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Landscape genetics and hierarchical genetic structure in Atlantic salmon: the interaction of gene flow and local adaptation

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Abstract

Disentangling evolutionary forces that may interact to determine the patterns of genetic differentiation within and among wild populations is a major challenge in evolutionary biology. The objective of this study was to assess the genetic structure and the potential influence of several ecological variables on the extent of genetic differentiation at multiple spatial scales in a widely distributed species, the Atlantic salmon, *Salmo salar*. A total of 2775 anadromous fish were sampled from 51 rivers along the North American Atlantic coast and were genotyped using 13 microsatellites. A Bayesian analysis clustered these populations into seven genetically and geographically distinct groups, characterized by different environmental and ecological factors, mainly temperature. These groups were also characterized by different extent of genetic differentiation among populations. Dispersal was relatively high and of the same magnitude within compared to among regional groups, which contrasted with the maintenance of a regional genetic structure. However, genetic differentiation was lower among populations exchanging similar rates of local as opposed to inter-regional migrants, over the same geographical scale. This raised the hypothesis that gene flow could be constrained by local adaptation at the regional scale. Both coastal distance and temperature regime were found to influence the observed genetic structure according to landscape genetic analyses. The influence of other factors such as latitude, river length and altitude, migration tactic, and stocking was not significant at any spatial scale. Overall, these results suggested that the interaction between gene flow and thermal regime adaptation mainly explained the hierarchical genetic structure observed among Atlantic salmon populations.

Keywords: gene flow, landscape genetics, local adaptation, population structure, salmon, temperature

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Introduction

Genetic similarities and differences among populations or groups of populations represent key sources of information to assess the evolutionary history of a species while also providing insights into both its contemporary state and future evolutionary potential. In addition to describing patterns of neutral genetic structure, documenting adaptive genetic differentiation at the individual and population levels is also increasingly considered for management and conservation purposes (Palsbøll *et al*. 2006; Schwartz *et al*. 2006). Moreover, understanding which evolutionary or ecological factors influence genetic structure and quantifying their spatial scale of influence is becoming as important as the mere description of genetic differentiation, although the task represents a major challenge. Landscape genetics is an emerging field addressing this problem that integrates genetic variation, biological and environmental variation, as well as spatial statistics, to understand which factors are structuring genetic variation at both the individual and population levels (Manel *et al*. 2003; Storfer *et al*. 2007).

Gene flow generally constrains the amount of structuring observed among populations (but see Garant & Kruuk 2005) by promoting exchanges and genetic homogeneity (Slatkin 1985). In the wild, however, gene flow can be con-

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strained by the intrinsic migration capability of the species and by physical barriers present in the environment (e.g. Angers *et al*. 1999; Crispo *et al*. 2006; Giordano *et al*. 2007). On the other hand, long-distance migration capabilities often observed in anadromous and marine fishes can be expected to enhance genetic homogeneity in the absence of obvious physical barriers. However, a reduction in gene flow may still occur if divergent local adaptation develops in response to the utilization of habitats characterized by different selection regimes (Schluter 2000). In this context, dispersal (movement of individuals) could be greater than gene flow (movement of genes) among locally adapted populations, as previously reported for chum salmon (Tallman & Healey 1994). This would result in the maintenance of a stable genetic structure if dispersers have a lower fitness in their novel environment than residents, a phenomenon referred to as 'selection against immigrants' (e.g. Hendry 2004; Nosil *et al*. 2005; Bolnick & Nosil 2007).

Anadromous Atlantic salmon, *Salmo salar*, is a long-distance migratory species that colonized a wide range of habitats along the North American and European Atlantic coasts. During any given year, Atlantic salmon may travel thousands of kilometres between its reproductive and feeding grounds (Hansen *et al*. 1993). Its homing behaviour, the propensity to return to spawn in natal rivers (Stabell 1984), is thought to be responsible for the genetic differentiation generally observed among rivers (e.g. O'Reilly *et al*. 1996; Fontaine *et al*. 1997; McConnell *et al*. 1997; Spidle *et al*. 2003; Castric & Bernatchez 2004; Verspoor *et al*. 2005; Dillane *et al*. 2007). However, it is not clear to what extent genetic differentiation reflects the influence of gene flow, genetic drift or the extent of local adaptation of this species. Indeed, evidence of straying from tagging experiments suggests that up to 6% of adult Atlantic salmon disperse to rivers other than, but most often near their natal river (Jonsson *et al*. 2003). Gene flow would then be most likely to occur among neighbouring populations, a situation best described by a stepping-stone model of population structure (Kimura 1953) and resulting in a pattern of isolation by distance (Wright 1943). However, the fate of these strays in the new environment as well as their evolutionary influence on population structure is not well known. Atlantic salmon has also adopted different life history and migration tactics varying within and among river systems (Caron *et al*. 2005) that could influence dispersal opportunities. One-sea-winter salmon travel smaller distances to feed at sea for one winter while multisea-winter salmon swim over longer distances to reach oceanic feeding grounds for multiple winters (reviewed in Fleming 1998), which might increase chances of straying for the latter. An association between migration tactic and genetic diversity or divergence has been documented in Atlantic salmon within a river system (Vähä *et al*. 2007). A similar tendency has been observed among rivers in Newfoundland, Canada (Palstra *et al*. 2007), but this relationship remains to be tested and quantified at a large spatial scale. In salmonids, gene flow may also be induced artificially by humans through stocking. Although the translocation of Atlantic salmon from one river to another was an extensive practice for several years in eastern Canada, the cumulative impact of these non-native salmon along with the potential homogenizing effect on large-scale population structure is unknown. Moreover, as some regions are more heavily stocked than others, we could expect that intensive stocking would favour genetic distinction of that region compared to others. Stocking of non-native fish had a homogenizing effect on genetic structure among and within native populations of coho salmon, *Oncorhynchus kisutch* (Eldridge & Naish 2007) but a moderate or limited impact on genetic structure of brown trout populations, *Salmo trutta* (Ruzzante *et al*. 2004). Local adaptation has also been recognized as an important evolutionary process in salmonids (reviewed in Taylor 1991; Garcia de Leaniz *et al*. 2007). In a recent study, temperature regime was specifically identified as a putative selective agent involved in local adaptation of Atlantic salmon in the wild by way of its influence on bacterial diversity (Dionne *et al*. 2007). Temperature or the length of the growing season has also been shown to be positively correlated with meristic traits such as fin size (Claytor *et al*. 1991) and negatively correlated with life-history traits such as smolt age (Power 1981; Metcalfe & Thorpe 1990). Even though no direct evidence exists, river length and size have also long been hypothesized to represent selective factors influencing life-history traits, with a tendency for salmon to be heavier and older in rivers with a high difficulty of upstream migration (Schaffer & Elson 1975; Power 1981; but see Claytor *et al*. 1991 for the reverse relationship). Local adaptation involving any or all of these factors may influence genetic differentiation by decreasing the dispersal success of immigrants and limiting gene flow among environments with divergent selective pressures.

The general objective of this study was to investigate which factors potentially interact to determine the magnitude and pattern of genetic structure in Atlantic salmon. More specifically, the first objective was to characterize genetic population structure of Atlantic salmon at a large spatial scale in eastern Canada by testing for a hierarchical organization of populations in regional groups, as proposed in previous studies (Power 1981; Verspoor 2005; Verspoor *et al*. 2005). The second objective was to evaluate, using a landscape genetic approach, the potential influence of gene flow (natural and human induced) and ecological factors potentially linked to natural selection, namely temperature regime and the difficulty of upstream migration in freshwater, on genetic differentiation at different spatial scales. A complementary objective was to evaluate the potential influence of local adaptation on population structure by comparing the genetic influence of migrants travelling within vs. among geographical regions differing in their environmental characteristics.

