A genetic assessment of single versus double origin of landlocked Atlantic salmon (*Salmo salar*) from Lake Saint-Jean, Québec, Canada

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Abstract: We assessed the genetic relationships among landlocked populations and parapatric anadromous populations of Atlantic salmon (*Salmo salar*) from the Saguenay River system to infer the alternative scenarios of single versus double origin of salmon in Lake Saint-Jean, Québec, Canada. We predicted that the hypothesis of double origin would be supported by the closer relationship of some landlocked populations to anadromous ones than to other landlocked populations. Alternatively, the single-origin scenario would be supported if landlocked populations were genetically closer to each other than to anadromous populations. Contrasting results of population differentiation at microsatellite loci were obtained with statistical treatments involving allelic frequency alone (θ, D_{CE}) or incorporating mutational differences (R_{ST}, $(\delta\mu)^2$). A closer relationship among all landlocked populations of Lake Saint-Jean compared with anadromous ones was observed in analyses that only incorporated allele frequency information. In contrast, analyses incorporating allelic size variance all supported a closer relationship between the Métabetchouane population and other populations from outside Lake Saint-Jean. We discuss the possible factors responsible for these apparently contradictory results and propose alternative historical scenarios potentially responsible for the salmon population structure in Lake Saint-Jean.

Résumé : Nous avons comparé les relations génétiques entre populations résidantes et populations parapatriques anadromes de saumon atlantique (*Salmo salar*) situées le long de la rivière Saguenay, dans l'optique de vérifier les hypothèses alternatives d'une seule origine versus une double origine des saumons résidants (ouananiches) du lac Saint-Jean, Québec, Canada. L'hypothèse d'une double origine serait supportée si certaines populations résidantes étaient plus près génétiquement des populations anadromes qu'entre elles-mêmes, alors que celle d'une origine unique serait supportée si les populations résidantes sont plus près génétiquement les unes des autres qu'avec les populations anadromes. Des résultats opposés quant à la différenciation génétique aux locus microsatellites ont été obtenus, selon que les traitements statistiques étaient basés seulement sur les fréquences alléliques (θ, D_{CE}) ou incorporaient la variance de taille allélique (R_{ST}, $(\delta\mu)^2$). Toutes les populations résidantes étaient plus près génétiquement en considérant les seules fréquences alléliques, alors que la population Métabetchouane étaient plus semblables aux populations anadromes sur la base de la variance de taille. Nous discutons des facteurs pouvant potentiellement expliquer ces résultats apparemment contradictoires et proposons des scénarios historiques alternatifs pouvant expliquer la structure populationnelle contemporaine de la ouananiche du lac Saint-Jean.

Introduction

Members of the north temperate freshwater fish fauna comprise young populations issued from postglacial recolonization approximately 18 000 and 8000 years ago. Of particular interest is the occurrence of lacustrine sympatric populations that have been most frequently reported in salmonid fishes but also exist in phylogenetically remote families. Genetic analyses generally provided evidence for restricted, yet variable, genetic exchange among such populations, which indicated that evolutionary forces are promoting their reproductive isolation in the face of gene flow and that the process of speciation has not been completely achieved in most cases (Taylor and Bentzen 1993; McVeigh et al. 1995). Genetic analyses have also been of particular interest for elucidating the origin of sympatric populations. Namely, these have revealed that both allopatric and sympatric modes of population divergence have been involved in the evolution of different species (Taylor and Bentzen 1993; Pigeon et al. 1997). Sympatric lacustrine populations thus represent unique systems for studying processes potentially involved in the early steps of population divergence and, ultimately, speciation.

We recently documented the existence of four genetically distinct sympatric populations of landlocked Atlantic salmon (*Salmo salar*) in Lake Saint-Jean, Québec, Canada (Tessier et al. 1997; Tessier and Bernatchez 1999). The extent of genetic differentiation was highly variable among these populations. Namely, the genetic divergence between the Métabetchouane River and others based on microsatellite analysis was more pronounced than that reported among anadromous Atlantic salmon populations over a much larger geographical

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scale. While these data were suggestive of multiple origins of salmon populations in Lake Saint-Jean, a comparison with populations from outside the lake was needed to refute the alternative hypothesis of a postglacial sympatric divergence followed by limited gene flow and pronounced genetic drift.

