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Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analyses in land-locked Atlantic salmon *Salmo salar* L.*

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Abstract

Four tributaries of Lake St-Jean (Québec, Canada) are used for spawning and juvenile habitat by land-locked Atlantic salmon. Spawning runs have drastically declined since the mid-1980s, and consequently, a supportive-breeding programme was undertaken in 1990. In this study, we analysed seven microsatellite loci and mtDNA, and empirically estimated effective population sizes to test the hypotheses that (i) fish spawning in different tributaries form genetically distinct populations and (ii) the supportive breeding programme causes genetic perturbations on wild populations. Allele frequency distribution, molecular variance and genetic distance estimates all supported the hypothesis of genetic differentiation among salmon from different tributaries. Gene flow among some populations was much more restricted than previously reported for anadromous populations despite the small geographical scale (40 km) involved. Both mtDNA and microsatellites revealed a more pronounced differentiation between populations from two tributaries of a single river compared with their differentiation with a population from a neighbouring river. The comparison of wild and F_1-hatchery fish (produced from breeders originating from the same river) indicated significant changes in allele frequencies and losses of low-frequency alleles but no reduction in heterozygosity. Estimates of variance and inbreeding population size indicated that susceptibility to genetic drift and inbreeding in one population increased by twofold after only one generation of supplementation.

Keywords: conservation, stocking, population genetics, gene flow, effective population size, fish

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Introduction

It has been proposed that the number of populations within aquatic species is a function of the number of geographical or physical structures within which a species' life cycle can be completed (Sinclair 1988). This can lead to the evolution of local populations which may enhance survival and/or reproductive success of individuals in a particular environment (Carvalho 1993). One well-documented species, in terms of genetic diversity and population structure throughout its distribution range, is the Atlantic salmon *Salmo salar* (Ståhl 1987; Verspoor 1988; Davidson *et al*. 1989; Koljonen 1989; Elo 1993). Anadromous populations reproduce in rivers where juveniles will spend 1–7 years before migrating to ocean feeding grounds. After 1–3 years, sexually mature salmon generally return to their natal river to complete their life cycle. Such life history favours the formation of high numbers of local populations exhibiting a large degree of differentiation across contrasting environments (Ståhl 1983; Crozier & Moffett 1989; Koljonen 1989; Hindar *et al*. 1991; Verspoor *et al*. 1991; King *et al*. 1993), which may be adaptive (Ricker 1972; Ros 1981; Hindar *et al*. 1991; Taylor 1991). For example, inherited functional differences in body shape and paired fins length have been documented between juve-

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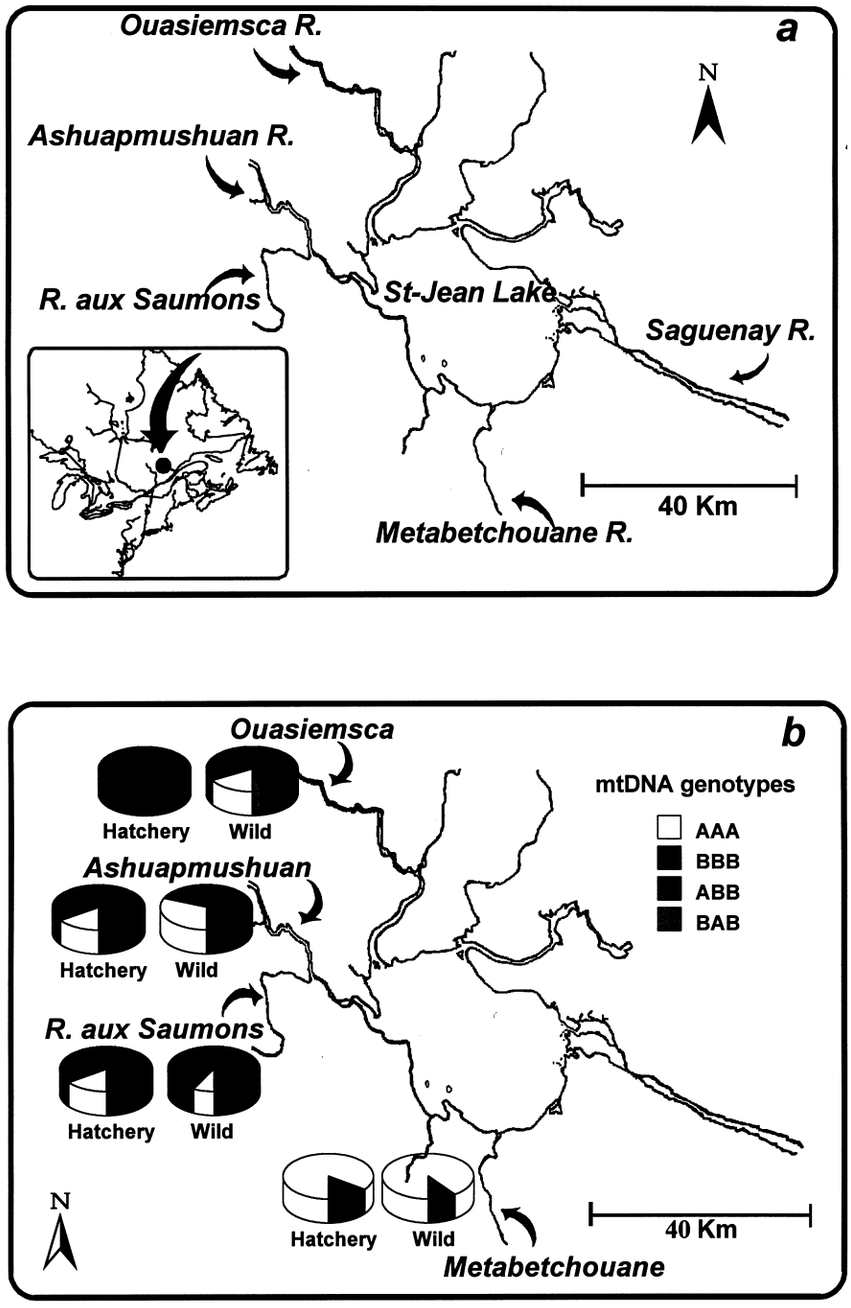

Fig. 1 (a) Location map of Lake St-Jean, and allelic frequency distribution at three selected loci for wild and F_1-hatchery land-locked salmon from four tributaries; (b) mtDNA, (c) μ79.1 and (d) Sfo-23.

nile salmon inhabiting fast-flowing or headwater streams and those living in streams with lower water velocities or being closer to the sea (Riddell & Leggett 1981).

Atlantic salmon also occur in land-locked populations both in North America and Europe (Dahl 1928; Power 1958; Havey & Warner 1970; Koch 1983). These display a life history similar to that of anadromous ones, except that the ocean phase is replaced by a lacustrine phase (Barbour & Garside 1983; Chernitskiy & Loenko 1983; Berg 1985; Berg & Gausen 1988). Literature about the genetic structure of land-locked populations is scarce and mainly concerns their genetic distinction from anadromous ones (e.g. Birt & Green 1986; Palva *et al*. 1989; Birt *et al*. 1991).

