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Genetic assignment identifies farm of origin for Atlantic salmon *Salmo salar* escapees in a Norwegian fjord

K. A. Glover*, O. T. Skilbrei, and Ø. Skaala

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This study represents the first example of genetic assignment being used to identify the farm of origin for escaped Atlantic salmon. Following reports by local fishers of escaped farmed salmon in Romsdalfjord, western Norway, baseline samples were collected from 16 cages located on seven operational farms. The baseline samples, in addition to 29 escapees, were screened for 15 microsatellite loci. Pairwise F_{ST} values between baseline samples varied from <0.001 to 0.154, indicating variable but significant genetic differences among them. Direct assignment of the escapees (data from 13 informative loci) demonstrated that the most likely origin of 21 of them was from a single baseline sample (5I) collected at one farm. At a probability of 0.01, between 25 and 29 of the escapees were rejected in 12 baseline samples, 19–21 escapees were rejected from another three baseline samples, and only seven of the escapees were rejected from baseline sample 5I. Consequently, these data demonstrate that most of the escapees most likely originated from a single farm, and importantly, that 15 of the 16 baseline samples could with high probability be excluded as donors for most of the escapees recaptured in the area.

Keywords: assignment, Atlantic salmon, escapees, farm, microsatellite, *Salmo salar*.

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*K. A. Glover, O. T. Skilbrei and Ø. Skaala: Institute of Marine Research, PO Box 1870, Nordnes N–5817, Bergen, Norway. *Correspondence to K. A. Glover: tel: +47 55 236357; fax: +47 55 238531; e-mail: kevin.glover@imr.no.*

Introduction

Worldwide aquaculture production of Atlantic salmon (*Salmo salar* L.) has increased rapidly since the industry was started in the late 1960s, and in Europe, the species is the most significant cultured one in terms of both gross production and economic value. Norway, followed by the United Kingdom, Faroe Islands, and Ireland, are the major European producers (FAO, 2007).

A major challenge with cage-rearing fish in the marine environment is containment. According to official statistics from the Norwegian Directorate of Fisheries, the official number of farmed escaped salmon in Norway has varied between 260 000 and 715 000 fish per year in the period 2001–2005 (www.statistics.no). However, it is generally accepted that this number underreports the true numbers of farmed fish escaping (Baarøy *et al.*, 2004), although farmers are legally obliged to report fish losses. The unknown number of minor and occasionally larger escapements can occur for a variety of reasons, ranging from the farmer not being aware of damage to a cage, to deliberately withholding information following an incident. In some instances, the first reports of salmon escapees in the wild originate in local fishers and anglers, who experience a sudden increase in the abundance of escapees (Skilbrei and Wennevik, 2006).

It has been argued that the numbers of farmed escapees need to be significantly reduced to protect wild salmon populations from potentially damaging genetic introgression from domesticated salmon (Hindar *et al.*, 1991, 2006; McGinnity *et al.*, 2003; Skaala *et al.*, 2006). Clearly, there is a need to tackle this problem on several fronts. First and foremost to be addressed is to reduce the number of escapees. However, it is also important to gain information on the causes of unreported episodes of escapements. Therefore, there is also a requirement to develop a method by which unreported farmed escaped salmon can be traced back to the cages and farms from which they escaped. This is especially important in instances where large numbers of unidentified farmed salmon escapees are observed in a region.

Genetic assignment tests for identification of individual(s) to population(s) of origin have been used to answer a variety of biological questions in various species (e.g. Paetkau *et al.*, 1995; Hansen *et al.*, 2000, 2006; Koljonen *et al.*, 2005; Norén *et al.*, 2005; Hauser *et al.*, 2006; Regnmark *et al.*, 2006). In a novel study, Primmer *et al.* (2000) used genetic assignment to detect a case of angling competition fraud in Finland. These last authors were able to demonstrate with a very high degree of probability that the winning fish in the competition could not have arisen from the landlocked Atlantic salmon population in which the competition was held, forcing a confession from the angler that he had indeed purchased the winning salmon from a local fish market. Since the early genetic assignment tests developed by Paetkau *et al.* (1995), a continually expanding list of tools and statistical techniques has been developed (reviewed by Hansen *et al.*, 2001, and Pearse and Crandall, 2004), permitting a variety of methods to be used for the purpose of identification of individuals to genetic groups.

Several genetic studies have been carried out on farmed Atlantic salmon, demonstrating reduced allelic variation compared with wild salmon populations (Mjølnerød *et al.*, 1997; Norris *et al.*,

1999; Skaala *et al.*, 2004). In one of the most comprehensive genetic studies of farmed salmon so far, Skaala *et al.* (2004) observed significant genetic differentiation among five major farmed strains, with pairwise F_{ST} values as high as 0.153. They observed 93–98% accuracy in assigning fish among the five farmed strains, demonstrating the potential for genetic assignment of escapees.