In this study, we used mtDNA and microsatellite analyses to assess the relationships among landlocked populations and parapatric anadromous populations from the Saguenay River system to infer the alternative scenarios of single versus double origin of salmon in Lake Saint-Jean. We predicted that the hypothesis of double origin would be supported if there was a closer relationship between either the Métabetchouane or the other three landlocked populations and the anadromous populations than among themselves. Alternatively, the single-origin scenario would be supported if landlocked populations were genetically closer to each other than to anadromous populations.

Materials and methods

Biological materials

Lake Saint-Jean (48°40′N, 72°00′W) is connected to the Saint Lawrence estuary via the Saguenay River where four tributaries harbour anadromous Atlantic salmon populations (Fig. 1). The Saguenay River was inundated by the Laflamme Sea, a large periglacial sea, between 10 300 and 8700 years BP (Elson 1969) and was probably the most important colonisation route of Lake Saint-Jean used by anadromous fish following the last glacial retreat (Bernatchez 1997). Landlocked salmon samples analysed by Tessier et al. (1997) consisted of approximately 40 sexually mature fish collected in 1994 in each of the four tributaries of Lake Saint-Jean harbouring salmon: Rivière aux Saumons and the Ashuapmushuan, Ouasiemsca, and Métabetchouane rivers (Fig. 1). Genetic diversity observed among these samples (detailed in Tessier et al. 1997) was compared with that obtained from comparable size samples of four anadromous populations collected in the Mars, St-Jean, Petit-Saguenay, and Sainte-Marguerite rivers draining into the Saguenay River (Fig. 1). We also included a sample of 20 fish from an isolated landlocked population at Grand Lake, Maine, U.S.A. (Fig. 1), as a geographic outgroup population to put into perspective the extent of genetic difference among the Lake Saint-Jean and Saguenay populations.

Mitochondrial DNA and microsatellite polymorphism

mtDNA RFLP analysis was performed as described in Tessier et al. (1997) on cytochrome *b*, D-loop, and ND1 regions with 10 restriction enzymes (*Alu*I, *Ava*II, *Cfo*I, *Hae*II, *Hae*III, *Hpa*II, *Mbo*I, *Msp*I, *Rsa*I, *Taq*I). Composite mtDNA genotypes were defined by distinct combinations of polymorphic restriction sites observed across all restriction enzymes.

Microsatellite polymorphism among anadromous and Grand Lake samples was quantified by amplification of the same loci used for landlocked populations (Table 1) (also see Tessier et al. (1997) for details). Electrophoresis, autoradiography, and allele scoring were as outlined in Tessier and Bernatchez (1999).

Data analysis

The GENEPOP computer package (version 3.1) (Raymond and Rousset 1995) was used to compute haplotype diversity (h) for mtDNA and observed heterozygosity (H_O) and gene diversity (H_E) for microsatellites and to estimate departures from Hardy–Weinberg equilibrium globally over all loci using the U test. This implied the use of the Markov chain method (Guo and Thompson 1992) to obtain unbiased estimates of Fisher's exact test through 2000 iterations to test the alternative hypothesis of heterozygote deficiency or excess (Rousset and Raymond 1995). Exact tests were also used to perform pairwise comparisons of allele frequencies at individual loci and mtDNA haplotypes among populations. No additional phylogenetic analyses were required for mtDNA data, as only the two main haplotypes previously described for landlocked populations were observed in anadromous ones.

We compared the extent of genetic differentiation among populations by computing pairwise fixation indices based on allelic frequencies (θ of Weir and Cockerham 1984) using the analysis of molecular variance procedure (AMOVA, Michalakis and Excoffier 1996) available in the program Arlequin (version 1.1) (Schneider et al. 1997). We also computed pairwise standardised R_{ST} using the program $\mathrm{R_{ST}}$ Calc (version 2.2) (Goodman 1997). The different properties of these two measures of divergence may potentially provide a way to obtain information about the time scale involved in population divergence (Angers and Bernatchez 1998; Goodman 1998; Estoup and Angers 1998; Widmer and Schmid-Hempel 1999). For both θ and R_{ST} estimates, departures from the null hypothesis (estimates = 0) were statistically assessed by nonparametric permutation procedures (2000 iterations) in both Arlequin and $\mathrm{R_{ST}}$ Calc. Probability values in homogeneity tests of allele frequency distribution and pairwise estimates of genetic differentiation were adjusted to minimise type I errors for multiple simultaneous tablewide tests with sequential Bonferroni adjustments. Arlequin was also used to compute the components of genetic variance due to both allelic frequency and allelic size variance at two hierarchical levels: between groups and among populations within groups. Significant departure from the null hypothesis of no differentiation was tested by performing 2000 permutations of multilocus haplotypes within and among groups.

