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Transatlantic secondary contact in Atlantic Salmon, comparing microsatellites, a single nucleotide polymorphism array and restriction-site associated DNA sequencing for the resolution of complex spatial structure

IAN R. BRADBURY,* LORRAINE C. HAMILTON,† BRIAN DEMPSON,* MARTHA J. ROBERTSON,* VINCENT BOURRET,‡ § LOUIS BERNATCHEZ§[1] and ERIC VERSPOOR¶[1]

*Science Branch, Department of Fisheries and Oceans Canada, 80 East White Hills Road, St. John's, Newfoundland, Canada A1C 5X1, †Aquatic Biotechnology Laboratory, Bedford Institute of Oceanography, Dartmouth, Halifax, Nova Scotia, Canada B2Y 4A2, ‡Département de Biologie, Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval, 1030 avenue de la Médecine, Québec, Québec, Canada G1V 0A6, §Direction de la faune aquatique, Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs, Québec, Québec, Canada G1S 4X4, ¶Rivers and Lochs Institute, Inverness College University of the Highlands and Islands, Inverness IV2 5NA, UK

Abstract

Identification of discrete and unique assemblages of individuals or populations is central to the management of exploited species. Advances in population genomics provide new opportunities for re-evaluating existing conservation units but comparisons among approaches remain rare. We compare the utility of RAD-seq, a single nucleotide polymorphism (SNP) array and a microsatellite panel to resolve spatial structuring under a scenario of possible trans-Atlantic secondary contact in a threatened Atlantic Salmon, *Salmo salar*, population in southern Newfoundland. Bayesian clustering indentified two large groups subdividing the existing conservation unit and multivariate analyses indicated significant similarity in spatial structuring among the three data sets. mtDNA alleles diagnostic for European ancestry displayed increased frequency in southeastern Newfoundland and were correlated with spatial structure in all marker types. Evidence consistent with introgression among these two groups was present in both SNP data sets but not the microsatellite data. Asymmetry in the degree of introgression was also apparent in SNP data sets with evidence of gene flow towards the east or European type. This work highlights the utility of RAD-seq based approaches for the resolution of complex spatial patterns, resolves a region of trans-Atlantic secondary contact in Atlantic Salmon in Newfoundland and demonstrates the utility of multiple marker comparisons in identifying dynamics of introgression.

Keywords: Atlantic Salmon, RAD-seq, secondary contact

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Introduction

The identification of discrete and unique assemblages of individuals or populations is central to the conservation and management of exploited species (Moritz 1994; Waples 1995; de Guia & Saitoh 2007; Schindler *et al.* 2010). Advances in population genomics (e.g. Baird *et al.* 2008; Davey & Blaxter 2010; Etter *et al.* 2011; Peterson *et al.* 2012) have recently revolutionized this task allowing increased numbers of loci to be surveyed, and both neutral and putatively adaptive genetic variation to be considered in defining management units (e.g.

Correspondence: Ian R. Bradbury, Fax: (709) 772-4188;
E-mail: ibradbur@me.com

[1]Authors contributed equally order alphabetical.

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Lamichhaney *et al.* 2012; Bradbury *et al.* 2013; Hohenlohe *et al.* 2013; Moore *et al.* 2014; Candy *et al.* 2015). Despite the obvious potential, rapidly increasing options for population genomic study in nonmodel organisms (e.g. Gonen *et al.* 2014; Houston *et al.* 2014; Benestan *et al.* 2015) contrast a lack of comparative studies (although for microsatellite—SNP comparisons see: Hohenlohe *et al.* 2013; Larson *et al.* 2014; Moore *et al.* 2014; Candy *et al.* 2015). Ultimately, these comparative examinations employing multiple approaches are needed to both explore limitations and biases and to evaluate the application of these technologies to questions of wildlife conservation and management, particularly under complex demographic scenarios.

Atlantic Salmon (*Salmon salar*) is a species of significant social, ecological and economic importance throughout the North Atlantic, characterized by large-scale ocean migrations (Reddin 1988; Thorstad *et al.* 2010; Reddin *et al.* 2012) and fine-scale homing behaviour (Stabell 1984; Keefer & Caudill 2014). Atlantic Salmon has been the subject of extensive population genetic (e.g. Dionne *et al.* 2009; Ozerov *et al.* 2012; Bradbury *et al.* 2014) and increasingly population genomic studies (e.g. Bourret *et al.* 2013b; Moore *et al.* 2014). Populations are commonly structured at various spatial scales, ranging from across the North Atlantic (McConnell *et al.* 1995; King *et al.* 2001; Bourret *et al.* 2013b), to among river systems (Castric & Bernatchez 2004; Verspoor 2005; Palstra *et al.* 2007; Moore *et al.* 2014), and even among tributaries of larger rivers (e.g. Primmer *et al.* 2006; Dionne *et al.* 2009).

Discerning the ultimate scale of neutral and adaptive population structuring in Atlantic Salmon is focal to the conservation of endangered populations and the identification of evolutionarily significant units (i.e. Designatable Units, COSEWIC 2008). At present, these conservation units are likely coarse and poorly resolved in Canadian waters (COSEWIC 2011) and this is of immediate concern as many regions are experiencing record lows and continued declines in abundance (ICES 2015). In the southern Newfoundland population, which is located along the southern coast of the island, continued declines over several decades led to a 'threatened' status in 2011. Management and conservation of the region is complicated by significant fine-scale differentiation and a surprising lack of genetic isolation by geographic distance (Palstra *et al.* 2007; Bradbury *et al.* 2014) suggesting significant heterogeneity and that further subdivision of this conservation unit may be warranted (COSEWIC 2011). However, a clear rationale for subdivision, including data on neutral and possibly adaptive variation, has remained elusive.

Here, we evaluate and compare the ability of RAD-seq, a single nucleotide polymorphism (SNP) array and a microsatellite panel to resolve spatial structuring and management units under a complex scenario of post-glacial recolonization and possible trans-Atlantic secondary contact in Atlantic Salmon along Newfoundland's south coast (<500 km). Specifically, the objectives are (i) to delineate population structure in southern Newfoundland using genetic and genomic approaches to provide guidance to management and conservation of Atlantic Salmon; (ii) to compare genetic and genomic approaches to inform future applications; and (iii) finally to explore the potential influence of trans-Atlantic secondary contact on contemporary spatial structure in the region. We build directly on previous studies documenting the presence of both complex spatial structuring (e.g. Palstra *et al.* 2007; Bradbury *et al.* 2014) and of European alleles (e.g. King *et al.* 2007) in salmon populations found in Newfoundland. This work highlights the utility of RAD-seq based approaches for the resolution of complex spatial patterns, explores a region of trans-Atlantic secondary contact in Atlantic Salmon in Newfoundland and demonstrates the utility of multiple marker comparisons in identifying dynamics of introgression.

Materials and methods

Sampling

River stocks utilized here represent a subset of rivers described elsewhere (see Bradbury *et al.* 2014) with additional ones. Tissue samples were collected in coastal rivers or tributaries (Fig. 1, Table 1) generally <5 m in width and 2 m depth. Tissue samples were obtained from parr of various age groups (0–3 years old) captured by electro-fishing during the period of July to September 2008–2010 and generally at two locations per river. For each salmon, fin clips were stored in 95% ethanol. The total number of river stocks analysed varied by approach and ranged from 31 for the microsatellite analysis, 17 for RAD-seq analysis, to 10 for SNP array (See Fig. 1 and Table 1 for locations for each analysis).