Lake St-Jean is a large lake (1100 km²) located in central Québec, Canada (72°00′W, 48°40′N) (Fig. 1a). Four of its tributaries drain within 40 km from each other and are used for spawning and nursery habitat by land-locked Atlantic salmon. Differences in egg size, growth, age at

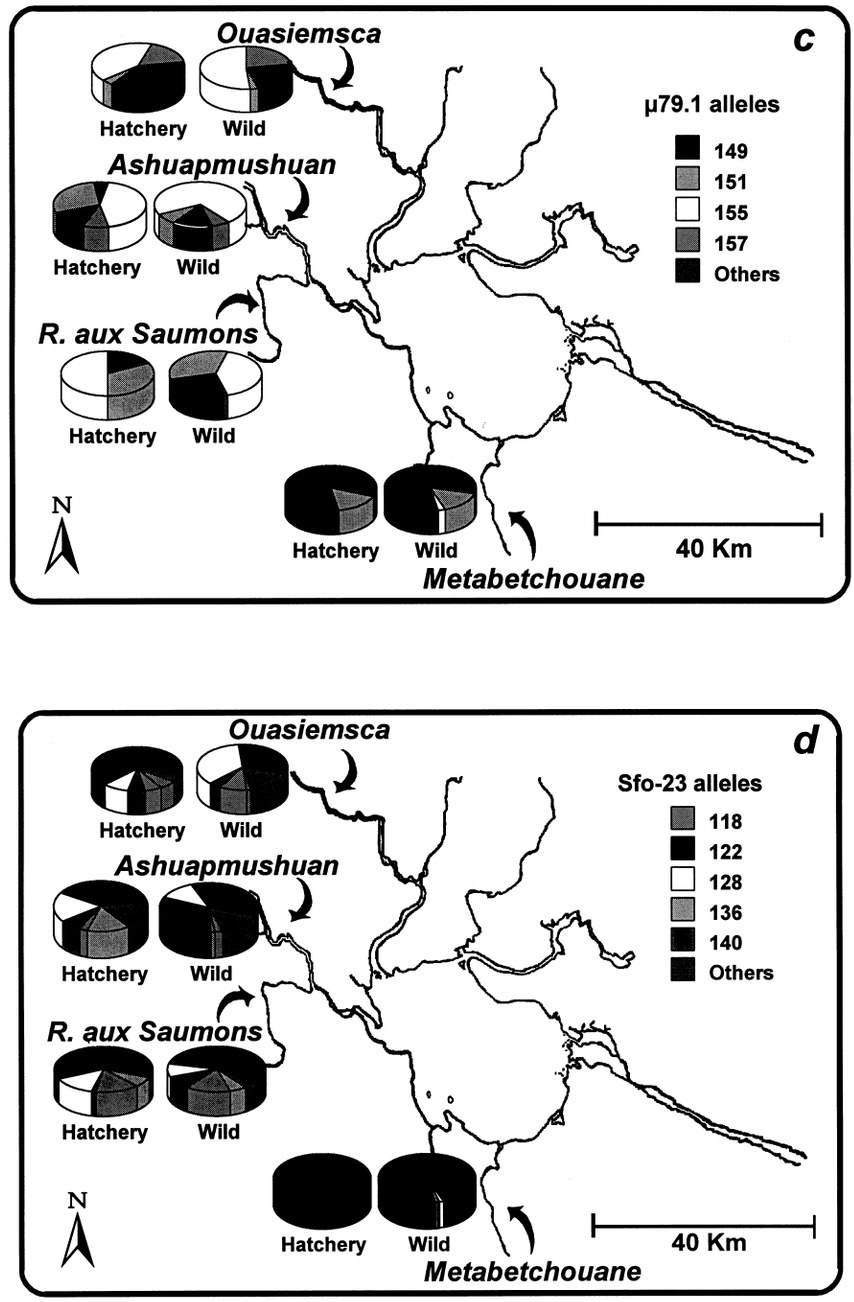

Fig. 1 *continued*

smoltification, and time of return to the river were observed among salmon from the different rivers, indirectly suggesting that they composed distinct populations (O. Gauthier, Ministère Environnement et Faune, Québec, personal communication).

Since the mid-1980s, spawning runs have declined drastically, and consequently a supportive-breeding programme was initiated in 1990. A fraction of wild parental fish from each river are brought into a hatchery for artificial reproduction, and the offspring are released into their river of origin where they can potentially interbreed with wild fish at completion of their life cycle. Depending on the number of captive spawners and their relative contribution to the overall progeny, such practice may result in increased inbreeding, reduction of heterozygosity, loss of rare alleles, leading to detrimental effects on wild populations (reviewed in Campton 1995). Using a small fraction of the wild parental fish for hatchery production may

favour the reproductive rate of one segment of the overall population, thus increasing the total variance of family size, a parameter of critical importance to either the variance (N_{eV}) or inbreeding (N_{eI}) effective size of the population. While such a programme may increase the absolute abundance of wild populations in the short term, they may threaten their genetic diversity through reduction of effective population sizes (Ryman & Laikre 1991; Ryman *et al*. 1995), and ultimately reduce population size in the long term (Frankham 1996). Waples & Do (1994) referred to this phenomenon as the 'Ryman–Laikre effect'.

We have recently shown that a combined analysis of mitochondrial and microsatellite DNA was best suited for characterizing genetic diversity of salmon from Lake St-Jean (Tessier *et al*. 1995). In the present study, we used this approach to compare genetic diversity among wild fish from four tributaries, and between wild and F_1-hatchery fish originating from the same river in order to test the hypotheses that: (i) fish spawning in different tributaries form genetically distinct populations and, (ii) the current supportive breeding programme causes genetic perturbations by reducing heterozygosity, number of rare alleles and both variance and inbreeding effective population size.

Materials and methods

Samples

Forty wild spawning fish (not issued from stocking programmes) were collected in 1994, either randomly throughout the spawning run or later during the autumn of 1994 on spawning grounds in four rivers draining into Lake St-Jean (Fig. 1a). We also randomly collected 40 F_1-hatchery parrs whose captive parents originated from each river and belonged to the same cohort as the wild spawning fish. The number of parents used to make hatchery crosses varied between 18 and 31 (Table 5). Adipose fins were clipped and preserved in 95% ethanol until total DNA extraction was performed according to Bernatchez *et al*. (1992).

Mitochondrial and microsatellite analysis

Mitochondrial DNA RFLP analysis was performed on two polymerase chain reaction (PCR) amplified segments using primers developed by Cronin *et al*. (1993) and Bernatchez & Danzmann (1993), encompassing the cytochrome *b* gene/D-loop (2.1 kb) and the NADH dehydrogenase subunit 1 (ND-1) (2.0 kb) regions, respectively. PCR conditions were as described in Bernatchez *et al*. (1995) except that the annealing temperature was 45 °C for the ND-1 segment. Pooled aliquots of the two PCR products were digested with 10 restriction enzymes (*Alu*I, *Ava*II, *Cfo*I, *Hae*II, *Hae*III, *Hpa*II, *Mbo*I, *Msp*I, *Rsa*I, *Taq*I). Electrophoresis and detection procedures were as described previously (Bernatchez *et al*. 1995). Composite mtDNA genotypes were defined by distinct combinations of polymorphic restriction sites observed across all restriction enzymes.