Relatedness among populations was estimated with neighbour-joining phenograms constructed with two different genetic distance estimates. We first used the pairwise Cavalli-Sforza and Edwards (1967) chord distance (D_{CE}), which assumes pure genetic drift. A second tree was constructed with $(\delta\mu)^2$ distances (Goldstein et al. 1995), which take into account deviations of allelic size variance under the hypothesis of the stepwise mutation model. Comparisons of both phenograms thus provided additionnal insight into the relative roles of genetic drift and mutation in population divergence. Bootstrapped confidence values on branches were obtained by resampling loci within samples and were given as percentages over 2000 replications using the program NJBPOP developed by J.M. Cornuet (Laboratoire de Modélisation et de Biologie Évolutive, INRA-URLB, Montpellier, France).

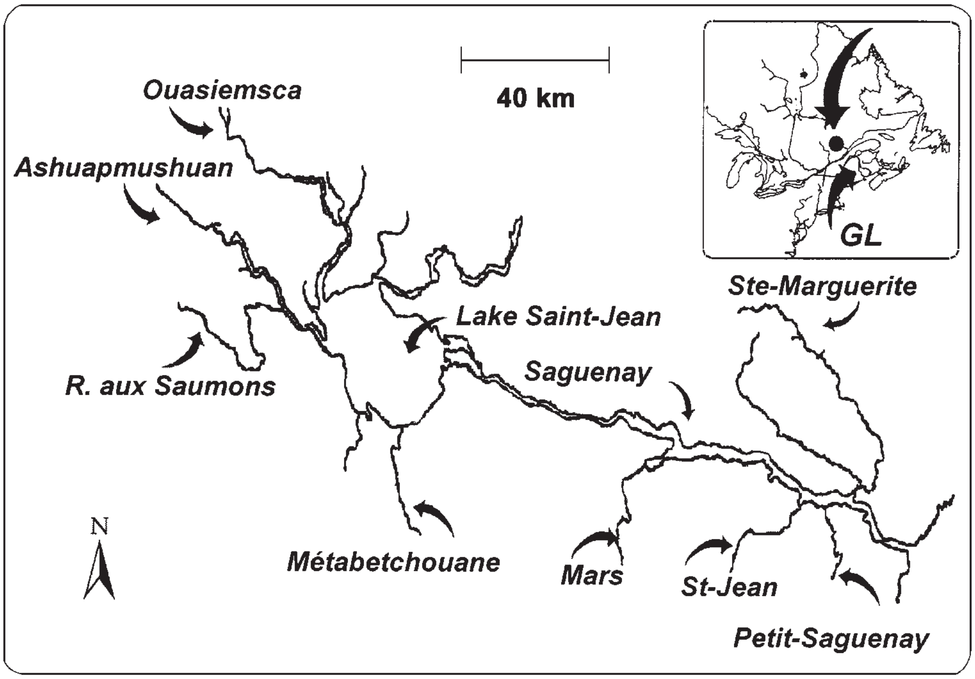
Results

No significant departures from Hardy–Weinberg equilibrium were detected by multilocus probability U test in landlocked and anadromous Atlantic salmon samples. We therefore cannot reject the null hypothesis of random mating for any of these samples. For microsatellite data, a higher genetic diversity was observed in anadromous samples, the number of alleles ranging from 64 to 69 compared with 32 to 48 for landlocked ones, and with a mean expected heterozygosity of 0.70 and 0.63, respectively (Table 1). mtDNA diversity was low in all landlocked or anadromous samples, with only three different haplotypes being observed.