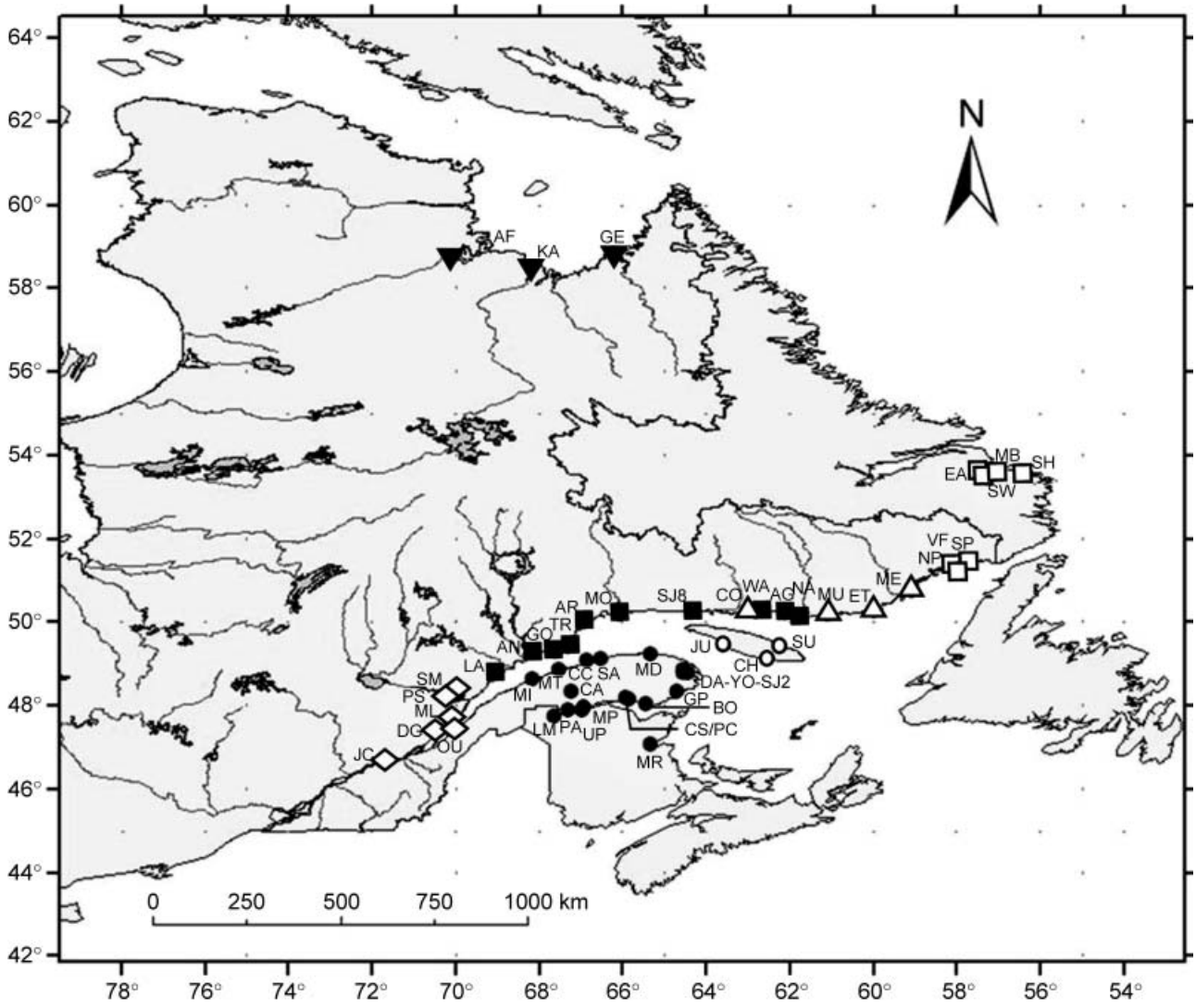

Fig. 1 Location of Atlantic salmon rivers in the provinces of Québec, New Brunswick and the region of Labrador (Canada) where sampling occurred. Each two- or three-digit code represents a different river. Symbols represent different regional groups according to GENELAND analysis: ▼, Region 1; □, Region 2; △, Region 3; ■, Region 4; ◇, Region 5; ●, Region 6; ○, Region 7. Rivers from top to bottom along the coast are: Aux Feuilles (AF), Koksoak (KA) and George (GE) from the province of Québec; Eagle (EA), Southwest Brook (SW), Muddy Bay (MB) and Sand Hill (SH) from the Labrador region; Saint Paul (SP), Vieux Fort (VF), Napitipi (NP), Gros Mécatina (ME), Étamamiou (ET), Musquaro (MU), Natashquan (NA), Aguanus (AG), Watshishou (WA), Corneille (CO), Saint Jean (SJ8), Moisie (MO), Aux Rochers (AR), Trinité (TR), Godbout (GO), Aux Anglais (AN), Laval (LA), Sainte-Marguerite (SM), Petit Saguenay (PS), Malbaie (ML), Du Gouffre (DG), Jacques Cartier (JC), Ouelle (OU), Mitis (MI), Matane (MT), Cap-Chat (CC), Sainte Anne (SA), Madeleine (MD), Dartmouth (DA), York (YO), Saint Jean (SJ2), Gros Pabos (GP), Bonaventure (BO), Grande Cascapédia (CS), Petite Cascapédia (PC), Matapédia (MP), Causapscal (CA), Patapédia (PA), Jupiter (JU), Aux Saumons (SU) and Chaloupe (CH) from the province of Québec; Little Main (LM) and Upsalquitch (UP) tributaries of the Restigouche river and Miramichi (MR) from the province of New Brunswick.

Materials and methods

Sampling and microsatellite genotyping

A total of 2775 adult salmon were sampled during summer 2004 through angling in 51 rivers located along the Atlantic coast and the Saint Lawrence Estuary in the provinces of Québec, New Brunswick, and the region of Labrador, Canada (Fig. 1). An average of 54 salmon was collected per river (Table S1, Supplementary material). For each salmon, the adipose fin was clipped and stored in 95% ethanol. DNA was extracted from fin clips using the QIAGEN DNeasy Tissue Kit following the guidelines of the manufacturer. Microsatellite polymorphism was quantified at 13 loci as

detailed in Appendix S1 of Dionne *et al*. (2007): Ssa85, Ssa202, Ssa197 (O'Reilly *et al*. 1996), Ssosl417 (Slettan *et al*. 1995), SsaD85 (T. King, unpublished), SsaD71, SsaD144 (King *et al*. 2005), MST-3 (Presa & Guyomard 1996), Sssp1605, Sssp2210, Sssp2215, Sssp2216 and SsspG7 (Paterson *et al*. 2004).

Statistical analyses

The potential occurrence of null alleles and scoring errors due to stuttering or large allele dropout in the data set was assessed using the software MICRO-CHECKER (van Oosterhout *et al*. 2004). Global allelic richness (A_r), adjusted for the sample size of the smallest population (35 individuals), was calculated for each population using FSTAT 2.9.3.2 (Goudet 2001). Deviation from Hardy–Weinberg expectations and linkage disequilibrium were tested for each locus and for each river sampled using GENEPOP 3.4 (Raymond & Rousset 1995). For each microsatellite locus, the LnRH neutrality test was performed to verify the neutrality of each marker (Schlötterer 2002; see Dionne *et al*. 2007 for calculation details). Temporal stability of allelic frequency was assessed by comparing the adult samples to 50 juvenile salmon (1+ and 2+ years of age) sampled the following year in seven rivers from different localities: Dartmouth (DA), St-Jean (SJ2), Causapscal (CA), Moisie (MO), Trinité (TR), Laval (LA) and Ste-Marguerite (SM) rivers. The extent of temporal genetic variation was estimated using the F_{ST} estimator θ_{ST} (Weir & Cockerham 1984) in GENETIX 4.05.2 (Belkhir *et al*. 2000) and significance was assessed using resampling methods, based on 1000 permutations.

Large scale genetic structure. Large scale population structure was investigated using the software GENELAND, which uses a Bayesian approach with Markov chain Monte Carlo algorithms to identify genetic spatial discontinuities (Guillot *et al*. 2005). This approach was favoured as opposed to non-spatial clustering methods to take into account the geographical clustering of individuals within defined rivers and the intrinsic homing behaviour of the species. The most probable number of clusters (K) was found using five replicates with 1×10^5 Markov chain Monte Carlo iterations and using Dirichlet as the selected model for allele frequencies. For each run, the maximum rate of the Poisson process was fixed at 1000 and the number of nuclei in the Poisson–Voronoi tessellation was fixed at 3000 to better reflect the number of individuals in the data set. Finally, an analysis of molecular variance (AMOVA) was conducted using ARLEQUIN 3.1 (Excoffier *et al*. 2005) to partition the genetic variance among the different hierarchical levels of structure observed in the previous analysis.