Microsatellite polymorphism was analysed by specific PCR at seven loci using primers developed for *Salmo trutta* L. (μ3, μ79.1, μ79.2; Presa 1995), *Salvelinus fontinalis* Mitchill (Sfo-23, Angers *et al*. 1995) and *S. salar* (SSOSL85, Ssa171, Ssa197; Slettan *et al*. 1995; O'Reilly *et al*. 1996). PCR conditions for amplifying *S. trutta* loci were as detailed in Tessier *et al*. (1995), and those for Sfo-23 followed Angers *et al*. (1995). PCRs for SSOSL85, Ssa171 and Ssa197 were performed in 15 μL reaction volume containing 2 units of *Taq* polymerase, 1.56 μL reaction buffer (10 mM Tris-HCl [pH 9.0], 1.5 mM $MgCl_2$, 0,1% Triton X-100, 50 mM KCl), 1.33 μM of each primer, 75 μM of each dGTP, dCTP, dTTP, 5 μM dATP, 0.15 μL of ^{35}S-dATP and 1 μL (50–100 ng) of total DNA. We used the following PCR profile: one denaturation at 95 °C for 5 min; 35 cycles of 1 min at 94 °C, 40 s at annealing temperature 55 °C, 1 min at 72 °C and a last elongation temperature at 72 °C for 5 min. Electrophoresis, fixation, drying and autoradiography followed standard procedures (Sambrook *et al*. 1989). Alleles were sized by comparison with the standard M13 sequence.

Gene diversity analysis among wild fish

Intrasample gene diversity was estimated by computing observed (H_O) and expected (H_E) heterozygosity at nuclear loci and by haplotype diversity index (h) for mtDNA (Nei 1987). Departure from Hardy–Weinberg equilibrium was estimated using the GENEPOP computer package, version 1.2 (Raymond & Rousset 1995). This uses the Markov chain method to obtain unbiased estimates of the exact Fisher test through iterations (1000 in this study) in order to test the alternative hypotheses of deficiency or excess of heterozygotes.

Genetic differentiation based on allelic and mtDNA genotype frequency differences among wild fish was performed for all pairwise comparisons of rivers at all individual locus by χ^2 randomization tests (Roff & Bentzen 1989) with 1000 permutations using the MONTE program of the REAP software package (McElroy *et al*. 1992).

Gene diversity among salmon from the different rivers for each locus was also assessed using the analysis of molecular variance model (AMOVA) of Excoffier *et al.* (1992). This procedure calculates standard variance components, and an array of allelic correlation measures, referred to as PHI statistics (Φ). Φ is a parameter analogous to F_{ST} which, however, takes into account variance in allele size between pairs of genes from different populations (Michalakis & Excoffier 1996). Φ-values were estimated for each individual locus, including mtDNA, and averaged for pooled microsatellite loci. No attempt was made to randomly allocate microsatellite alleles to nuclear haplotypes.

Significance of the observed values was tested using a random allelic permutation procedure available in the winAMOVA computer package.

Hardy–Weinberg, χ^2 and Φ probability values were adjusted for multiple simultaneous tablewide tests using the sequential Bonferroni adjustments (Rice 1989), in order to minimize type-I errors.

The extent of gene flow (Nm) among the different rivers was evaluated from overall Φ estimates of microsatellites by the equation: $Nm = ((1/\Phi) - 1)/4$ (Slatkin 1995; Michalakis & Excoffier 1996). Estimates of the effective number of females exchanged per generation was estimated from mtDNA Φ-values according to the approximation $N_fm = ((1/\Phi)–1)/2$. Although the veracity of the absolute gene flow estimates depends on several assumptions that may not be met in the present situation (population in equilibrium with respect to genetic drift and migration, island model of population structure), they nevertheless provide a basis for comparing gene flow among populations.

Because of the uncertainty regarding what constitutes the most appropriate method for quantifying population differences based on microsatellite polymorphism, relatedness among populations was estimated using two genetic distances; chord distance (Cavalli-Sforza & Edwards 1967), assuming pure genetic drift, and the stepwise weighted genetic distance measure (D_{SW}), which takes into account size differences between alleles and fit linearity with time better than IAM-based distance measures, when microsatellites follow a strict stepwise mutation model (Shriver *et al*. 1995). We then built neighbour-joining (NJ) phenograms from the resulting distance matrices. Bootstrap values on branching pattern were obtained by resampling loci within samples and given as percentages over 1000 replications using a program developed by JM Cornuet (INRA, Laboratoire de Neurobiologie comparée des invertébrés, Bures-sur-Yvette, France).

Gene diversity between hatchery and wild fish

Genetic changes between F_1-hatchery and wild stock of each river were quantified in terms of allele frequency differences, χ^2, Φ, D_c, and D_{SW} estimates as described above. Differences in expected heterozygosity (H_E) estimates and number of low-frequency alleles (frequency < 0.10) between wild and F_1-hatchery groups at all loci were tested by non-parametric Wilcoxon signed-rank tests (Statistica 1994).

The potential impact of releasing F_1-hatchery fish in wild population was estimated by computing both N_{eI} and N_{eV} of the Metabetchouane and R. aux Saumons populations (incomplete data for the other two rivers). N_{eI} quantifies the population susceptibility to inbreeding and N_{eV} that to genetic drift (Ryman *et al*. 1995). When the population size is constant, both parameters are identical. However, when populations are manipulated, as in the case of supportive breeding, the potential differences between the two must be considered (Ryman *et al*. 1995).

N_{eI} was estimated using the equation of Ryman & Laikre (1991),

$$\frac{1}{N_{eI}} = \frac{x^2}{N_c} + \frac{(1–x)^2}{N_w}$$

where N_c and N_w refer to the effective numbers of captive and wild parents, x is the relative contribution of offspring from the captive parents, and $(1–x)$ that of the wild ones. N_c was corrected for unequal sex ratio according to the relation $N_c = (4N_m N_f)/(N_m + N_f)$, where N_m and N_f are the actual number of male and female breeders (Wright 1931). No correction was made for wild fish as sex ratio is approximately close to 1.0 in those populations (O. Gauthier, Ministère de l'Environnement et de la Faune, Québec). No adjustment for variance in reproductive success among individuals was attempted as the data was not available. The relative offspring contribution of captive parents was estimated from the exact number of 0+ hatchery parrs released in each river. The relative offspring contribution of wild fish was approximated by the formula: $N_{Fw} \times N_E \times SR$, where N_{Fw} is the number of wild adult females enumerated at a counting fence during the entire spawning migration, N_E corresponds to the number of eggs produced by female, approximated at 3200 (O. Gauthier, Ministère de l'Environnement et de la Faune, Québec), and SR is the survival rate until 0 +, estimated to 15% for salmon of R. aux Saumons (Valentine 1991).

N_{eV} was estimated using the equation of Ryman *et al*. 1995),

$$N_{eV} = \frac{1–1/2N}{1/(N')^2[N'+(N'_c(N'_c–1/2)/N_c)+(N'_w(N'_w–1/2)/N_w)]–1/N}$$

where N is the total number of breeders ($N_c + N_w$) and N' is the summation of offsprings from captive (N'_c) and wild (N'_w) parents.