Genetic divergence among Atlantic salmon populations

Most loci showed highly significant differences (following sequential Bonferroni adjustments) in allele frequencies (114

Fig. 1. Location map of Lake Saint-Jean (48°40′N, 72°00′W), Saguenay River, and Grand Lake (GL) (45°41′N, 67°48′W). Landlocked Atlantic salmon populations: Ouasiemsca, Ashuapmushuan, Rivière aux Saumons, Métabetchouane; anadromous populations: Mars, Ste-Marguerite, St-Jean, Petit-Saguenay.



significant comparisons out of 128 for eight loci; exact test, $P < 0.001$; data not shown) between all pairwise comparisons involving landlocked versus anadromous populations. The Grand Lake population was also very different from the other landlocked populations and from the anadromous ones (51 significantly different comparisons out of 64). However, the pattern of population differentiation was very different among landlocked or anadromous samples. The landlocked populations were much more differentiated among themselves in terms of allele frequencies (35 significant comparisons out of 48) than were the anadromous samples (only one significant comparison out of 48), showing that the latter ones were very similar genetically. Consequently, the null hypothesis of population panmixia could not be rejected for the anadromous samples.

No distinct pattern of differentiation between andromous and landlocked populations was observed for mtDNA. The most frequent haplotype (BBB) in landlocked Rivière aux Saumons, Ashuapmushuan, and Ouasiemsca populations was also prevalent in samples from anadromous fish. The Grand Lake population included equal proportions of BBB and AAA haplotypes, the latter being the most frequent in the Métabetchouane population (Table 1). The sharing of the same common mtDNA haplotypes in all populations (although to a lesser extent in the Métabetchounae population) did not provide any additional information that could have helped in elucidating population origin, and consequently, these data were not considered further.

Contrasting results of population differentiation at microsatellite loci were obtained with statistical treatments involving allelic frequency alone (θ, D_{CE}) or incorporating mutational differences among loci (R_{ST}, $(\delta\mu)^2$). Based on allele frequency alone, highly significant genetic divergences (θ estimates, $P < 0.001$) between landlocked and anadromous populations were observed, and the Métabetchouane population was as divergent from the other landlocked ones as these were from the anadromous populations (means: Métabetchouane = 0.177, other landlocked = 0.134) (Table 2). The D_{CE} gave similar results. Accordingly, the anadromous samples clustered together and separately from all Lake Saint-Jean landlocked populations in the neighbour-joining phenogram (Fig. 2*a*). The Métabetchouane population occupied a basal position within the landlocked group. The branch lengths separating the Grand Lake population from any others were approximately the same as those observed between anadromous and landlocked populations. In contrast, results based on allelic size variance revealed that the Métabetchouane population was genetically less divergent from the anadromous and Grand Lake populations than from the other landlocked populations of Lake Saint-Jean (means: Métabetchouane anadromous = 0.068, Métabetchouane landlocked = 0.260) (Table 2). In the neighbour-joining phenogram based on $(\delta\mu)^2$, the Métabetchouane population did not cluster with the other landlocked populations of Lake Saint-Jean but rather with the anadromous ones and the Grand Lake population (Fig. 2*b*).

The hierarchical analysis of gene diversity further illustrated the dichotomy of results obtained from allele frequency or size information (Table 3). When the top level of grouping involved landlocked versus anadromous, both components of genetic variance, between groups and among populations within groups, were relatively similar. Thus, the ratio of variance proportions between groups/among populations within groups was 1.83 (9.36/5.11) for allelic and 1.28 (5.47/4.26) for molecular variance, respectively. However, in a second hierarchical analysis that included the Méta-

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Table 1. Range of allele size or haplotype identity for mtDNA (*S*), total number of alleles (*A*), sample size (*N*), observed (H_O) and expected (H_E) heterozygosity (*h*, haplotype diversity for mtDNA) by locus for landlocked Atlantic salmon from Rivière aux Saumons (Rs), Ashuapmushuan (As), Ouasiemsca (Oua), and Métabetchouane (Met) rivers and Grand Lake (GL) and for anadromous salmon from the Petit-Saguenay (P. Sag), Mars, St-Jean (St-J), and Sainte-Marguerite (Ste-M) rivers.