Regional structure and dispersal. For the seven regional groups identified by GENELAND with high posterior probabilities (see Results section), differences in ecological characteristics were assessed using a multivariate analysis of variance (MANOVA) approach (Proc GLM, sas software 9.1, SAS Institute). Wilks' lambda (Λ) as well as Pillais' trace (T) were examined to test for global significance among regional groups. Multiple pairwise-comparison tests using least-square means were performed for post-hoc comparisons, and a sequential Bonferroni correction was applied to evaluate pairwise significance (Rice 1989). A canonical variates analysis (CVA), a multigroup discriminant analysis, was conducted following the MANOVA to identify which of the factors tested explained most of the variability among regional groups. Factors considered in this analysis were available environmental, life history and human impact factors potentially implicated in local adaptation and/or influencing genetic population structure. First, a 'temperature index' per river, represented the number of degree-days above 5 °C between April and October (temperature that is encountered from spring to fall in the study area, and that covers the growth season of salmon), cumulated over 30 years (1971–2000, Environment Canada, http://climate.weatheroffice.ec.gc.ca/climateData/canada_f.html, see also Dionne *et al*. 2007). Second, an index for the 'difficulty of upstream migration' for adults returning to spawn in freshwater was computed for each river by multiplying the 'geographical distance' a fish must ascend in each river by the 'altitude' of the maximally accessible upstream river section. A long and steep river was then assumed to represent a higher level of difficulty during upstream migration than a shorter and less sloping river (Schaffer & Elson 1975; Power 1981). Distance data were obtained from the Ministère des Ressources Naturelles et de la Faune du Québec (MRNF). Altitude data were extracted from the Canada3D database available through the GéoGratis web site of the Ministry of Natural Resources Canada (http://geogratis.cgdi.gc.ca) using the ARCMAP module of ARCGIS 9.0 (ESRI inc.). Third, migration tactic of anadromous salmon was represented by the 'proportion of one-sea-winter' salmon in each river in 2004, available from the MRNF (Caron *et al*. 2005). The proportion of one sea-winter salmon, although variable through time, still remains relatively stable temporally compared to the spatial variability found among rivers (F. Caron, unpublished data). Finally, 'stocking' was represented by the cumulative number of non-native salmon stocked per river between 1990 and 2002, taking into account their river of origin, information readily available from the MRNF. All factors included in the analysis were standardized for a mean of zero and a variance of one.

The genetic structure within regional groups was first quantified by calculating the mean pairwise F_{ST} estimator θ_{ST} (Weir & Cockerham 1984) between population pairs within each regional group using GENETIX 4.05.2 (Belkhir *et al*. 2000) and the significance of differences observed was tested using resampling methods, based on 1000 permutations.

The distinctiveness of each regional group was then assessed by comparing mean F_{ST} per region, available from GESTE 1 (Foll & Gaggiotti 2006). This index differs from pairwise differentiation estimators (e.g. θ_{ST}, Weir & Cockerham 1984) since it is estimated for each population individually and represents how distinct a population is compared to the study area as a whole (Foll & Gaggiotti 2006). Contemporary connectivity among regional groups was then assessed by estimating the proportion of first generation immigrants in each river as well as their most probable origin using the assignment method in GENECLASS 2 (Piry *et al*. 2004). Here, our objective was to compare patterns of immigration within vs. among regional groups rather than estimate the absolute number of immigrants per river. Immigrants were detected using the L_home/L_max likelihood computation, the frequency-based method of Paetkau *et al*. (1995) as the likelihood calculation criteria and the Monte Carlo simulation algorithm of Paetkau *et al*. (2004) with 1000 simulations. Salmon identified as immigrants were only considered as such if their probability of belonging to another river was at least 100 times higher than the probability of belonging to the river where it was sampled (log-likelihood home–log-likelihood max ≥ 2). We used this very conservative threshold in order to minimize the detection of false immigrants (type I error). The global error rate under this stringent criterion was estimated using simulations. Through 100 simulations, we generated 20 000 individuals from four populations by sampling alleles based on real allele frequencies of four representative populations in the studied system (N = 50 simulated individuals per population for each simulation for concordance with our sampling design, E. Milot, M. Dionne, L. Bernatchez, H. Weimerskirch, unpublished). Allele frequencies of the Moisie (MO), Nathasquan (NA), Saint-Jean (SJ8) and Watshichou (WA) rivers were used as a baseline to simulate individuals as these populations have θ_{ST} values between 0.013 and 0.033 (mean θ_{ST} = 0.023), which is representative of the low to moderate genetic differentiation found within regional groups, where error rate is more susceptible to be high. All 100 simulated data sets were submitted to the assignment test using the same conditions as outlined above. All simulated individuals from the four virtual populations were assumed to be residents and any 'immigrant' identified by the assignment test was considered as a false immigrant (type I error). The genetic influence of immigrants travelling within vs. between regional groups (local vs. inter-regional immigration respectively) was then compared by estimating mean θ_{ST} between populations for both immigrant categories over a comparable coastal distance range of 100–700 km using Mantel tests. All Mantel tests included in this study were conducted with R 2.5.0 (R Development Core Team 2007) using the package VEGAN and significance was assessed with 1000 permutations.

Landscape genetics. A landscape genetic approach was used to link genetic differentiation with potential factors that could influence the observed genetic structure at different spatial scales. We used GESTE 1 (Foll & Gaggiotti 2006) to estimate F_{ST} values for each local population and to relate them to ecological factors using a generalized linear model. Posterior probabilities associated with each factor, estimated from the number of times the algorithm visited each model, identified the factors most influencing genetic structure. We used a burn-in of 2000 iterations to attain convergence and a total chain length of 1×10^5 iterations. These parameters were sufficient for convergence and were adequate under a wide range of scenarios (Foll & Gaggiotti 2006). Each analysis was conducted for three replicates at each of these three spatial scales: (i) a large spatial scale of 4580 km of coastal distance, representing the overall studied system; (ii) an intermediate spatial scale of 2, 262 km of coastal distance around the Saint Lawrence water system; and (iii) a small regional scale of 915 km and 652 km of coastal distance representing the Southern Québec and the Higher North Shore regional groups respectively, identified by GENELAND (see Results section). These regions were selected because they included at least 10 populations, a minimal condition to achieve sufficient power in simulated data sets (Foll & Gaggiotti 2006). Six factors were considered in the landscape genetic analyses including the four environmental, life history and human impact factors mentioned earlier. Additionally, two types of distance were considered: 'latitude' and 'coastal distance' (river mouth of each population) to take into account the restricted movement that can occur at different spatial scales and to consider the geographical location of rivers around the Saint Lawrence water system. Coastal distance was calculated from the Miramichi River, the southernmost river included in this study, using CANMAP WATER 2005.3 integrated in the ARCMAP module of ARCGIS 9.0 (ESRI Inc.). Based on data availability, the 'difficulty of upstream migration' index could only be tested at the Saint Lawrence and regional spatial scales. To complement the results of the Bayesian analyses, the association between genetic differentiation [$F_{ST}/(1 - F_{ST})$, Rousset 1997] and pairwise differences in the ecological factors mentioned above was tested using Mantel tests and partial Mantel tests, two tests widely used in landscape genetics (Storfer *et al*. 2007) for which, however, an unresolved controversy exists on their statistical validity (Rousset 2002 and references therein). It has to be noted that absolute values for each of the above-mentioned factor were used in the GESTE analysis while pairwise values were used for Mantel tests. For this reason, 'coastal distance' represents coastal distance from the southernmost river in the former analysis (hereafter referred to as 'coastal distance from the South') while it represents 'pairwise coastal distance' in the latter (hereafter referred to as 'pairwise coastal distance').

Source of variation	d.f.	Percentage of variance	*P*
Among regional groups	6	2.54	< 0.001
Among populations within regional groups	44	2.02	< 0.001
Among individuals within populations	5495	95.44	< 0.001

Table 1 Analysis of molecular variance (AMOVA) partitioning genetic variance among individual Atlantic salmon, among populations within regional groups and among regional groups identified by GENELAND

Results

Genetic polymorphism

Mean adjusted allelic richness per river, averaged over all 13 microsatellites, ranged from 9.1 to 14.5 alleles, while mean observed heterozygosity was 0.84 (range: 0.74–0.89) (Table S1). Deviations from Hardy–Weinberg equilibrium were identified in 42 out of 663 comparisons (all heterozygote deficits), while 33 significant tests were expected by chance at $\alpha = 0.05$. There was no evidence of significant deviations associated with either particular loci or populations. No significant linkage disequilibrium was detected between pairs of loci for any population. The LnRH neutrality test revealed that microsatellite Sssp1605 was a significant outlier in terms of gene diversity relative to other loci ($P < 0.0001$). This locus was therefore excluded from the analyses requiring neutral markers, but was retained in the assignment tests. Null alleles were potentially implicated in only five population-loci combinations out of 663, with the estimated null allele frequency varying between 0.03 and 0.13. No evidence for scoring errors due to stuttering or large allele dropouts were found in the whole data set. We therefore concluded that neither potential null alleles nor scoring errors affected the outcome of the results. Pairwise comparisons between temporal samples indicated no significant genetic differentiation after Bonferroni correction, and the θ_{ST} ranged from – 0.0005 to 0.006. This suggested that the signal of genetic differentiation detected at microsatellite loci in this study was relatively stable over a short time period, at least for the sub-sample of rivers compared.