Results

mtDNA and microsatellite polymorphism

Low polymorphism was observed in mtDNA. Only three of the 10 restriction enzymes used were variable. The ND-1 segment was polymorphic for *Cfo*I and *Hae*II as was the cytochrome *b*/D-loop segment for *Alu*I (Table 1). Each of these enzymes revealed two fragment patterns that generated a total of four composite genotypes for an overall haplotypes diversity of 0.502 (Table 2). All microsatellites were polymorphic, the number of alleles per locus varying between two and 17, and overall heterozygosity at each locus ranging between 0.49 and 0.91 (Table 2, Table 3).

Table 1 Fragment patterns generated by polymorphic restriction enzymes used to screen pooled mtDNA segments of ND1 and cytochrome *b*/D-Loop mtDNA of wild and F_1-hatchery landlocked salmon from R. aux Saumons, Ashuapmushuan, Metabetchouane and Ouasiemsca rivers.

Enzymes	Fragments size (pb)										
*Alu*I	953	585	536	536	450	393	393	376	327	269	218
A	+	+	+	-	-	+	+	+	+	+	+
B	-	+	+	+	+	+	+	+	+	+	+
*Cfo*I	845	809	704	520	421	387	318	243	237	211	
A	+	+	+	+	-	+	+	+	+	+	
B	+	+	+	+	+	+	+	-	+	-	
*Hae*II	2012	1726	943	661	507	270	265				
A	-	+	+	+	+	+	+				
B	+	-	+	+	+	-	+				

Genetic divergence among wild populations

Gene-diversity analyses supported the hypothesis that land-locked Atlantic salmon from the different tributaries form genetically distinct populations. Highly significant differences ($P < 0.001$) in mtDNA genotype frequency were observed between the population of Metabetchouane river and the three other rivers (Table 4). The most common genotype (AAA, 90%) in Metabetchouane was observed at much lower frequency in the other rivers (Table 3, Fig. 1b). That population was also characterized by the presence of genotype ABB (8%) which was not observed elsewhere. The most frequent mtDNA genotype (BBB) in R. aux Saumons and Ashuapmushuan and Ouasiemsca rivers was observed in only one fish in the Metabetchouane river (Fig. 1b).

The degree of mtDNA differentiation between the Metabetchouane river population and the others was also illustrated by the highly significant ($P < 0.001$) Φ-values (Table 4). This suggested relatively weak female effective migration rates between this population and others, as reflected by the $N_f m$ values ranging from 0.065 to 0.339. Although Φ was not significantly different among the three other rivers, its value was more than twice as large between R. aux Saumons and Ashuapmushuan rivers than between the Ashuapmushuan and the Ouasiemsca rivers, suggesting that female-mediated gene flow between the Ashuapmushuan salmon and those from its tributary was lower than that with salmon from the neighbouring Ouasiemsca river (Table 4).

For microsatellites, the intrapopulation diversity was generally higher than for mtDNA, the expected heterozygosity ranging from 0.05 to 0.91 depending on locus and population (Table 3). No significant departures from Hardy–Weinberg equilibrium was detected. Following the Bonferroni sequential adjustments, most loci showed highly significant differences ($P < 0.001$) in allele frequency between all pairwise population comparisons (Table 4). As for mtDNA, the Metabetchouane population was most different from the other three rivers. For example, it was characterized at locus μ79.1 by the common allele 149 and the almost complete absence of allele 155 most common in the other populations (Table 3, Fig. 1c). At locus Sfo-23, the Metabetchouane population was almost fixed for the allele 122 which was observed at moderate frequencies in the other populations. That population also lacked several alleles (e.g. 118, 130, 136) shared by the others. R. aux Saumons population differed from those of Ashuapmushuan and Ouasiemsca rivers at the frequency of alleles 151, 155 and 157 of locus μ79.1 and that of allele 122 of Sfo-23 (Table 3, Fig. 1c,d). The Ouasiemsca river population differed from the other populations by the frequency of allele 157 at locus μ79.1 and that of 128 and 138 at Sfo–23. Additional differences in allele frequency distribution can be observed at locus μ3, SSOL85, Ssa71, and Ssa197 (Table 3).

Differences in allele frequency distribution translated into highly significant ($P < 0.001$) Φ-values at two to four loci depending on pairwise comparisons (Table 4). In congruence

Table 2 Sample size (*N*), number of alleles or mtDNA genotypes (*A*), alleles size in base pairs, and overall expected heterozygosity (H_E) or haplotype diversity (*h*) for mtDNA for the different loci used to analyse wild and F_1-hatchery landlocked salmon from R. aux Saumons, Ashuapmushuan, Metabetchouane and Ouasiemsca rivers

Loci	*N*	*A*	Size	H_E or *h*
MtDNA	285	4	–	0.502
μ3	313	6	204-216	0.672
μ79.1	308	6	149-161	0.687
μ79.2	302	2	120-122	0.491
SSOSL85	305	10	174-206	0.751
Ssa171	305	17	233-267	0.908
Ssa197	309	10	164-200	0.733
Sfo-23	274	12	114-144	0.621

Table 3 Allele and mtDNA genotype frequencies, total number of alleles or mtDNA genotypes (*A*), sample size (*N*), observed (H_O) and expected (H_E) heterozygosity (haplotype diversity for mtDNA) by locus for wild and F_1-hatchery landlocked salmon from R. aux Saumons (Rs), Ashuapmushuan (As), Metabetchouane (Met) and Ouasiemsca (Oua) rivers. Allele designation is expressed in base pairs. Order of enzymes for mtDNA: *Alu*I, *Cfo*I, *Hae*II

Locus\	Wild				F_1-hatchery			
Allele	Rs	As	Met	Oua	Rs	As	Met	Oua
MtDNA								
AAA	0.071	0.316	0.900	0.167	0.150	0.154	0.850	0.000
ABB	0.000	0.000	0.075	0.000	0.000	0.000	0.150	0.000
BBB	0.929	0.684	0.025	0.806	0.850	0.846	0.000	1.000
BAB	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000
A	2	2	3	3	2	2	2	1
N	14	38	40	36	40	39	40	38
h	0.138	0.438	0.186	0.327	0.258	0.264	0.258	0.000
μ3								
204	0.042	0.000	0.207	0.000	0.000	0.000	0.412	0.000
208	0.319	0.200	0.305	0.181	0.300	0.188	0.125	0.125
210	0.417	0.675	0.329	0.597	0.450	0.775	0.237	0.550
212	0.000	0.000	0.000	0.167	0.000	0.000	0.025	0.313
214	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000
216	0.222	0.125	0.159	0.042	0.250	0.038	0.200	0.013
A	4	3	4	5	3	3	5	4
N	36	40	41	36	40	40	40	40
H_O	0.667	0.450	0.634	0.417	0.575	0.300	0.475	0.525
H_E	0.683	0.495	0.740	0.589	0.653	0.364	0.726	0.592
μ79.1								
149	0.236	0.128	0.821	0.236	0.171	0.138	0.837	0.375
151	0.333	0.077	0.154	0.028	0.329	0.287	0.162	0.038
153	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000
155	0.417	0.718	0.026	0.514	0.500	0.438	0.000	0.412
157	0.000	0.064	0.000	0.222	0.000	0.087	0.000	0.175
161	0.014	0.013	0.000	0.000	0.000	0.000	0.000	0.000
A	4	5	3	4	3	5	2	4
N	36	39	39	36	38	40	40	40
H_O	0.556	0.410	0.256	0.528	0.579	0.850	0.225	0.560
H_E	0.669	0.464	0.306	0.639	0.621	0.706	0.276	0.666
μ79.2								
120	0.424	0.667	0.697	0.657	0.346	0.550	0.632	0.587
122	0.576	0.333	0.303	0.343	0.654	0.450	0.368	0.412
A	2	2	2	2	2	2	2	2
N	33	39	38	35	39	40	38	40
H_O	0.606	0.462	0.553	0.457	0.385	0.600	0.421	0.475
H_E	0.496	0.450	0.428	0.457	0.459	0.501	0.472	0.491
SSOSL85								
174	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000
182	0.043	0.000	0.000	0.000	0.000	0.000	0.000	0.000
186	0.057	0.190	0.250	0.014	0.075	0.087	0.359	0.029
194	0.414	0.357	0.600	0.400	0.350	0.475	0.513	0.235
196	0.343	0.048	0.125	0.114	0.375	0.250	0.128	0.265
198	0.029	0.012	0.013	0.057	0.100	0.087	0.000	0.250
200	0.029	0.036	0.000	0.100	0.013	0.000	0.000	0.000
202	0.029	0.190	0.013	0.071	0.087	0.063	0.000	0.015
204	0.043	0.131	0.000	0.143	0.000	0.013	0.000	0.088
206	0.000	0.036	0.000	0.100	0.000	0.025	0.000	0.118
A	9	8	5	8	6	7	3	7
N	35	42	40	35	40	40	39	34
H_O	0.711	0.691	0.600	0.800	0.650	0.850	0.564	0.735
H_E	0.771	0.787	0.569	0.789	0.723	0.701	0.599	0.801