In autumn 2006 (20 September on), local gillnet and sports-fishers in Romsdalfjord in mid-Norway reported a sudden increase in catches of farmed salmon to the Norwegian Directorate of Fisheries. Acting immediately upon this information, the Directorate of Fisheries contacted all companies operating salmon farms in the region. However, no losses of fish were reported from sea cages. As a consequence, and following dialogue with scientists at the Institute of Marine Research (IMR) in Bergen, the Directorate of Fisheries collected adipose fin tissue samples from all active salmon farms in the region and secured genetic samples from a group of recaptured escapees. This study presents the results of the genetic analysis of these samples.

Material and methods

Baseline samples from farms

Inspectors working for the Norwegian Directorate of Fisheries sampled seven salmon farms in Romsdalfjord in the period 6–7 October 2006 (Figure 1). This represents 100% sampling of all active salmon farms in the fjord that were registered as containing salmon that were transferred to the sea in 2005. It was decided before sampling that because of the size range of most of the escapees in the area then (Table 1), it was unnecessary to sample farms rearing salmon that were transferred to the sea in 2006. All companies owning these marine sites consented to and were present during sampling. At most of the farms, salmon that were delivered by different smolt producers were reared in adjacent cages. Consequently, 50 adipose fin clips from one cage per smolt delivery per farm was sampled and stored in ethanol. In all, 16 cages from the seven farms were sampled, representing smolts delivered from 12 producers (Table 2). These 16 samples are hereafter referred to as the baseline samples.

Samples of escapees

Five fishers who had reported capturing farmed escaped salmon in Romsdalfjord in this period (Figure 1) were contacted by the Directorate of Fisheries on 12 October 2006. An inspector from the Directorate of Fisheries visited each of these fishers in turn and took adipose tissue and scale samples from the escapees that were stored in the fishers' personal freezers. In addition, information on capture site, date, fish size, state of maturity, observations of sea lice, and any other relevant information were collected. For many of the escapees, only partial biological information could be collected because some had been gutted and had had their tails removed (Table 1). In all, samples from 32 escapees were secured. These samples represent just a fraction of the total number of escapees captured by these fishers during this period, because many of the recaptured salmon had already been consumed, sold, or otherwise distributed. All 32 recaptured escapees were easily classified as farmed salmon as opposed to wild fish based upon morphological characteristics (reviewed by Fiske *et al.*, 2005). In addition, scale samples from all 32 recaptured escapees were sent for further analysis (Rådgivende Biologer AS). Scales were read on a microfiche print-reader by a technician experienced in using scale characteristics to distinguish farmed from wild salmon based upon smolt length, presence of winter zones, and accelerated early marine growth (Lund and Hansen, 1991). Except for one farmed salmon that was captured on 11 October, all escapees had been captured in the period 11–30 September. All tissue samples were delivered to IMR in Bergen.

Genotyping

Upon arrival at IMR, samples were given a unique number during entry to a genetic database. DNA was extracted using a Qiagen DNAeasy 96-well extraction kit according to the manufacturer's protocol. The number of individuals extracted for each baseline sample ranged from 47 to 48 to fit in with 96-well plate format, and DNA from all 32 recaptured escapees was extracted. From the DNA extraction stage on, each 96-well plate contained two randomly assigned blank wells, allowing unique identification of the plate and allowing for identification of potential contamination. Aliquots of these DNA samples were sent to Prokaria in Iceland in 96-well plates. In all, 15 microsatellite markers (*SSsp*3016, *SSsp*2210, *SSsp*G7, *Ssa*197, *Ssa*171, *Ssa*202, *SSsp*2201, *Ssa*D157, *Ssa*F43, *Ssa*D486, *Sp*1605, *Sp*2216, *Ssa*D144, *Ssa*14, and *Ssa*289) were amplified in two multiplexes and a singleplex PCR (amplification details are available from the authors upon request). PCR products were run on an ABI 3730 Genetic Analyser and size-called according to the 500LIZ^{TM} standard. Alleles were automatically called and manually checked in GeneMapper V4.0.

Data analysis

Tests of Hardy–Weinberg equilibrium for each marker in each sample, and exact tests for pairwise sample genetic differentiation using Fishers method (Markov chain parameters: demorization 1000, batches 100, iterations per batch 1000), were computed in the program Genepop V3.3 (Raymond and Rousset, 1995). After enumeration, data were imported into the program FSTAT (Goudet, 2001) for computation of numbers of alleles and pairwise F_{ST} values. The program TFPGA (Miller, 1999) was used to compute pairwise genetic distances (Nei, 1978) and to create the UPMGA diagram with percentage support for nodes (1000 permutations).