Microsatellite genotyping

The total number of individuals sampled and genotyped for the 15 microsatellite loci was 2439 with an average of 78.7 individuals per population (Table 1). Microsatellite polymorphisms were quantified at 15 loci as follows: Ssa85, Ssa202, Ssa197 (O'Reilly *et al.* 2000), SSOSL417 (Slettan *et al.* 1995), SsaD85 (T. King, unpublished data), SsaD58, SsaD71, SsaD144, SsaD486 (King *et al.* 2005), MST-3 (hereafter referred to as U3) (Presa & Guyomard 1996), SSsp2201, SSsp2210, SSsp2215,

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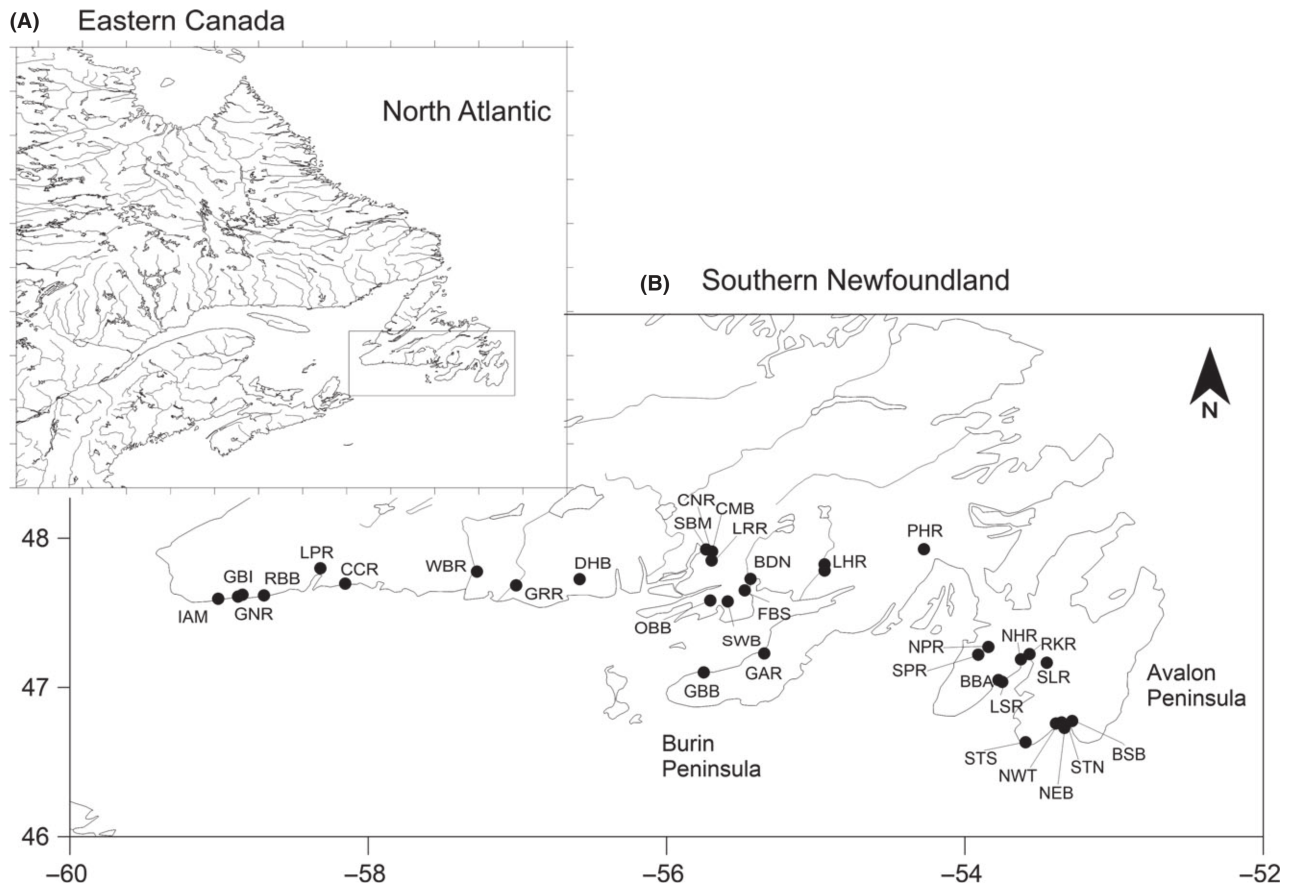

Fig. 1 Map of sample locations, (A) inset shows southern Newfoundland in relation to eastern North America. (B) Southern Newfoundland. See Table 1 for sample sizes and Materials and methods for sampling details.

SSsp2216 and SSspG7 (Paterson *et al.* 2004). Details regarding the microsatellite genotyping have been described elsewhere (e.g. Bradbury *et al*. 2014). In brief, DNA was extracted using the Qiagen DNeasy 96 Blood and Tissue extraction kit (Qiagen) following the guidelines of the manufacturer. DNA was quantified using QuantIT PicoGreen (Life Technologies) and diluted to a final concentration of 10 ng/μL in 10 mM Tris (Buffer EB; Qiagen). Loci were multiplexed (Bradbury *et al.* 2014), and PCR reactions were set up in a 10 μL volume containing 10 ng DNA, 1× Type-it Microsatellite PCR master mix (Qiagen) and the primer mix for that panel. PCR products were size separated on an ABI 3130xl (Life Technologies) capillary electrophoresis system using Gene Scan 500 as the internal size standard (labelled in LIZ; Life Technologies). The resulting electropherograms were analysed using GENE MAPPER 4.0 (Life Technologies). On each extraction plate, two types of control samples were included, both redundants and cross-plate controls. Individuals that failed to amplify for more than two loci were removed from further analysis.

SNP array genotyping

A subset of individuals and populations (Table 1) genotyped at the microsatellite loci were also analysed using 5568 SNP loci with the SNP array developed by the Centre for Integrative Genetics (CIGENE, Norway) using the Illumina infinium assay (Illumina, San Diego, CA, USA) following the manufacturer's instructions (see Bourret *et al.* 2013a,b for details). A total 203 individuals were genotyped from 10 populations, with an average of 20 individuals per population. This array included primarily nuclear loci, as well as eight mtDNA loci. Loci were visually classified into different categories including single locus SNPs, paralogous sequence variants (PSVs) and multisite variants (MSVs), arising from genome duplication (for further details see Lien *et al.* 2011; Bourret *et al.* 2013b). We filtered for true SNPs and genotypes with a >0.95 call rate (*CR*: proportion of genotyped SNPs) and removed SNPs with minor allele frequencies <5% (MAF < 0.05) or missing in more than 15% of individuals. Previous analysis suggests that ascertainment bias using this panel

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Table 1 Locations for all samples with sample sizes for each data type. Sample codes refer to locations on Fig. 1

