Determinants of hierarchical genetic structure in Atlantic salmon populations: environmental factors *vs.* anthropogenic influences

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Abstract

Disentangling the effects of natural environmental features and anthropogenic factors on the genetic structure of endangered populations is an important challenge for conservation biology. Here, we investigated the combined influences of major environmental features and stocking with non-native fish on the genetic structure and local adaptation of Atlantic salmon (*Salmo salar*) populations. We used 17 microsatellite loci to genotype 975 individuals originating from 34 French rivers. Bayesian analyses revealed a hierarchical genetic structure into five geographically distinct clusters. Coastal distance, geological substrate and river length were strong predictors of population structure. Gene flow was higher among rivers with similar geologies, suggesting local adaptation to geological substrate. The effect of river length was mainly owing to one highly differentiated population that has the farthest spawning grounds off the river mouth (up to 900 km) and the largest fish, suggesting local adaptation to river length. We detected high levels of admixture in stocked populations but also in neighbouring ones, implying large-scale impacts of stocking through dispersal of non-native individuals. However, we found relatively few admixed individuals suggesting a lower fitness of stocked fish and/or some reproductive isolation between wild and stocked individuals. When excluding stocked populations, genetic structure increased as did its correlation with environmental factors. This study overall indicates that geological substrate and river length are major environmental factors influencing gene flow and potential local adaptation among Atlantic salmon populations but that stocking with non-native individuals may ultimately disrupt these natural patterns of gene flow among locally adapted populations.

Keywords: Atlantic salmon, genetic structure, landscape genetics, introgression, local adaptation, stocking

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Introduction

Patterns of population genetic structure are increasingly considered for the conservation of endangered species

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(Palsboll *et al.* 2007; Schwartz *et al.* 2007). A major issue is to determine how landscape-level environmental factors are involved in the spatial distribution of genetic variation (Manel *et al.* 2003; Storfer *et al.* 2007). The role of ecological variables on mediating gene flow across populations has been investigated in a wide range of plants (Sork *et al.* 1999; Albaladejo *et al.* 2009; Schmidt *et al.* 2009) and animals (Sokal & Thomson 1998; Keyghobadi *et al.* 1999; Castric *et al.* 2001) using a growing

variety of dedicated statistical tools (Manel *et al.* 2003; Balkenhol *et al.* 2009; Guillot *et al.* 2009). Landscape genetics studies usually focus on the distribution of neutral genetic variation and attempt to identify landscape features that constrain or facilitate dispersal (McRae 2006). However, landscape heterogeneity can ultimately result in divergent selection among habitats, which can be detected by analysing adaptive genetic variation (Hendry 2004; Nosil *et al.* 2005; Bolnick & Nosil 2007). Indirect evidence for local adaptation can also be uncovered by genome scans that identify putatively selected loci (Storz *et al.* 2004; Oleksyk *et al.* 2010). Alternatively, pattern of dispersal vs. gene flow can provide some evidence for local adaptation: dispersal is expected to be higher than gene flow among locally adapted populations (Currat *et al.* 2008; Rasanen & Hendry 2008). Such a selection against immigrants has been suggested in certain chum salmon (*Oncorhynchus keta*) and Atlantic salmon (*Salmo salar*) populations (Tallman 1994; Dionne *et al.* 2008).

Atlantic salmon is a long-distance migratory and philopatric species that returns to spawn in its natal river after a growing period at sea (Stabell 1984; Quinn 1993). This homing behaviour is not fully strict but is often invoked to explain the genetic differentiation among salmon populations (King *et al.* 2001; Verspoor *et al.* 2005). Accordingly, the amount of gene flow among populations follows a pattern of isolation by distance (IBD) (Dionne *et al.* 2008; Tonteri *et al.* 2009) also observed within rivers (Primmer *et al.* 2006; Vaha *et al.* 2007; Dillane *et al.* 2008). The recognition of river-specific odours linked to the local geological substrate might be an important mechanism allowing salmon to return to their natal river (reviewed in Stabell 1984 and Dittman *et al.* 1996). Consequently, dispersal could be constrained among populations located on different geological substrates or facilitated among populations with similar geologies (Stabell 1984; Grandjean *et al.* 2009). Furthermore, geological variations may be associated with other factors involved in salmonids' local adaptation including water's pH (Schom 1986; Donaghy & Verspoor 1997; Fraser *et al.* 2008) and gravel size (Quinn *et al.* 1995). Temperature regime is also assumed to influence local adaptation in *S. salar* (Taylor 1991; Garcia de Leaniz *et al.* 2007), and it can be related to neutral genetic differentiation among populations (Dionne *et al.* 2008; Valiente *et al.* 2010). Finally, some studies suggested a positive correlation between the difficulty to upstream migration and size or age of returning adults (Schaffer & Elson 1975; Power 1981). River length could thus restrict gene flow among populations with big individuals being selected for in large river systems.

Supplementation of wild salmonids' populations with hatchery-reared fish has been widely used to stop their dramatic decline and favour recreational fishing (Aprahamian *et al.* 2003). However, such practices can have major impacts on the genetic structure (Hansen *et al.* 2009; Marie *et al.* 2010; Pearse *et al.* 2011) and evolutionary potential of natural populations (Araki *et al.* 2007; Fraser 2008; Williams & Hoffman 2009). First, stocking can lead to variable admixture rates in wild populations (Campos *et al.* 2008; Finnegan & Stevens 2008; Hansen *et al.* 2009). It can also produce a decrease in the genetic structure among supplemented and donors stocks (Ayllon *et al.* 2006; Lage & Kornfield 2006; Hansen *et al.* 2009; Marie *et al.* 2010) and a disruption of IBD in wild populations (Pearse *et al.* 2011). Alterations in adaptive genetic variation may occur in recipient populations if supplemented individuals experienced different selection regimes (Dionne *et al.* 2007). Captive bred individuals could be selected for traits that are disadvantageous in nature explaining their lower fitness than wild conspecifics once released (Fleming & Einum 1997; Jonsson & Jonsson 2006; Araki *et al.* 2008; Williams & Hoffman 2009; Theriault *et al.* 2011). Ultimately, the release of captive bred individuals may reduce the fitness of recipient populations (Hindar *et al.* 1991; McGinnity *et al.* 2003; Ford & Myers 2008) and increase their risks of extinction (Levin *et al.* 2001; McGinnity *et al.* 2009). Despite a large body of research on consequences of stocking on recipient populations, few studies have investigated the impact of such practices on the links between population genetic structure and environmental features especially at large spatial scales.

