to calculate allele frequencies, and observed and unbiased expected heterozygosities. Exact tests for genotypic disequilibrium between loci, departure from Hardy–Weinberg equilibrium and for genotypic differentiation between population pairs were computed using GENEPOP 3.1b (Raymond & Rousset 1995). The same program was used to quantify the genetic differentiation between samples by calculating unbiased F_{ST} values (Weir & Cockerham 1984). In all cases of multiple tests, significance levels were adjusted using the sequential Bonferroni technique (Rice 1989). To determine the genetic origin of founders of the river Selja salmon population, the Bayesian approach of assignment test implemented in the GENECLASS 1.0.02 program (Cornuet *et al.* 1999) was used. Although the computation of this likelihood-based assignment test assumes Hardy–Weinberg and linkage equilibrium, it has been reported that at least in the case of homozygote excess, the Bayesian approach still performs better than the distance-based assignment methods that are not restricted to these assumptions (Cornuet *et al.* 1999). To measure the confidence that the individual to be assigned truly belongs to any of the reference populations, a simulation option (with 10 000 simulated individuals per population) of the GENECLASS 1.0.02 program was used (Cornuet *et al.* 1999). The individual was excluded from a given candidate population if the individual probability of

belonging to the particular population was lower than 5% (as the result, the individual can be assigned to more than one population). To avoid the possible bias in self-assignment of the individuals to their population of origin, the 'leave one out' procedure was applied, excluding the tested individual when calculating the allele frequency distribution of their own population (see Cornuet *et al.* 1999 for details). The hybrid origin of the Selja 1999 sample (interbreeding between wild recolonizers and stocked hatchery fish) was visualized by applying factorial correspondence analysis described in She *et al.* (1987) and Berrebi *et al.* (2000) using GENETIX 4.0 (Belkhir *et al.* 2000).

Results

Allelic variation and population differentiation

The total number of alleles per locus ranged from nine (*Ssa202*) to 25 (*SSOSL311*) and the expected heterozygosity among loci varied between 0.335 and 0.932 in different populations. The mean number of alleles per locus ranged from 3.8 (Selja 1995) to 12 (Neva). In total, 22 private alleles were found, all with a frequency below 0.06, except one allele in the Narva sample with a 0.1 frequency. No private alleles were detected in the Selja 1995 sample. Multiple probability tests by population revealed significant deviations from Hardy–Weinberg expectations at the 5% level in Loobu, Neva and both Selja samples. An exact test for linkage disequilibrium among loci showed zero to four deviations ($P < 0.05$) in 15 pairwise combinations of loci. However, linkage was not consistent between the same pair of loci among samples. A possible exception was the locus pair *SSOSL311* and *SSOSL417*, for which linkage was detected in four samples out of eight. Pairwise comparisons of genotypic frequencies combining probabilities over all loci indicated highly significant ($P < 0.001$) differentiation between all pairs of samples. Multilocus F_{ST} values ranged from 0.06 to 0.2 (mean 0.13) with the smallest differentiation between the Kunda and Selja 1995 samples.

Analysis of individual multilocus genotypes

To identify the probable source population of the founders, multilocus genotype likelihoods of the salmon from the river Selja were compared to the six possible donor populations using the assignment test (Table 1). Direct assignment classified correctly 91.6% of individuals of known samples to their population of origin, indicating that sufficient genetic differentiation exists for individual classification using six microsatellites. The individuals of unknown origin from the Selja 1995 sample were assigned with the highest frequency to the wild Kunda population (85% and 73% by the direct and simulated approach,

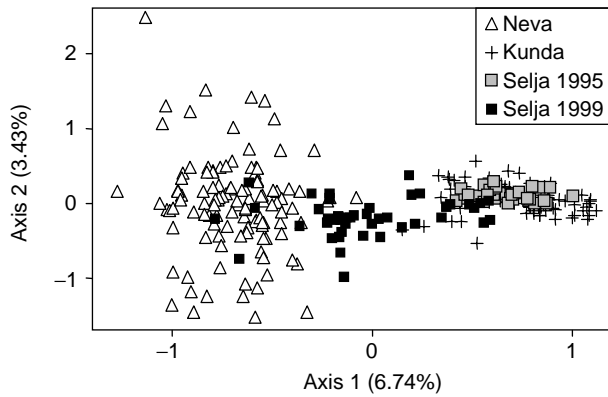


Fig. 2 Factorial correspondence analysis between individuals of Neva, Kunda, Selja 1995 and Selja 1999 samples.

respectively), followed by the river Loobu population (12% and 30%, respectively) (Table 1). After 3 years of stocking hatchery salmon (Neva) to the river Selja, a high proportion of individuals (58%) from the Selja 1999 sample were rejected from all simulated baseline samples. To reveal their origin, a factorial correspondence analysis was applied. Most individuals of the Selja 1999 sample had intermediate component scores compared to the hatchery fish (Neva) and initial recolonizers (Selja 1995), indicating that these fish may have a hybrid origin (Fig. 2). The recolonizers, however, had very similar scores to the individuals in the Kunda sample, which suggests their very close genetic relationship (Fig. 2).