Location	Region	Code	Microsatellite	RAD-seq	SNP array
Biscay Bay River	Trepassey Bay	BSB	78	20	20
Stoney Brook	Trepassey Bay	STN	51	–	–
Northeast Brook	Trepassey Bay	NEB	95	20	20
Northwest Brook	Trepassey Bay	NWT	88	–	–
St. Shott's River	Trepassey Bay	STS	75	–	–
Big Barachois River	St. Mary's Bay	BBA	68	–	–
Little Salmonier River	St. Mary's Bay	LSR	75	20	19
North Harbour	St. Mary's Bay	NHR	57	–	–
Rocky River	St. Mary's Bay	RKR	100	20	40
Salmonier River	St. Mary's Bay	SLR	84	20	–
Northeast River	Placentia Bay	NPR	111	20	20
Southeast River	Placentia Bay	SPR	74	20	–
Bay du Nord River	Fortune Bay	BDN	76	20	15
Garnish River	Fortune Bay	GAR	100	–	–
Grand Bank Brook	Fortune Bay	GBB	100	–	–
Long Harbour River	Fortune Bay	LHR	56	20	9
Southwest Brook	Fortune Bay	SWB	71	–	–
Simms Brook Fortune Bay	Fortune Bay	FBS	74	–	–
Old Bay Brook	Great Bay de l'Eau	OBB	69	–	–
Conne River	Bay d'Espoir	CNR	99	20	20
Conne River Mill Brook	Bay d'Espoir	CMB	77	–	–
Little River	Bay d'Espoir	LRR	82	–	–
Southwest Brook	Fortune Bay	SWB	71	–	–
Southwest Brook Milltown	Bay d'Espoir	SBM	76	–	–
Cinq Cerf Brook	Southwest Coast	CCR	80	20	–
Dolland Brook	Southwest Coast	DHB	78	20	–
Garndy's Bay of Islands	Southwest Coast	GBI	75	–	–
Grandys Brook	Southwest Coast	GNR	77	20	
Grey River	Southwest Coast	GRR	82	20	20
Isle aux Morte	Southwest Coast	IAM	75	–	–
La Poile River	Southwest Coast	LPR	70	20	20
White Bear River	Southwest Coast	WBR	66	13	–
Total			2439	313	203

SNP, single nucleotide polymorphism.

appears minimal for North American populations (Bourret *et al.* 2013b). The distribution of these SNPs across the genome was evaluated using a previously published linkage map for North American Atlantic Salmon (Brenna-Hansen *et al.* 2012), and we conducted a sliding window analysis using a LOWESS second-order filter and locus-specific F_{ST} values.

RAD-seq analysis

For RAD sequencing, again a subset of individuals and populations were analysed. A total of 313 individuals were genotyped from 16 populations, with an average of 19.5 individuals per population (Table 1). Total genomic DNA was extracted from fin clips using the DNeasy Kit (Qiagen) according to the manufacturer's protocols. Subsequently, 2.5 μg of spectrophotometrically quantified DNA was submitted to FLORAGENEX, Oregon, who generated and sequenced RAD tags following the methods outlined by Baird *et al.* (2008), Hohenlohe *et al.* (2010) and Emerson *et al.* (2010). In brief, sequencing adaptors and individual barcodes were ligated to *Sbf* I-digested total genomic DNA, and the resulting fragments were sequenced from the restriction sites. Individually barcoded RAD samples were jointly sequenced on the Illumina GAIIx platform with single-end 1 × 100-bp chemistry. Reads were separated by individual, and sequencing barcodes were removed after the sequencing run, resulting in RAD tags of 90–100 bp. The available *S. salar* genome (http://genomicasalmones.dim.uchile.cl) was used for RAD reference mapping and to identify SNP candidates. SNP calling was based on output from the BOWTIE (version 0.11.3; Langmead *et al.* 2009) and SAMTOOLS (0.1.12a; Li *et al.* 2009) algorithms and custom scripts to identify SNP candidates. Reference mapping with

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BOWTIE took sequence quality information into account, allowed for up to three mismatches (4.28%) between each read and the reference sequence and ignored reads which mapped against more than a single position in the genome. SAMTOOLS tabulated SNP results for all individuals (using the 'pileup' module) and retained information on the number of reads covering each SNP. Finally, SNP candidates were filtered for possible PSVs or MSVs by the removal of all SNPs with three or more alleles, and additionally, we restricted our analysis to those SNPs which were unambiguously mapped to the *S. salar* reference genome (Davidson *et al.* 2010). We also filtered and removed SNPs with a MAF < 5%, Phred scores < 15, sample depth of <14×.

Data analysis

Microsatellite data were checked for the presence of null alleles and scoring errors using MICRO-CHECKER (van Oosterhout *et al.* 2004). Genetic polymorphism was quantified by examination of the number of alleles (microsatellites) or minor allele frequency (SNPs) and observed heterozygosity using MSA (Dieringer & Schlötterer 2003) and GENODIVE (Meirmans & Van Tienderen 2004). Global and pairwise *F*-statistics and significance were calculated using MSA (microsatellites, Dieringer & Schlötterer 2003) and GENODIVE (SNP, Meirmans & Van Tienderen 2004). Isolation by distance was examined using the relationship between linearized F_{ST} ($F_{ST}/(1-F_{ST})$), and coastal geographic distance measured as the shortest distance between all locations within 5 km of the coast. Bayesian clustering was performed using STRUCTURE v.2.2.4 (Pritchard *et al.* 2000) to provide an estimate of the number of distinct groups present. This approach assumes Hardy–Weinberg equilibrium and linkage equilibria among loci, introduces population structure and assigns populations that are not in linkage equilibrium using a MCMC (Markov chain Monte Carlo) algorithm to estimate the number of populations (*K*). The algorithm was run 10 times for each *K* to ensure convergence of values, and with a burn-in of 100 000 repetitions, 300 000 repetitions after burn-in and *K* = 1–25. Structure was initially run with 1 million repetitions to ensure 300 000 was sufficient. The optimal *K* was determined visually from the value at which $L\ P(D)$ plateaus as well as using the ΔK method (Evanno *et al.* 2005). All structure results were amalgamated among replicates using CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and summarized graphically using DISTRUCT 1.1 (Rosenberg 2004). We explored the variation explained by the STRUCTURE clusters using AMOVA and all three data sets in ARLEQUIN version 3.5.1.3 (Excoffier & Lischer 2010). Comparisons among data sets were restricted to locations in common. We used two approaches to explore similarity in spatial structuring observed in each data set. First, we used Mantel tests in PASSAGE (Rosenberg 2001) using the pairwise F_{ST} matrices for each data set to explore similarity in spatial structure. Second, we conducted principal component analysis and compared the first principal component for each data set.

We explored the influence of trans-Atlantic secondary contact using several mitochondrial SNPs present on the CIGENE SNP array that have previously been shown to be diagnostic for European or North American Atlantic Salmon. We focus on one SNP in particular which appears to be most polymorphic and informative in this region. This SNP, 10 879, is widely polymorphic in Europe but not in North America and shows similar trends as SNPs 365, 5768 (Bourret *et al.* 2013b, E. Verspoor and D. Knox, in preparation). We compared differences in the proportion of alleles at 10 879 among pairs of populations to pairwise F_{ST} values in all three data sets to explore possible influences of secondary contact on spatial structure. Finally, allele frequencies of the diagnostic loci were compared with range wide mtDNA data on the distribution of North American and European types.