	Landlocked				Anadromous				
Locus	Rs	As	Oua	Met	P. Sag	Mars	St-J	Ste-M	GL
MST-3									
S	208–216	208–216	204–216	208–216	200–216	200–216	200–216	200–212	204–216
A	4	3	5	4	6	6	6	6	6
N	36	40	36	41	40	40	40	40	20
H_O	0.667	0.450	0.417	0.634	0.650	0.700	0.925	0.775	0.750
H_E	0.683	0.495	0.577	0.740	0.771	0.656	0.752	0.731	0.660
MST-79.1									
S	149–157	149–159	149–151	145–155	149–155	139–157	137–157	149–155	139–155
A	4	5	4	4	5	4	6	4	3
N	35	39	36	41	40	39	40	38	20
H_O	0.571	0.410	0.611	0.317	0.450	0.538	0.575	0.421	0.300
H_E	0.662	0.464	0.611	0.294	0.478	0.520	0.568	0.465	0.272
MST-79.2									
S	120–122	120–122	120–122	120–122	120–122	120–122	120–122	120–122	120–122
A	2	2	2	2	2	2	2	2	2
N	36	39	36	39	40	39	40	40	20
H_O	0.583	0.359	0.444	0.538	0.200	0.179	0.150	0.275	0.350
H_E	0.504	0.466	0.468	0.441	0.222	0.207	0.141	0.240	0.450
SSOSL85									
S	182–206	186–206	186–198	184–204	182–208	182–206	182–206	178–206	182–202
A	9	8	8	5	10	11	13	13	8
N	35	41	35	40	40	40	40	40	20
H_O	0.771	0.732	0.800	0.600	0.775	0.950	0.900	0.900	0.750
H_E	0.711	0.805	0.782	0.591	0.826	0.884	0.877	0.888	0.787
Ssa171									
S	235–261	237–265	237–265	237–253	217–273	225–271	225–279	225–273	235–261
A	10	14	13	9	17	17	15	20	10
N	37	41	36	40	40	38	40	40	20
H_O	0.892	0.951	0.917	0.850	0.875	0.947	0.825	0.975	0.800
H_E	0.811	0.911	0.888	0.792	0.889	0.888	0.862	0.922	0.837
Ssa197									
S	172–200	172–200	168–188	172–200	160–212	156–212	160–220	156–220	164–208
A	6	7	5	5	13	13	15	13	11
N	37	41	36	42	40	40	40	40	20
H_O	0.703	0.610	0.639	0.429	0.875	0.900	0.878	0.925	0.850
H_E	0.732	0.597	0.681	0.575	0.881	0.876	0.913	0.879	0.873
SFO-23									
S	116–144	118–144	118–122	114–144	110–144	112–140	110–144	110–144	116–122
A	8	9	9	3	12	11	11	11	3
N	30	39	36	37	40	40	40	40	19
H_O	0.533	0.769	0.694	0.027	0.675	0.775	0.775	0.850	0.158
H_E	0.669	0.743	0.759	0.054	0.841	0.814	0.769	0.816	0.152
Total *A*	43	48	46	32	65	64	68	69	43
Mean H_O	0.674	0.612	0.646	0.485	0.643	0.713	0.718	0.732	0.565
Mean H_E	0.682	0.640	0.681	0.498	0.701	0.692	0.697	0.706	0.576
mtDNA									
S	AAA, BBB	AAA, BBB	AAA, BBB, BAB	AAA, ABB, BBB	BBB	AAA, BBB	AAA, BBB	AAA, BBB	AAA, BBB
A	2	2	3	3	1	2	2	2	2
N	14	38	36	38	30	39	39	35	19
h	0.138	0.438	0.371	0.195	1.000	0.298	0.051	0.109	0.512

Note: Allele designation is expressed as base pairs. The order of restriction enzymes for mtDNA haplotype definition is *Alu*I, *Cfo*I, and *Hae*II.

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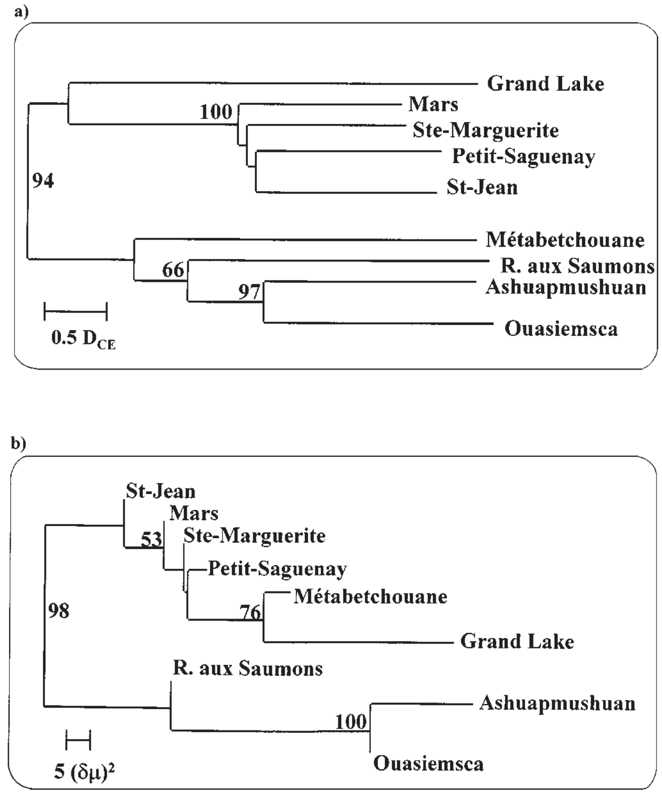
Table 2. Multilocus θ (below the diagonal) and standardised R_{ST} estimates (above the diagonal) for landlocked Atlantic salmon from Rivière aux Saumons (Rs), Ashuapmushuan (As), Ouasiemsca (Oua), and Métabetchouane (Met) rivers and Grand Lake (GL) and for anadromous salmon from the Petit-Saguenay (P. Sag), Mars, St-Jean (St-J), and Sainte-Marguerite (Ste-M) rivers.