In France, *S. salar* populations are distributed in contrasted habitats and some of them have been stocked with non-native individuals during the last decades allowing inferences on the respective roles of ecological factors and stocking on population genetic structure. In this study, we genotyped 975 individuals from 34 French populations using 17 microsatellite loci to investigate three hypotheses. First, we tested whether major environmental factors including coastal distance, river length, geological substrate and temperature influence the genetic structure and potential local adaptation of salmon populations. Second, we tested three predictions related to the effects of stocking with non-native fish on the genetic make-up of wild populations: (i) stocked populations should display higher levels of admixture than unstocked populations, (ii) stocked fish could disperse into nearby unstocked populations resulting in significant levels of admixture in these populations and (iii) overall genetic structure should theoretically increase when excluding stocked populations. Third, we investigated the interplay between stocking and

environmental factors on population genetic structure. Specifically, we tested whether stocking may disrupt patterns of IBD and diminish the relationships between genetic distances and environmental factors among Atlantic salmon populations.

Materials and methods

Study populations and sampling

We studied 34 Atlantic salmon populations (Fig. 1, Table 1) representing the main stocks of this species in France (Baglinière & Porcher 1994) along 1662 km of coastline. Only very few populations inhabiting small coastal rivers were not sampled. Our sampling is representative of the former distribution of the species in France except for populations from the Gironde region (Garonne and Dordogne), which had been extirpated and have been recently restocked (Baglinière & Dumas 1988; Baglinière *et al.* 1990). Adult fish were collected by angling or trapping, and scales were stored by the INRA laboratory in Rennes and by the *Office National de l'Eau et des Milieux Aquatiques* (ONEMA). Sampling and angling sites were generally located downstream, except for the Allier R. where individuals were trapped at 650 km from the river mouth. Scales were kept in 95% ethanol or in paper envelopes and used to determine the age of each individual (Baglinière *et al.* 1991). We

focused on cohorts 2002–2003 and collected between eleven and 80 samples per population except in five small populations where only between five and nine samples were available (Table 1). Whenever possible, we added some samples from cohorts 1998 to 2001 and 2004–2006 to increase sample size of reduced populations.

Proportions of multi-sea-winter fish and data concerning stocking practices were available from reports and databases from INRA, ONEMA and Fédération Nationale pour la Pêche en France (FNPF). Supplementation operations were performed in some rivers using non-native or native fish as summarized in table 1 (Baglinière & Dumas 1988; Baglinière *et al.* 1990; INRA and ONEMA, unpublished data). The Orne River (ORN) was stocked with fish from Gave d'Oloron R. (GAV) in 1995 (Table 1). The Sélune R. (SEL) was stocked with individuals from the Aulne R. (AUL) from 1989 to 1997, except in 1995 where Gave d'Oloron individuals were used. The Couesnon R. (COU) has been stocked with fish from the Aulne since 1989, except in 1995 where Gave d'Oloron individuals were used. Garonne (GAR) and Dordogne (DOR) rivers had been extirpated and were stocked with non-native individuals from Scotland, Allier and Gave d'Oloron from 1980 and 1977, respectively, to 1991 and have been stocked with native individuals since 1992. Elorn (ELO), Aulne, Allier (ALL) and Gave d'Oloron have been stocked with non-
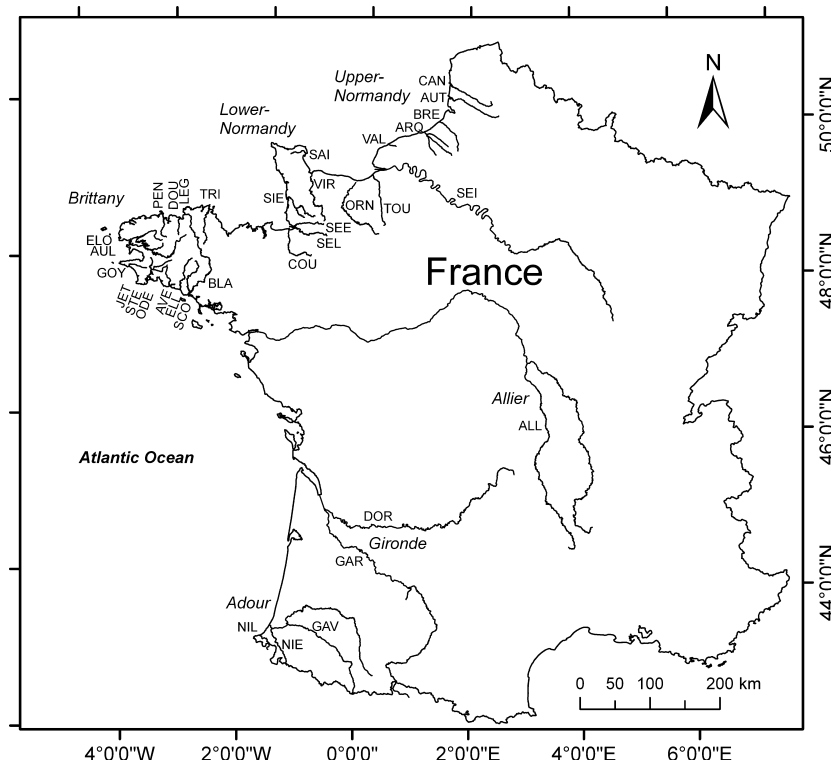

Fig. 1 Map with locations of the study populations (see also Table 1).

Table 1 Geographical characteristics of the 34 sampled rivers and description of populations' characteristics, sampling and genetic diversity indices. N is the number of alleles, A_R is allelic richness (based on samples of 11 individuals), H_E is the unbiased expected heterozygosity, H_O is the observed heterozygosity, and F_{IS} is the inbreeding coefficient (significance is indicated by asterisk). The origin of fish stocked since 1990 is given (either non-native or native), and the mention 'nearby' is indicated for 'unstocked' populations in which we expected significant straying from nearby (<100 km) stocked populations