Large-scale genetic structure

Seven population clusters (K) were consistently identified by GENELAND. Each population had a probability of 0.90–1.00 of belonging to their regional group (white area surrounded by the 0.90 isocline, Fig. 2), which provided strong support to the clustering result. The posterior probabilities of cluster membership defined these regional groups named as follows: Region 1, 'Ungava' (rivers AF, KA and GE); Region 2, 'Labrador' (rivers EA, SW, MB, SH, SP, VF and NP); Region 3, 'Lower North Shore' (rivers ME, ET, MU and CO); Region 4, 'Higher North Shore' (rivers NA, AG, WA, SJ8, MO, AR, TR, GO, AN and LA); Region 5, 'Québec City' (rivers SM, PS, ML, DG, JC and OU); Region 6, 'Southern Québec' (rivers MI, MT, CC, SA, MD, DA, YO, SJ2, GP, BO, CS, PC, MP, CA, UP, PA, LM and MR) and Region 7, 'Anticosti' (rivers JU, SU, CH) (Fig. 2). Each regional group included geographically proximate rivers. The Corneille River (CO) was an exception to this general pattern as it clustered with the Lower North Shore regional group (Region 3) while geographically closer to rivers of the Higher North Shore regional group (Region 4). In the AMOVA, a significant and higher net proportion of the total genetic variance was found among regional groups than among populations within regions (2.54% and 2.02% respectively, Table 1), supporting the regional structure found in the Bayesian analysis. These values indicated that on average, the extent of differentiation between populations from different regional groups was more than twice that observed within any region. Still, most populations within regions harboured significant genetic differences with 1238 out of 1275 pairwise θ_{ST} comparisons significant after Bonferroni correction. Global θ_{ST} was 0.051 and pairwise θ_{ST} between all pairs of populations ranged from – 0.0001 to 0.125 (pairwise θ_{ST} table available upon request). Exceptions occurred between rivers of Anticosti Island which showed very low and non-significant genetic differentiation (θ_{ST} range = 0.001–0.003), as well as between several rivers in Southern Québec.

Regional structure and dispersal

The multivariate analysis of variance revealed a significant overall difference among the seven regional groups of populations in terms of environmental characteristics, life-history traits and stocking ($P < 0.0001$, Table 2). The temperature index was on average 1132.9 degree-days (range: 531.8–1797.8 degree-days per river) and was significantly different for all regional comparisons following sequential Bonferroni correction (18/21 significant comparisons), except between the regions of Anticosti, Higher North Shore and Lower North Shore where differences were not significant. Mean accessible distance in each river for salmon was 68.6 km (range: 3.0–262.2 km) and mean altitude was 211.6 m (range: 5.0–618.0 m). Mean difficulty of upstream migration was on average 20.5 and varied from 0.02 to 153.2 per river but was not significantly different among regional groups ($P > 0.05$). The proportion of one-sea-winter salmon was on average 50.3% (range: 3.0–94.7%) and was significantly greater for the Labrador and Lower North Shore compared to the Higher North Shore, Québec City and Southern

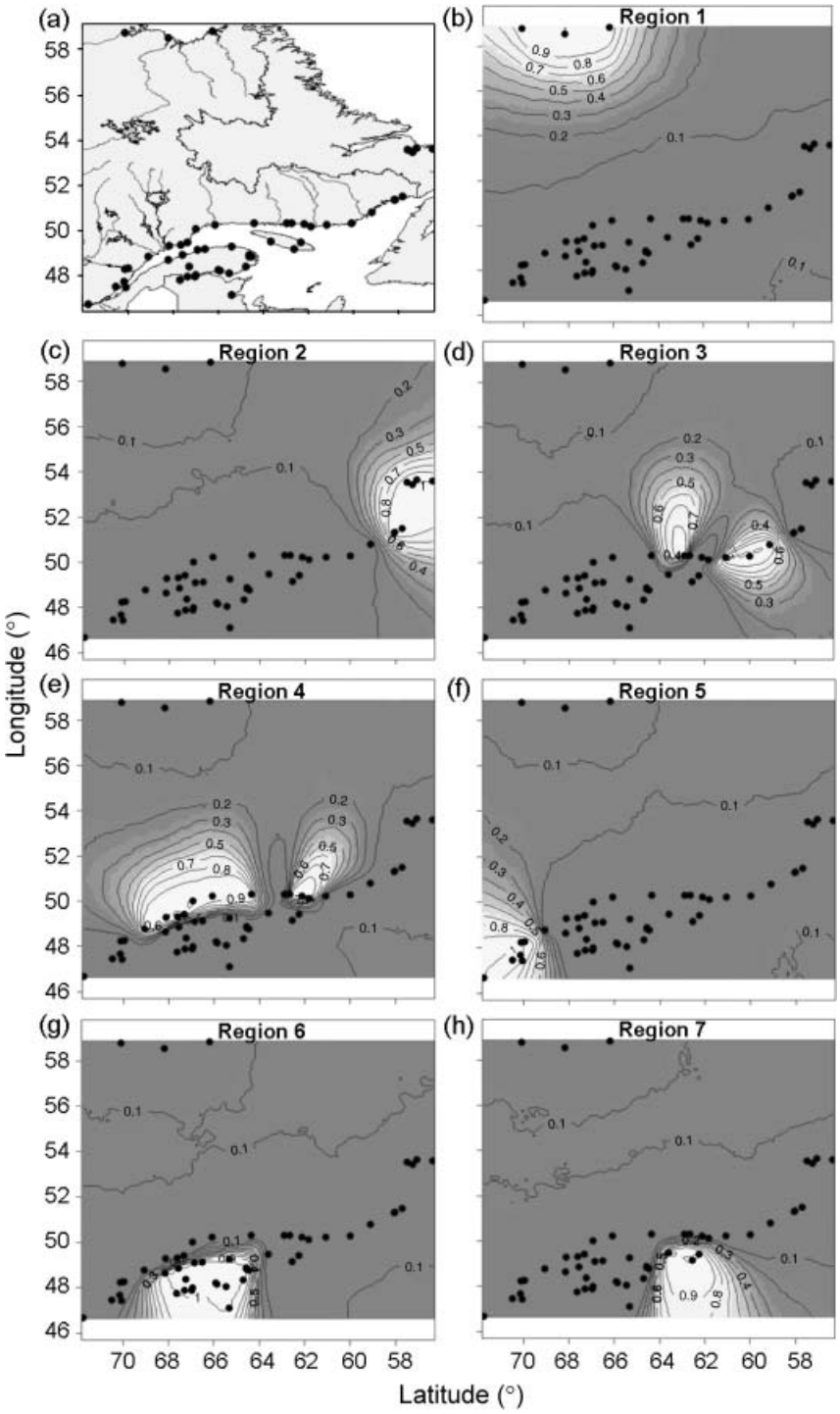

Fig. 2 Posterior probabilities for each Atlantic salmon river to belonging to each of the seven regional groups identified by GENELAND. The white area represents a probability between 90% and 100% for rivers to belong to their respective regional group. (a) Map of the rivers studied for comparison with other panels. (b) Regional group 1: 'Ungava' including AF, KA and GE rivers; (c) Regional group 2: 'Labrador' including EA, SW, MB, SH, SP, VF and NP rivers; (d) Regional group 3: 'Lower North Shore' including ME, ET, MU and CO rivers; (e) Regional group 4: 'Higher North Shore' including NA, AG, WA, SJ8, MO, AR, TR, GO, AN and LA rivers; (f) Regional group 5: 'Québec City' including SM, PS, ML, DG, JC and OU rivers; (g) Regional group 6: 'Southern Québec' including MI, MT, CC, SA, MD, DA, YO, SJ2, GP, BO, CS, PC, MP, CA, PA, LM, UP and MR rivers; (h) Regional group 7: 'Anticosti' including JU, SU and CH rivers. See Fig. 1 for river code description.