Table 3 *continued*

Ssa171								
233	0.000	0.012	0.000	0.069	0.000	0.000	0.000	0.000
235	0.000	0.049	0.013	0.000	0.000	0.000	0.000	0.000
237	0.149	0.098	0.000	0.222	0.000	0.038	0.000	0.279
239	0.257	0.037	0.000	0.014	0.313	0.150	0.000	0.235
241	0.068	0.183	0.000	0.014	0.162	0.075	0.013	0.015
243	0.081	0.085	0.128	0.056	0.063	0.150	0.039	0.118
245	0.014	0.037	0.051	0.083	0.000	0.050	0.066	0.015
247	0.054	0.024	0.103	0.056	0.000	0.000	0.039	0.191
249	0.027	0.085	0.115	0.000	0.000	0.013	0.013	0.000
251	0.189	0.110	0.103	0.167	0.275	0.150	0.079	0.044
253	0.027	0.012	0.000	0.014	0.000	0.013	0.039	0.000
255	0.041	0.098	0.410	0.056	0.000	0.063	0.434	0.029
257	0.000	0.012	0.000	0.069	0.000	0.000	0.105	0.015
259	0.081	0.037	0.051	0.139	0.188	0.150	0.013	0.000
261	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000
263	0.000	0.110	0.000	0.042	0.000	0.087	0.013	0.044
267	0.014	0.012	0.026	0.000	0.000	0.050	0.145	0.015
A	12	16	9	13	5	13	12	11
N	37	41	39	36	40	40	38	34
H_O	0.865	0.951	0.872	0.917	0.900	0.875	0.737	0.882
H_E	0.864	0.912	0.785	0.888	0.771	0.897	0.774	0.823
Ssa197								
164	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000
168	0.000	0.000	0.024	0.000	0.000	0.000	0.038	0.000
172	0.257	0.037	0.286	0.111	0.400	0.025	0.321	0.074
176	0.419	0.598	0.583	0.458	0.338	0.363	0.436	0.309
180	0.162	0.183	0.012	0.306	0.188	0.025	0.026	0.456
184	0.027	0.012	0.000	0.000	0.000	0.000	0.000	0.000
188	0.000	0.000	0.095	0.000	0.000	0.000	0.179	0.000
192	0.000	0.134	0.000	0.111	0.000	0.237	0.000	0.088
196	0.081	0.024	0.000	0.000	0.000	0.213	0.000	0.059
200	0.054	0.012	0.000	0.000	0.075	0.138	0.000	0.015
A	6	7	5	5	4	6	5	6
N	37	41	42	36	40	40	39	34
H_O	0.703	0.610	0.429	0.639	0.475	0.925	0.744	0.559
H_E	0.732	0.597	0.575	0.681	0.694	0.756	0.682	0.690
Sfo-23								
114	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.000
116	0.019	0.000	0.000	0.000	0.014	0.000	0.000	0.000
118	0.148	0.014	0.000	0.097	0.143	0.029	0.000	0.061
122	0.574	0.365	0.973	0.319	0.429	0.382	1.000	0.682
126	0.000	0.014	0.000	0.000	0.000	0.015	0.000	0.000
128	0.093	0.122	0.014	0.361	0.143	0.191	0.000	0.091
130	0.019	0.027	0.000	0.028	0.157	0.074	0.000	0.000
134	0.000	0.027	0.000	0.028	0.000	0.029	0.000	0.015
136	0.056	0.027	0.000	0.014	0.071	0.147	0.000	0.061
138	0.000	0.000	0.000	0.056	0.014	0.000	0.000	0.030
140	0.093	0.324	0.014	0.042	0.014	0.074	0.000	0.061
144	0.000	0.081	0.000	0.056	0.014	0.000	0.000	0.000
A	7	9	3	9	9	9	1	7
N	27	37	37	36	35	34	35	33
H_O	0.593	0.784	0.027	0.694	0.857	0.941	0.000	0.546
H_E	0.639	0.748	0.054	0.759	0.756	0.791	0.000	0.523
Mean A	6.286	7.143	4.429	6.571	3.714	6.429	4.286	5.857
Mean H_O	0.672	0.623	0.482	0.636	0.632	0.763	0.452	0.612
Mean H_E	0.693	0.636	0.494	0.686	0.668	0.674	0.504	0.655

Table 4 Pairwise and global Φ estimates, effective number of migrant per generation estimated from microsatellites (Nm) and mtDNA (N_fm) for R. aux Saumons (Rs), Ashuapmushuan (As), Metabetchouane (Met) and Ouasiemsca (Oua) and between wild (w) and F_1-hatchery (h) groups of landlocked salmon originating of the same river. **:significant Φ estimates ($P < 0.001$); bold: pairwise comparisons with significant allele frequency differences ($P < 0.001$) following sequential Bonferroni adjustments for simultaneous tests