| Sampling sites | | | | | | | | | Samples | | | Genetic diversity indices | | | | |
Region	River	Longitude	Latitude	Distance from canche (km)	River length (km)	Proportion of multi-sea-winter fish	Geological substrate	Stocking sources since 1990	Abbreviation	Sample size	Cohorts	N	A_R	H_E	H_O	F_{IS}
Upper Normandy	Canche	1°36	50°32	0	88	18%	1	–	CAN	8	1999–2006	6.1	–	0.76	0.74	0.03
	Authie	1°34	50°22	19	103		1	–	AUT	11	2003–2006	7.6	6.7	0.76	0.68	0.11*
	Bresle	1°22	50°03	57	72		1	–	BRE	30	1998–2004	8.7	5.5	0.72	0.72	0
	Arques	1°05	49°56	82	67		1	–	ARQ	31	2003	7.3	5.3	0.71	0.7	0.02
	Valmont	0°22	49°45	138	14		1	nearby	VAL	5	2003–2005	5.4	–	0.82	0.72	0.13*
Lower Normandy	Seine	0°07	49°26	185	777	13%	1	nearby	SEI	7	1998–2006	6.9	–	0.79	0.75	0.06
	Touques	0°04	49°21	193	108		1	nearby	TOU	11	2007	8.4	7.5	0.83	0.75	0.11*
	Orne	0°14	49°17	218	170		1	GAV	ORN	31	2001	11.7	7.4	0.83	0.81	0.02
	Vire	1°07	49°21	284	128		2	nearby	VIR	19	1998–2004	10.4	7.3	0.83	0.78	0.06
	Saire	1°14	49°36	315	31		2	nearby	SAI	9	2005–2006	7.1	–	0.8	0.8	0
	Sienne	1°34	49°00	465	93		2	nearby	SIE	37	2002–2003	11.6	6.8	0.78	0.76	0.02
	Sée	1°29	48°39	505	78		2	nearby	SEE	66	2002–2003	11.5	6.1	0.79	0.79	0.01
	Sélune	1°29	48°39	505	91		2	AUL & GAV	SEL	80	2002–2003	13.4	6.8	0.79	0.8	0.01
	Couesnon	1°30	48°37	508	101		2	AUL & GAV	COU	34	2002–2003	10.7	6.7	0.81	0.8	0.01
Brittany	Trieux	3°04	48°49	649	72	17%	2	–	TRI	26	2002	9.8	6.9	0.82	0.81	0.02
	Leguer	3°33	48°43	716	60		2	–	LEG	27	2002–2003	10.4	7.3	0.82	0.79	0.04
	Douron	3°38	48°40	726	27		2	–	DOU	27	2002–2003	9.8	6.8	0.8	0.8	0.01
	Penzé	3°56	48°40	755	30		2	–	PEN	26	2002–2003	10.2	6.6	0.81	0.81	0
	Elorn	4°21	48°24	881	57		2	native	ELO	33	2003	10.4	6.4	0.79	0.78	0.01
	Aulne	4°15	48°17	903	140		2	native	AUL	34	2003	11.5	6.9	0.81	0.8	0.02
	Goyen	4°32	48°00	966	36		2	–	GOY	34	2003	10.1	6.5	0.8	0.77	0.04
	Steir	4°06	47°52	1018	62		2	–	STE	20	2002	9	6.8	0.81	0.81	0
	Jet	4°06	47°52	1018	62		2	–	JET	20	2000–2004	8.6	6.3	0.78	0.78	0
	Odet	4°06	47°52	1018	62		2	–	ODE	19	2003	8.4	6.4	0.78	0.77	0.01
	Aven	3°44	47°48	1052	37		2	–	AVE	34	2003	10.2	6.4	0.79	0.79	0.01
	Ellé	3°32	47°46	1069	76		2	–	ELL	34	2003	10.2	6.4	0.78	0.79	–0.01
	Scorff	3°22	47°42	1087	78		2	–	SCO	64	2002–2003	11.6	6.3	0.79	0.78	0.01
	Blavet	3°22	47°42	1087	149		2	–	BLA	64	2002–2003	12.5	6.5	0.79	0.78	0.02
Allier	Allier	2°10	47°16	1197	1013	95%	3	native	ALL	35	2001–2002	8.1	5.4	0.74	0.74	0
Gironde	Dordogne	1°06	45°34	1412	483	na	4	ALL & GAV	DOR	15	2002	8.1	6.5	0.79	0.83	–0.05
	Garonne	1°06	45°34	1412	647		4	ALL & GAV	GAR	30	2002	10.2	6.6	0.8	0.82	–0.02
Adour	Gave d'Oloron	1°31	43°31	1642	309	19%	4	native	GAV	29	2003	11.6	7.2	0.81	0.77	0.06
	Nive	1°31	43°31	1642	80		4	–	NIE	8	2001–2006	5.5	–	0.77	0.75	0.04
	Nivelle	1°40	43°23	1662	45		4	–	NIL	17	1998–2004	7.9	6.4	0.8	0.8	–0.01

native fish before 1980 and then with native fish. The populations from Authie (AUT), Canche (CAN), Valmont (VAL), Touques (TOU) and Saire (SAI) rivers were very low at the time of sampling and were considered as extinct or residual. In the Seine (SEI) river, although no stocking was performed, more than 162 adult salmon were observed in 2008 by video-counting, and this recolonization event involved individuals from multiple origins (Perrier *et al.* 2010). In several rivers, no stocking was performed but their geographic proximity (<100 km) with certain stocked populations suggests that their genetic make-up may have been influenced by stocking in nearby populations (Table 1).

We investigated potential relationships between some key environmental factors including coastal distance, river length, local geology and temperature on the population genetic structure. Geographic coastal distances between estuaries of the study rivers were calculated following coastline using Google Earth (http://www.google.com/earth/index.html) and ranged from 0 to 1662 km with a median value of 741 km (Table 1). River lengths were calculated as the distance between sources and mouths of rivers using ESRI® ArcGIS 9.2 and ranged from 14 to 1013 km with a median value of 78 km (Table 1). Local air temperatures were available from https://public.meteofrance.com. For each population, we computed the annual average air temperature at the river mouth over the last 10 years as air and water temperatures are usually highly correlated (Sahoo *et al.* 2009). Finally, the 34 rivers are distributed among four distinct geological areas according to geological maps from the BRGM (http://www.brgm.fr/preCarte50.do). We thus classified each river into one of these four areas: (i) 'Bassin Parisien' with mostly sedimentary calcareous rocks; (ii) 'Massif Armoricain' with mostly metamorphic siliceous rocks; (iii) 'Massif Central' with metamorphic and volcanic rocks and (iv) 'Bassin Aquitain' with metamorphic and sedimentary rocks (Table 1).

Molecular analyses

Genomic DNA was extracted using a Chelex procedure, and individuals were genotyped at 17 microsatellites (Table S1, Supporting information) as described in Perrier *et al.* (2010).

Within-population genetic diversity

We used MICRO-CHECKER 2.2.3 (Van Oosterhout *et al.* 2004) to assess the frequency of null alleles and scoring errors owing to stuttering or large allelic dropout. Allele number and allelic richness per population were obtained using FSTAT 2.9.3.2 (Goudet 1995), and expected heterozygosity, H_E, (Nei 1978) and observed heterozygosity (H_O) were calculated with GENETIX 4.05.2 (Belkhir *et al.* 1996). Tests for linkage and Hardy–Weinberg disequilibria were conducted with FSTAT 2.9.3.2 based on 1000 permutations. GENALEX 6 (Peakall & Smouse 2006) was used to calculate the number and frequency of private alleles in each population. FDIST 2 (Beaumont & Nichols 1996) was employed to validate the neutrality of the markers used. BOTTLENECK 1.2.02 (Cornuet & Luikart 1996; Piry *et al.* 1999) was used to investigate the occurrence of recent bottlenecks in each population. We followed authors' recommendations by assuming a two-phase model of mutation (TPM) with 95% single-step, 5% multistep mutations, 12% variance using 1000 iterations and a Wilcoxon sign-rank test.