	Rs	As	Oua	Met	P. Sag	Mars	St-J	Ste-M	GL
Rs		0.084*	0.061*	0.172*	0.163*	0.133	0.152*	0.158*	0.238
As	0.065*		0.018	0.316*	0.238*	0.215*	0.212*	0.235*	0.371*
Oua	0.063*	0.029*		0.291*	0.241*	0.206*	0.203*	0.231*	0.351*
Met	0.139*	0.185*	0.172*		0.068*	0.065*	0.078*	0.061*	0.094*
P. Sag	0.149*	0.163*	0.136*	0.176*		0.000	0.000	0.000	0.054*
Mars	0.131*	0.120*	0.096*	0.165*	0.015*		0.000	0.000	0.070*
St-J	0.152*	0.151*	0.125*	0.195*	0.006	0.007		0.000	0.080*
Ste-M	0.135*	0.139*	0.113*	0.170*	0.003	0.000	0.004		0.066*
GL	0.231	0.278*	0.230*	0.277*	0.112*	0.114*	0.108*	0.018*	

*Significant θ and R_{ST} estimates following sequential Bonferroni adjustments for simultaneous tests (α = 0.05, k = 36).

Fig. 2. (*a*) Chord (D_{CE}) and (*b*) $(\delta\mu)^2$ distances phenogram illustrating relationships among landlocked and anadromous Atlantic salmon populations. Percentages of replications of the observed topology based on 2000 bootstraps on loci are given along the branches when more than 50% (majority-rule consensus tree). Landlocked populations: Ouasiemsca, Ashuapmushuan, Rivière aux Saumons, Métabetchouane; anadromous populations: Mars, Ste-Marguerite, St-Jean, Petit-Saguenay.



betchouane population in the anadromous group (Fig. 2), this ratio was much higher based on allelic size variance (11.08/1.33 = 8.33) than based on allele frequency alone with the allelic one (7.90/6.33 = 1.25). We also performed the hierarchical AMOVA by sequentially including each of the three other landlocked populations with the anadromous ones. In all cases, the ratio based on allelic size variance was much lower than observed for the Métbabetchouane (ratios: 0 for Ouasiemsca, 0 for Ashupmushuane, and 0.933 for Rivière aux Saumons compared with 8.33 for Métabetchouane). This indicated that the Métabetchouane population was more similar to the anadromous ones when considering mutational information, which was not the case for the other landlocked populations.

Discussion

The main objective of this study was to assess the relationships among landlocked populations and parapatric anadromous populations from the Saguenay River system to infer the alternative scenarios of single versus double origin of Atlantic salmon in Lake Saint-Jean. The salient feature of our results was the contrasting patterns of population differentiation and relationships observed between those populations with different types of analyses. Thus, closer relationships between all landlocked populations of Lake Saint-Jean compared with anadromous ones were observed in analyses that only incorporated allele frequency information. These results thus apparently supported the hypothesis of a postglacial sympatric divergence of landlocked populations followed by limited gene flow and pronounced genetic drift. In contrast, analyses incorporating allelic size variance all supported a closer relationship between the Métabetchouane population and other populations from outside Lake Saint-Jean, including Grand Lake, than with other sympatric populations found in the lake. These results thus tended to support the hypothesis of a double origin of sympatric landlocked populations.