Genetic assignment tests were performed in the program Geneclass V. 1.02.01 (Cornuet *et al.*, 1999). To estimate the potential for genetic assignment among the 16 baseline samples, self-assignment was performed using the direct assignment leave-one-out option with the Bayesian method of computation. After self-assignment, 29 recaptured escapees were assigned/excluded from the 16 baseline samples. DNA samples from three of the escapees were excluded from the analysis for failing to produce readable genotypes. Direct assignment of escapees was performed using the Bayesian method, which assigns each individual to the closest baseline sample, even in situations where the true baseline population may not have been sampled. This technique is applicable to assignment where all possible populations have been sampled, or where there is good reason to believe this. However, in the present study, it is not possible to exclude the possibility that some of the recaptured salmon may have escaped from a farm not sampled. Consequently, the probability of exclusion from each baseline sample was computed for the 29 genotyped escapees. For this study, we decided to utilize the Bayesian method of simulation, setting frequency level to 0.01 and 0.05, identical with the

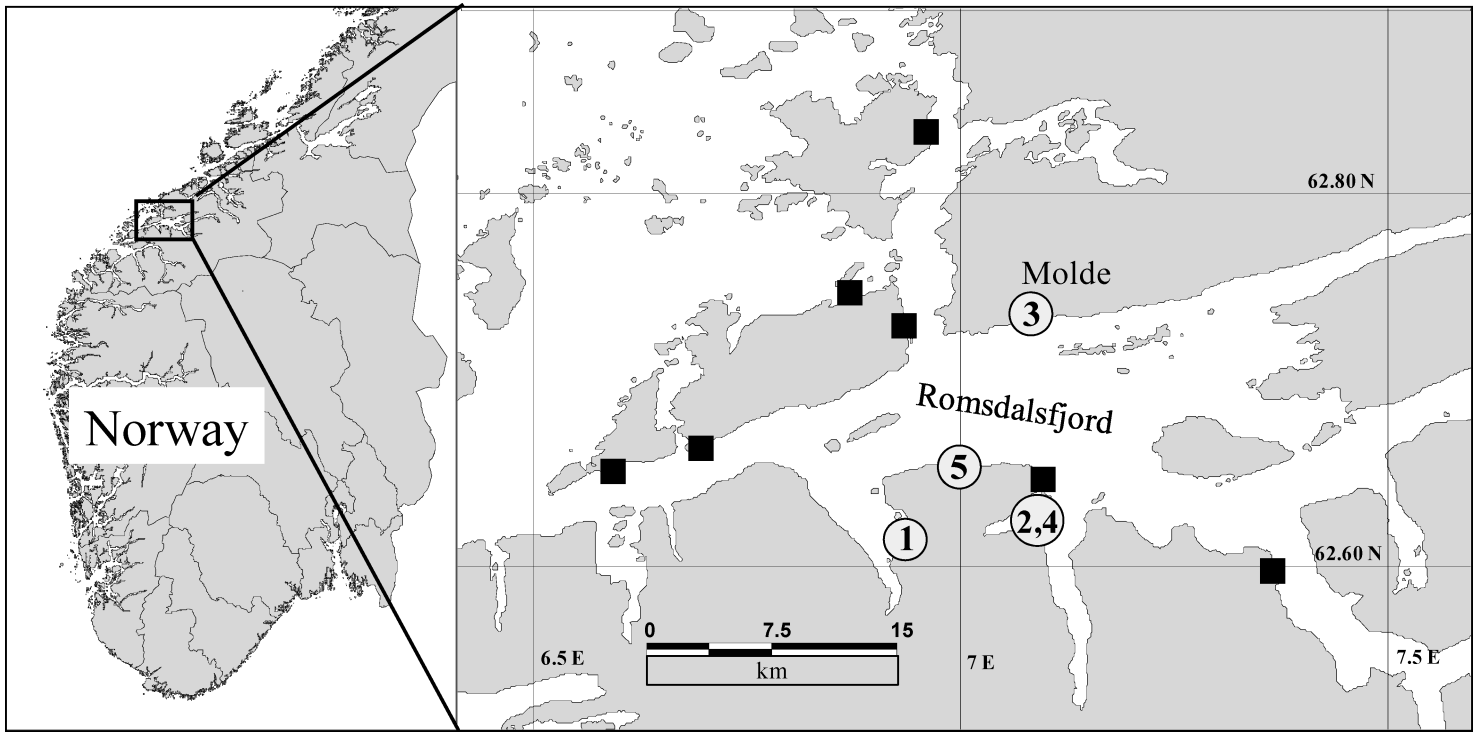

Figure 1. Location of the farms (black squares) from which the 16 baselines samples were collected, and location of the five fishers who recaptured the escapees (the fisher numbers in circles correspond to Table 1).