Québec regional groups (6/21 significant comparisons following sequential Bonferroni correction). Québec City was the region most heavily stocked with non-native salmon compared to all other regional groups (6/21 significant comparisons, $P < 0.0001$). The first axis of the canonical variates analysis explained 85.1% of the total variability among the seven regional groups. Temperature index had the highest loading on this axis (eigenvector: 0.88) and was opposed to the proportion of one-sea-winter salmon (eigenvector: −0.42). Low loadings were found for the difficulty of upstream migration index and for stocking on this axis (eigenvectors: 0.005 and 0.02 respectively).

Mean θ_{ST} for each of the seven regional groups was variable and ranged from 0.002 to 0.039 (Table 3), while pairwise coastal distance between rivers was similar within each regional group (ANOVA: $F = 1.68$, $P = 0.13$). Mean F_{ST}

Table 2 Characteristics associated with each of the seven Atlantic salmon regional group identified by GENELAND. Mean values and standard deviation for each ecological factor are indicated per regional group: T°, historical temperature index in degree-days; difficulty index, difficulty of upstream migration in freshwater; 1SW, proportion of one-sea-winter salmon; stocking, number of non-native salmon stocked from 1990 to 2002. For each factor, dissimilar superscripts indicate significant statistical differences in the MANOVA analysis

Regional group	T°	Difficulty Index	1SW (percentage)	Stocking
1. Ungava	531.8 ± 0.0[a]	—	58.0 ± 14.7[ab]	0.0 ± 0.0[a]
2. Labrador	784.2 ± 109.5[b]	4.2 ± 3.4[a]	86.3 ± 12.3[a]	0.0 ± 0.0[a]
3. Lower North Shore	938.1 ± 48.9[c]	2.2 ± 3.0[a]	72.5 ± 25.1[a]	0.0 ± 0.0[a]
4. Higher North Shore	1066.5 ± 132.4[c]	35.0 ± 48.2[a]	29.2 ± 17.6[b]	40888.5 ± 109658.8[a]
5. Québec City	1542.4 ± 159.9[d]	14.5 ± 30.8[a]	42.9 ± 21.7[b]	606173.8 ± 406122.2[b]
6. Southern Québec	1343.4 ± 77.2[e]	23.6 ± 14.7[a]	42.6 ± 13.2[b]	51.9 ± 241.4[a]
7. Anticosti	945.8 ± 0.0[c]	8.0 ± 7.1[a]	59.8 ± 10.3[ab]	0.0 ± 0.0[a]

Table 3 Genetic differentiation and immigration statistics associated with each of the seven Atlantic salmon regional group identified by GENELAND. θ_{ST} (Weir & Cockerham 1984) indicates mean pairwise differentiation between rivers while mean F_{ST} (GESTE) indicates mean genetic distinctiveness of all rivers within each regional group. Mean proportion of immigrants per river detected in each regional group is indicated (mean immigrants) as well as the proportion of these immigrants belonging to another regional group (inter-regional immigrants). Regions of origin refer to the regions (numbers from column 1) in which immigrants belong according to the assignment test

Regional group	Mean θ_{ST} (± SD)	Mean F_{ST} (± SD)	Mean immigrants (percentage of ± SD)	Inter-regional immigrants (percentage)	Regions of origin
1. Ungava	0.025 ± 0.010	0.086 ± 0.015	2.2 ± 2.3	0.0	1
2. Labrador	0.039 ± 0.019	0.049 ± 0.027	9.3 ± 5.6	62.9	1,2,3,4,5,6
3. Lower North Shore	0.037 ± 0.017	0.058 ± 0.016	6.9 ± 3.8	71.4	2,3,4,5,6
4. Higher North Shore	0.027 ± 0.017	0.034 ± 0.015	7.9 ± 4.2	50.0	2,3,4,5,6
5. Québec City	0.013 ± 0.012	0.043 ± 0.012	9.0 ± 5.9	60.0	2,3,4,5,6
6. Southern Québec	0.011 ± 0.009	0.020 ± 0.008	13.6 ± 6.7	50.4	2,3,4,5,6,7
7. Anticosti	0.002 ± 0.001	0.041 ± 0.004	6.4 ± 2.4	44.4	3,4,6,7

SD, standard deviation.

per regional group, calculated using GESTE, ranged from 0.020 to 0.086 and Ungava was a region significantly more differentiated than Southern Québec in the study area (ANOVA, $F = 12.39$, $P < 0.0001$). Other comparisons were nonsignificant. A total of 255 putative immigrants were detected in the whole system (9.2% of the salmon analysed) and the mean proportion of immigrants per river was 9.7% (range: 0–28%). The mean proportion of immigrants per regional group ranged from 2.2% to 13.6% (Table 3). A proportion of 53.9% of all immigrants were assigned to a river from a regional group other than the region of origin. These inter-regional immigrants varied in proportion between 44.4% and 71.4% depending on the regional group considered and were assigned to various regions without any apparent geographical pattern (Table 3). The Ungava region was an exception as immigrants identified in this region were only assigned to local rivers. A total of 100 pairs of populations exchanged migrants over distances ranging from 100 to 700 km. From these, mean genetic differentiation (θ_{ST}) was significantly lower between populations exchanging local migrants (0.015 ± 0.012; mean of 1.15 migrants/population pair) than between populations exchanging inter-regional migrants (0.034 ± 0.009; mean of 1.13 migrants/population pair; *t*-test: $P < 0.0001$), for a similar coastal distance range (mean: 418.2 ± 141.6 km and 432.2 ± 146.5 km respectively, *t*-test: $P = 0.13$).

According to the simulations, 21 of these immigrants (8%) may represent falsely detected immigrants (error rate = 0.76%) and could therefore inflate these estimates. This error rate is most probably overestimated as simulated individuals were obtained from sampling alleles according to the allele frequencies of each population, instead of sampling gametes, a method that was found to overestimate error rates in other simulations (Paetkau *et al*. 2004). Power at correctly identifying immigrants in this system can then be defined as 1 – type I error. The overall power was then 0.92 which means that at least 92% of the immigrants detected in this study can be considered as true immigrants with high confidence. The distribution of the estimated 8% error rate might not be homogeneous among regional groups, with a high number of source populations potentially increasing the risk of detecting false immigrants. To verify

Table 4 Posterior probabilities of nine possible models associated with the two factors selected by GESTE: temperature and coastal distance from the southernmost sampling site (Miramichi River). Posterior model probabilities illustrate the degree of association between these factors and genetic differentiation of Atlantic salmon populations at a large, intermediate (Saint Lawrence water system) and small regional scale (Southern Québec and Higher North Shore regional groups). The constant parameter indicates that the selected regression model do not pass through the zero intercept. 'All' represents the combined influence of the constant, both factors and their interaction

		Probability			
Model	Factors	Large	Intermediate	Small (Southern Québec)	Small (HNS)
1	Constant	0	0.11	0.53	0.72
2	Temperature	0	0	0	0
3	Constant and temperature	0	0.21	0.40	0.17
4	Coastal distance	0	0	0	0
5	Constant and coastal distance	0.95	0.65	0.04	0.09
6	Temperature and coastal distance	0	0	0	0
7	Constant, temperature and coastal distance	0.05	0.03	0.03	0.02
8	Temperature, coastal distance and interaction	0	0	0	0
9	All	0	0	0	0

Table 5 Results of Mantel tests and partial Mantel tests between genetic differentiation ($F_{ST}/1–F_{ST}$) of Atlantic salmon populations and pairwise differences in coastal distance or temperature at the large, intermediate (Saint Lawrence water system) and small regional scale (Southern Québec and Higher North Shore regional groups). The controlled variable in the partial Mantel tests is indicated in parentheses. Significant tests are denoted by an asterisk (*)

	Large		Intermediate		Small (Southern Québec)		Small (HNS)	
Mantel/Partial Mantel tests	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Coastal distance	0.54	< 0.001*	0.38	< 0.001*	0.36	0.05	0.39	0.004*
Temperature	0.21	0.007*	0.36	0.002*	0.63	0.01*	0.47	0.10
Coastal distance (temperature)	0.51	< 0.001*	0.36	< 0.001*	0.05	0.28	0.22	0.10
Temperature (coastal distance)	0.03	0.32	0.34	0.005*	0.56	0.007*	0.34	0.18

this potential bias, a regression was conducted between the number of detected immigrants and the number of source populations. A positive association was detected between the proportion of immigrants travelling within regions and the number of rivers in each regional group ($r = 0.47$, $P = 0.0005$), but this association was negative between the number of inter-regional immigrants and the number of rivers outside each group ($r = –0.32$, $P = 0.02$). Both associations were mostly driven by the high immigration rate found in the Southern Quebec regional group.