Discussion

The results of assignment tests suggest that the initial recolonizers of the river Selja in the mid-1990s originated from the river Kunda population that was geographically the nearest (7 km). This was also supported by the factorial correspondence analysis. However, it cannot be completely excluded that a small number of native fish may have survived in the river Selja and the juveniles detected in 1995 were the result of recovery of the population, instead of recolonization. For example, Nielsen *et al.* (1997) demonstrated that the river Skjern salmon population in Denmark was close to extinction, but a few breeders found in one tributary clustered together to the historical samples of the same river. However, short distance to the first obstacle (12 km), limited reproduction area (5–8 ha) and absence of tributaries together with the water-quality data (Kangur & Viilmann 2001) and results of electrofishing surveys from the 1970s and 1980s strongly support the hypothesis of extinction of the native salmon population in the river Selja.

Identification of the wild Kunda population as the most likely source of the recolonizers is surprising considering

that the proportion of hatchery releases compared to wild production is even more pronounced (approximately 37 : 1) in the Gulf of Finland (ICES 1998), compared to the total Baltic where the stocked fish outnumber the wild salmon approximately nine-fold. It is also unlikely that the founders came from the Gulf of Bothnia or from the Baltic Main Basin population(s) not included in this study. It has been shown with mitochondrial DNA and allozymes that the Gulf of Bothnia populations belong to a different phylogeographic lineage compared to the southern populations and the salmon stocks of the Main Basin cluster apart from the populations in the Gulf of Finland (Koljonen *et al.* 1999; Nilsson *et al.* 2001). There are no wild salmon populations left on the northern coast of the Gulf of Finland. In addition, the absence of private alleles in the Selja 1995 sample and the low F_{ST} value between the Selja 1995 and Kunda samples suggests close relationships between the geographically adjacent wild population and the recolonized population. Recolonization from the geographically nearest population is consistent with the correlation between genetic and geographical distances found in wild salmon populations (Koljonen *et al.* 1999; Nielsen *et al.* 1999), indicating that the spawners tend to stray and successfully reproduce in adjacent rivers. Surprisingly fast recolonization of the extinct river may indicate a higher level of straying to the unoccupied habitats than the present gene flow between extant salmon populations. Rapid colonization of the new areas, followed by smaller straying rates after the populations have become established, has been shown for example in several Pacific salmon species (*Oncorhynchus*) (Quinn 1993). We have presumed that the colonizers could come only from one population. However, it is possible that the founders were from different genetic sources, e.g. from both the Kunda and the Loobu populations, although this is less likely because of the threatened situation of the Loobu population, where no natural reproduction has been reported in 1983, 1987, 1990 and during 1996–97 (Kangur & Viilmann 2001).

Interbreeding between the recolonizers and the hatchery individuals was not directly evident from the individual assignments, but visualization of the multilocus genotypes using factorial correspondence analysis indicated the probable occurrence of first-generation hybrids between wild colonizers and hatchery fish. To evaluate the relative success of the hatchery and naturally recolonized individuals, monitoring of the genetic structure over a longer timescale is necessary.

Acknowledgements

We thank Michael Hansen, Jens Carlsson and two anonymous reviewers for many useful suggestions on the manuscript and the managers of Pömla Fish Farming Centre for providing hatchery

samples. The laboratory work was carried out at the Department of Inland Fisheries (Danish Institute for Fisheries Research), at the Department of Animal Science (University of Helsinki) and at the Department of Fish Farming (Estonian Agricultural University). This work was supported by the Royal Swedish Academy of Agriculture and Forestry and grants 2615, 3155, 4095 and 4826 from the ESF.