We evaluated two different measures to examine the degree of introgression or the proportion of alleles present in hybrid individuals from each of the two groups, and how this differed among the marker types. First, we compared the frequency distributions of the *Q*-values from the STRUCTURE analysis for each data set, run with *K* = 2, again limiting the analysis to the 10 common populations among the data sets. However, in certain scenarios, a simplified model may be preferred (Buerkle 2005) and as such we also calculated a maximum-likelihood hybrid index using the method of Buerkle (2005) implemented in the R package INTROGRESS (Gompert & Alex Buerkle 2010; R Development Core Team 2010). This analysis assumes parental forms can be identified in advance, and the index scales from 0 to 1, with 0 corresponding to one parental form and 1 to the other. Individuals identified as parental forms were selected based on *Q*-values >0.9 or <0.1 in the STRUCTURE analysis above. Similarly, hybrid individuals for examination were selected based on *Q*-values ranging from 0.1 to 0.9. Again frequency distributions of hybrid index values were compared among data sets and were also compared among methods using Pearson's correlation coefficient.

Results

Microsatellite mean allelic richness ranged from 11.3 to 18.4 and observed heterozygosity ranged from 0.70 to 0.88. No evidence of linkage disequilibrium was

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detected among loci, and deviations from Hardy–Weinberg equilibrium were rare (<5% of locus population comparisons). For the SNP array, the average call rate was 0.99, with 4060 SNPs classified as true SNPs, 271 as monomorphic, 1099 as MSVs and 48 failed. Of the total 5568 SNPs assayed, 2574 were informative and displayed a MAF > 0.05 and included in further analysis. Average observed heterozygosity was 0.230, and the proportion of loci declined with increasing MAF (Fig. 2). For the RAD-seq SNPs, the average number of sequence reads per individual was 6.29 million (2.9–7.7 million) with an average depth of coverage per individual overall SNPs of 34.2× (individual mean depths 19× to 51×). This resulted in 283K variant loci which provided ~80K SNPs with unique alignments to the genome, of which 8495 SNPs remained following removal of loci characterized by a MAF < 0.05. See Table S1 (Supporting information) for genotyping and filtering results. Average observed heterozygosity was 0.231, and the proportion of loci again peaked at MAF values < 0.10 a second peak was visible at MAF values of 0.40–0.50 (Fig. 2). There was no significant difference in the observed heterozygosity of the two SNP data sets (Mann–Whitney rank sum test, $P = 0.065$). Among the three data sets, 10 populations were in common including 199 individuals used for comparative analysis, see below.

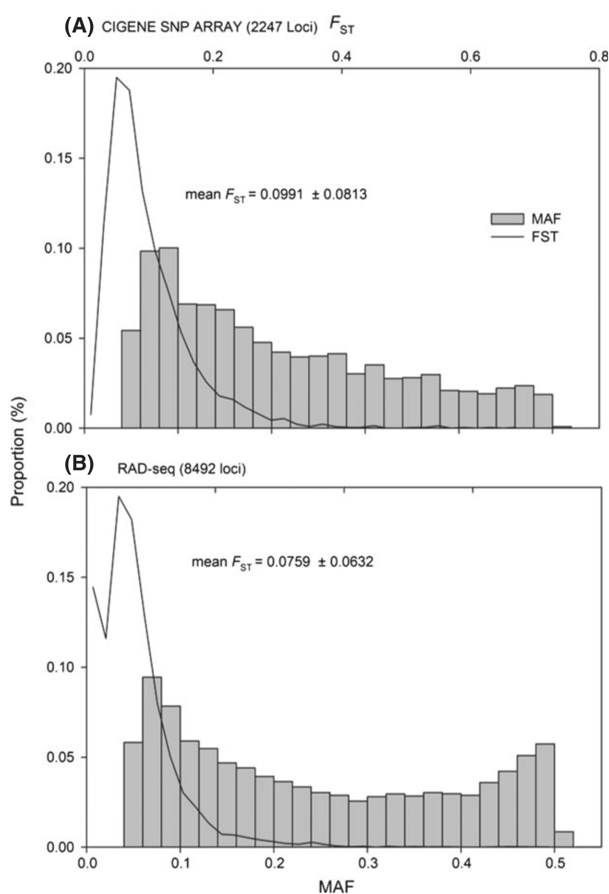

Fig. 2 Frequency distributions of minor allele frequency (MAF) and F_{ST} values for (A) single nucleotide polymorphism (SNP) array data and (B) RAD-seq data. Grey bars represent MAF, and solid lines represent F_{ST} frequency distributions.

Spatial structure

The magnitude of spatial structure observed generally differed among marker types. The microsatellite loci displayed a global F_{ST} of 0.055, the SNP array data displayed a global F_{ST} of 0.099, and finally, the RAD-seq loci displayed a global F_{ST} of 0.080. Locus-specific values for the SNPs similarly displayed peaks between 0.05 and 0.10 with the a few SNP loci displaying F_{ST} values as high as 0.20 (Fig. 2). Bayesian clustering with the microsatellite loci revealed two main clusters, with an additional single isolated population (i.e. Northeast Brook Trepassey). The boundary between the two large clusters was located near the Burin Peninsula (Fig. 1) but was characterized by significant overlap and gradual transition (Fig. 3A). Using the SNP array data, two clusters were also best supported again with the transition near the Burin Peninsula. In the west, individual admixture coefficients were largely low (<0.05) in contrast to the east, where with the exception of a single population which displayed high admixture coefficient (>0.9), the other populations showed *q*-values of 0.7–0.8. A similar pattern was evident in the RAD-seq SNPs, with two groups best supported, and again with the transition point near the Burin Peninsula. Admixture coefficients are uniformly low in the west, but this time with two populations displaying very high admixture coefficients in the east and the others slightly lower (0.7–0.8). We explored the spatial variation explained by these clusters using AMOVA (Table 2). This east–west division explained a significant component of the variation in all three data sets; however, more variation was explained by this grouping in the SNP data sets (8.7–14.1%) than the microsatellites (5.4%).