	ADNmt	μ3	μ79.1	μ79.2	SSOSL85	Ssa171	Ssa197	Sfo-23	Global	Nm	N_fm
Wild-Wild											
Rs(w)As(w)	0.103	0.000	**0.188****	0.100	**0.031**	**0.024**	**0.000**	**0.265****	0.122	1.799	4.354
Rs(w)Met(w)	**0.885****	0.057	**0.491****	**0.129****	**0.079**	**0.286****	**0.082****	**0.083**	0.226	0.856	0.065
Rs(w)Oua(w)	0.000	**0.000**	**0.124****	0.090**	**0.224****	**0.021**	**0.000**	**0.071**	0.071	3.271	infinite
As(w)Met(w)	**0.596****	**0.065**	**0.750****	0.000	**0.188****	**0.104**	**0.175****	**0.497****	0.317	0.539	0.339
As(w)Oua(w)	0.041	**0.000**	0.000	0.000	0.049	**0.000**	0.007	**0.097**	0.041	5.848	11.695
Met(w)Oua(w)	**0.763****	**0.062**	**0.667****	0.000	**0.468****	**0.104**	**0.101**	**0.318****	0.292	0.606	0.155
Mean	0.398	0.031	0.370	0.053	0.173	0.090	0.061	0.222			
Wild-Hatchery											
Rs(w)Rs(h)	0.000	0.000	0.000	0.000	0.012	**0.004**	0.015	0.000	0.004		
As(w)As(h)	0.046	0.021	0.067	0.016	**0.006**	**0.021**	**0.293****	**0.119****	0.083		
Met(w)Met(h)	0.000	0.000	0.000	0.000	0.019	**0.148****	0.000	0.007	0.077		
Oua(w)Oua(h)	**0.114**	0.000	0.033	0.000	**0.000**	**0.099**	0.042	**0.053**	0.065		
Mean	0.040	0.005	0.025	0.004	0.009	0.068	0.088	0.045			

with the previous results, the highest overall Φ-values were observed between the Metabetchouane river and the other populations. As with mtDNA, highly restricted gene flow ($Nm < 1$) between Metabetchouane and other populations was indicated. A higher overall Φ-value was observed between the Ashuapmushuan river population and that of its tributary R. aux Saumons than with the Ouasiemsca river population, again corroborating mtDNA results. Gene flow between R. aux Saumons and Ashuapmushuan river is estimated to be one-third that between the Ashuapmushuan and Ouasiemsca rivers (Table 4).

Relationships among populations are illustrated by the neighbour-joining phenogram built from the matrix of pairwise chord-distance genetic distance estimated from microsatellite data (Fig. 2). The Metabetchouane river population clearly clustered separately from the other three populations. The Ashuapmushuan river population clustered closer to that of the Ouasiemsca river than that of its tributary R. aux Saumons. This relationship was supported by a bootstrap value higher than that of the majority-rule criterion of 50%. A similar topology for the relationships among wild populations was obtained based on D_{SW} distance.

Genetic divergence between wild and hatchery fish

Genetic diversity analyses revealed changes in the genetic composition between F_1-hatchery and wild populations of the different rivers. Thus, a significant difference in mtDNA genotype frequency was observed between wild and F_1-hatchery fish from the Ouasiemsca river, implying the absence of genotypes AAA and BAB in hatchery fish (Table 3, Fig. 1b). The change in mtDNA genotype frequencies translated into a higher Φ-value (although not significant) between these two fish groups than was observed between Ouasiemsca, Ashupmushuan, and R. aux Saumons wild populations (Table 4).

For microsatellites, significant differences in allele frequency ($P < 0.001$) were observed between F_1-hatchery and wild fish at three loci in Ouasiemsca, four in Ashupmushuan, and one in each R. aux Saumons and Metabetchouane river populations (Table 4). For example, significant changes in the frequency of alleles 151 and 155 at locus μ79.1, alleles 136 and 140 at Sfo-23, and alleles 196 and 200 at Ssa197 were observed between the two Ashuapmushuan fish groups (Table 3, Fig. 1c,d). Similarly, important changes in the frequency of alleles 122 and 128 at Sfo-23, alleles 194, 196 and 198 at locus SSOL85, and alleles 239, 247 and 251 were observed between the Ouasiemsca groups. Numerous other differences can be found in Table 3.

Changes in allele frequencies translated into significant Φ-values only between hatchery and wild fish groups of the Ashuapmushuan and the Metabetchouane rivers (Table 4). It is nevertheless noteworthy that three out of four overall Φ estimates were as high or higher than some observed among the Ouasiemsca, Ashuapmushuan and R. aux Saumons wild populations. Genetic distances among wild and F_1-hatchery fish from a given river were generally important, as illustrated by branch lengths of the NJ phenogram, and in the case of Ashuapmushuan, wild and F_1-hatchery groups did not cluster together (Fig. 2). The topology of the NJ phenogram based on D_{SW} only differed by the position of the Ouasiemsca F_1-hatchery group which clustered closer to the Ashuapmushuan F_1-hatchery group than to its parental population.

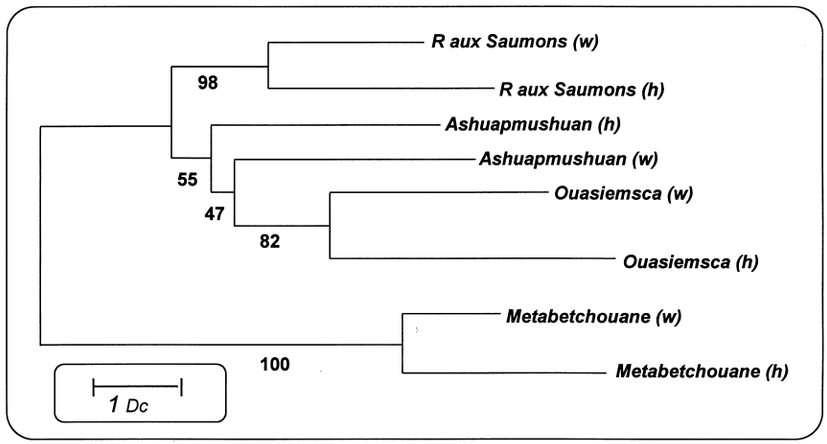

Fig. 2 Neighbour-joining phenogram constructed from the matrix of pairwise of chord distance estimates clustering wild (w) and F_1-hatchery (h) land-locked salmon originating of R. aux Saumons, Ashuapmushuan, Metabetchouane and Ouasiemsca rivers. Bootstrap estimates (in percentage) are given along branches.

No clear tendency (Wilcoxon; $P > 0.05$) of reduced expected heterozygosity was observed from wild to hatchery fish (Table 3). However, a significantly lower number of low-frequency alleles was detected in the F_1-hatchery fish in all populations (Wilcoxon; $P = 0.016$) (Table 3). For example, several alleles, such as SSOSL85-200 for Ouasiemsca, Ssa171-237 for R. aux Saumons and Sfo23-144 for Ashuapmushuan, with an observed frequency between 0.08 and 0.15 in wild populations were absent in hatchery groups. Also, the number of alleles with a frequency less than 0.10 that were observed in wild populations but absent in hatchery groups was always higher than the reciprocal: R. aux Saumons, 13 vs. two; Ashuapmushuan and Ouasiemsca, eight vs. three; Metabetchouane, seven vs. four.

The potential impact of releasing F_1-hatchery parr on both inbreeding (N_{eI}) and variance (N_{eV}) effective population sizes of wild populations is described in Table 5. Both (N_{eI}) and (N_{eV}) estimates were comparable for a given population, although N_{eV} was slightly higher in both cases. However, results were quite different between the two populations. For the Metabetchouane, release of hatchery fish would result in a nearly 50% reduction in effective population size, passing from an estimate of 607 ($N_{eI} = N_{eV}$) for a natural situation without stocking impact to estimates of 323 and 359 for N_{eI} and N_{eV}. In contrast, very similar values were observed with or without stocking for the R. aux Saumons population.