Table 1. Information available for the 32 recaptured farmed escapees, including assignment to baseline sample at $p = 0.05$.

Fish	Fisher	Capture date (week)	Sex	Maturity	Weight (kg)[a]	Length (cm)[b]	Winter zones	Capture method	Baseline sample
1	1	41	–	Immature	3.5	58	No	Net	1B
2	1	39	–	Immature	3.9	59	No	Net	1B
3	1	38	–	Immature	3.7	56	No	Net	4G
4	1	38	–	Immature	11.9	84	Yes	Net	Excluded all
5	1	38	–	Immature	4.9	59	No	Net	5I
6	1	38	–	Immature	3.5	57	No	Net	Not genotyped
7	1	38	–	Immature	2.4	52	No	Net	5I
8	1	37	–	Immature	3.7	58	No	Net	5I
9	1	37	–	Immature	3.7	61	No	Net	5I
10	1	37	–	Immature	3.3	59	No	Net	5I
11	1	37	–	Immature	2.8	54	No	Net	5I
12	1	37	–	Mature	2.4	53	No	Net	5I
13	1	37	–	–	4.4	62	No	Net	5I
14	2	39	♀	Immature	–	59	No	Angling	4G
15	2	38	♀	Immature	–	57	No	Angling	5I
16	2	38	♀	Immature	3.4[c]	62	No	Angling	Not genotyped
17	2	38	♀	Immature	3.2[c]	60	No	Angling	5I
18	2	38	♀	Immature	3.7[c]	63	No	Angling	5I
19	2	38	♀	Immature	2.7[c]	57	No	Angling	Excluded all
20	3	39	–	–	3.8	55	No	Angling	1A
21	3	39	–	–	3.2	56	No	Angling	5I
22	3	39	–	–	4.6	61	No	Angling	5I
23	4	38	–	Immature	–	42[d]	No	Net	5I
24	4	39	–	Immature	–	36[d]	No	Net	Not genotyped
25	4	40	–	Immature	–	29[d]	No	Net	Excluded all
26	5	38	–	Immature	3.1[c]	62	No	Net	5I
27	5	38	–	Immature	3.3[c]	58	No	Net	5I
28	5	38	–	Immature	1.5[c]	47	No	Net	5I
29	5	38	–	Immature	2.5[c]	59	No	Net	5I
30	5	38	–	Immature	3.4[c]	60	No	Net	4G/5I/5B
31	5	38	–	Immature	1.8[c]	50	No	Net	Excluded all
32	5	38	–	Immature	2.5[c]	54	No	Net	4G/5I

[a]Weight of fish excluding tail.
[b]Length of fish excluding tail.
[c]Gutted and tailed weight.
[d]Length without head or tail.

parameters utilized by other authors using this method of assignment (e.g. Hansen *et al.*, 2000).

Results

Basic information for escapees

Available information for the 32 recaptured escapees is presented in Table 1. Although biological information is fragmented, and some of the fish had been gutted and/or had their tails removed before weighing, it is apparent that most of the escapees' round weight would have been in the range 3–5 kg. Where reported, all fish, except one, were recorded as immature. The results of scale reading indicated a lack of any winter zones on all fish, except escapee 4. This fish was coincidently 11.9 kg and, from the biological information, did not appear to belong to the year class of smolts transferred to the sea in 2005.

Basic statistics

Of the 32 recaptured farmed escapees, three failed to produce readable genotypes and were consequently excluded from the study. One of the loci, *Sp*1605, provided irregular amplification in the remaining 29 farmed escaped fish, so was also removed from this part of the dataset.

The allelic variation observed in the 16 baseline samples and the 29 recaptured escapees is presented in Table 3. All markers were polymorphic in all samples, except *Ssa*D486, which was monomorphic in all fish except a single heterozygote in baseline sample 5J. Despite consisting of only 29 genotyped individuals, and lacking any genetic data on locus *Sp*1605, together the recaptured escapees displayed the joint second highest total number of alleles of any sample. Despite this, only one individual in this sample contained a single allele not observed in any of the baseline samples.

Out of 255 independent tests of Hardy–Weinberg equilibrium, 22 displayed significant deviations. Deviations were distributed

Table 2. Origin of the salmon groups reared on the seven marine farms sampled in Romsdalfjord.