The genomic distribution of differentiation among these groups was examined using the SNP array data and a previous published linkage map for North American Atlantic Salmon (Brenna-Hansen *et al.* 2012). Overall, 2180 (89%) of the SNP array SNPs could be placed on the existing linkage map, and mapped SNPs were distributed across the 27 linkage groups with significant variation in the number of SNPs per linkage group ranging from 29 to 157 (Fig. S1, Supporting information). Differentiation among the two dominant groups varied across this linkage map and was consistent with

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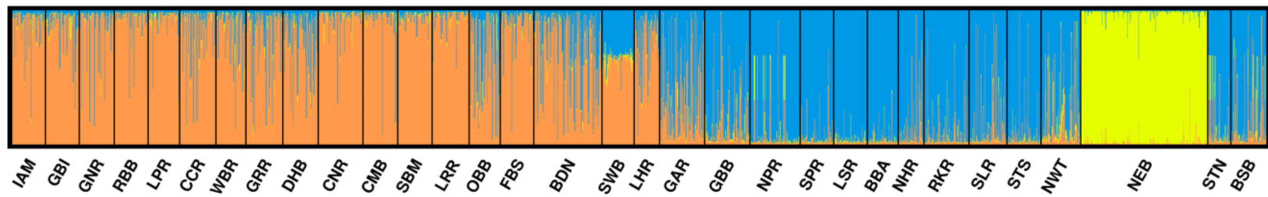

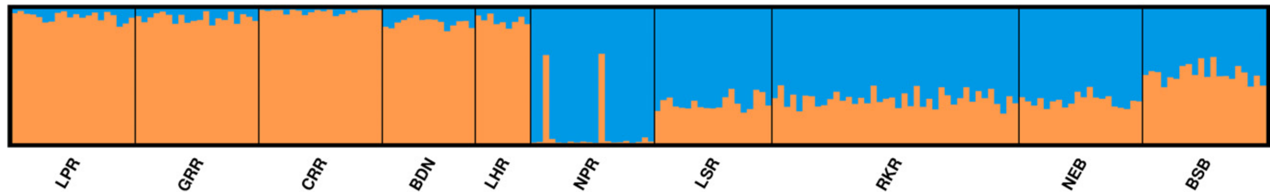

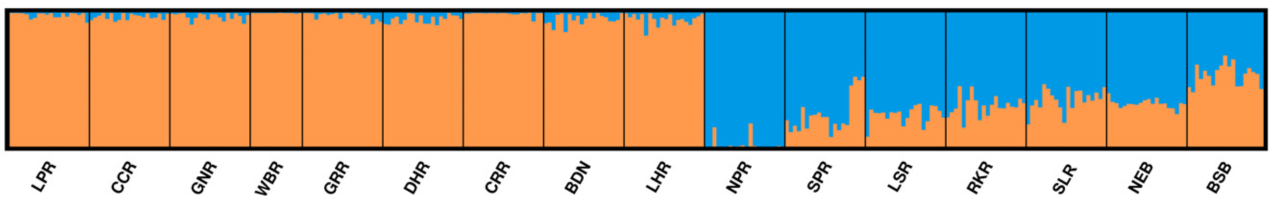

Fig. 3 Bayesian clustering (i.e. STRUCTURE) analysis of the microsatellite and single nucleotide polymorphism (SNP) data sets: (A) microsatellite, (B) SNP array, (C) RAD-seq SNPs. All analyses shown represent optimal value of k in each instance. See Figure 1 and Table 1 for location details.

Table 2 Analysis of molecular variance (AMOVA) results comparing two groups of Atlantic Salmon present in southern Newfoundland identified through STRUCTURE analysis

Source of variation	d.f.	Sum of squares	Variance component	Percentage of total	F_{ST} (P-value)
(A) Microsatellites					
Among populations	33	2076.1	0.3496	5.40	0.054 (<0.001)
Within populations	5502	33612.9	6.1092	94.5	
(B) SNP Array					
Among populations	9	13577.7	29.855	8.67	0.087 (<0.001)
Within populations	396	124483.8	314.35	91.3	
(C) RAD-seq					
Among populations	15	30073.35	44.478	14.1	0.141 (<0.001)
Within populations	608	164857.2	271.15	85.9	

SNP, single nucleotide polymorphism.

the hypothesis that group-associated differentiation is widespread across the genome.

Spatial patterns were directly compared among the different marker types using several approaches. First, we used principal component analysis of each data set and directly compared the first principal components generated using Pearson's correlation coefficient. SNP array and RAD-seq data sets were highly associated ($R^2 = 0.99$, $P < 0.001$, Fig. 4), as were SNP and microsatellite data, although to a lesser degree (SNP array $R^2 = 0.62$, $P < 0.001$; RAD-seq $R^2 = 0.51$, $P < 0.001$, Fig. 4). Furthermore, Mantel tests were used to compare matrices of F_{ST} values generated using the different markers. Again, all comparisons among the different marker types were significant, with microsatellite and SNP comparisons characterized by correlation coefficients of 0.83 ($P < 0.001$, RAD-seq) to 0.89 ($P < 0.001$, SNP array) and the SNP comparisons of 0.98 ($P < 0.001$, Fig. 4).

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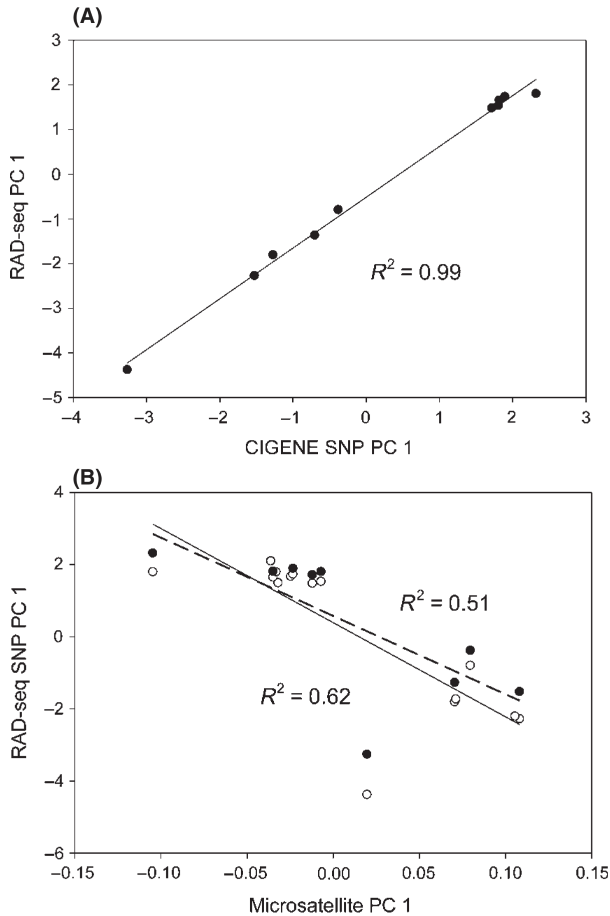

Fig. 4 Comparison of first principal component from the analysis of each data set. (A) Comparing RAD-seq and single nucleotide polymorphism (SNP) array PCs, (B) comparing SNP and microsatellite PCs. Closed circles represent comparisons with SNP array and open circles the RAD-seq data.

The possible influence of secondary contact between eastern and western Atlantic populations on regional structuring was explored using the diagnostic mtDNA SNPs genotyped as part of the SNP array. First, we compared the distribution of European haplotypes in southern Newfoundland with entire range using data from E. Verspoor and D. Knox (in preparation) to demonstrate the diagnostic nature of these alleles for continental type (Fig. S2, Supporting information). We observed a clear association between diagnostic mtDNA alleles in Newfoundland and those found in the eastern Atlantic supporting their usage in this context and there was a clear tendency for European alleles to occur in the southeast and not the southwest of Newfoundland (Fig. S2, Supporting information). In fact, the frequency of the eastern Atlantic allele was clearly elevated in eastern Newfoundland although the population-specific values were variable. Changes in marker diversity seemed to coincide with this transition across the south coast with microsatellite observed heterozygosity decreasing to the east and SNP observed heterozygosity increasing (Fig. 5). To evaluate the role of secondary contact vs. dispersal or straying as structuring agents in this region, we used all three data sets and compared the isolation-by-distance relationships with the relationship between the proportion of European alleles present and F_{ST}. The portion of European alleles per population was significantly associated with F_{ST} and similar in each marker type ($R > 0.80$, $P < 0.001$) despite a lack of isolation by distance in the region again in all three data sets ($R < 0.20$, $P > 0.05$) (Fig. 6).