Divergence among populations

Pairwise F_{ST} was computed in FSTAT 2.9.3.2 and tested with 1000 permutations. Neighbor-joining dendrograms based on pairwise Nei (Da) genetic distances (Nei *et al.* 1983) and confidence estimates of tree topology calculated by bootstrap resampling of loci 1000 times were made with POPULATIONS 1.2.30 (http://bioinformatics.org/~tryphon/populations/). Neighbor-joining dendrograms were visualized using TreeView (Page 1996). Analysis of molecular variance (AMOVA) was performed using ARLEQUIN (Excoffier *et al.* 2005). For each AMOVA, we tested the significance of variance components and fixation indices among groups using 10 000 permutations. We performed three AMOVAs: (i) with the 34 populations distributed in six groups: Upper Normandy, Lower Normandy, Brittany, Allier, Gironde and Adour (see Fig. 2 for geographic localization of populations), (ii) with a subset of 29 populations 'unstocked' with non-native fish and distributed into five groups (Upper Normandy, Lower Normandy, Brittany, Allier and Adour) and (iii) with a subset of 22 populations not stocked and at a minimal coastal distance of 100 km from populations stocked with non-native fish and distributed into four groups (Upper Normandy, Brittany, Allier and Adour). These analyses aimed at testing potential effects of stocking using non-native fish on the distribution of genetic variance among hierarchical levels of genetic structure. The five populations stocked with non-native fish were the following: Orne, Sélune, Couesnon, Dordogne and Garonne (Table 1). The seven unstocked populations located near stocked populations include the Valmont, Seine, Touques, Vire, Saire, Sienne and Sée rivers.

Individual Bayesian clustering

To estimate the number of genetic clusters in our data set without taking into account any predefined populations, we used the Bayesian clustering method
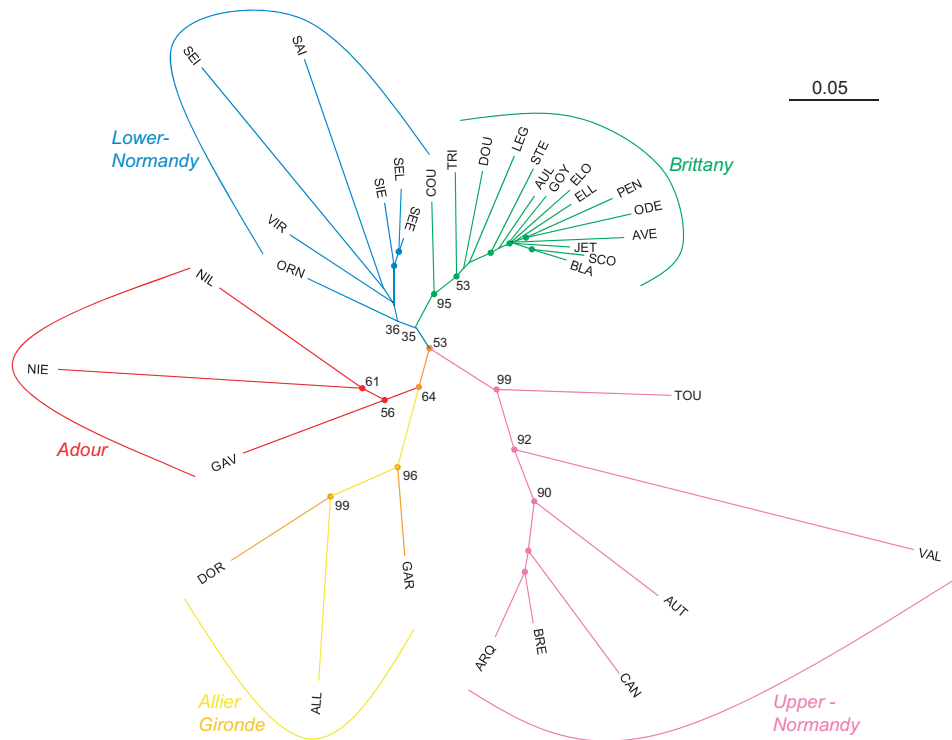
Fig. 2 Neighbor-joining tree based on Nei genetic distances among the 34 populations. Key bootstrap values are given, and nodes supported by bootstrap values >50% in 1000 pseudoreplicates are indicated with filled circles.

implemented in STRUCTURE 2.2 (Pritchard *et al.* 2000). This analysis also allowed us to compute admixture proportions among the inferred clusters at both the population and individual levels. STRUCTURE analyses were performed assuming an admixture model and a number of genetic clusters (*k*) from 1 to 14 (15 replicates for each *k*). Each run started with a burn-in period of 50 000 steps followed by 300 000 Markov Chain Monte Carlo (MCMC) replicates. To determine the best number of clusters, we inspected likelihood values computed by STRUCTURE and we used the Δk statistic (Evanno *et al.* 2005). We used DISTRUCT to plot STRUCTURE output data (Rosenberg 2004). To investigate the impact of non-native stocking on population admixture, we calculated average population admixtures. Then, within each population, we used individual membership values (*q*) to classify individuals as belonging to one of the three categories: the local cluster (*q* > 0.70 of the local cluster), a nonlocal cluster (*q* > 0.70 of nonlocal cluster) or with 'hybrid' characteristics (0.30 ≤ *q* ≤ 0.70). We then compared the average proportions of individuals from each category among three groups of populations: unstocked, stocked with non-native fish or unstocked but with a nearby (<100 km) stocked population. Differences among groups of populations were tested with Mann–Whitney tests implemented in GenStat.

Landscape genetic analyses

To test the effect of coastal distance (between river mouths), local geological substrate, river length and temperature on genetic differentiation, we used Mantel tests and partial Mantel tests implemented in PASSAGE (http://www.passagesoftware.net/index.php). Pairwise population genetic differentiation was estimated by the $F_{ST}/(1 - F_{ST})$ quantity using Fstat (Goudet 1995). Pairwise river length was implemented as the sum of the lengths of rivers considered in each pair. Four geological substrates were identified, and each pair of population was assigned a '0' or '1' score indicating whether populations were on the same substrate or not, respectively. Temperature was implemented as the difference in annual average air temperature among populations. The significance of correlation coefficients was estimated with a two-tailed test and 9999 permutations in PASSAGE. We investigated potential effects of stocking on correlations between environmental factors and genetic structure by analysing three subsets of populations: the entire data set (*n* = 34), a subset of 29 populations considered as unstocked and a subset of 22 unstocked populations but with a nearby stocked population. To examine the role of the Allier population in driving the observed patterns, we carried out Mantel tests without

this population ($n = 24$). Lastly, to test the effects of environmental factors independently from geology, we computed Mantel tests within a single cluster (Brittany) including 14 populations with the same geology.

