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Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.)

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Abstract

It may often be necessary to perform genetic analyses of temporal replicates to estimate the significance of spatial variation independently from that of temporal variation in order to ensure the reliability of estimates of a defined population structure. Nevertheless, temporal studies of genetic diversity remain scarce in the literature relative to the plethora of empirical studies of population structure. In vertebrates, a limited number of studies have specifically assessed the temporal stability of population structure for more than one generation. In this study, we performed a microsatellite analysis of DNA obtained from archived scales to compare the population structure among four sympatric landlocked populations of Atlantic salmon (*Salmo salar*) over a time frame of three to five generations. The same patterns of allele frequency distribution, θ, R_{ST} and genetic distance estimates were observed among populations for two time periods, confirming the temporal stability of the population structure. Despite population declines and stocking during this period, no statistically significant changes in intrapopulation genetic diversity were apparent. This study illustrates the feasibility and usefulness of microsatellite analysis of temporal samples, not only to infer changes of intrapopulation genetic diversity, but also to assess the stability of population structure over a time frame of several generations.

Keywords: ancient DNA, microsatellites, population genetics, *Salmo salar*, temporal variation

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Introduction

Empirical studies of genetic population structure typically aim at defining the number of distinct populations of a species, quantifying the amount of divergence among those populations, and inferring their relationships. Genetically distinct populations are usually defined on the basis of statistically significant heterogeneity (e.g. the probability of rejecting the null hypothesis of no differentiation, $P<0.05$) in allele frequencies among samples, implying nonrandom mating. It is also most often implicitly accepted that the observed genetic pattern is stable over time, and that other factors potentially causing genetic differences among samples are negligible. However, stochastic temporal fluctuations in allelic frequency due to genetic drift may be important in small populations (Waples & Teel 1990; Waples 1990). Variation in reproductive success may also lead to changes in genotypic frequencies via demographic stochasticity (e.g. Hedgecock 1994). Significant allele-frequency differences among samples may also result from nonrandom sampling of populations, for instance in cases where samples differ in age composition (Allendorf & Phelps 1981). Consequently, performing genetic analyses of temporal replicates to estimate the magnitude of spatial variation independently of temporal variation may be required to ensure the reliability of estimates of population structure (Jordan *et al*. 1992).

Despite their potential benefits, studies of temporal genetic diversity have often been neglected and remain

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scarce in the literature relative to the plethora of empirical studies of population structure. Most detailed studies focus on invertebrate species with short generation times (e.g. Lessios *et al*. 1994 and references therein). In vertebrates, temporal changes in genetic diversity have seldom been the main objective of empirical studies. They have more typically included temporal replicates of a subset of samples to assess within-population stability in the time frame of less than one generation (e.g. Grant *et al*. 1980; Gharrett *et al*. 1987; Beacham *et al*. 1989; Crozier & Moffett 1989; Moffett & Crozier 1996; but see Jorde & Ryman 1996; Laikre *et al*. 1998). Few studies have specifically been designed to assess the temporal stability of population structure and even fewer have been performed over a time frame exceeding one generation (but see Gaines *et al*. 1978; Gyllensten 1985; McClenaghan *et al*. 1985; Gyllensten & Ryman 1988; Lacson & Morizot. 1991; Jordan *et al*. 1992; Brown *et al*. 1996).

By providing access to DNA analyses from ancient or archived tissue samples, the advance of PCR-based methods in the 1990s has facilitated the assessment of genetic diversity over longer time periods (Thomas *et al*. 1990; Ellegren 1991; Taylor *et al*. 1994; Hardy *et al*. 1995). In fish, dried scales have routinely been collected for ageing purposes, particularly in economically important species, and are a good source of DNA to perform genetic analyses at highly variable loci such as microsatellites and mitochondrial DNA (e.g. Estoup *et al*. 1996; Purcell *et al*. 1996; Miller & Kapuscinski 1997; Nielsen *et al*. 1997).