Farm	Smolt producer											
	A	B	C	D	E	F	G	H	I	J	K	L
1	X	X	X	–	–	–	–	–	–	–	–	–
2	–	–	–	X	–	–	–	–	–	–	–	–
3	–	–	–	–	X	X	–	–	–	–	–	–
4	–	–	–	–	–	–	X	X	–	–	–	–
5	–	X	–	–	–	–	X	–	X	X	X	–
6	–	–	–	–	X	X	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	X

Smolt producers may rear more than one strain (data unavailable).

Table 3. Total and mean number of alleles observed in 15 microsatellites screened in 16 baseline samples collected on seven farms and 29 recaptured escapees (14 loci).

Sample	Number of individuals	Allele summary	
		Total	Mean
1A	48	93	6.2
1B	47	97	6.5
1C	48	106	7.1
2D	47	89	5.9
3E	48	93	6.2
3F	47	85	5.7
4G	48	115	7.7
5B	48	132	8.8
5H	47	103	6.9
5I	48	119	7.9
5J	47	122	8.1
5G	48	112	7.5
5K	47	99	6.6
6E	48	96	6.4
6F	47	102	6.8
7L	47	91	6.1
Escapees	29	122	8.7
Total	789	196	13.1

among the baseline samples and loci, with neither any of the 15 loci nor any of the 16 baseline samples accounting for more than three or four significant deviations, respectively. When Bonferroni correction for multiple independent tests was used to recalculate the significance level (15 repeat tests per baseline sample; revised $p = 0.003$), only two deviations remained significant. These were observed in baseline sample 5J for the locus *Ssa*D144 ($p < 0.0001$) and in baseline sample 5B for locus *SSsp*2201 ($p = 0.0007$). Within the sample of 29 escapees, before adjustment of the significance level, the following three loci were out of equilibrium: *Ssa*202, *Ssa*F43, and *Sp*2216. However, after Bonferroni correction (revised $p = 0.003$), none of these deviations remained significant.

Genetic differentiation among samples

Exact tests for pairwise genetic differentiation among the baseline samples, and genetic distances as computed by F_{ST} and Nei's standard genetic distance (Nei, 1978) are presented in Table 4. Among the 16 baseline samples, pairwise F_{ST} values ranged from <0.001 to 0.154. A UPMGA diagram illustrating relationships among the baseline samples is presented in Figure 2. The extent of genetic differentiation between pairs of baseline samples derived from the same smolt producer, but collected from different farms, varied substantially. To illustrate, samples 4G and 5G displayed a pairwise F_{ST} value of less than 0.001, indicative of a high degree of genetic similarity. However, other baseline samples consisting of salmon delivered by the same smolt producer showed moderate differences (F_{ST} between 3E and 6E = 0.029 and F_{ST} between 1B and 5B = 0.081), and, for the pair of baseline samples 3F and 6F, the differentiation between them was large ($F_{ST} = 0.149$).

On farms that reared salmon from different smolt producers in adjacent cages, differences between baseline samples were also highly variable. For example, farm 5 contained cages of salmon derived from five different smolt producers, and all samples displayed variable degrees of genetic differentiation among them (Figure 2). In contrast, farm 1 consisted of smolt groups having arisen from three separate smolt producers, two of which groups were almost genetically identical (1A and 1B), whereas the third was more distinct (1C) (Figure 2).

Assignment tests

Results of self-assignment among the 16 baseline samples indicated an overall correct assignment of 62.5% (Table 5). For specific baseline samples, correct self-assignment varied from a low of 28% in 5G to a high of 100% correct assignment in baseline samples 1C and 6F. Baseline samples displaying very low pairwise F_{ST} values from other baseline samples tended to display a greater degree of mis-assignment to each other. This is best illustrated by looking at assignment for the pair of baseline samples 1A and 1B collected on the same farm, but consisting of salmon delivered by two different smolt producers. In this instance, these baseline samples displayed correct self-assignment of 51 and 59%, respectively, and of the individuals mis-assigned, all but two were mis-assigned between them, reflecting their overall genetic similarity.

Following self-assignment simulations, the 29 recaptured escaped salmon were directly assigned to the baseline samples (Table 6). Here, each individual is assigned to the closest baseline sample irrespective of the absolute value of similarity. Of the 29 escapees, 21 were directly assigned to baseline sample 5I. However, direct assignment does not consider the possibility that not all potential donor populations are represented within the baseline. Exclusion-based simulations were calculated with two levels of rejection. At a probability of 0.01, 25–29 of the escapees tested were rejected from 12 of the baseline samples, whereas three of the escapees were rejected from all baseline samples. Between 19 and 21 of the escapees were rejected from a further three of the remaining baseline samples. However, baseline sample 5I stood out insofar as just 7 of the 29 escapees were rejected at this level of probability. When the level of stringency was increased, to provide a more conservative estimate of the potential origin of the fish, between 22 and 29 of the 29 escapees were rejected from 15 of the 16 baseline samples, and only nine of the escapees could be rejected from baseline sample 5I. At this level of probability, just four escapees were rejected from all samples.

