Detection of European ancestry in escaped farmed Atlantic salmon, *Salmo salar* L., in the Magaguadavic River and Chamcook Stream, New Brunswick, Canada

Patrick T. O’Reilly, Jonathan W. Carr, Frederick G. Whoriskey, and Eric Verspoor


The use of European Atlantic salmon strains for commercial culture by the salmon farming industry has never been permitted in Nova Scotia or New Brunswick, Canada. Despite this, varying levels of European ancestry were detected in escaped farmed salmon in the Magaguadavic River (in 1999 and 2000) and in Chamcook Stream (in 2003), New Brunswick. Of the 53 escaped farmed salmon smolts from the Magaguadavic River and 17 escaped farmed parr from Chamcook Stream analysed, a single European “type” allele was observed at a single locus in two escaped farmed salmon smolts from the Magaguadavic River and in two escaped farmed parr from the Chamcook Stream. Of the 35 escaped farmed salmon adults analysed, two captured at the Magaguadavic fishway had European “type” microsatellite alleles at multiple loci and one also exhibited European “type” mitochondrial DNA. These results highlight the need for better containment strategies for freshwater hatcheries and genetic screening programmes for farmed salmon broodstock to minimize the likelihood of the introgression of non-local genetic material into severely depressed wild Atlantic salmon populations in the Bay of Fundy region.

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Introduction

Many concerns have been expressed about the possible genetic and ecological impact of escaped farmed salmon on wild salmonid populations (Hindar et al., 1991; Saunders, 1991; Carr et al., 1997). These concerns have intensified in North America with the decline of wild salmonid populations in areas where salmon farming is concentrated (Parrish et al., 1998). It is believed that genetic interactions between wild and farmed fish could reduce population size and resilience by diminishing the ability of future generations to survive and reproduce in the wild (Fleming et al., 2002; McGinnity et al., 2003).

Escaped Atlantic salmon may differ genetically from wild populations as a result of intentional selection for traits of interest, unintentional selection for captive rearing conditions, and genetic drift during domestication (Gjedrem et al., 1991; Gjoen and Bentsen, 1997; Johnsson et al., 2001; Fleming et al., 2002; McGinnity et al., 2003). The fact that an unknown number of escaped farmed salmon may be from strains of non-local origin is a further concern vis-à-vis the potential genetic impacts of farmed salmon on wild Atlantic salmon populations in North America. Salmon milt of European (Norwegian) origin was imported into Maine, USA, for use by the salmon farming industry in the mid-1980s, and in 1998, 30—50% of farmed salmon in Maine were thought to exhibit some level of European ancestry (Baum, 1998). The use of European strains of Atlantic salmon in commercial culture in Maine has been prohibited since 1993. European Atlantic salmon have...
European ancestry in aquaculture escapees in Canada

not been imported into New Brunswick or Nova Scotia under license for culture in sea cages, although some controlled experimental work has been permitted (Glebe, 1998). However, the salmon farming industry on the east coast of North America is concentrated along the border between Maine and New Brunswick (DFO, 1999; Figure 1). The same aquaculture companies operate in both jurisdictions and may transfer broodstock, eggs, or milt among their facilities across the border. In addition, escaped farmed fish can readily swim between Maine and New Brunswick (Figure 1). This has raised concerns that European genetic material may have been introduced inadvertently into aquaculture and wild populations in New Brunswick.

The introgression of Norwegian-origin farmed salmon into wild North American populations is potentially a greater threat than the introgression of local-origin salmon for two reasons. First, in experiments in culture and in the wild, Norwegian strains of farmed fish grew faster than wild salmon, and the offspring of F1 hybrids exhibited considerably higher mortality relative to salmon from controlled parental crosses (McGinnity et al., 2003, 2004). The authors of this multi-year study suggested that the high mortality observed in this F2 hybrid generation resulted from genetic divergence between the farmed and wild strains, probably associated with selection for faster growth and break-up of co-adaptive gene complexes. In New Brunswick, local farmed salmon strains are mostly derived from fish from the province's St John River, which have undergone fewer generations of domestic selection than the commercial Norwegian strains and exhibit rates of growth intermediate between two local wild populations tested (Lawlor, 2003). Second, except for a few isolated populations in Newfoundland and Russia, salmon from the two continents appear highly distinct genetically (Cutler et al., 1991; Taggart et al., 1995; Verspoor and McCarthy, 1997; King et al., 2001; Nilsson et al., 2001; Gilbey et al., 2005). In fact, analyses of mitochondrial DNA sequence divergence indicate that most salmon from Europe and