Results

Within-population diversity

Successive cohorts analysed in Sienne, Sée, Sélune, Scorff and Blavet populations were not significantly differentiated. We thus combined genotypes from successive cohorts of the same population in our analyses. Based on MICRO-CHECKER 2.2.3 analyses, we found evidence of null alleles or large allele dropout for 18 of 578 tests, but we detected no association with any particular marker or population. Considering the full data set, there was no evidence of departure from Hardy–Weinberg equilibrium associated with a particular marker (Table S2, Supporting information). Analyses carried out with FSTAT 2.9.3.2 and FDIST 2 did not suggest any evidence of linkage disequilibrium or significant signature of selection, respectively. Significant heterozygote deficits were detected in Authie, Valmont and Touques populations (Tables 1 and S2, Supporting information). Results from BOTTLENECK 1.2.02 suggested an evidence of recent bottleneck in the Saire population only ($P < 0.05$). Indices of genetic diversity per population are presented in Table 1. Average gene diversity (HE) over all populations was 0.79, ranging from 0.71 to 0.83. Allelic richness and HE were the lowest in the Bresle, Arques and Allier populations and the highest in the Touques, Orne, Vire and Leguer populations.

Divergence among populations

F_{ST} among the 34 populations was 0.043 (CI: 0.038–0.049), and pairwise F_{ST} ranged from −0.006 to 0.162 (Table S3, Supporting information). F_{ST} was low and often not significant among populations from the same cluster (e.g. 105 values of 166 were not significant among Brittany populations). Pairwise F_{ST}s including the Allier population were particularly high, ranging from 0.09 to 0.16 (0.10 on average).

The AMOVAS revealed a significant and higher proportion of the total genetic variance among clusters than among populations within clusters (4.8% [95% CI for F_{CT}: 4.2–5.5%] and 0.87%, respectively, Table 2). When removing stocked populations from this analysis, the proportion of variance among groups was higher (5.6% [95% CI for F_{CT}: 5.0–6.4%]) while the proportion of variance within groups was similar (0.83%). Finally, when further removing populations in the vicinity of stocked populations, the proportion of variance among groups increased (7.4% [95% CI for F_{CT}: 6.5–8.5%]) and the proportion of variance within groups remained stable (0.72%).

The Neighbor-joining phylogram revealed the organization of populations into five clusters (Fig. 2). However, in this analysis, Touques and Couesnon populations were not found in the Lower Normandy cluster but respectively closer to the Upper Normandy and Brittany clusters. Garonne and Dordogne populations clustered with Allier.

Bayesian individual clustering

Using STRUCTURE, the highest likelihood was observed for $k = 6$, and the Δk statistic (Evanno et al. 2005) showed multiple peaks for $k = 2$, 4 and 6 (Fig. S1, Supporting information). The six clusters inferred corresponded to five geographic regions: Upper Normandy, Lower Normandy, Brittany, Allier and Adour (Fig. 3). Populations from the Gironde region appeared as an admixture between Adour and Allier clusters. Individuals from Brittany were distributed into two clusters not clearly associated with the spatial locations of populations. We also separately analysed samples from Brittany with STRUCTURE without detecting any clear substructure in this region. Therefore, the two clusters from Brittany were pooled into a single one in the subsequent analyses. A similar analysis with the BAPS soft-

Table 2 Analyses of molecular variance (AMOVA) partitioning genetic variance within populations, among populations within regional groups and among regional groups for three data sets: 34 populations grouped in six clusters (including Gironde) by geographic location (from Fig. 2), 29 populations unstocked with non-native fish including populations near stocked ones (five clusters) and 22 populations unstocked with non-native fish, excluding populations near stocked ones (four clusters)

	Among groups	Among populations within groups	Within populations	F_{CT} [5–95%]
For all populations	4.84%	0.87%	94.29%	0.048 [0.042–0.055]
For 'unstocked' populations and populations close to stocked ones	5.63%	0.83%	93.54%	0.056 [0.050–0.064]
For 'unstocked' populations	7.47%	0.72%	91.81%	0.075 [0.065–0.085]
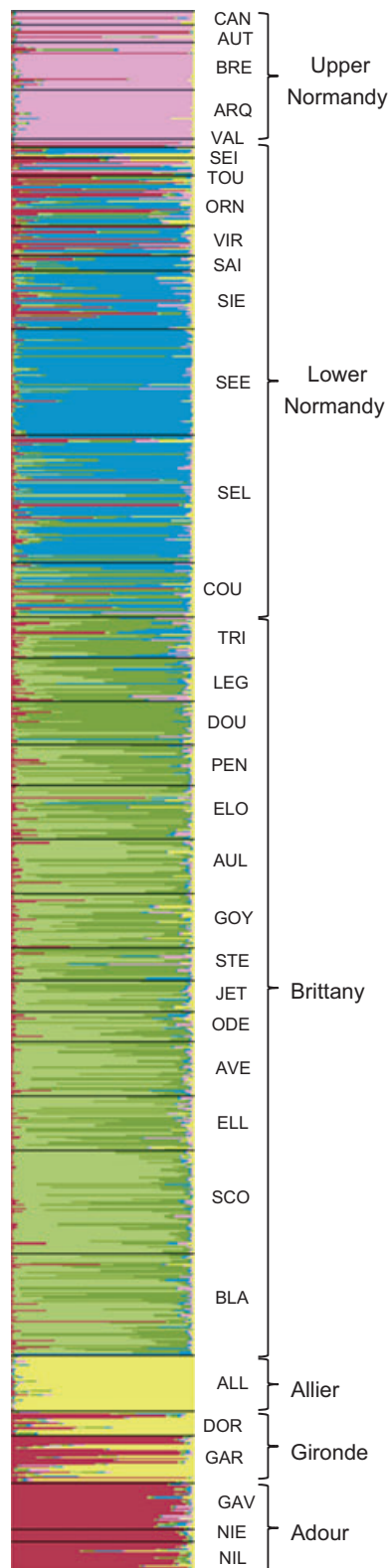
Fig. 3 Bayesian individual clustering results with SMALL CAPS STRUCTURE for *k* = 6. Coloured bars represent proportions of membership of each individual to each cluster.
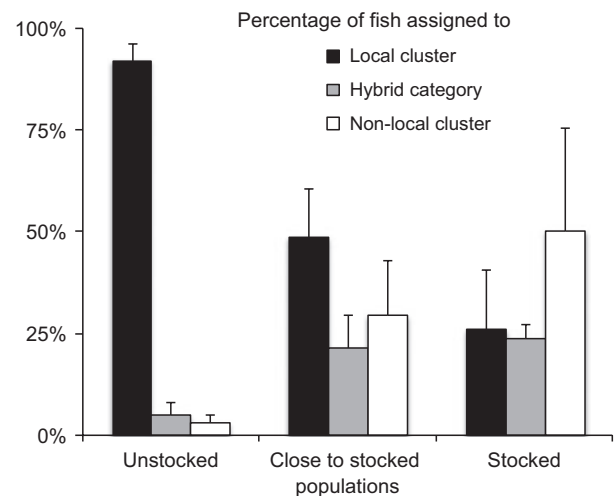
Fig. 4 Percentages (±SD) of fish assigned to the local cluster, 'hybrid' category or nonlocal cluster for three categories of populations: unstocked, close to stocked ones or stocked.