Looking more closely at the assignment of the 29 escapees (Table 1), the 11.9 kg salmon displaying clear winter zones on

Table 4. Pairwise genetic distances observed between 16 baseline farm samples from Romsdalfjord, as estimated by Nei's (1978) genetic distance (lower diagonal) and F_{ST} (upper diagonal), based upon data from 15 microsatellite loci.

Baseline sample	Baseline sample															
	1A	1B	1C	2D	3E	3F	4G	4H	5I	5J	5G	5K	6E	6F	5B	7L
1A		<0.001	0.073	0.093	0.080	0.108	0.089	0.096	0.090	0.060	0.078	0.080	0.093	0.105	0.074	0.085
1B	<0.001		0.078	0.088	0.073	0.098	0.091	0.097	0.089	0.051	0.080	0.072	0.084	0.101	0.081	0.078
1C	0.174	0.187		0.115	0.099	0.135	0.092	0.105	0.097	0.080	0.082	0.112	0.115	0.074	0.066	0.119
2D	0.197	0.183	0.253		0.032	0.015	0.146	0.132	0.114	0.064	0.130	0.018	0.020	0.132	0.130	0.035
3E	0.177	0.161	0.227	0.055		0.032	0.140	0.134	0.118	0.051	0.125	0.028	0.029	0.118	0.121	0.009
3F	0.229	0.201	0.301	0.023	0.052		0.154	0.141	0.126	0.070	0.138	0.025	0.008	0.149	0.144	0.046
4G	0.236	0.241	0.236	0.368	0.379	0.382		0.023	0.044	0.088	<0.001	0.128	0.138	0.128	0.046	0.153
4H	0.233	0.237	0.254	0.294	0.324	0.311	0.048		0.062	0.098	0.016	0.125	0.132	0.139	0.064	0.148
5I	0.249	0.245	0.263	0.270	0.312	0.298	0.111	0.146		0.071	0.037	0.104	0.112	0.093	0.049	0.125
5J	0.155	0.123	0.211	0.129	0.109	0.137	0.257	0.260	0.208		0.078	0.041	0.055	0.092	0.086	0.057
5G	0.200	0.208	0.205	0.312	0.326	0.329	<0.001	0.031	0.093	0.222		0.114	0.125	0.112	0.038	0.142
5K	0.179	0.157	0.264	0.029	0.051	0.040	0.334	0.298	0.265	0.084	0.288		0.016	0.122	0.117	0.023
6E	0.206	0.183	0.264	0.032	0.052	0.010	0.360	0.309	0.278	0.114	0.315	0.027		0.134	0.126	0.040
6F	0.263	0.254	0.166	0.297	0.276	0.338	0.356	0.361	0.242	0.245	0.299	0.289	0.320		0.099	0.136
5B	0.196	0.219	0.166	0.324	0.324	0.361	0.117	0.153	0.132	0.263	0.095	0.311	0.330	0.263		0.133
7L	0.183	0.166	0.277	0.059	0.015	0.076	0.415	0.361	0.322	0.119	0.372	0.039	0.070	0.322	0.355	

All exact tests for pairwise population differentiation using Fisher's method were $p < 0.0001$, except 1A vs. 1B ($p = 0.3$), 3F vs. 6E ($p = 0.001$), and 4G vs. 5G ($p = 0.97$).

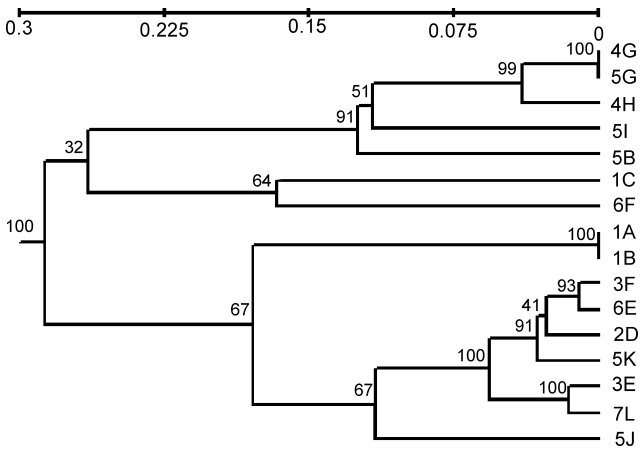

Figure 2. UPMGA diagram based upon Nei's (1978) genetic distance for 15 microsatellite markers combined. The numbers on nodes indicate the percentage of bootstraps supporting that node.

its scales was rejected from all samples. Of the three other salmon rejected at a probability of 0.05, two were very small fish, and fish 19 was within the size range of most of the other escapees.