Figure 1. Map showing the location of Magaguadavic River and Chamcook Stream, the commercial salmon hatcheries, and salmon smolt and adult sampling sites.
North America may have been reproductively isolated nearly one million years (Nilsson et al., 2001).

Given the genetic differences between Norwegian farmed and wild salmon associated with intense intentional selection for faster growth in the former and the underlying genetic differences between North American and European Atlantic salmon that have accrued through selection and drift during long periods of reproductive isolation, there is cause for concern about the possible presence and increased effects of European farmed salmon on wild populations in Atlantic Canada. The objectives of this study were (i) to test for the presence of alleles that are common in European salmon but rare or absent in reference populations from the Bay of Fundy, in both escaped farmed and wild Atlantic salmon obtained from the Magaguadavic River and Chamcook Stream, New Brunswick; and (ii) to assess whether any European-type alleles found are likely to have resulted from historic introgression of natural transoceanic strays or from introduction through salmon farming-related activity in recent years.

Methods

Study area

The Magaguadavic River is the sixth largest river system in New Brunswick with a drainage area of 1812 km². A trap in the fish ladder in the hydroelectric dam at the head of the tide allows the capture of all fish moving into the river from the sea (Figure 1). Since 1996, all escaped farmed salmon entering the river have been removed at the fish ladder, having been distinguished from wild salmon using external morphology and scale circuli characteristics (Carr et al., 1997). Three commercial salmon hatcheries are located within the Magaguadavic River watershed (Figure 1). More than 70% of the Bay of Fundy sea cage facilities are situated within a 10 km radius of the river’s mouth.

Chamcook Stream drains from Chamcook Lake, emptying into Passamaquoddy Bay after 0.4 km (Figure 1). A dam at the lake’s outlet has blocked upstream fish passage for more than 100 years. No indigenous anadromous Atlantic salmon have been reported in Chamcook Stream. In 1974, a salmon genetics research and production facility was sited along the stream (Figure 1), but it was closed in 2004.

Collection of samples

Fin tissues collected from a sample of 58 wild and 53 escaped farmed salmon smolts captured in the Magaguadavic River in 1999 and 2000, either in a smolt wheel located 24 km above the head of the tide at Lee Settlement or in a smolt fence in the bypass stream of the hydroelectric dam (Figure 1), were screened for the presence of European alleles. In addition, fin tissue collected from a sample of 30 wild and 35 escaped farmed salmon, captured ascending the fish ladder in the Magaguadavic River during 1999 and 2000, was genetically screened. Finally, 17 presumably farmed salmon parr collected during electrofishing in Chamcook Stream in September 2003 were genetically screened. Before completion of any molecular genetic analyses, two of these fish were identified as being morphologically atypical for the species. Both specimens exhibited a unique spotting pattern and pectoral fin structure and colouration (short with white leading edges) resembling those of brown trout (Salmo trutta). The remaining 15 fish from Chamcook Stream were morphologically similar to Atlantic salmon from the area.

European and North American reference populations

Microsatellite variation was surveyed among salmon from 18 rivers in Europe. The average sample size per river was 43 (range 24–50). In all, more than 776 salmon contributed to the European salmon reference database. For North America, allele reference information was obtained from nearby populations within the Bay of Fundy and comprised 100 salmon each from the St John, Stewiacke, and Big salmon rivers, for a total of 300 Atlantic salmon collected between 2001 and 2004. The year of sample collection reflects the availability of samples and existing microsatellite genotype information. Samples of wild Atlantic salmon collected after 2003 were not available for most Bay of Fundy rivers.