To explore the presence of possible hybridization and introgression among the groups present on the south coast, we used two approaches. First, we evaluated the distribution of Q-values from STRUCTURE analysis ($k = 2$) for each marker type (Fig. 7). For the microsatellite loci, the Q-value distribution clearly showed two peaks associated with the two groups, but little evidence of individuals with intermediate Q-values. This was in contrast to the SNP data sets, which displayed Q-values associated with the two groups, but also a significant proportion of individuals with intermediate Q-value consistent with introgression (Fig. 7). In both instances (i.e. SNP data sets), the intermediate Q-values were not evenly distributed, but skewed towards the eastern regions 'European-' type individuals. Second, we also used a maximum-likelihood-based hybrid index to quantify the presence of introgression in the different data sets (Fig. 8). Again, the SNP data sets showed a significant proportion of intermediate hybrid index values and again they are skewed towards the individuals from the southeast or European type (Fig. 8).

Discussion

The identification of discrete and unique assemblages of individuals or populations is central to the management and conservation of exploited or threatened species. Recent advances in population genomics utilizing next generation sequencing provide opportunities for re-evaluating existing management units but comparisons among approaches remain rare. Here, we report significant similarity in the utility of RAD-seq, a SNP array and a microsatellite panel to resolve spatial structuring in a threatened Atlantic Salmon population and provide evidence of significant subgroups within an existing conservation unit. Interestingly, these groups seem defined by secondary contact between European and North American origin Atlantic Salmon, and analysis of the SNP data sets indicates some evidence of asymmetrical introgression with gene flow towards the European type. This work builds directly on previous

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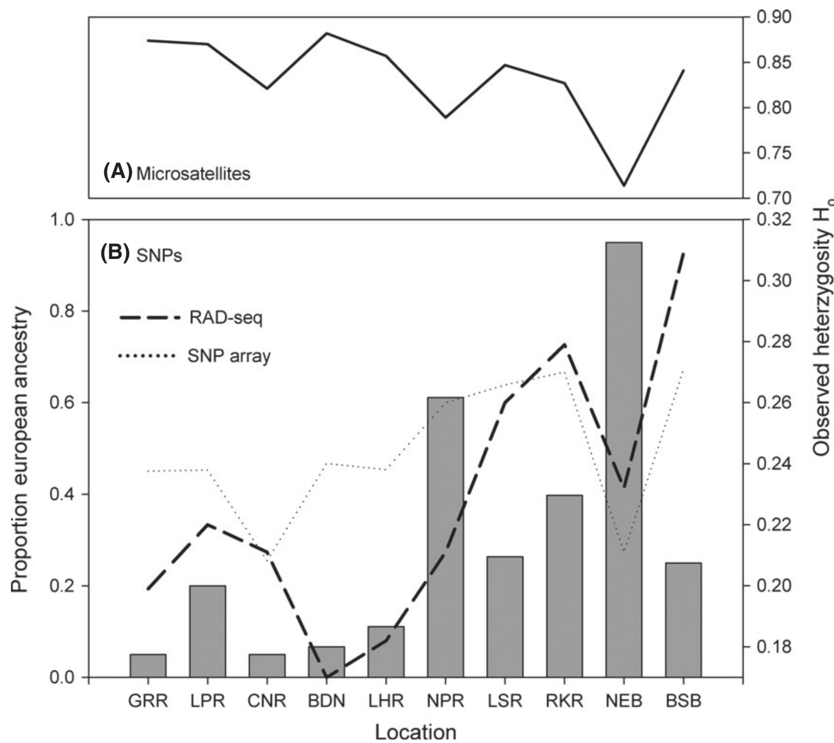

Fig. 5 Population observed heterozygosity (H_O) for each data set (A) Microsatellite and (B) single nucleotide polymorphisms (SNPs) and the proportion of individuals with European-type mtDNA alleles in each population. Populations are ordered from west to east.

studies suggesting complex spatial structure in this region (e.g. Verspoor 2005; Palstra *et al.* 2007; Bradbury *et al.* 2014; Moore *et al.* 2014), as well as the influence of European colonization in eastern Canada (King *et al.* 2007; Verspoor *et al.* 2015). Furthermore, our results support the utility of RAD-seq based approaches for the resolution of complex spatial patterns and in the conservation and management of exploited or at risk species.

Regional population structure

Atlantic Salmon throughout its natural range are characterized by complex hierarchical spatial genetic structure driven by a mix of both evolutionary and ecological processes (e.g. Verspoor *et al*. 2005; King *et al.* 2007; Dionne *et al.* 2008; Vähä *et al.* 2011). Previous studies in southern Newfoundland have reported significant spatial variation in genetic structuring with both regions of little structure (100 km) and of unexpectedly high fine-scale spatial structure (1–10 km) (Palstra *et al.* 2007; Bradbury *et al.* 2014) challenging attempts to identify conservation units (e.g. COSEWIC 2011). In contrast to previous studies, we report consistent evidence of subdivision of salmon populations into eastern and western southern Newfoundland with a boundary in the region of the Burin Peninsula. These two groups are evident in the microsatellite data, as well as an additional single eastern isolated population, Northeast Brook Trepassey. Northeast Brook has been observed previously to be unusually distinct (e.g. Palstra *et al.* 2007; Bradbury *et al.* 2014). This population is characterized by a very small run of anadromous salmon (<100 anadromous adults, Robertson *et al.* 2013) and a high proportion of precocial males (e.g. Dalley *et al.* 1983; Johnstone *et al.* 2013). Analysis of both SNP data sets supports the presence of two large groups with a boundary again along the Burin Peninsula. Examination of the SNP array data suggest that (i) divergence among the east and west is widespread across the genome and (ii) involves a variety of independent genes. Similar east–west groupings in southern Newfoundland have been reported in Rainbow Smelt, *Osmerus mordax*, which also display a boundary near the Burin Peninsula (Bradbury *et al.* 2011). In smelt, this structuring has been suggested to be associated with Pleistocene vicariance and secondary contact (Bradbury *et al.* 2011). The coherence in spatial structure among these two anadromous species suggests a more generalizable regional pattern of differentiation may be present.