The relative performance of statistics for microsatellites assuming either a stepwise mutation model, infinite allele model, or no mutations at all has been adressed on several occasions (e.g., Takezaki and Nei 1996; Paetkau et al. 1997; Gaggiotti et al. 1999). A general observation of these studies was that because of the larger variance around distance estimates derived from measures incorporating allelic size variance, these were less prone to recover the correct relationships among populations. Such measures apparently only perform well if a large number of loci are used. Given these facts and the relatively low number of loci used, it thus appears that the most conservative conclusion for the results of this study is that they support the hypothesis of single ori-

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Table 3. Hierarchical analysis of Atlantic salmon based on microsatellite loci (Grand Lake population omitted). Listed are test statistics including fixation indices (θ and Φ) with probability values (*P*), variance components (*V*), and the percentage of total genetic variance for two different groupings based on the tree topology in Fig.2.

Source of variation	Fixation indices	*P*	*V*	%
First grouping				
Between landlocked and anadromous				
θ	0.094	<0.0001	0.246	9.36
Φ	0.055	0.082	3.479	5.47
Among populations within groups				
θ	0.056	<0.0001	0.134	5.11
Φ	0.045	<0.0001	2.710	4.26
Second grouping				
Between landlocked and anadromous + Métabetchouane				
θ	0.079	0.018	0.207	7.90
Φ	0.111	0.019	7.258	11.08
Among populations within group				
θ	0.069	<0.0001	0.166	6.33
Φ	0.015	<0.0001	0.871	1.33

gin for salmon in Lake Saint-Jean. As such, the strong divergence between the Métabetchouane population and the others would be the result of pronounced genetic drift, perhaps due to a population bottleneck, combined with very restricted gene flow.

On the other hand, it has also been shown that completely ignoring mutational information, even when derived from a relatively low number of loci, may potentially lead to loss of historical information and possibly incorrect interpretations (e.g., Angers and Bernatchez 1998; Goodman 1998). Several lines of evidence suggest that this could be the case in this study. First, the pattern of genetic differentiation (based on allelic size variance) observed between the Métabetchouane and anadromous populations strikingly differed from that of other landlocked populations. Second, previous studies in which poor topologies were recovered when using measures such as $(\delta\mu)^2$ implied very low or no bootstrap support on branching patterns, unlike the present case. Third, an ongoing study on genes of the major histocompatibility complex (MHC) variation performed on the same populations studied here also revealed more similarity between anadromous populations and the Métabetchouane population than between those and other landlocked populations (C. Landry, unpublished data). Finally, there would be an obvious contradiction in, on one hand, tacitly accepting that the strong divergence observed between the Métabetchouane and other landlocked populations is the result of its pronounced genetic drift and, on the other hand, concluding that this same process also explains its similarity to the anadromous ones (the presumed ancestral group). Indeed, reduced genetic diversity due to the drift effect is expected to inflate any distance measures (e.g., Paetkau et al. 1997). Consequently, the Métabetchouane population should always be the most genetically distant from the anadromous populations.

Using computer simulations, Slatkin (1995) compared the performance of R_{ST} and F_{ST} to estimate the extent of population differentiation from microsatellite data and argued that discrepancies between both parameters will vary with the net colaescence time of gene copies drawn from a collection of populations. Consequently, Slatkin (1995) and Rousset (1996) also suggested that comparing estimates of demographic parameters obtained by using F_{ST} and R_{ST} can give insights into differring demographic processes affecting natural populations. Thus, the value of R_{ST} is mainly determined by extra mutations that accumulate within each population. The opportunity for having such mutations will largely depend on the time of divergence of populations. The R_{ST} estimates will generally be larger than F_{ST} estimates when divergence time has been sufficiently long for mutations to accumulate. In contrast, both parameters should yield relatively similar values when the time of divergence among populations has been too short for neutral mutations to rise at high frequencies. In such cases, genetic drift and gene flow are likely to be more important than mutation in determining the extent of population divergence. If a typical microsatellite mutation rate is on the order of 10^{-4} (reviewed in Estoup and Angers 1998), a divergence time of a few thousand generations (a cutoff of 2000 generations proposed by Estoup and Angers 1998) could be considered as an upper limit above which mutation at microsatellite loci should have a substantial effect on population divergence. Recent empirical studies comparing both parameters corroborated this view (Angers and Bernatchez 1998; Estoup et al. 1998; Goodman 1998; Lugon-Moulin et al. 1999). In the case of salmon with a mean generation time of 5 years, this means that the R_{ST}/F_{ST} ratio should be close to 1 for populations that diverged postglacially (over the last 10 000 years) and higher than 1 for populations that diverged in more ancient times. The fact that the R_{ST}/F_{ST} ratio observed between the Métabetchouane and either the Ouasiemsca or Ashupmushuan population (1.7) was 2.8 times higher than that observed beween these two (0.62), coupled with its closer relationship to other populations outside Lake Saint-Jean when considering mutational information, is therefore suggestive that these populations evolved from two historical groups that were already differentiated before the last glacier retreat. Considering all evidence at hand, we tentatively propose that the double-invasion hypothesis appears more compatible with the observed pattern of genetic population structure than that of a single origin. Admittedly, however, a firmer conclusion must await a reanalysis of this system using a higher number of loci.