Landscape genetics

Landscape genetic analyses in GESTE revealed the predominant influence of coastal distance from the South and temperature regime on the genetic differentiation of populations depending on the spatial scale of observation. No other factors were retained as they showed a posterior probability smaller than 0.10. These two factors were represented by nine different models including the effect of a constant, the two factors and their interactions (Table 4). All significant models included a constant, as expected from its strong effect easily detectable by the method (Foll & Gaggiotti 2006). The presence of a constant in the selected model indicates that the regression models do not pass through the zero intercept. At the larger spatial scale over the entire study area, coastal distance from the South was the predominant factor influencing genetic differentiation with a posterior probability of 0.95 and a relatively low mean σ^2 value of 0.26 ± 0.06. A low σ^2 value is indicative of a good fit between the model and the F_{ST} values. At the intermediate spatial scale of the Saint Lawrence, both coastal distance from the South and temperature were retained (posterior probability: 0.65 and 0.21, mean σ^2: 0.34 ± 0.10 and 0.37 ± 0.11, respectively). Finally, at the small regional scale, temperature was the only factor retained by the analysis in Southern Québec (posterior probability: 0.40, mean σ^2: 0.36 ± 0.15) and less so in Higher North Shore (posterior probability: 0.17, mean σ^2: 0.40 ± 0.22). For partial Mantel tests, pairwise coastal distance and temperature were the only factors significantly associated with genetic differentiation (Table 5), providing complementary and

concordant information with the GESTE analyses. Pairwise coastal distance was significantly correlated with genetic differentiation when controlling for temperature at a large and intermediate spatial scales ($r = 0.51$ $P < 0.001$ and $r = 0.36$ $P < 0.001$ respectively). Temperature was significantly correlated with genetic differentiation when controlling for pairwise coastal distance at the intermediate ($r = 0.34$ $P = 0.005$) and small regional scale in Southern Québec ($r = 0.56$ $P = 0.007$) but not in Higher North Shore, even though the proportion of the variance was relatively high ($r = 0.34$ $P = 0.18$).

Discussion

Our results revealed a hierarchical genetic structure in Atlantic salmon populations along most of its latitudinal distribution in eastern Canada. Seven regional groups were identified based on their degree of genetic differentiation and geographical location, each group generally composed of genetically distinct populations, albeit to a weaker extent than what was observed among regions. Each regional group was also defined according to either environmental, life-history or stocking characteristics. A regional structure was observed despite a relatively high connectivity among regions and a similar proportion of immigrants detected within and among regional groups, raising the hypothesis of a lower success of inter-regional immigrants relative to local immigrants. Landscape genetic analyses underlined the interaction of coastal distance, either from the southern-most sampling site or between rivers, and temperature regime in determining the pattern of genetic structure at different spatial scales. We therefore propose that the interaction between gene flow and thermal regime adaptation best explains the observed genetic structure at the regional and river scales. Different levels of genetic-temperature association depending on the regional group considered further suggest that such interaction between gene flow and local adaptation is not uniform over the entire Atlantic salmon distribution area but instead specific to a particular region.

Hierarchical genetic structure

The regional structure inferred by the Bayesian analyses revealed the presence of seven genetically defined regional salmon groups each including rivers with a posterior probability of 90% to 100% of belonging to their respective group. The net proportion of genetic variance associated with the regional structure was significant and higher than that associated with population structure within regions, providing additional support for the existence of a significant regional structure. This indeed revealed that the extent of genetic differentiation between rivers from different regions was on average the double of that observed between rivers within any given region. While these results corroborated previous reports of a regional structure in North American Atlantic salmon, they were substantially different in the details of population structure. In accordance with previous results on genetic differentiation among seven rivers in Québec (Fontaine *et al*. 1997), our results did not support the existence of three groups of Atlantic salmon populations as proposed by Power (1981) based on differences in life-history traits between the Ungava, North Shore and South Shore-Anticosti regions. Our results also contrasted with those of Verspoor (2005) who identified, based on allozyme data, Ungava–Labrador salmon populations as a distinct regional group from Québec and New Brunswick populations. This discrepancy was most probably due to the greater spatial coverage of our sampling design in the province of Québec and possibly also to the use of different types of molecular markers. However, the identification of the Southern Québec and Labrador regional groups in this study was concordant with the morphometric differences (mainly head length) and the malate dehydrogenase *Mdh-3,4* frequency discontinuity observed between Gaspésie-Maritimes and Labrador-Newfoundland populations. Both regions were indeed previously suggested as representing two different regional stocks (Claytor & MacCrimmon 1988).

Regional structure and dispersal

Contemporary dispersal was observed among all regional groups, except Ungava. The relatively high genetic distinctiveness of Ungava, along with the absence of immigrants from other regions, indicated that salmon populations from this region are isolated from other regional groups. The geographical isolation of this region and the fact that no rivers were sampled between Ungava and lower Labrador may have contributed to this marked distinction compared to other regions. A proportion of approximately 20% of adult salmon in Ungava complete their life cycle in nearby estuaries (Robitaille *et al*. 1986), a characteristic that may also have contributed to the isolation of salmon from this region. In contrast, the proportion of detected immigrants in all other regions was relatively high with a mean proportion of immigrants per river of 9.7%. This immigration rate was slightly higher than straying rate estimates of up to 6% per year detected for Atlantic salmon in European rivers using capture–mark–recapture techniques (Jonsson *et al*. 2003) but fell within the range of straying rates observed for Pacific salmon (1% to 27% per year, Quinn 1993; Tallman & Healey 1994; Adkison 1995). Theory predicts that moderate or high dispersal rate among populations could confer multiple advantages for philopatric species with small populations experiencing variable conditions in time and space, namely the avoidance of poor conditions at local sites, the spread of beneficial alleles, the increase in adaptive potential through increased genetic variation and

the reduction of kin competition and inbreeding (reviewed in Garant *et al*. 2007).

Absolute values of immigration rate per river or regional group should be considered with caution, as these estimates could be underestimated when using a stringent criterion. Moreover, although no evidence of bias was found because of the number of source populations at the inter-regional scale, immigration rate estimated within large regional groups such as Southern Quebec might be inflated relative to other regions due to the high number of source populations from which type I error rate can accumulate. However, the power at detecting real immigrants estimated through simulations was high (92%) and we could therefore expect that type I error should be relatively low. Another potential limitation of assignment methods is that power in detecting immigrants could decrease with decreasing genetic differentiation (Paetkau *et al*. 2004; Manel *et al*. 2005). While we cannot completely rule out this possibility, we found little evidence for the effect of population differentiation in influencing the detection of immigrants, as the number of detected immigrants did not decrease but rather increased (within regions) or was not associated with (among regions) a decrease in genetic differentiation (data not shown). Also, our sample size averaged 50 individuals per population genotyped at 13 loci, conditions which allowed avoiding this bias in simulated data sets with comparable levels of population divergence (Paetkau *et al*. 2004). Another potential limitation is that the difficulty of correctly assigning detected immigrants to their population of origin could increase in weakly differentiated populations (Manel *et al*. 2005). In this study, however, assignment was interpreted according to the immigrant category only (local vs. inter-regional immigrants) rather than at the river scale, which reduces the importance of this potential limitation. Indeed, errors in assignments would be expected to occur mainly between genetically similar rivers within regional groups.

Our results did not indicate distance-restricted gene flow based on limited dispersal capability but rather suggested significant interconnection among regional groups, except Ungava, which contrasted with the regional genetic structure observed in the system. This corroborated the results of a previous capture–mark–recapture study which provided evidence for movements of hundreds of kilometres around the Gulf of Saint Lawrence (Belding & Préfontaine 1937). Although a regional structure might be maintained with high genetic connectivity, the comparison of the impact of local vs. inter-regional immigrants on realized gene flow suggested that inter-regional immigrants had a lower success in their new environment. Indeed, lower genetic differentiation among populations exchanging local as opposed to inter-regional migrants was found over a comparable geographical distance despite similar dispersal rates. Lower success of immigrants relative to residents as a consequence of selection against immigrants in the wild has previously been reported in salmonids (Tallman & Healey 1994; Hendry *et al*. 2000) and other organisms (e.g. Nosil *et al*. 2005; Bolnick & Nosil 2007). However, further work is needed to test this hypothesis more rigorously. Namely, computer simulations could be used to further evaluate the influence of such a dispersal pattern and the potential role of selection in defining a hierarchical population structure.