Laboratory analysis

Genomic DNA was extracted from 5–25 mg of fin tissue and purified as described by Carr et al. (2004). Polymerase Chain Reaction (PCR) amplifications at the microsatellite loci Ssa197 and Ssa202 were carried out as in O’Reilly et al. (1996), and at the SSsp1605 locus as in Paterson et al. (2004). The sizes of unknown fragments (microsatellite alleles) were determined by acrylamide gel electrophoresis using internal ladder and external allele size standards, as discussed in Carr et al. (2004).

A portion of the mitochondrial DNA locus (3914–4546 bp, as given in Hurst et al., 1999) was PCR amplified using the forward 5’ ACGTGAATCTGACTTCAGAAGCGG 3’ and reverse 5’ TATTCCGGTAGAAGAGAAGGGGC 3’ primers, as described in Knox et al. (2002). Cycle-sequencing reactions were carried out using the oligonucleotide 5’ CGGTTTCTCACCCATTGACTGAACGG 3’ and Applied Biosystem’s Big Dye sequencing chemistry, following the manufacturer’s recommended procedures, and products were size-fractionated and detected using an MJ automated DNA sequencer/fragment analyser. European ancestry was indicated by the observation of a C at site 4079 and corroborated by the observation of a T at site 4082. North American ancestry was indicated by a T at site 4079 and a C at site 4082 (Nilsson et al., 2001; Verspoor et al., 2002).
Results

Of the 53 escaped farmed salmon smolts from the Magaguadavic River and 17 escaped farmed salmon parr from Chamcook Stream analysed, a single European-type allele was observed at a single locus in two escaped farmed smolts from the Magaguadavic River (MagSmt1 and MagSmt2) and in two escaped farmed salmon parr from the Chamcook Stream (ChamParr1 and ChamParr2) (Table 1). The smolt MagSmt1 was captured in May 2000 at the smolt counting fence in the bypass channel below the dam, and MagSmt2 was caught on the Magaguadavic River in a rotary screw trap situated 24 km above the head of the tide in May 1999 (Figure 1, Table 2). Both parr from Chamcook Stream (ChamParr1 and ChamParr2) were captured in 2003 (Table 2). Of the 35 escaped farmed salmon analysed, one European-type allele was observed at the two loci surveyed in a single farmed salmon adult (MagAdt1) captured in 1999 at the Magaguadavic fish ladder (Table 1). One other smolt from this collection (post-smolt MagPstSmt1), captured in 1999 at the Magaguadavic fish ladder, exhibited five European-type alleles: two European-type alleles at both the SSspl605 and Ssa202 microsatellite loci and a single European-type allele at the Ssa197 locus (Table 1). No European-type alleles were observed in any wild salmon obtained from the Magaguadavic River at any of the three microsatellite loci surveyed.

Discussion

The two escaped farmed salmon smolts obtained from the Magaguadavic River in 1999 and 2000 and the two escaped farmed salmon parr obtained from Chamcook Stream in 2003 exhibited a single microsatellite allele from a size class that occurs at very low frequency in reference populations from the Bay of Fundy (<1.5%). This could reflect the chance occurrence of rare North American alleles in the individual salmon surveyed, but it is worth noting that, although a similar number of escaped farmed (n = 53) and wild salmon (n = 58) smolts were analysed from the Magaguadavic River, all four fish in which European alleles were observed were from samples of farmed salmon (two farmed salmon smolts and two farmed salmon adults). Furthermore, the two parr collected from Chamcook Stream, before the microsatellite analyses, were identified, before the microsatellite analysis, as being morphologically distinct from other parr observed in that stream in terms of their spot patterns, body size, and fin colour; all the other 15 parr were morphologically similar to local wild salmon parr and exhibited only North-American-type alleles (data not shown). It is unlikely that these two individuals are brown trout or salmon–trout hybrids because allele sizes at the Ssa197 locus in brown trout generally range from 119 to 162 bp (Carlsson et al., 1999; Hansen, 2002), whereas both alleles in these two Chamcook Stream salmon parr were larger than 162 bp, typical in size for salmon from the Bay of Fundy area (Table 1; see also McConnell et al., 1997; Jones, 2001).