Discussion

To the authors' knowledge, this study represents the first "real-life" example of genetics being used to assign farmed escaped Atlantic salmon, or any other recaptured escaped aquaculture species, to their farm of origin. A combination of direct assignment and probability-based exclusion indicated that 20 or 21 of the 29 escapees originated from a single farm in Romsdalfjord. In addition, the data were used to acquit the other six farms operating in the fjord from being the source of the escapement. The design of this study can provide governing bodies with a framework upon which to develop a management tool to trace farmed escaped salmon.

In Norway, it is not an offence for farmers to lose fish from an aquaculture installation unless the farm fails to comply with regulations. Nevertheless, farmers are legally obliged immediately to file a report of escapement, or even suspicion of escapement, to the Norwegian Directorate of Fisheries (FKD, 2004). In this case, no reports were filed despite the Directorate contacting all fish farms operating in the area. Upon completion of the analyses, genetic data were presented to the Norwegian Directorate of Fisheries and the Norwegian National Authority for Investigation and Prosecution of Economic and Environmental Crime. However, although samples were collected from the farm, staff from the company operating the farm acknowledged, before DNA analysis, that there had been an incident with the cage to which most of the escapees were traced, which is consistent with the results of this study.

In Norwegian aquaculture, >95% of the salmon produced originate in four major breeding programmes (O. M. Rødseth, pers. comm.). From the breeding stations where selection is practiced, this genetic material is numerically up-scaled and distributed to commercial marine farms through intermediate producers, some of which may also practice some additional form of selection, and strain crossing may occur. In a genetic study of some of the major Norwegian breeding strains, Skaala *et al.* (2004) reported interstrain pairwise F_{ST} values as high as 0.149, and demonstrated the potential for tracing escaped salmon through self-assignment simulations. However, the samples in Skaala *et al.* (2004) were collected directly from breeding stations. Consequently, until the present study, it was not known what level of genetic variation existed among groups of salmon reared in commercial production cages and whether opportunities for genetic assignment are retained. However, the pairwise F_{ST} values observed among production cages were as

Table 5. Results of self-assignment tests using the direct assignment Bayesian approach and the leave-one-out procedure in Geneclass 1.0 (Cornuet *et al.*, 1999).

Baseline sample	Baseline sample															
	1A	1B	1C	2D	3E	3F	4G	4H	5I	5J	5G	5K	6E	6F	5B	7L
1A	**24**	19	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1B	21	**27**	–	–	–	–	–	–	–	1	–	–	–	–	–	–
1C	–	–	**48**	–	–	–	–	–	–	–	–	–	–	–	11	–
2D	–	–	–	**25**	2	5	–	–	–	1	–	7	6	–	–	5
3E	1	–	–	3	**26**	4	–	–	–	–	–	4	3	–	–	12
3F	–	–	–	7	2	**23**	–	–	–	–	–	–	10	–	–	–
4G	–	–	–	–	–	–	**17**	2	1	1	27	1	–	–	–	–
4H	1	–	–	–	–	–	3	**39**	–	–	5	–	–	–	–	–
5I	–	–	–	–	–	–	2	2	**46**	–	1	–	–	–	1	–
5J	–	–	–	–	–	–	–	–	1	**38**	–	–	–	–	–	–
5G	–	–	–	–	–	–	26	3	–	–	**13**	–	–	–	2	–
5K	–	–	–	8	3	1	–	–	–	4	–	**21**	3	–	–	4
6E	–	–	–	2	4	9	–	–	–	1	–	11	**23**	–	–	1
6F	–	–	–	–	–	–	–	–	–	–	–	–	–	**47**	1	–
5B	–	–	–	–	–	–	–	–	–	–	1	–	–	–	**33**	–
7L	–	–	–	2	11	4	–	–	–	1	–	3	2	–	–	**25**
Percentage assigned correctly	51	59	100	53	54	50	35	85	96	81	28	45	49	100	69	53

Emboldened numbers represent the numbers of fish correctly assigned to a cage sample, and the other numbers represent misclassified individuals. Overall assignment = 62.5%.

Table 6. Results of direct assignment and exclusion of 29 farmed escapees from Romsdalfjord to 16 baseline samples collected from seven farms within the same fjord.