In this study, we assessed the stability of population structure over time (three to five generations), using microsatellite analysis of DNA obtained from old scales, and by comparing genetic diversity among four sympatric landlocked populations of Atlantic salmon (*Salmo salar*) from Lake St-Jean, Québec, Canada (Fig. 1). We recently showed (Tessier *et al*. 1997) that these populations are genetically distinct but, unexpectedly, we observed that the genetic divergence of one population with the three others was substantially higher than that reported to date using similar markers among anadromous populations over a much greater geographical distance (e.g. Fontaine *et al*. 1997; McConnell *et al*. 1997). We also observed more pronounced differentiation between populations associated with two tributaries in a single river system than between populations in two neighbouring river systems.

Recent demographic changes may have contributed to the modification of both the intrapopulation genetic diversity as well as the population genetic structure, thus potentially providing an explanation for this unusual population structure. Based on fishing records, these previously unstocked populations apparently remained demographically stable until the early mid-1980s when signs of population decline became evident. Fishing effort/capture increased fivefold between 1985 and 1993, and the number of adults counted during spawning runs drastically declined. For instance, the number of spawners in River aux Saumons declined from 900 in 1971 to 62 in 1985, and in the Ouasiemsca from 1634 to 137 between 1987 and 1993. To compensate for these population reductions, the River aux Saumons was stocked between 1980 and 1983 with 15 000–50 000 salmon fry originating from a non-native stock (Baldwin) and 30 000 fry from the Métabetchouane River. Similarly, the Ashuapmushuan was stocked between 1990 and 1994 with 2400–92 000 fry or 1-year-old salmon parr produced by a mixture of fish of the four Lake St-Jean populations (O. Gauthier, Ministère Environnement et Faune, Québec, personal communication).

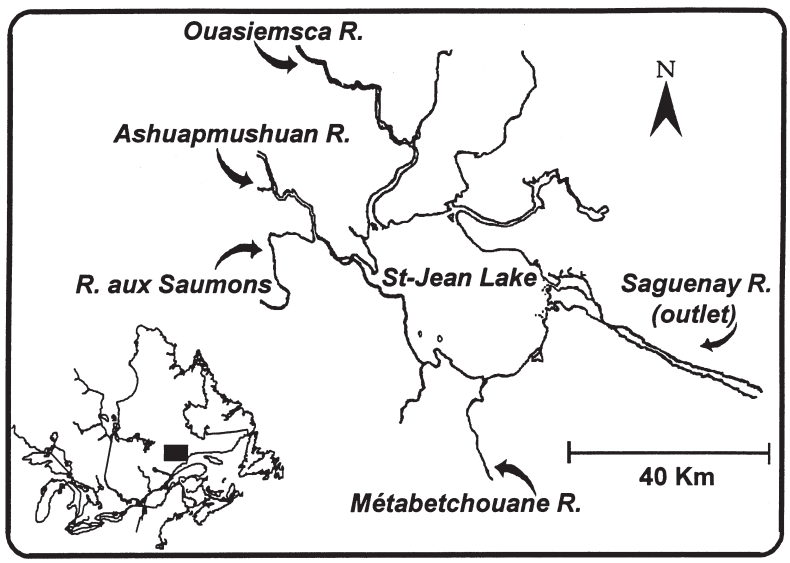

Fig. 1 Location map of Lake St-Jean.

In this context, we compared the genetic structure of these populations prior to and following demographic changes: (i) to ensure that genetic differences among rivers were not purely stochastic but reflected the existence of persistent populations; and (ii) to assess the temporal stability of population structure and genetic diversity in the face of demographic disturbance. In doing so, we also aimed to provide an example of the potential usefulness of performing temporal studies of microsatellite variation not only to describe changes of intrapopulation diversity but also to address specific questions related to genetic population structure.

Materials and methods

Samples

Contemporary samples analysed in Tessier *et al*. (1997) consisted of about 40 sexually mature fish collected in 1994 throughout their spawning run or later during the fall in spawning grounds, in each of the four tributaries of Lake St-Jean harbouring salmon (Fig. 1). Genetic diversity observed among these samples (detailed in Tessier *et al*. 1997) was compared to that obtained among similar samples collected in 1970 from the River aux Saumons, in 1978 from the Ouasiemsca, in 1980 from the Ashuapmushuan and in 1981 from the Métabetchouane. Years of collection were selected on the basis of availability of at least 40 individuals for the oldest year possible. Thus, the time frame between contemporary and old samples represented about three to five generations.