Landscape genetics and the potential interaction between gene flow and local adaptation

Landscape genetic analyses identified coastal distance, either from the South or between rivers, and temperature regime as the main factors influencing genetic differentiation in Atlantic salmon populations. The latitude, the difficulty of upstream migration, the non-native salmon stocking and the migration tactic were not retained in these analyses as being good predictors of genetic differentiation. However, it is noteworthy that the proportion of one-sea-winter salmon was greater in populations from Labrador and Lower North Shore where a tendency for a lower difficulty of upstream migration was also observed compared to rivers from other regional groups. This corroborated previous observations of a negative association between the proportion of one-sea-winter salmon and the difficulty of upstream migration (river size, length or discharge, Shaffer & Elson 1975; Power 1981). Overall, while negative results do not allow us to completely rule out possible effects of these factors on population differentiation in Atlantic salmon, the clear evidence we observed for others, namely coastal distance and temperature regime, established their predominant roles in population differentiation.

The influence of coastal distance from the South on the genetic structure documented here at the large and intermediate spatial scales could represent the historical footprint of the North American colonization process. This process is hypothesized to have occurred progressively from South to North between 14 000 and 7000 years ago following the last glacial retreat (Dyke & Prest 1987), from a southern Atlantic glacial refugium located off the New England coast (Schmidt 1986). Coastal distance from the South could then represent the influence of a stepwise colonization process along the Atlantic coast and the Gulf of Saint Lawrence. Historical colonization was also identified as being implicated in defining population structure in other North American salmonids, namely the brook charr, *Salvelinus fontinalis* (Castric & Bernatchez 2003) and the lake cisco, *Coregonus artedi* (Turgeon & Bernatchez 2001). Pairwise coastal distance between rivers, on the other hand, was significantly associated with genetic structure at the large and intermediate spatial scales, when temperature was taken into account. The integration of multiple genetically differentiated regional groups most probably created this

association at a large spatial scale and could explain the weaker association at smaller spatial scales. The importance of pairwise coastal distance still emphasized, however, the influence of contemporary gene flow on population structure. Indeed, although large water systems exist such as the Miramichi in Canada and the Varzuga in Russia, Atlantic salmon populations are generally characterized by relatively small effective population sizes and moderate to high migration rates between populations (Jonsson *et al*. 2003, Fraser *et al*. 2007; Palstra *et al*. 2007; this study). These represent ideal conditions to reach migration–drift equilibrium over brief evolutionary time (Slatkin 1994). Other factors such as the absence of physical barriers to dispersal, the long-distance migration behaviour and dispersal ability also favour gene flow (Crispo & Hendry 2005) and further promote a state of equilibrium. Under these circumstances and assuming a migration–drift equilibrium, contemporary more than historical gene flow would influence Atlantic salmon population structure (Hutchison & Templeton 1999).

Temperature regime was also found to significantly influence genetic differentiation in Atlantic salmon populations at both intermediate (Saint Lawrence water system) and regional scales. Sea surface temperature was identified as a factor associated with genetic structure in other fishes, such as herring in the Baltic Sea, and may have been involved in limiting gene flow through local adaptation of populations (Jørgensen *et al*. 2005). In Atlantic salmon, temperature was previously suggested as a factor potentially involved in local adaptation based on its associations with morphological and life-history traits (see reviews in Taylor 1991 and Garcia de Leaniz *et al*. 2007) and based on its association with malic enzyme (*MEP-2**) allele frequency at different spatial scales (Verspoor & Jordan 1989). More recently, temperature regime was shown to be involved in local adaptation through its probable influence on bacterial diversity (Dionne *et al*. 2007). The latter study indicated that Atlantic salmon were locally adapted to the thermal regime and associated pathogenic bacterial diversity prevailing in their environment by way of their genetic diversity at the major histocompatibility complex (MHC) class IIβ gene, involved in pathogen resistance. These recent findings along with (i) the positive association of temperature with genetic structure in the landscape genetic analyses, (ii) the temperature differences observed between most regional groups that best characterized regions compared to other ecological variables, and (iii) the evidence of restricted gene flow but not dispersal among regional groups found in this study supports the hypothesis that temperature regime may represent a major factor promoting genetic divergence by constraining gene flow between different thermal regimes to which salmon are locally adapted. Furthermore, the extent of local adaptation might vary among regional groups because of a different balance between gene flow, genetic drift and selection pressure (Adkison 1995, Hansen *et al*. 2002, Hansen *et al*. 2007). This could explain for instance the stronger association of temperature and genetic structure in the Southern Québec compared to the Higher North Shore regional group. While our study identified thermal regime as an important factor influencing population structure in Atlantic salmon through its potential implication in local adaptation, the exact underlying adaptive mechanism remains to be elucidated, for instance, using common garden experiments to test how temperature regime could influence individual fitness.

In conclusion, our findings are consistent with previous observations concerning the interacting influence of gene flow and local adaptation in defining genetic structure in wild populations (Garant *et al*. 2007). Indeed, our results suggest that gene flow and thermal regime adaptation can interact to define a hierarchical genetic structure in a widely distributed and long-distance migratory species in the wild. Also, our study emphasizes the need of documenting genetic structure at multiple spatial scales in order to better identify influential evolutionary processes. The complex evolutionary mechanisms underlying genetic structure may be, however, only fully understood through a combination of field studies, computer simulations and common garden experiments, which should be prioritized in future studies.

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References

Adkison MD (1995) Population differentiation in Pacific salmon: local adaptation, genetic drift, or the environment? *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 2762–2777.

Angers B, Magnan P, Plante M, Bernatchez L (1999) Canonical correspondence analysis for estimating spatial and environmental effects on microsatellite gene diversity in brook charr (*Salvelinus fontinalis*). *Molecular Ecology*, **8**, 1043–1053.

Belding DL, Préfontaine G (1937) *Études sur le saumon de l'Atlantique (Salmo Salar* L.*) I*, PhD Thesis. Université de Montréal, Quebec, Canada.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2000) GENETIX *4.02, logiciel sous WindowsTM pour la génétique des populations*. CNRS UPR 9060, Université Montpellier II, Montpellier, France.

Bolnick DI, Nosil P (2007) Natural selection in populations subject to a migration load. *Evolution*, **61**, 2229–2243.

Caron F, Fontaine P-M, Cauchon V (2005) *États des stocks de saumon au Québec en 2005*. Ministère des Ressources naturelles et de la Faune, Québec, Canada.

Castric V, Bernatchez L (2003) The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). *Genetics*, **163**, 983–996.

Castric V, Bernatchez L (2004) Individual assignment test reveals differential restriction to dispersal between two salmonids despite no increase of genetic differences with distance. *Molecular Ecology*, **13**, 1299–1312.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution*, **32**, 550–570.

Claytor RR, MacCrimmon HR (1988) Morphological and meristic variability among North American Atlantic salmon (*Salmo salar*). *Canadian Journal of Zoology*, **66**, 310–317.

Claytor RR, MacCrimmon HR, Gots BL (1991) Continental and ecological variance components of European and North American Atlantic salmon (*Salmo salar*) phenotypes. *Biological Journal of the Linnean Society*, **44**, 203–229.

Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP (2006) The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology*, **15**, 49–62.

Crispo E, Hendry AP (2005) Does time since colonization influence isolation by distance? A meta-analysis. *Conservation Genetics*, **6**, 665–682.

Dillane E, Cross MC, McGinnity P *et al*. (2007) Spatial and temporal patterns in microsatellite DNA variation of wild Atlantic salmon, *Salmo salar*, in Irish rivers. *Fisheries Management and Ecology*, **14**, 209–219.

Dionne M, Miller KM, Dodson JJ, Caron F, Bernatchez L (2007) Clinal variation in MHC diversity with temperature: evidence for the role of host-pathogen interaction on local adaptation in Atlantic salmon. *Evolution*, **61**, 2154–2164.

Dyke AS, Prest VK (1987) Late Wisconsinian and Holocene history of the Laurentide Ice Sheet. *Géographie Physique et Quaternaire*, **41**, 237–263.

Eldridge WH, Naish KA (2007) Long-term effects of translocation and release numbers on fine-scale population structure among coho salmon (*Oncorhynchus kisutch*). *Molecular Ecology*, **16**, 2407–2421.

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fleming IA (1998) Pattern and variability in the breeding system of Atlantic salmon (*Salmo salar*), with comparisons to other salmonids. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 59–76.

Foll M, Gaggiotti O (2006) Identifying the environmental factors that determine the genetic structure of populations. *Genetics*, **174**, 875–891.

Fontaine P-M, Dodson JJ, Bernatchez L, Slettan A (1997) A genetic test of metapopulation structure in Atlantic salmon (*Salmo salar*) using microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2434–2442.

Fraser D, Hansen MM, Ostergaard S *et al*. (2007) Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Molecular Ecology*, **16**, 3866–3889.