References

- Beacham TD, Dempson JB (1998) Population structure of Atlantic salmon from the Conne river, Newfoundland as determined from microsatellite DNA. *Journal of Fish Biology*, **52**, 665–676.
- Belkhir K, Borsa P, Chikhi L, Goudet J, Bonhomme F (2000) *GENETIX 4.0, logiciel sous Windows™ pour la génétique des populations*. Laboratoire Génome, Populations, Interactions. CNRS UPR 9060, Université de Montpellier II, Montpellier (France).
- Berrebi P, Povz M, Jesenek D, Cattaneo-Berrebi G, Crivelli AJ (2000) The genetic diversity of native, stocked and hybrid populations of marble trout in the Soca river, Slovenia. *Heredity*, **85**, 277–287.
- Bogdanowicz SM, Mastro VC, Prasher DC, Harrison RG (1997) Microsatellite DNA variation among Asian and North American gypsy moths (*Lepidoptera: Lymantriidae*). *Annals of the Entomological Society of America*, **90**, 768–775.
- Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.
- Estoup A, Rousset F, Michalakis Y *et al.* (1998) Comparative analysis of microsatellite and allozyme markers: a case study investigating microgeographic differentiation in brown trout (*Salmo trutta*). *Molecular Ecology*, **7**, 339–353.
- Garant D, Dodson JJ, Bernatchez L (2000) Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.). *Molecular Ecology*, **9**, 615–628.
- Hansen MM, Ruzzante DE, Nielsen EE, Mensberg K-LD (2000) Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). *Molecular Ecology*, **9**, 583–594.
- ICES (1998) *Report of the Baltic salmon and trout assessment working group*. ICES Doc. CM 1998/ACFM: 17. ICES, Copenhagen.
- Kangur M, Viilmann M-L (2001) Habitat, fish and fisheries. In: *Present and Potential Production of Salmon in Estonian Rivers* (eds Wahlberg B, Kangur M), pp. 32–69. Estonian Academy Publishers, Tallinn.
- Kazakov RV, Titov SF (1993) Population genetics of salmon, *Salmo salar* L., in Northern Russia. *Aquaculture and Fisheries Management*, **24**, 495–506.
- Koljonen M-L, Jansson H, Paaver T, Vasin O, Koskiniemi J (1999) Phylogenetic lineages and differentiation pattern of Atlantic salmon (*Salmo salar*) in the Baltic Sea with management implications. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1766–1780.
- Kyle CJ, Davies CS, Strobeck C (2000) Microsatellite analysis of North American pine marten (*Martes americana*) populations from the Yukon and Northwest Territories. *Canadian Journal of Zoology*, **78**, 1150–1157.
- Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A (1991) Simplified mammalian DNA isolation procedure. *Nucleic Acids Research*, **19**, 4293.
- Loigu N, Leisk Ü, Hannus M, Blinova I (2001) Water quality. In: *Present and Potential Production of Salmon in Estonian Rivers* (eds Wahlberg B, Kangur M), pp. 17–31. Estonian Academy Publishers, Tallinn.
- Luikart G, England PR (1999) Statistical analysis of microsatellite data. *Trends in Ecology and Evolution*, **14**, 253–256.
- Muir G, Fleming CC, Schlotterer C (2000) Taxonomy: species status of hybridizing oaks. *Nature*, **405**, 1016.
- Nielsen EE, Hansen MM, Loeschcke V (1997) Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: a comparison of genetic composition over 60 years. *Molecular Ecology*, **6**, 487–492.
- Nielsen EE, Hansen MM, Loeschcke V (1999) Genetic variation in time and space: microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution*, **53**, 261–268.
- Nilsson J, Gross R, Asplund T *et al.* (2001) Matrilineal phylogeography of Atlantic salmon (*Salmo salar* L.) in Europe and postglacial colonization of the Baltic Sea area. *Molecular Ecology*, **10**, 89–102.
- O'Reilly PT, Hamilton LC, McConnell SK, Wright JM (1996) Rapid analysis of Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2292–2298.
- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.
- Pálsson S (2000) Microsatellite variation in *Daphnia pulex* from both sides of the Baltic Sea. *Molecular Ecology*, **8**, 1075–1088.
- Primmer CR, Koskinen MT, Piironen J (2000) The one that did not get away: individual assignment using microsatellite data detects a case of fishing competition fraud. *Proceedings of the Royal Society of London B*, **267**, 1699–1704.
- Quinn TP (1993) A review of homing and straying of wild and hatchery-produced salmon. *Fisheries Research*, **18**, 29–44.
- Raymond M, Rousset F (1995) *GENEPOP* (Version 1.2): a population genetics software for exact tests and ecumenism. *Journal of Heredity*, **86**, 248–249.
- Reed J, Tollit D, Thompson P, Amos W (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular Ecology*, **6**, 225–234.
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.
- Roques S, Duchesne P, Bernatchez L (1999) Potential of microsatellites for individual assignment: the North Atlantic redfish (genus *Sebastes*) species complex as a case study. *Molecular Ecology*, **8**, 1703–1718.
- Sefc KM, Lopes MS, Lefort F *et al.* (2000) Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. *Theoretical and Applied Genetics*, **100**, 498–505.
- She J-X, Autem M, Kotoulas G, Pasteur N, Bonhomme F (1987) Multivariate analysis of genetic exchanges between *Solea aegyptiaca* and *Solea senegalensis* (Teleosts, Soleidae). *Biological Journal of the Linnean Society*, **32**, 357–371.
- Slettan A, Olsaker I, Lie Ø (1995) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics*, **26**, 277–285.
- Tessier N, Bernatchez L, 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. *Molecular Ecology*, **6**, 735–750.

- Verspoor E, Fraser NHC, Youngson AF (1991) Protein polymorphism in Atlantic salmon within a Scottish river: evidence for selection and estimates of gene flow between tributaries. *Aquaculture*, **98**, 217–230.
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Young KA (1999) Managing the decline of Pacific salmon: metapopulation theory and artificial recolonization as ecological mitigation. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 1700–1706.

This study was carried out with the co-operation of several institutions and is part of Anti Vasemägi's PhD thesis that deals with the genetic structure and interactions of wild and reared Atlantic salmon populations in the Baltic Sea. Riho Gross is a senior research scientist working on various aspects of population genetics and phylogeography in fish. Tiit Paaver is a head of the Department of Aquaculture at the Estonian Agricultural University and his research is focused on problems of biology and aquaculture of salmonid fish.