Discussion

MtDNA and microsatellite polymorphism

Very low polymorphism was detected in mtDNA despite the screening of two mtDNA segments totalling 4.1kb with 10 restriction enzymes that resolved 87 restriction sites on average per in fish. In a preliminary study of land-locked salmon that also included the PCR-RFLP analysis of the ND5 and ND6 regions (≈ 2.4 kb), no polymorphism was detected in those regions using the same enzymes. A subset of nine enzymes that resolved 63 additional restriction sites were also invariant for the mtDNA segments studied here (Tessier *et al*. 1995). It is noteworthy that despite its reduced polymorphism, mtDNA was among the most discriminating loci as reflected by its high overall Φ estimate (Table 4). This corroborates theoretical predictions of more restricted gene flow ($N_f m$) and increased genetic drift at mtDNA resulting from smaller effective population size imposed by haploidy and maternal transmission (Takahata & Slatkin 1984).

Low mtDNA diversity in salmon from Lake St-Jean corroborates other mtDNA studies in anadromous and land-locked Atlantic salmon which all reported low levels of polymorphism compared with many other fishes (e.g. Birt & Green 1986; Birmingham *et al*. 1991; King *et al*. 1993; O'Connell *et al*. 1995; Nielsen *et al*. 1996). Similarly, unusually low polymorphism at protein loci has generally been reported in this species (Verspoor 1994). Together,

Table 5 Number of wild (N_w) and captive (N_c) adults that participated to the production of offspring in 1995, production of 0+ salmon in wild and hatchery, contribution of hatchery in total offspring production (x), predicted inbreeding and variance effective population size for Metabetchouane (Met) and R. aux Saumons (Rs) populations following release of hatchery offspring

	N_w	N_c	No. of 0+ in wild	No. of 0+ in hatchery	x	N_{eI}	N_{eV}
Met	607	31	145 920	51 753	0.26	323	359
Rs	194	18	46 560	17 815	0.28	141	176

these observations are indicative of historical genetic bottlenecks in Atlantic salmon, as observed in other northern fishes, such as lake whitefish *Coregonus clupeaformis* (Bernatchez *et al*. 1989) and Arctic charr *Salvelinus alpinus* (Wilson *et al*. 1996).

The high levels of polymorphism observed at most microsatellite loci in land-locked Atlantic salmon is in sharp contrast with the above observations but is congruent with the few available microsatellite studies conducted on anadromous populations. In a study involving the analysis of 104 individuals representing four populations, McConnell *et al*. (1995) reported a total number alleles per locus varying between four and 34, and heterozygosity estimates ranging from 0.30 to 0.89. Similar levels of variation were reported in two other studies involving comparable sampling effort (Sanchez *et al*. 1996; Fontaine *et al*. in press). While other factors, such as differential selective pressures among types of loci, may potentially be invoked, the high level of polymorphism observed in microsatellites likely reflect higher mutation rates that may have been sufficient to replenish intraspecific diversity at those loci following the postglacial range expansion of Atlantic salmon over the last 15 000 thousands years. Such an argument has also recently been proposed to explain discrepancies in polymorphism between microsatellites and other types of loci in other northern species such as brook char *Salvelinus fontinalis* (Angers *et al*. 1995), Atlantic cod *Gadus morhua* (Bentzen *et al*. 1996), and Arctic charr (P. C. Brunner *et al*. pers. comm.).

Population structure in land-locked Atlantic salmon

All measures employed to assess genetic differentiation revealed significant differences among salmon sampled from the four tributaries, thus supporting the hypothesis that they each harbour genetically differentiated populations. Alternative hypotheses could conceivably result in genetic variation among samples, such as natural selection, temporal variation in allele frequencies, and nonrandom sampling of fish. Selective effects imposed by the local environment on specific alleles cannot empirically be ruled out but appear unlikely in the present case, given the noncoding nature of microsatellite loci and their high degree of polymorphism. That all loci might be indirectly involved in selection through linkage to functional genes also appears unlikely by probability alone. Because our analysis was based on spawning adults, it is also difficult to imagine a scenario involving selection and no permanent population differentiation that would produce the results obtained here. Similarly, the sampling of adult salmon in the same year for all tributaries insured that the same cohorts were compared, thus minimizing the potential effect of temporal variation among samples. Because spawners were captured either randomly throughout the spawning runs or later during the autumn on spawning grounds, departure from randomness due to sampling of only a fraction of the spawning run cannot explain the magnitude of genetic divergence we observed among populations. It appears more plausible that genetic differences truly reflect the effects of drift and mutation acting on populations among which gene flow has been restricted. This suggests that mechanisms, such as homing ability, that drive population divergence among anadromous populations also operate in land-locked salmon populations. This also implies that land-locked populations have the potential to develop river-specific local adaptations and as such should be considered distinct management units (MU, *sensu* Moritz 1994).

Our results indicated various levels of genetic divergence among river populations. The most salient feature of this study was the demonstration of highly restricted gene flow between the Metabetchouane and the other three populations. Nearly alternate fixation of mtDNA genotypes and highly different allele frequency distribution in microsatellites resulted in Nm estimates < 1 in all pairwise comparisons involving that population. Partial reproductive isolation among lacustrine populations living sympatrically for parts of or their entire life cycle is a common feature of many northern fishes (e.g. Hartley *et al*. 1992; Taylor & Bentzen 1993; McVeigh *et al*. 1995; Bernatchez *et al*. 1996). However, the extent of isolation between the Metabetchouane river population and the other three populations is unusual, being surpassed only by sympatric dwarf and normal ecotypes of lake whitefish *Coregonus clupeaformis* occurring in Cliff lake (Maine) in which alternate fixation of mtDNA genotypes and alternate allelic occurrence at microsatellites were observed (Bernatchez & Dodson 1990, A. Chouinard & L. Bernatchez, unpublished data).

The fact that the Metabetchouane river is the most geographically distant population from others may suggest that isolation by distance is partly responsible for restricting gene flow between that population and others. However, the scale of 40 km appears relatively small to act as a strong isolating barrier for such a migratory species. This is supported by the observation that gene flow estimates reported here are much lower that those obtained by the analysis of the same microsatellites among anadromous populations separated by hundreds of kilometres (Fontaine *et al*. in press). A more obvious cause of reproductive isolation is the differential timing of spawning migration, which occurs in early September for the Metabetchouane river compared with July and August for the other populations.

At this time, the ultimate causes responsible for the development of such reproductive isolation are only hypothetical and their elucidation must await more

detailed studies. Later timing of the spawning run in the Metabetchouane river could simply be a consequence of the much shorter length of its river migration (7 km) compared with that of the other rivers which vary between 40 and 100 km. The development of reproductive isolation mechanisms could also be a consequence of directional selective pressures imposed by the need to maintain trophic specialization; this has recently proposed to explain the correlation between morphological and genetic divergence among sympatric ecotypes in whitefish (Bernatchez *et al*. 1996; Chouinard *et al*. 1996; Pigeon *et al.* 1997). Unlike whitefish, however, sympatry among salmon populations occurs only during the lacustrine feeding phase which begins at the smolt (2 years and more) stage, and no obvious morphological specialization, as commonly observed in many northern species complexes (Schluter & McPhail 1993; Skulason & Smith 1995) distinguishes the Metabetchouane population from the others. This suggests that factors promoting reproductive isolation in land-locked salmon may not be only trophically based as generally inferred for other lacustrine sympatric population complexes (Schluter & McPhail 1993). Taylor *et al.* (1997) recently came to similar conclusions to explain the sympatric occurrence of two reproductive ecotypes of kokanee *Oncorhynchus nerka* in Okanagan Lake (British Columbia) in absence of apparent differences in trophic ecology.