One escaped farmed salmon adult obtained from the Magaguadavic River in 1999 exhibited one European allele at each of two microsatellite loci analysed (SSspl605 and Ssa197). As all loci analysed here appear genetically unlinked (Gilbey et al., 2004) and, therefore, can be treated as independent characters, the likelihood of observing one

<table>
<thead>
<tr>
<th>Locus</th>
<th>Sample</th>
<th>SSspl605</th>
<th>Ssa197</th>
<th>Ssa202</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>218 + X(bp)</td>
<td>204 + Y(bp)</td>
<td>&gt;225 bp</td>
<td>&lt;225 bp</td>
</tr>
<tr>
<td>Europe</td>
<td>100 (100–100)</td>
<td>0 (0–0)</td>
<td>8 (0–21.9)</td>
<td>92 (78.1–100)</td>
</tr>
<tr>
<td>North America</td>
<td>0.5 (0–1)</td>
<td>99.5 (99.0–100)</td>
<td>0 (0–0)</td>
<td>100 (100–100)</td>
</tr>
<tr>
<td>Mag. Escapes</td>
<td>2.9</td>
<td>97.1</td>
<td>0.6</td>
<td>99.4</td>
</tr>
<tr>
<td>Mag. Wild</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>MagSmt1</td>
<td>258</td>
<td>NA</td>
<td>NA</td>
<td>311</td>
</tr>
<tr>
<td>MagSmt2</td>
<td>254</td>
<td>255</td>
<td>191</td>
<td>311</td>
</tr>
<tr>
<td>MagPstSmt1</td>
<td>250</td>
<td>183</td>
<td>171</td>
<td>299</td>
</tr>
<tr>
<td>MagAdt1</td>
<td>256</td>
<td>255</td>
<td>187</td>
<td>239</td>
</tr>
<tr>
<td>ChamParr1</td>
<td>236</td>
<td>167</td>
<td>179</td>
<td>247</td>
</tr>
<tr>
<td>ChamParr2</td>
<td>232</td>
<td>240</td>
<td>167</td>
<td>179</td>
</tr>
</tbody>
</table>

Suspect alleles of direct European origin from Magaguadavic and Chamcook samples are underlined. X, integers 1 through 12; Y, integers 1 through 18; NA, Not available; MagSmt1, Magaguadavic smolt #1; MagSmt2, Magaguadavic smolt #2; MagPstSmt1, Magaguadavic post-smolt #1; MagAdt1, Magaguadavic adult #1; ChamParr1, Chamcook Stream parr #1; ChamParr2, Chamcook Stream parr #2.
rare allele, occurring at a maximum frequency of 1.5%, of North American origin at both loci is relatively low (approximately 1:1000). One post-smolt captured ascending the river in 1999 exhibited five European-type alleles, including two at each of loci SSp1605 and Ssa202, and a single allele at Ssa197. The likelihood of observing five low frequency alleles (<1.5%) at the three loci surveyed under this scenario is less than 1.5 x 10^-9. A portion of the ND1 mitochondrial DNA locus (3914-4546; Hurst et al., 1999) in this individual was sequenced to further test this individual’s continent of origin. The mitochondrial DNA ND1 base 4079 was C and 4082 was T. The observation of a single European allele at either of these two sites could be explained by a rare mutation in a North American mitochondrial variant, but mutations at both informative sites to European-type states are unlikely.