ware (Corander *et al.* 2003) confirmed the hierarchical structure into five clusters without any substructure in Brittany and no specific cluster corresponding to the Gironde region (Fig. S2, Supporting information).

While populations located in Brittany, Allier and Adour were very weakly admixed (Fig. 3, Table S4, Supporting information), we detected low to high admixture rates in some populations from Upper Normandy, Lower Normandy and Gironde. The contribution of Brittany clusters to the Couesnon population was 0.49, and contributions of the Adour cluster to Touques, Orne and Vire populations were 0.15, 0.18 and 0.24, respectively. In Upper Normandy, we found a relatively high contribution of the Adour cluster into Canche, Authie and Valmont (0.12, 0.23 and 0.54, respectively) but a low one into Bresle and Arques populations (0.08 and 0.01, respectively). Finally, the respective contributions of Allier and Adour clusters were 0.63 and 0.27 in Garonne and 0.42 and 0.49 in Dordogne.

At the population level, the contribution of non-native clusters increased as a function of stocking (Figs 3 and 4, Table S4, Supporting information). Percentages of individuals assigned to nonlocal clusters or classed as admixed individuals were higher within stocked than unstocked populations ($U = 109$, $P < 0.001$ and $U = 106$, $P < 0.001$, respectively). We found on average a small percentage of individuals assigned to nonlocal clusters (3%) or to 'hybrid' category (5%) in unstocked populations. In populations that did not directly experience stocking but were close to stocked ones (<100 km), we found a large percentage of fish assigned to nonlocal clusters (30%) or to 'hybrid' category (22%). As expected, high percentages of fish

assigned to nonlocal clusters (50%) or to 'hybrid' category (24%) were observed in populations stocked with non-native individuals. The proportion of admixed individuals appeared relatively low compared to proportions of individuals assigned to both other categories (Fig. 4). For instance, in the Couesnon population, we identified 10 native fish and 16 non-native fish (mainly from Brittany), but only eight admixed individuals (Fig. 3, Table S4, Supporting information). In Dordogne population, we found 11 fish assigned to Allier and 12 assigned to Adour, but only seven admixed individuals among these two clusters. In contrast, looking at the 462 individuals collected in the 14 populations from Brittany which were not stocked with non-native fish, we found seven migrants and 27 admixed individuals. Similarly, we detected one migrant and two admixed individuals among 51 fish from the Adour region, seven migrants and three admixed individuals among 85 fish from Upper Normandy, and no migrant nor admixed individual among the 35 samples of the Allier R.

Landscape genetic analyses

Considering the entire data set, we found significant correlations between coastal distance, river length, local geological substrate and temperature with pairwise genetic distances (Table 3, Fig. 5). The three best models included either geological substrate ($r = 0.65$, $P < 0.0001$), costal distance and river length kept constant ($r = 0.60$, $P < 0.0001$), or coastal distance (IBD, $r = 0.55$, $P < 0.0001$).

Coastal distance, river length and geological substrate seemed more correlated with pairwise genetic distances when both stocked populations and populations near stocked ones were excluded (Table 3). In this configuration, the three best models included either geological substrate ($r = 0.88$, $P < 0.0001$) and local geological substrate with river length kept constant ($r = 0.88$, $P < 0.0001$) or coastal distance with river length kept constant ($r = 0.86$, $P < 0.0001$).

As mentioned earlier, pairwise genetic distances including the Allier population were higher than most other pairwise comparisons, and we also found a significant effect of coastal distance (IBD) for this particular set of comparisons ($r = 0.38$, $P < 0.001$) (Fig. 5). When considering the 22 populations considered as not impacted by stocking and further removing the Allier population, the effect of river length was not significant anymore ($r = 0.18$, $P = 0.32$), whereas the effect of coastal distance, geological substrate and temperature remained significant (Table 3). In this case, the four

Table 3 Results of Mantel tests and partial Mantel tests for correlations between genetic distance ($F_{ST}/(1 − F_{ST})$), geological substrate (GS), coastal distance (CD), river length (RL) and temperature (T) for four data sets including respectively: i) the 34 French populations ii) 29 populations not stocked with non-native fish, iii) this same last data set excluding populations close to stocked ones (<100 km), iv) this same last data set excluding Allier ($n = 21$) and v) the Brittany populations ($n = 14$). Brackets indicate the variable kept constant in a partial Mantel test. The effect of geological substrate was not tested (indicated by 'na') for the data set including the 14 populations from Brittany located in the same geological area