Microsatellite analysis

Total DNA was extracted from one to 16 uncleaned dried scales stored in envelopes according to a protocol using proteinase K (Estoup *et al*. 1996). Microsatellite polymorphism among samples of old scales was quantified by amplification of the same loci used in Tessier *et al*. (1997): MST-3, MST-79.1, MST-79.2 (Presa & Guyomard 1996), SF0–23 (Angers *et al*. 1995), SSOSL85 (Slettan *et al*. 1995), Ssa171 and Ssa197 (O'Reilly *et al*. 1996). Electrophoresis, gel fixation, drying, and autoradiography followed Sambrook *et al*. (1989). Alleles were sized by comparison with the standard M13 sequence and with standard controls consisting of two samples run on all gels. Each gel was also scored independently three times to minimize errors in assigning allelic size, which was always determined from at least two congruent scores.

Gene diversity within population

The temporal variation of genetic diversity was quantified in terms of heterozygosity, number of alleles per locus and allele frequencies observed in the two temporal samples of a given population. Observed (H_O) and expected (H_E) heterozygosity were computed using the GENEPOP computer package, version 3.0 (Raymond & Rousset 1995). GENEPOP was also used to estimate departures from Hardy–Weinberg equilibrium at each locus and globally over all loci. This implied the use of the Markov chain method (Guo & Thompson 1992) to obtain unbiased estimates of Fisher's exact test through 1000 iterations, in order to test the alternative hypotheses of deficiency or excess of heterozygotes (Rousset & Raymond 1995). Exact tests were also used to perform pairwise comparisons of allele frequencies at individual locus between temporal samples of a given population. To quantify the temporal stability of allele frequencies, we computed Pearson's correlation coefficients between frequencies for each allele observed in the two temporal samples for each population. Differences in expected heterozygosity (H_E) estimates and number of alleles per locus between temporal samples of each population were tested with a nonparametric Wilcoxon signed-rank test.

Temporal stability of population structure

We first compared the heterogeneity in allele frequency distribution among rivers observed for old samples to that reported in Tessier *et al*. (1997) using exact tests as described above. We then compared the extent of genetic differentiation among populations by computing pairwise fixation indices based on allelic frequencies (θ of Weir & Cockerham 1984) using an analysis of molecular variance (AMOVA, Michalakis & Excoffier 1996) available in the program ARLEQUIN version 1.1 (Schneider *et al*. 1997). The 95% confidence intervals were calculated by bootstraps with the program FSTAT version 1.2 provided by J. Goudet (Institut de Zoologie et d'Ecologie Animale, Dorigny, Switzerland). While potential differences in genetic diversity over the period of three to five generations are probably influenced more by drift than by mutation, we wanted to quantify how such changes would effect statistics that consider mutational differences. This was done by computing pairwise standardized R_{ST} using the program R_{ST} CALC (Goodman 1997). This program corrects the initial Slatkin's (1995) R_{ST} formula for differences in sample size between populations and for differences in the magnitude of variance in allele size between loci. This procedure weights R_{ST} by allele size, standardizing alleles across the whole data set, to make different loci more directly comparable. It then averages a variance component over loci before calculating R_{ST}, as recommended by Slatkin (1995), rather than averaging R_{ST} estimates over loci. We also calculated the 95% confidence intervals on R_{ST} estimates using R_{ST} CALC. For both θ and R_{ST} estimates, departure from the null hypothesis (estimates = 0) was

statistically assessed by permutation procedures (1000 iterations) in both Arlequin and R_{ST} CALC.