Clearly, European genetic material was present among the escaped farmed salmon samples obtained from the Magaguadavic River, though at relatively low frequencies (~4% of escaped farmed smolts and 6% of escaped farmed adults). The presence and recent use of European genetic material by the local (US) salmon farming industry, the absence of European genetic material in the wild Magaguadavic River samples, and the occurrence of European-type alleles at multiple loci are consistent with a recent farmed origin of European genetic material, as opposed to natural transoceanic straying of European salmon in the recent or distant past. The two escaped farmed smolts may be second generation progeny (F2) of an original European-North American cross, progeny of F1 hybrids backcrossed to North American salmon, or possibly salmon from long generations removed from the original hybrid cross. The occurrence of a single European-type allele at both SSp1605 and Ssa202 in MagAdt1 is consistent with the escaped farmed adult being an F1 of an original hybrid cross; the absence of a European-type allele at the Ssa197 locus is not unexpected, given the moderate frequency of alleles of this size in European salmon. The escaped farmed post-smolt (MagPstSmtl), however, exhibited European mtDNA and two European alleles at both the SSp1605 and Ssa202 loci and a single European allele at the Ssa197 locus, consistent with this individual being a wholly European salmon, produced through the spawning of European parents. The distribution of nuclear alleles could not be explained by multiple backcrossing of hybrid salmon to wholly European males (e.g. via cryopreserved European milt) because this would not account for the European mitochondrial DNA, which is almost always maternally inherited. The high frequency of European nuclear and mitochondrial DNA within a single individual further suggests that pure breeding European lines of salmon were being maintained somewhere in the vicinity. The low frequency of occurrence of salmon exhibiting European ancestry may reflect the overall rarity of such individuals across the local industry. However, given the presence of so many possible sources in the area, the pattern observed may also reflect low levels of leakage from one tank or facility where European alleles are common, and higher levels of leakage from facilities exhibiting only or mostly salmon of North American origin. The high prevalence of European alleles in some individual fish analysed is consistent with the latter scenario.

The alternative hypothesis for the presence of European-type alleles in these samples is the successful spawning of transoceanic European strays in the Magaguadavic River in the recent past. European-type nuclear and mitochondrial DNA variants have been observed in salmon populations from eastern Newfoundland (Gilbey et al., 2005), likely reflecting low-level post-glacial gene flow during early colonization (Knox et al., 2002). However, this explanation is unlikely here for two reasons. First, if transoceanic strays had spawned successfully in the past and the frequency of European alleles in the area was very low, as appears to be the case, then one would expect to see a more diffuse pattern of European alleles in Magaguadavic salmon, and certainly not two or five nuclear alleles within single individuals at the three nuclear loci surveyed. Second, the frequency of alleles would be expected to be higher in the wild salmon samples relative to the farmed samples surveyed. Additionally, no European-type alleles have been identified in thousands of salmon surveyed from dozens of rivers along the east coast of Nova Scotia (unpublished data), which separates the Newfoundland and southwestern New Brunswick populations. Given the recent arrival and

Table 2. The location of capture, date of capture, life stage, size, and age information for Atlantic salmon suspected of exhibiting partial European ancestry.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Drainage</th>
<th>Site</th>
<th>Date</th>
<th>Stage</th>
<th>Fork length (cm)</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagSmt1</td>
<td>Mag. H. T.</td>
<td>Dam</td>
<td>May 2000</td>
<td>Smolt</td>
<td>17.5</td>
<td>1</td>
</tr>
<tr>
<td>MagSmt2</td>
<td>Mag</td>
<td>Lee Settle.</td>
<td>May 1999</td>
<td>Smolt</td>
<td>22.7</td>
<td>1</td>
</tr>
<tr>
<td>MagPstSmt1</td>
<td>Mag. H. T.</td>
<td>Dam</td>
<td>Oct 1999</td>
<td>Post-smolt</td>
<td>48.0</td>
<td>1</td>
</tr>
<tr>
<td>MagAdt1</td>
<td>Mag. H. T.</td>
<td>Dam</td>
<td>Oct 1999</td>
<td>Adult</td>
<td>57.0</td>
<td>2</td>
</tr>
<tr>
<td>ChampParr1</td>
<td>Chamcook</td>
<td>By hatchery</td>
<td>Sep 2003</td>
<td>Parr</td>
<td>20.9</td>
<td>0</td>
</tr>
<tr>
<td>ChampParr2</td>
<td>Chamcook</td>
<td>By hatchery</td>
<td>Sep 2003</td>
<td>Parr</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

Mag, Magaguadavic River; H. T., Head of tide; Lee Settle., Lee Settlement; NA, Not available.
proximity of intense salmon farming activity in the region (DFO, 1999) and the known use of European Norwegian milt in farmed salmon produced in the area (Baum, 1998), a more recent anthropogenic explanation would seem more likely. Evidence of European genetic material of probable aquaculture origin in one of the few monitored rivers in the area indicates that better containment and monitoring strategies are required to protect endangered salmon populations.

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