| Variable	i) all populations, $n = 34$		ii) (i)-stocked populations, $n = 29$		iii) (ii)-populations close to stocked ones, $n = 22$		iv) (iii)-Allier, $n = 21$		v) Brittany populations only, $n = 14$	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
CD	0.55	<0.001	0.58	<0.001	0.74	<0.001	0.90	<0.001	0.48	<0.01
CD(GS)	0.25	<0.001	0.24	<0.001	0.19	<0.05	0.57	<0.001	na	na
CD(RL)	0.54	<0.001	0.61	<0.001	0.86	<0.001	0.89	<0.001	0.50	<0.001
CD(T)	0.31	<0.001	0.39	<0.001	0.57	<0.001	0.82	<0.001	0.37	<0.05
GS	0.65	<0.001	0.72	<0.001	0.88	<0.001	0.91	<0.001	na	na
GS(CD)	0.47	<0.001	0.55	<0.001	0.73	<0.001	0.66	<0.001	na	na
GS(RL)	0.60	<0.001	0.68	<0.001	0.88	<0.001	0.91	<0.001	na	na
GS(T)	0.50	<0.001	0.61	<0.001	0.83	<0.001	0.85	<0.001	na	na
RL	0.37	<0.01	0.41	<0.01	0.53	<0.01	0.18	0.32	0.15	0.40
RL(CD)	0.36	<0.01	0.46	<0.01	0.77	<0.001	0.05	0.70	0.20	0.27
RL(GS)	0.21	0.11	0.29	<0.05	0.53	<0.001	0.13	0.32	na	na
RL(T)	0.30	<0.05	0.43	<0.01	0.12	0.46	0.09	0.57	0.14	0.43
T	0.51	<0.001	0.50	<0.001	0.55	<0.001	0.64	<0.001	0.35	<0.05
T(CD)	0.22	<0.05	0.16	0.19	0.03	0.84	0.06	0.58	0.09	0.55
T(GS)	0.21	<0.05	0.17	0.17	0.11	0.34	0.63	<0.001	na	na
T(RL)	0.47	<0.001	0.51	<0.001	0.58	<0.001	0.00	0.98	0.34	<0.05
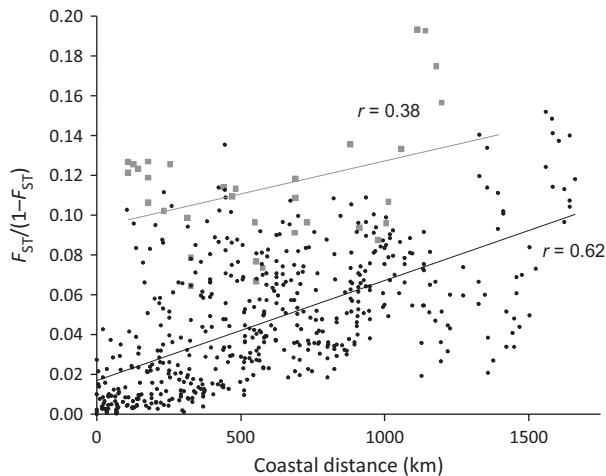
Fig. 5 Pairwise genetic distances ($F_{ST}/(1 - F_{ST})$) as a function of coastal distance among populations ($n = 34$). Pairs including the Allier population (grey squares) or excluding this population (black points) are presented.

best models were obtained with geological substrate ($r = 0.91$, $P < 0.0001$), geological substrate with river length kept constant ($r = 0.91$, $P < 0.0001$), coastal distance ($r = 0.90$, $P < 0.0001$) and coastal distance with river length kept constant ($r = 0.89$, $P < 0.0001$).

Finally, among populations from Brittany with the same geology, we detected a significant effect of coastal distance and temperature but no effect of river length (Table 3). The best model included coastal distance and river length kept constant ($r = 0.50$, $P < 0.001$).

We found a significant correlation between temperature and coastal distance ($r = 0.84$, $P < 0.0001$). Most models including temperature and coastal distance kept constant revealed no significant effect of temperature with pairwise genetic distances (Table 3). Conversely, the effect of coastal distance remained significant in all models with temperature as a constant (Table 3).

Discussion

We detected a hierarchical genetic structure into five clusters corresponding to different geographic regions. Geological areas fitted well with clusters of populations suggesting higher gene flow among populations with the same geology. As expected, we found major effects of stocking with non-native fish on the distribution of genetic diversity within and among populations. Stocked populations were highly admixed but admixed individuals were relatively rare, suggesting a low fitness of stocked fish or some degree of reproductive isolation between wild and hatchery individuals. Stocking was also linked to a reduced genetic differentiation among populations and to a decreased correlation

between genetic structure and environmental factors. The admixture observed in unstocked populations further indicates that stocking influences untargeted populations.

Genetic structure

Overall, the level of genetic differentiation among our study populations was similar to those previously observed in east Atlantic populations (King *et al.* 2001; Saisa *et al.* 2005; Verspoor *et al.* 2005; Grandjean *et al.* 2009; Tonteri *et al.* 2009). The proportion of genetic variance among the five clusters was much higher than among populations within clusters, suggesting high gene flow within clusters. Interestingly, some populations were geographically close but belonged to different clusters (e.g. Loire-Allier and Blavet). Such a hierarchical genetic structure has been described among Atlantic salmon populations from Baltic Sea (Saisa *et al.* 2005), Russia (Tonteri *et al.* 2009) and Canada (Dionne *et al.* 2008). However, the observed level of variation among clusters appeared higher than those reported in these studies.

The genetic differentiation among Brittany populations was low despite the large spatial distribution of this cluster (up to 438 km among river mouths). We suspect that the significant IBD combined with the low genetic differentiation and the high relative number of individuals found in Brittany may explain why STRUCTURE detected two clusters in this area. Indeed, recent studies demonstrated that Bayesian clustering methods could overestimate genetic structure between weakly differentiated populations in case of IBD (Frantz *et al.* 2009; Schwartz & McKelvey 2009).

The level of structure found in Upper Normandy and Lower Normandy clusters was low, suggesting a metapopulation structure and/or recent recolonization of some rivers by nearby populations (Grandjean *et al.* 2009; Perrier *et al.* 2010). Some sample sizes were low in these regions, especially for small rivers with populations thought to have been extirpated and recolonized (Baglinière & Dumas 1988; Baglinière *et al.* 1990). However, these samples were important for identifying the populations of origin of individuals recolonizing the rivers. Most of these populations exhibited high levels of genetic diversity and admixture, suggesting the occurrence of natural recolonization by individuals of various origins (Perrier *et al.* 2010). An evidence of bottleneck was only found in Saire R., but it may be due to low sample sizes. These results support the assumption that straying of wild or stocked individuals could play an important role in the recolonization of depopulated but restored river systems (Stabell 1984; Vasemagi *et al.* 2001; Jonsson *et al.* 2003; Grandjean *et al.* 2009; Perrier *et al.* 2010).

Landscape genetics analyses

Our analyses revealed that coastal distance influences the genetic differentiation among Atlantic salmon populations. This IBD is consistent with the previous observations on Atlantic salmon populations from across the species range (King *et al.* 2001), eastern Atlantic (Verspoor *et al.* 1999), western Atlantic (McConnell *et al.* 1997), northern Atlantic (Tonteri *et al.* 2009), Baltic sea (Saisa *et al.* 2005) and at smaller geographic scale, among Canadian (McConnell *et al.* 1997; Dionne *et al.* 2008), Irish (Dillane *et al.* 2007) or some French populations (Grandjean *et al.* 2009). The pattern of IBD was strong at a large spatial scale but much lower within the Brittany cluster, where populations were weakly differentiated despite the large range of distances among Breton populations. This could suggest that dispersal and/or gene flow were facilitated within this cluster in which rivers have similar lengths and the same geology.