Comparison among markers

The comparison of multiple marker types here provides an opportunity to explore differences and potential biases inherent in each approach. The striking similarity and significant association in the spatial signal among the three data sets supports the hypothesis that each

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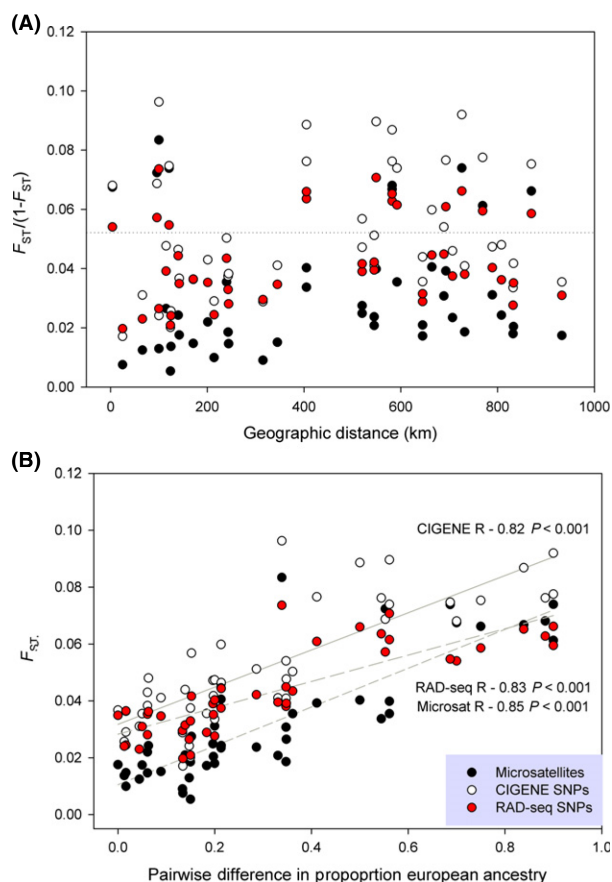

Fig. 6 (A) Isolation by distance for each data set comparing linearized F_{ST} with geographic distance (km), (B) comparison of F_{ST} with the pairwise difference in the proportion of individuals with European-type mtDNA alleles in each population. Closed black filled circles represent microsatellite data, open circles the single nucleotide polymorphism (SNP) array data, and closed red filled circles the RAD-seq data.

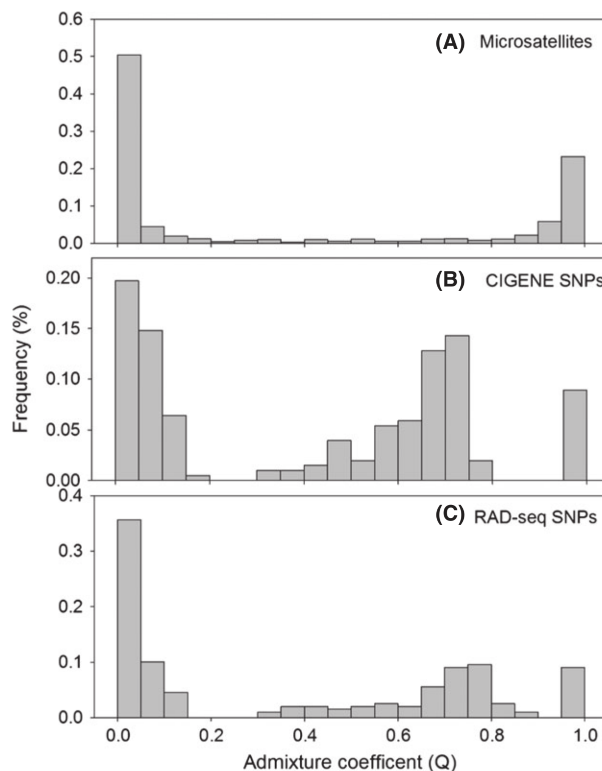

Fig. 7 Q-value distributions from STRUCTURE analysis of each data set, (A) microsatellite, (B) single nucleotide polymorphism (SNP) array, (C) RAD-seq. All analyses based on $k = 2$.

marker type is effective in resolving a common spatial genetic signal. Given that the SNP array was developed using European Atlantic Salmon (Bourret *et al.* 2013b), it is possible that ascertainment bias may influence inferences, particularly with both European and North American ancestry present. Bourret *et al.* (2013b) reported that diversity measured using the SNP array was elevated in the east Atlantic consistent with possible ascertainment bias. However, similar trends were also noted in a range wide microsatellite survey (King *et al.* 2001) suggesting ascertainment bias may be difficult to distinguish from historical contingency (Bourret *et al.* 2013b). However, the opposite has been observed in mtDNA variation resolved at the ND1 gene region (Verspoor *et al.* 2002). Interestingly, we observed contrasting trends along the south coast in heterozygosity among the marker types. Both SNP data sets display increases in diversity in the southeast with similar levels of heterozygosity observed suggesting a role for historical processes. However, it is worth noting that the SNP array data set was based on the subset of loci which were polymorphic (~46.2%). As such, for the subset of loci on the SNP array that were polymorphic, trends in diversity did not appear to differ substantially from those in the RAD-seq data set supporting the hypothesis of historical contingency as the dominant agent and minimal influences of ascertainment bias. The microsatellite panel on the other hand displayed clear declines in diversity from west to east, previously associated with habitat area and increased genetic drift in smaller populations (Bradbury *et al.* 2014). The cause of the different trends observed here among marker types ultimately remains unclear, although some influence of historical (secondary contact) and contemporary (i.e. drift) processes seems likely. The marker types also differed in their ability to detect evidence of hybridization or introgression. In contrast to the SNP data sets, the microsatellite panel detected little or no introgression (see Discussion below). Ultimately, the comparison of marker types demonstrates the utility of multiple marker comparisons in identifying processes associated with secondary contact and the dynamics of introgression.

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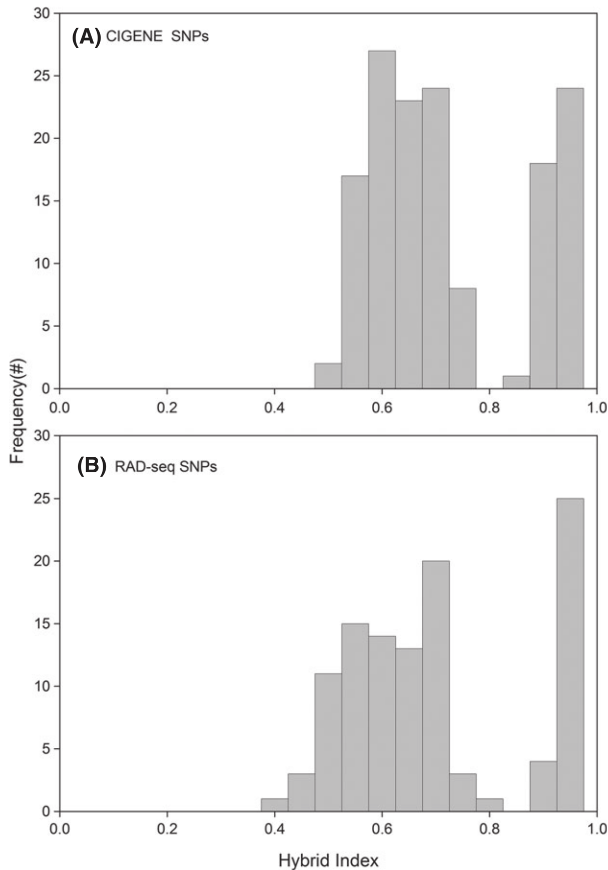

Fig. 8 Hybrid index values from INTROGRESS for both single nucleotide polymorphism (SNP) data sets (A) SNP array and (B) RAD-seq.