Hypothetical historical scenarios for the origin of Atlantic salmon in Lake Saint-Jean

Based on current paleogeographic knowledge of the area, we propose alternative hypothetical scenarios to explain the contemporary salmon population structure in Lake Saint-Jean, based on the hypothesis of either a double or a single invasion. The whole Lake Saint-Jean and Saguenay River area was inundated by the Laflamme Sea between 10 300 and 8700 years BP (Elson 1969). The water level reached 180–197 m above the present level (Dionne 1968). This means that all tributaries draining into the Saguenay River, the Métabetchouane River, and Rivière aux Saumons were completely inundated and not available to salmon. The longer rivers situated in the northern part of the lake, such as the Ashuapmushuan and the Ouasiemsca, could, however, potentially provide spawning habitat. Thus, Dionne and Laverdière (1969) showed that the northern part of the Laflamme Gulf had a very low salinity compared with the southern and southeastern parts (near the Métabetchouane River) of the lake. They explained this phenomenon by the voluminous glacial melted water of the Scheffer ice sheet that discharged into these rivers. They also found freshwater mollusc fossils in the northern part of the lake but an important concentration of marine mollusc fossils in the southern and southeastern parts, all dating between 9930 and 8630 years BP. With the retreat of the Laflamme Sea and the isostatic rebound, the Métabetchouane River and Rivière-aux-Saumons became available for colonisation by salmon. Under the single-origin hypothesis, populations already present in the Ashuapmushuan and Ouasiemsca rivers would have then colonised those two rivers. The stronger divergence of these two new populations would thus be the result of founder events followed by very limited gene flow. Under the hypothesis of double origin, a first ancestral salmon group would have recolonised the Ashuapmushuan and Ouasiemsca rivers, whereas the Métabetchouane River and tributaries of the Saguenay River would have been colonised later by a second ancestral group. The intermediate level of divergence in Rivière aux Saumons observed for all parameters would thus reflect the admixture of both ancestral groups in that tributary draining into the Ashuapmushuan River (Tessier et al. 1997; Tessier and Bernatchez 1999).

The scenario of double invasion would imply the past existence of distinct glacial refugia for salmon in North America, contrary to the currently prevailing assumption of a single refugium (Schmidt 1986). However, native populations of Atlantic salmon, now extinct, were found in Lakes Ontario and Champlain until the end of the nineteenth century (Scott and Crossman 1973). Although hypothetical, it is possible that these populations originated from a glacial refugium distinct from a more northeastern one that could have recolonised this area via the Hudson River drainage (discussed in Bernatchez 1997). This ancestral group could have then recolonised suitable tributaries of the Lake Saint-Jean area via connections with the Champlain and Laflamme seas. This scenario would be very similar to the one described for rainbow smelt (*Osmerus mordax*) (Bernatchez 1997). The second ancestral salmon group could have originated from the northeastern bank refugium described by Schmidt (1986) and shown to have served as refugium for other anadromous and freshwater fishes (reviewed in Bernatchez 1997). This group would have recolonised the Lake Saint-Jean area via connections between the Goldthwait and Laflamme seas. As glacial retreats occurred more rapidly in more western than eastern parts of North America (Dyke and Prest 1987), the Goldthwait Sea was colder than the Champlain Sea due to the effect of voluminous glacial melted waters. Such conditions would likely not have been favourable for salmon, thus favouring an earlier colonisation via the Champlain Sea.

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