The genetic differentiation of the Metabetchouane population could also be explained by historical rather than ecological factors. For instance, the development of reproductive isolation between fish assemblages that evolved in separate glacial refugia and subsequently came into secondary contact have been documented in other fishes, such as whitefish (Bernatchez & Dodson 1990), smelt *Osmerus mordax* (Bernatchez & Martin 1996; Bernatchez 1997), and brown trout, *S. trutta* (McVeigh *et al*. 1995). The hypothesis that a similar scenario could be involved in St-Jean Lake Atlantic salmon is indirectly supported by the observation of alternate frequencies of mtDNA genotypes differing by three restriction sites (sequence divergence = 0.0053) between the Metabetchouane river and other populations. Firm demonstration of this historical hypothesis must however, await a more detailed phylogeographical study which was beyond the scope of the present study.

A second important feature of this study was the congruent demonstration by both mtDNA and microsatellite analyses of a more pronounced genetic differentiation between the Ashuapmushuan population and that of its tributary, the R. aux Saumons, than that observed between the Ashuapmushuan and the neighbouring Ouasiemsca river population. Population substructuring among branches of a same river system has previously been reported for anadromous Atlantic salmon (e.g. Crozier & Moffett 1989; Pollard 1992; King *et al*. 1993). To our knowledge, however, such differentiation has never reported to exceed that existing among populations from disjunct drainages. There is no obvious explanation at this time for the more pronounced differentiation of the R. aux Saumons population. Until detailed ecological studies are undertaken, one can only hypothesize that gene flow with other populations is more restricted due to ecological selection imposed by unique environmental features which remain to be identified.

Impact of supportive-breeding programme

The demonstration that the different tributaries harbour genetically distinct populations confirms that the strategy of supportive breeding established on a river basis is pertinent in order to maintain interpopulation genetic integrity. However, our results revealed that management practices have consequences on intrapopulation diversity, as indicated by important changes in allele frequencies and significant genetic divergence between wild and hatchery fish. Because we did not compare different cohorts of wild populations, it is not possible to assess firmly whether or not such changes are more important than those potentially occurring naturally due to drift in small populations. However, genetic divergence between wild and hatchery fish from the same population was in some cases as important as that observed among wild populations, which strongly indicates that genetic perturbations related to management practices is important.

While no reduction in heterozygosity (H_E) was detected in F_1-hatchery fish, significant losses of low-frequency alleles was detected in all populations at variable intensity. Such discrepancy between heterozygosity and allelic diversity is mainly imputable to the fact that alleles with low frequency contribute little to the overall heterozygosity as reflected by the asymptotic relationships between H_E and effective number of alleles for a given effective population size (Hartl & Clark 1989). Our results also corroborate previous empirical studies (e.g. Skaala 1992) and computer simulations suggesting that low-frequency alleles are subject to rapid extinction in populations with effective number of breeders less than 100 (Cross & King 1983), which was the case for all captive breeders populations in this study. While the loss of genetic variability has been discussed generally in terms of reduction in heterozygosity (e.g. Allendorf & Phelps 1980; Vuorinen 1984; Verspoor 1988), the present study suggests that allelic diversity may be a more sensitive measure of genetic perturbations. Waples *et al.* (1990) had previously reached similar conclusions on the basis of computer simulations relating loss of genetic variability with population sizes in Pacific salmon populations. Because of the importance of the reservoir that represents low-frequency alleles in terms

of genetic variation, allelic diversity may also constitute a more relevant measure of genetic health of populations (Ryman *et al*. 1995).

Changes in allelic composition at neutral loci may not be directly relevant to the health of populations, and consequently conclusions based on such analyses must be taken with caution. However, the behaviour of neutral alleles may mimic that of selected alleles, particularly in the case of small populations in which drift may counteract and even overwhelm the effect of selection depending on the relationships between effective size and selection intensity (Crow & Kimura 1970). This could be the case for populations of captive breeders in this study and even for wild populations in which spawning runs have in some cases declined to less than 30 breeders in recent years.

Several factors may potentially bias our estimates of supplementation effects on effective population sizes. First, consequences of supplementation on effective sizes may be more complex than approximated by the methods of Ryman & Laikre (1991) and Ryman *et al*. (1995). These methods deal with only one generation of supplementation and do not consider the effect of age structure and the temporal demography of wild populations. Also, both N_{eV} and N_{eI} estimates are potentially biased by uncertainties regarding the exact values of parameters such as the effective numbers of wild breeders (N_w) and their contribution of offsprings ($1–x$) that could be lower than approximated here. N_{eV} and N_{eI} estimates could also be affected to an unknown degree by the impossibility of quantifying variance in reproductive success in both wild and hatchery fish. If variance estimates were equal in both cases, then N_e/N ratio would also be equal and our interpretations would still hold true. On the other hand, the effect of supplementation on inbreeding would partially be offset if N_e/N ratio was larger in hatchery fish (resulting from more homogeneous family sizes). Another factor that may affect N_{eV} and N_{eI} values is that these were based on the relative abundance of hatchery-reared and wild fish estimated at the juvenile stage, assuming that the proportions would remain the same until sexual maturity. However, a lower adult survival of hatchery-reared progeny compared with wild fish would also reduce inbreeding effects due to supplementation.

Because of these potential caveats, our N_e estimates should not be considered absolute. They nevertheless provide a basis to quantify differential effects on inbreeding caused by different supplementation practices. Thus, both estimates of variance (N_{eV}) and inbreeding (N_{eI}) population size obtained in the present study indicated that susceptibility to genetic drift and inbreeding will increase after only one generation of supplementation. It is noteworthy, however, that this effect was much more pronounced for the Metabetchouane than the R. aux Saumons population, despite similar relative contribution (0.26 vs. 0.28) of progeny from captive breeders. The main difference between the two populations was the higher proportion of captive breeders relative to wild adults in R. aux Saumons river (9%) compared to Metabetchouane river (5%). Also, the mean number of released offsprings per captive breeder was lower for R. aux Saumons river (mean = 990) than for Metabetchouane river (mean = 1670). These differences and their impact on effective population size indicate that genetic perturbations caused by supportive breeding can be reduced by improving supplementation practices. For instance, there is no doubt that increasing the number of captive breeders to produce an identical proportion of progeny will reduce effects on effective population sizes. Such reduction will be more pronounced for N_{eV} than for N_{eI} (Ryman *et al*. 1995). These observations illustrate that, as recently pointed out by Campton (1995), genetic problems related to population supplementation may sometimes be more related to management practices than biological effects. It is, however, important to keep in mind that even though supplementation practices can be improved to reduce their effects on inbreeding, these may still entail substantial opportunities for directional ecological and genetic changes of wild populations we are trying to conserve (Waples & Do 1994).

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This study is part of N.T.'s PhD thesis on conservation genetics of *Salmo salar*, supervised by L.B. The major interests of L. B. and J. M. W. are in the understanding of patterns and processes of molecular and organismal evolution, as well as their significance to conservation.