The extent of gene flow (*Nm*) among populations was estimated for both temporal periods from either θ or R_{ST} estimates (Slatkin 1995). Although the accuracy of *Nm* estimates depends on several assumptions that are probably not met in the present situation (populations in equilibrium with respect to genetic drift and migration, and the island model of migration), they nevertheless provide a relative basis for comparing gene flow among populations for the two temporal periods. Probability values in all of the above tests were adjusted for multiple simultaneous tablewide tests using the sequential Bonferroni adjustments (Rice 1989) to minimize type-I errors.

Relationships among samples were estimated from a neighbour-joining (NJ) phenogram (Saitou & Nei 1987) constructed from a pairwise chord distance matrix (D_{CE}; Cavalli-Sforza & Edwards 1967). D_{CE} assumes that divergence between populations is due solely to genetic drift and is probably most appropriate for the framework of this study. It also appears to be one of the most efficient distance measures to obtain correct tree topology from allele frequency data (Takezaki & Nei 1996). Bootstrapped confidence values on branches were obtained by resampling loci within samples and given as percentages over 1000 replications, using the program NJBPOP developed by J. M. Cornuet (INRA, Laboratoire de Neurobiologie comparée des invertébrés, Bures-sur-Yvette, France).

Results

Genetic diversity within population

No significant departure from Hardy–Weinberg equilibrium was detected by multilocus probability tests (Fisher's method) in old or contemporary samples. We therefore cannot reject the null hypothesis of random mating for any of these populations. High levels of genetic diversity were observed in samples for each of the four rivers. However, the total numbers of alleles, mean numbers of alleles per locus, and heterozygosity values were more similar between temporal samples at the same location than they were between locations within the same temporal period (Table 1). No significant differences (Wilcoxon: $P > 0.05$) were observed in expected heterozygosity and number of alleles per locus between past and contemporary samples (Table 2). Similarly, no significant differences were observed in allele frequency distribution (Fisher's exact test: $P > 0.05$) between temporal samples of the same population, with the exception of River aux Saumons for one locus (see below). Figure 2 illustrates the overall similitude of allelic frequency distribution between temporal samples at the same location for the different loci. The frequencies of individual alleles for all loci were highly correlated between temporal samples, with correlated values ranging from 0.934 to 0.977 (Fig. 3).

Such stability of genetic composition between the two periods was also reflected in low and nonsignificant multilocus θ and standardized R_{ST} estimates, with values ranging from 0.000 to 0.017 (Table 3). This also resulted in low genetic distances between temporal samples from the same river, such that samples from the same location always clustered together with strong bootstrap support (Fig. 4).

The exception to the overall pattern of within-population stability of genetic diversity was observed in River aux Saumons for which the number of alleles or expected heterozygosities differed between temporal samples (Tables 1, 2). Temporal changes were particularly apparent at loci SSOSL85 and Ssa171, for which we observed alleles in the contemporary sample that were not observed in older samples. We also observed a shift in frequency of alleles SSOSL85–194 (0.70–0.41) and SSOSL85–196 (0.14–0.34; Fig. 2). The departures of observed differences from a one-to-one relationship of allele frequencies between temporal samples were also larger for River aux Saumons than for the other three populations (Fig. 3).

	N_S	A_T	A	H_E	H_O
Past (1970–81)					
River aux Saumons	39.29 (1.16)	38	5.42 (2.38)	0.62 (0.11)	0.60 (0.07)
Ashuapmushuan	37.14 (1.00)	49	7.00 (3.46)	0.64 (0.22)	0.64 (0.22)
Métabetchouane	37.57 (1.05)	30	4.29 (2.71)	0.51 (0.26)	0.54 (0.30)
Ouasiemsca	35.14 (1.64)	47	6.71 (3.49)	0.68 (0.12)	0.63 (0.16)
Contemporary (1994)					
River aux Saumons	35.00 (2.20)	43	6.14 (2.75)	0.68 (0.09)	0.68 (0.12)
Ashuapmushuan	40.29 (1.39)	48	6.86 (3.76)	0.64 (0.17)	0.61 (0.20)
Métabetchouane	40.00 (1.51)	32	4.57 (2.06)	0.51 (0.23)	0.50 (0.24)
Ouasiemsca	35.86 (0.35)	46	6.57 (3.42)	0.68 (0.13)	0.65 (0.17)