Test	Farm															
	1A	1B	1C	2D	3E	3F	4G	4H	5B	5I	5J	5G	5K	6E	6F	7L	Rejected all farms
Direct assignment to farm																	
	1 (0.03)	2 (0.07)	1 (0.03)	0	0	0	2 (0.07)	1 (0.03)	0	21 (0.72)	0	0	0	0	1 (0.03)	0	NA
Exclusion from farm																	
0.01	25	25	27	29	29	29	20	25	19	7	26	21	29	29	29	28	3
0.05	26	26	28	29	29	29	22	25	20	9	28	22	29	29	29	29	4

Exclusion is based upon a simulation with 10 000 individuals per population, and a probability that the escapee is below a threshold of 1 and 5% likelihood of having arisen from that baseline sample. NA, not applicable.

high as 0.151, and self-assignment of some of the baseline samples was as high as 100%, demonstrating excellent opportunities for genetic assignment.

A large variation in genetic distances was observed between pairs of baseline samples originating from the same smolt producer. For example, the lack of any significant genetic differentiation between baseline samples 4G and 5G ($F_{ST} < 0.001$) indicated that these farms received a very similar fraction of that smolt producer's (G) production. In other instances, such as between samples 3F and 6F ($F_{ST} = 0.149$), the smolt groups delivered to those farms were genetically distinct despite being delivered from the same producer (F). Although perhaps this is the result of receiving incorrect information on the origin of the fish, there are three operational practices which could explain these apparent discrepancies. First, smolt producers often receive eggs in batches throughout the breeding season. The numbers of families contributing to, and family composition of, each egg batch will differ because females are used just once, and males, if used on multiple occasions, may be rotated throughout the season. Second, because of egg availability, some smolt producers may rear fish derived from different genetic strains (i.e. they receive eggs from different sources). Third, although fish may be sorted and mixed throughout the fresh-water production stage, fish resulting from different egg batches are generally kept separate in consideration of potential disease issues and for traceability of their own production. Consequently, although it will vary, any given smolt producer may deliver a number of genetically distinct groups of smolts to cage producers.

In 2006, 921 licences for production of salmon and trout in marine cages were registered along the Norwegian coastline (Anon, 2007). Despite the demonstration of genetic differences among many of the baseline samples analysed in this study, there are likely to be a number of farms containing salmon with very similar genetic backgrounds throughout Norway. Consequently, rapid sampling of the escapees once they are observed, together with supplementary observations, may

provide an important component in the process of tracing the source of the escapees. In our study, supplementary observations of the escapees were used to demonstrate that the recaptured escapees assigned to the baseline samples most likely originated from a farm within the fjord. First, no official reports of losses of salmon by farmers outside the sampling area were registered, and no reports of recaptured farmed escapees by local fishers outside of the sampling area were reported following substantial efforts on the Directorate's part to collect such information (Norwegian Directorate of Fisheries, pers. comm.). Second, the present investigation was initiated after local fishers reported the sudden appearance of large numbers of escaped farmed salmon. This suggested that the salmon originated from within the same fjord. This is supported by the results of Skilbrei and Wennevik (2006), who observed that in areas where there is fishing pressure, even small-to-moderate escapement events are quickly detected and reported by local fishers. Third, in all but one case, no clear winter zones were observed on any of the scales taken from the escapees. This indicated that the salmon had not overwintered in the wild. Fourth, where maturation was recorded, only one fish was maturing. This indicates that the salmon were not mature individuals migrating into the fjord in search of a river to enter.

Genetic assignment tests have been implemented in many applications for a variety of species (e.g. Paetkau *et al.*, 1995; Hansen *et al.*, 2000, 2006; Koljonen *et al.*, 2005). Such tests are gaining importance in the management of mixed fisheries (Beacham *et al.*, 2004; Elfstrom *et al.*, 2007) and have been implemented in cases of fraud (Primmer *et al.*, 2000). Our study demonstrates a new and successful use for genetic assignment tests in the management of the Atlantic salmon. Although the escapees were successfully assigned to their cage of origin, the self-assignment accuracy varied greatly among the baseline samples and the average was 62.5%. Consequently, if fish had escaped from a different cage in the baseline, the identification of a single source of escapement may not be guaranteed. However, with the continued development of large numbers of single nucleotide polymorphisms in Atlantic salmon (Hayes *et al.*, 2007), the opportunity to screen large numbers of loci, and particularly the opportunity to identify strain-specific diagnostic loci, will lead to even greater precision in tracing the source of farmed escaped salmon in the future.

Summarizing, the results of this study have clearly demonstrated that genetic methods can be used to trace recaptured farmed escaped salmon back to a farm of origin with a high degree of accuracy. This can provide authorities with a tool for identification of farmed escapees where their source is not known. It is hoped that knowledge of the existence of this method will increase the likelihood of farmers sending reports of fish losses to the authorities. In turn, this will provide more accurate estimates of fish losses in production, and lead to a better understanding of the causes and solutions of, and hence ultimately to reduce, escapement.

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