We also found a significant correlation between temperature and genetic differentiation among populations. Temperature has been suggested to be a major factor influencing gene flow and local adaptation in Atlantic salmon (Taylor 1991; Garcia de Leaniz *et al.* 2007; Dionne *et al.* 2008; Valiente *et al.* 2010). However, variations in temperature among the considered rivers followed a north–south axis gradient and were highly correlated with coastal distance among populations. We also noticed that coastal distance clearly had a better predictive power of genetic structure than temperature regime. Therefore, we assume that the latitudinal distribution of our study populations does not allow rigorous investigations into the effect of temperature on neutral population genetic structure.

We found a significant influence of geological substrate on differentiation among populations. Each genetic cluster was related to a specific geology, except the Brittany cluster that has a substrate similar to some of the Lower Normandy populations. The weak genetic structure among Brittany populations could be linked to the homogeneity of the geological substrate within this cluster. This factor could also explain the differentiation between the nearby Lower and Upper Normandy clusters, which have a siliceous and limestone geology, respectively (Grandjean *et al.* 2009). The recognition of river-specific odours linked to the local geological substrate has long been recognized as an important mechanism allowing salmons to return to their natal river (reviewed in Stabell 1984 and Dittman *et al.* 1996). As a result, dispersal could be favoured among nearby rivers with similar geologies, whereas homing would be much higher in cases of nearby rivers running on distinct geological substrates. In addition, adaptation to, e.g., the local spawning substrate (Quinn *et al.* 1995) or water's pH may further decrease the fitness of immigrants and thus diminish gene flow among populations with different geologies (Schom 1986; Quinn *et al.* 1995; Donaghy & Verspoor 1997; Fraser *et al.* 2008).

We found a significant effect of river length on genetic differentiation among populations only when the Allier population was included in the analysis. Spawning grounds of this river are located up to 900 km from the sea, and the run timing occurs several months earlier than in other French rivers (Bachelier 1963; Baglinière & Porcher 1994). Furthermore, adults returning in the Allier R. are mostly multi-sea-winter fish, whereas in other French populations, there are mainly single-sea-winter fish. A positive correlation between the proportion of multi-sea-winter fish and the river length, water discharge or difficulty to upstream migration has been suggested (Schaffer & Elson 1975; Power 1981). In addition, some studies showed a high heritability of salmon size (Riddell *et al.* 1981) or sea age at maturity (Naevdal *et al.* 1978). Therefore, we believe that the strong genetic differentiation of the Allier population combined with its phenotypic and behavioural specificities is best explained by a process of local adaptation to a difficult upstream migration. Local adaptation has been frequently documented in salmonids (review in Fraser *et al.* 2011), but this interpretation needs to be supported by further analyses of adaptive traits or selected loci.

Impacts of stocking

In accordance with stocking data, some populations showed moderate to high levels of admixture with distant donor populations. In particular, Orne, Sélune and Couesnon were strongly admixed with Aulne and Gave d'Oloron. Dordogne and Garonne populations had gone through extinction before being stocked with fish from Allier and Gave d'Oloron, and accordingly, they appeared as mostly admixed with the two donor clusters. Similar examples of introgression of wild populations by stocked non-native fish have been described in Atlantic salmon, but no study was based on such a large number of microsatellite markers to accurately infer the admixture between wild and stocked individuals (Campos *et al.* 2008; Finnegan & Stevens 2008).

We also found relatively high admixture rates within some populations from Lower Normandy that had not been stocked but were geographically close to stocked ones. According to several studies indicating an important straying of stocked salmonids (Quinn 1993; Jonsson *et al.* 2003; Pedersen *et al.* 2007), these results strongly suggest that individuals stocked in Lower Normandy

rivers dispersed into nearby rivers. Additionally, wild-born non-native fish could also have higher dispersal rates than native ones (McGinnity *et al.* 2004), hence increasing admixture of neighbouring populations. These results could have major implications for the management of salmon populations by demonstrating that stocking can modify the genetic make-up of untargeted populations.

While we found some high admixtures of wild populations with the clusters they were stocked with, few admixed individuals were detected. This result is consistent with a low reproductive success of hatchery-reared individuals introduced in the wild (Araki *et al.* 2007; Theriault *et al.* 2011). This low success has been attributed to poor competitivity of hatchery-reared fish facing wild individuals (Fleming & Einum 1997; Johnsson *et al.* 2001; Jonsson & Jonsson 2006). The rarity of admixed individuals could also be due to their possible lower survival or to a temporal reproductive isolation (Hansen & Mensberg 2009) or spatial segregation of wild and hatchery-reared fish.

The higher genetic structure found among clusters when stocked populations were excluded strongly suggests that stocking resulted in a breakdown of genetic structure at a large geographic scale. Such a result can be explained by the introgression following intergroups translocations and is consistent with studies showing a genetic homogenization of populations at much smaller scales after stocking (Hansen *et al.* 2009; Marie *et al.* 2010). In addition, the fact that correlations between environmental features and genetic differentiation were higher when stocked populations were excluded provides to our knowledge the first evidence that such practices can alter the link between environment and population genetic structure. Such modifications may also ultimately be associated with an alteration in adaptive potential and a disruption of natural patterns of local adaptation. These results may have important implications for the conservation of salmonid populations and more broadly of wild populations in which translocations of non-native individuals are performed.

To conclude, this study illustrates the hierarchical genetic organization of Atlantic salmon populations in relation to the regional distribution of specific environmental features. In particular, our results indicate either enhanced or constrained gene flow among populations located on similar or different geological areas, respectively. In addition, the unique attributes of the Allier population suggest an adaptation to an exceptional difficulty of upstream migration. Finally, our study demonstrates that stocking with non-native individuals in some rivers leads to significant admixture rates also in neighbouring untargeted populations. However, even though some high population admixture rates were observed, surprisingly few admixed individuals were detected, suggesting a low fitness of hatchery fish or reproductive barriers between local and non-native individuals.

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Data accessibility

Data deposited at Dryad: doi:10.5061/dryad.72151.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Mean L(K) (±SD) and ΔK over 15 runs for each K from 1 to 14 based on Structure analysis.

Fig. S2 BAPS individual clustering for $k = 5$.

Table S1 list and characteristics of the microsatellite markers used

Table S2 Allelic richness (A_R) estimated for four and eight individuals, number of alleles (AN), observed (H_o) and expected heterozygoties (H_E), inbreeding coefficient (F_{IS}, significant values in bold) and presence of null alleles for every locus and population

Table S3 Pairwise Fst (above diagonal, non significant values in bold) and coastal distances among populations

Table S4 Table showing population admixture and individual assignment estimated with Structure for $k = 6$ (The two clusters found in Brittany were pooled to clarify the table), and individuals assignment to local, non-local, or hybrid categories (see materials and methods)

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