Garant D, Kruuk LEB (2005) How to use molecular marker data to measure evolutionary parameters in wild populations. *Molecular Ecology*, **14**, 1843–1859.

Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology*, **21**, 434–443.

Garcia de Leaniz C, Fleming IA, Einum S *et al*. (2007) A critical review of adaptive genetic variation in Atlantic salmon: implication for conservation. *Biological Review*, **82**, 173–211.

Giordano AR, Ridenhour BJ, Storfer A (2007) The influence of altitude and topography on genetic structure in the long-toed salamander (*Ambystoma macrodactulym*). *Molecular Ecology*, **16**, 1625–1637.

Goudet J (2001) FSTAT, *A Program to Estimate and Test Gene Diversities and Fixation Indices*, Version 2.9.3. Institut d'Écologie, Université de Lausanne, Lausanne, Switzerland.

Guillot G, Mortier F, Estoup A (2005) GENELAND: a computer package for landscape genetics. *Molecular Ecology Notes*, **5**, 712–715.

Hansen LP, Jonsson N, Jonsson B (1993) Oceanic migration in homing Atlantic salmon. *Animal Behaviour*, **45**, 927–941.

Hansen MM, Ruzzante DE, Nielsen EE, Bekkevold D, Mensberg K-LD (2002) Long-term effective population size, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology*, **11**, 2523–2535.

Hansen MM, Skaala O, Jensen LF, Bekkevold D, Mensberg K-LD (2007) Gene flow, effective population size and selection at major histocompatibility complex genes: brown trout in the Hardanger Fjord, Norway. *Molecular Ecology*, **16**, 1413–1425.

Hendry AP (2004) Selection against migrants contributes to the rapid evolution of ecologically dependent reproductive isolation. *Evolutionary Ecology Research*, **6**, 1219–1236.

Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science*, **290**, 516–518.

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.

Jonsson B, Jonsson N, Hansen LP (2003) Atlantic salmon straying from the river Imsa. *Journal of Fish Biology*, **62**, 641–657.

Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea. *Molecular Ecology*, **14**, 3219–3234.

Kimura M (1953) 'Stepping-stone' model of population. *Annual Report of the National Institute of Genetics*, **3**, 62–63.

King TL, Eackles MS, Letcher BH (2005) Microsatellite DNA markers for the study of Atlantic salmon (*Salmo salar*) kinship, population structure, and mixed-fishery analyses. *Molecular Ecology Notes*, **5**, 130–132.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, **18**, 189–197.

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology & Evolution*, **20**, 136–142.

McConnell SKJ, Ruzzante DE, O'Reilly PT, Hamilton L, Wright JM (1997) Microsatellite loci reveal highly significant genetic differentiation among Atlantic salmon (*Salmo salar* L.) stocks from the East coast of Canada. *Molecular Ecology*, **6**, 1075–1089.

Metcalfe NB, Thorpe JE (1990) Determinants of geographical variation in the age of seaward-migrating salmon, *Salmo salar*. *Journal of Animal Ecology*, **59**, 135–145.

Nosil P, Vines TH, Funk DJ (2005) Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, **59**, 705–719.

O'Reilly PT, Hamilton L, McConnell SKJ, Wright JM (1996) Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2292–2298.

van Oosterhout CV, Hutchinson WF, Willis DPM (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Paetkau D, Calvert W, IS.Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, **13**, 55–65.

Palsbøll PJ, Bérubé M, Allendorf FW (2006) Identification of management units using population genetic data. *Trends in Ecology & Evolution*, **22**, 11–16.

Palstra FP, O'Connell MF, Ruzzante DE (2007) Population structure and gene flow reversals in Atlantic salmon (*Salmo salar*) over contemporary and long-term temporal scales: effects of population size and life history. *Molecular Ecology*, **16**, 4504–4522.

Paterson S, Piertney SB, Knox D, Gilbey J, Verspoor E (2004) Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (*Salmo salar* L.) microsatellites. *Molecular Ecology Notes*, **4**, 160–162.

Piry S, Alapetite A, Cornuet J-M *et al*. (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, **95**, 536–539.

Power G (1981) Stock characteristics and catches of Atlantic salmon (*Salmo salar*) in Québec, and Newfoundland and Labrador in relation to environmental variables. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1601–1611.

Presa P, Guyomard R (1996) Conservation of microsatellites in three species of salmonids. *Journal of Fish Biology*, **49**, 1326–1329.

Quinn TP (1993) A review of homing and straying of wild and hatchery-produced salmon. *Fisheries Research*, **18**, 29–44.

R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http: // www.R-project.org

Raymond M, Rousset F (1995) GENEPOP (version 3.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Robitaille JA, Côté Y, Shooner G, Hayeur G (1986) Growth, maturation patterns of Atantic salmon, *Salmo salar*, in the Koksoak river, Ungava, Québec. *Canadian Special Publication of Fisheries and Aquatic Sciences*, **89**, 62–69.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Rousset F (2002) Partial mantel tests: reply to Castellano and Balletto. *Evolution*, **56**, 1874–1875.

Ruzzante DE, Hansen MM, Meldrup D, Ebert KM (2004) Stocking impact and migration pattern in an anadromous brown trout (*Salmo trutta*) complex: where have all the stocked spawning sea trout gone? *Molecular Ecology*, **13**, 1433–1445.

Schaffer WM, Elson PF (1975) The adaptive significance of variations in life history among local populations of Atlantic salmon in North America. *Ecology*, **56**, 577–590.

Schlötterer C (2002) A microsatellite-based multilocus screen for the identification of local selective sweeps. *Genetics*, **160**, 753–763.

Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, New York.

Schmidt RE (1986) Zoogeography of the northern Appalachians. In: *The Zoogeography of North American Freshwater Fishes* (eds Hocutt CH, Wiley EO), pp. 137–159. John Wiley and Sons, New York.

Schwartz MK, Luikart G, Waples RS (2006) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, **22**, 25–33.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Slatkin M (1994) Gene flow and population structure. In: *Ecological Genetics* (ed. Real LA), pp. 3–17. Princeton University Press, Princeton, New Jersey.

Slettan A, Olsaker I, Lie O (1995) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics*, **26**, 281–282.

Spidle AP, Kalinowski ST, Lubinski BA *et al*. (2003) Population structure of Atlantic salmon in Maine with reference to populations from Atlantic Canada. *Transaction of the American Fisheries Society*, **132**, 196–209.

Stabell OB (1984) Homing and olfaction in salmonids: a critical review with special references to the Atlantic salmon. *Biological Review*, **59**, 333–388.

Storfer A, Murphy MA, Evans JS *et al*. (2007) Putting the 'landscape' in landscape genetics. *Heredity*, **98**, 128–142.

Tallman RF, Healey MC (1994) Homing, straying, and gene flow among seasonally separated populations of chum salmon (*Oncorhynchus keta*). *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 577–588.

Taylor EB (1991) A review of local adaptation in salmonidae, with particular reference to Pacific and Atlantic salmon. *Aquaculture*, **98**, 185–207.

Turgeon J, Bernatchez L (2001) Clinal variation at microsatellite loci reveals historical secondary intergradation between glacial races of *Coregonus artedi* (teleostei: coregoninae). *Evolution*, **55**, 2274–2286.

Vähä J-P, Erkinaro J, Niemelä E, Primmer CR (2007) Life-history and habitat features influence the within-river genetic structure of Atlantic salmon. *Molecular Ecology*, **16**, 2638–2654.

Verspoor E (2005) Regional differentiation of North American Atlantic salmon at allozyme loci. *Journal of Fish Biology*, **67**, 80–103.

Verspoor E, Jordan WC (1989) Genetic variation at the Me-2 locus in the Atlantic salmon within and between rivers: evidence for its selective maintenance. *Journal of Fish Biology*, **35**, 205–213.
Verspoor E, Beardmore JA, Consuegra S *et al*. (2005) Population structure in the Atlantic salmon: insights from 40 years of research into genetic protein variation. *Journal of Fish Biology*, **67**, 3–54.
Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
Wright S (1943) Isolation by distance. *Genetics*, **28**, 114–138.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Sample statistics associated with each of the 51 salmon rivers sampled and analyzed at 13 microsatellite loci. Rivers are grouped in seven regional groups according to GENELAND. *N*, number of salmon sampled; distance, coastal distance in kilometres from the southernmost sampling site (Miramichi River); A_r, mean allelic richness adjusted for the sample size of the smallest population (35 individuals); H_E/H_O, expected/observed heterozygosities.

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