Secondary contact and introgression

A significant factor in the formation of the east and west groups along the south coast of Newfoundland seems to be secondary contact between two phylogeographic lineages representing North American and European Atlantic Salmon variants. Despite significant divergence between east and west Atlantic, previous work had suggested the presence of these alternate variants in a few locations both in North America (e.g. Verspoor *et al.* 2005) and northern Europe (e.g. Makhrov *et al.* 2005), but their fine-scale distribution and influence on spatial structure had not been resolved. In these southeastern Newfoundland populations, the proportion of diagnostic European alleles was elevated in comparison with southwestern Newfoundland, varied among locations sampled and was highest in two small rivers with partial obstructions to upstream migration, Northeast River Placentia and Northeast Brook Trepassey. Indeed, the unusual uniqueness of Northeast Brook Trepassey noted here and elsewhere (Palstra *et al.* 2007; Bradbury *et al.* 2014) may be partly due to the dominance of this European lineage. Dating of the divergence of Eastern and Western Atlantic Salmon suggests the isolation of these lineages for the last 600 000–700 000 years (King *et al.* 2007). Recent examination of the European lineage in Newfoundland suggests its isolation from Europe occurred *c.* 18 000–19 000 years ago (E. Verspoor and D. Knox, in preparation) implying that the North American European lineage was isolated from Europe around the end of the last glacial period. Recent range wide SNP analysis supports hypotheses of a deep genetic divergence between the east and west Atlantic stocks, although no loci displayed fixed differences between the regions (Bourret *et al.* 2013b) perhaps in part due to the presence of these alternate variants; this parallels observations for allozyme variation (Verspoor *et al.* 2005). Similar patterns of the influence of European lineages in the western Atlantic have been reported in Atlantic cod on the Flemish Cap (Cross & Payne 1978; Bradbury *et al.* 2013), and Atlantic wolfish on the Grand Banks (McCusker & Bentzen 2010). In addition to marine fish, several intertidal marine invertebrates display patterns of colonization of eastern North America from Europe following the last glacial period (Wares & Cunningham 2001). This growing body of work supports the hypothesis that periods of trans-Atlantic colonization and gene flow have been a common phenomenon. Admittedly, our ability to interpret these lineage-specific trends here depends on the use of the diagnostic mtDNA SNPs used in the analysis. The fact that these SNPs correlate with whole mtDNA patterns in lineage distribution generally supports their usage here.

The presence of both lineages in eastern North America raises the possibility of hybridization and introgression. Our analysis of both SNP data sets supports the hypothesis that introgression has occurred, as intermediate q-values and hybrid index values were observed. Although there was limited evidence of introgression in the microsatellite data set, it is possible that the microsatellite panel has less power to resolve introgression in comparison with the SNP panels; however, the east and west groups are clearly resolved in the clustering analysis using the microsatellites. It also possibly supports a hypothesis of differential introgression or perhaps the leakage of favourable gene-associated SNPs. This observation is in contrast to the patterns of differential introgression usually observed at hybrid zones during the early stages of speciation where divergence is only apparent at loci associated with reproductive isolation or ecological speciation (e.g. Payseur 2010; Larson *et al.* 2013).

Also worth noting is the observation that the intermediate q-values and hybrid index values in both SNP data sets were skewed towards the southeastern populations. This is consistent with a hypothesis of asymmetric introgression and gene flow from the North

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American lineage into the local European lineage. Asymmetrical or unidirectional hybridization and introgression has been reported among several other species of closely related salmonids (e.g. Garcia-Vazquez *et al.* 2004; Kirkpatrick *et al.* 2007; Álvarez & Garcia-Vazquez 2011) and is consistent with significant divergence and hypotheses of subspecies status of North American and European Atlantic Salmon (King *et al.* 2007). In fact, previous work has reported significant differences in chromosome number between North American and European Atlantic Salmon, and significant divergence at almost all marker types examined (King *et al.* 2007), again supporting hypotheses of significant divergence. Our observation of asymmetrical introgression is also consistent with a recent study exploring the viability of backcrosses between North American and European Atlantic Salmon. Cauwelier *et al.* (2012) report a complete lack of viability when F1 hybrids were back crossed to North American Salmon and hypothesize that this may be associated with Dobzhansky—Muller incompatibilities. Thus, our study supports early experimental and genetic work suggesting that hybridization can occur, that it appears to be asymmetric towards the European variant, and is consistent with subspecies level isolation observed among other salmonids.

Management and conservation implications

Dramatic increases in the number of loci surveyed and the inclusion of both functional and neutral genetic variation have revolutionized how genetics and genomics can contribute to management and conservation activities (Funk *et al.* 2012). Atlantic Salmon in southern Newfoundland have undergone significant declines in abundance the last few decades with some individual rivers declining by more than 50% (Robertson *et al.* 2013) and at present are designated as a single designatable or evolutionarily significant unit (COSEWIC 2011). Our results clearly demonstrate the presence of two groups characterized by significant differentiation both at neutral and gene-associated loci, warranting subdivision of this conservation unit. The identification of eastern Atlantic affinities in one of these groups and the possibility of limited introgression and even subspecies status only serve to strengthen this case. Furthermore, the similarities reported here among methods despite the complex demographic scenario support the utility of new approaches such as RAD-seq for resolving structuring in nonmodel species. Recent work in Atlantic Salmon is continuing to define conservation units both in North American and Europe (Bourret *et al.* 2013a; Moore *et al.* 2014). Similar results have been reported in marine fish where the identification of cryptic diversity using genome wide SNP surveys has the potential to change management and conservation practices (COSEWIC 2010; Bradbury *et al.* 2013; Milano *et al.* 2014). The growing body of examples including this work support the conclusion that population genomic tools will ultimately change management and conservation of wild populations by allowing a more accurate delineation of diversity in conservation units and providing fine-scale tools for assignment and individual identification.

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I.R.B. designed, and supervised the study and performed most of the analyses and wrote the manuscript. L.C.H. conducted the laboratory analyses including the microsatellite genotyping and participated in writing. B.D., M.J.R., V.B., L.B. and E.V. provided samples or existing data, and assisted with analysis interpretation and writing.

Data accessibility

DNA sequences: All raw sequence data have been uploaded to NCBI SRA. Bioproject # PRJNA291587, Biosample #s SAMN04054010-SAMN04054188, SAMN04053806-SAMN04053964, SAMN04090079-SAMN04090093. The SNP array, RAD-seq and microsatellite genotypes used in this study, and all distance matrices have been deposited in Dryad at http://dx.doi.org/10.5061/dryad.7tv21. All corresponding sample codes and locations are in Table 1 and Fig. 1, respectively.

Supporting information

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

Fig. S1 Genomic distribution of differentiation between the east and west groups of Atlantic Salmon populations in southern Newfoundland.

Fig. S2 Distribution of mtDNA alleles diagnostic for European lineage both in (A) Newfoundland and worldwide (B).

Table S1 Results of RAD-seq analysis and various filtering steps.

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