Table 1 Mean sample size per locus (N_S) and standard deviation in parentheses, total number of alleles (A_T) for all loci, mean number of alleles per locus (A), mean expected heterozygosity across loci (H_E) and mean observed heterozygosity across loci (H_O) for landlocked salmon in past and contemporary samples from River aux Saumons, Ashuapmushuan, Métabetchouane and Ouasiemsca rivers

Table 2 Range of allele size (*S*), total number of alleles (A_T), sample size (*N*), observed (H_O) and expected (H_E) heterozygosity by locus for landlocked salmon in past and contemporary samples from River aux Saumons (Rs), Ashuapmushuan (As), Métabetchouane (Met) and Ouasiemsca (Oua) rivers. Allele designation is expressed in base pairs

	Past				Contemporary			
Locus	Rs	As	Met	Oua	Rs	As	Met	Oua
MST-3								
S	208–216	208–216	204–216	208–216	204–216	208–216	204–216	208–216
A	3	4	4	4	4	3	4	5
N	40	37	38	34	36	40	41	36
H_O	0.600	0.487	0.763	0.588	0.667	0.450	0.634	0.417
H_E	0.629	0.577	0.704	0.609	0.683	0.495	0.740	0.577
MST-79.1								
S	145–155	149–159	149–151	149–157	149–161	149–161	149–157	149–157
A	4	5	2	4	4	5	4	4
N	40	36	38	37	35	39	41	36
H_O	0.600	0.194	0.211	0.514	0.571	0.410	0.390	0.611
H_E	0.676	0.209	0.232	0.633	0.662	0.464	0.352	0.611
MST-79.2								
S	120–122	120–122	120–122	120–122	120–122	120–122	120–122	120–122
A	2	2	2	2	2	2	2	2
N	37	37	38	37	35	39	39	36
H_O	0.487	0.595	0.526	0.378	0.600	0.359	0.539	0.444
H_E	0.424	0.507	0.472	0.489	0.501	0.466	0.441	0.468
SSOSL85								
S	184–204	186–206	186–198	182–206	174–204	186–206	186–202	186–206
A	8	8	4	9	9	8	5	8
N	40	36	38	34	35	43	40	35
H_O	0.525	0.806	0.553	0.824	0.771	0.721	0.600	0.800
H_E	0.490	0.822	0.677	0.771	0.711	0.812	0.591	0.782
Ssa171								
S	237–253	237–265	237–265	235–261	237–265	233–265	233–265	231–261
A	6	13	10	13	10	14	9	13
N	38	38	35	33	37	41	40	36
H_O	0.658	0.895	0.886	0.849	0.892	0.951	0.850	0.917
H_E	0.733	0.914	0.771	0.900	0.811	0.911	0.792	0.888
Ssa197								
S	172–200	172–200	168–188	172–200	172–200	172–200	168–188	164–192
A	6	7	6	5	6	7	5	5
N	40	39	38	37	37	41	42	36
H_O	0.725	0.718	0.816	0.730	0.703	0.610	0.429	0.639
H_E	0.758	0.661	0.677	0.651	0.732	0.597	0.575	0.681
SFO-23								
S	114–144	118–144	118–122	116–144	116–140	118–144	122–140	118–144
A	9	10	2	9	8	9	3	9
N	40	37	38	34	30	39	37	36
H_O	0.600	0.811	0.026	0.529	0.533	0.769	0.027	0.694
H_E	0.627	0.766	0.026	0.712	0.670	0.743	0.054	0.759

Temporal stability of population structure

The pattern of population differentiation was similar for the two temporal periods. Most loci (34 significant comparisons out of 42 for the old samples, and 32 significant comparisons out of 42 for the contemporary ones) showed highly significant differences in allele frequencies between populations within each period (Fisher's exact test; $P < 0.001$; data not shown).

The high degree of allele frequency differentiation among populations also translated into similar and significant (Fisher's exact test; $P < 0.001$) population divergence for both θ and standardized R_{ST} estimates (Table 3). Thus, the Métabetchouane was the most divergent from the

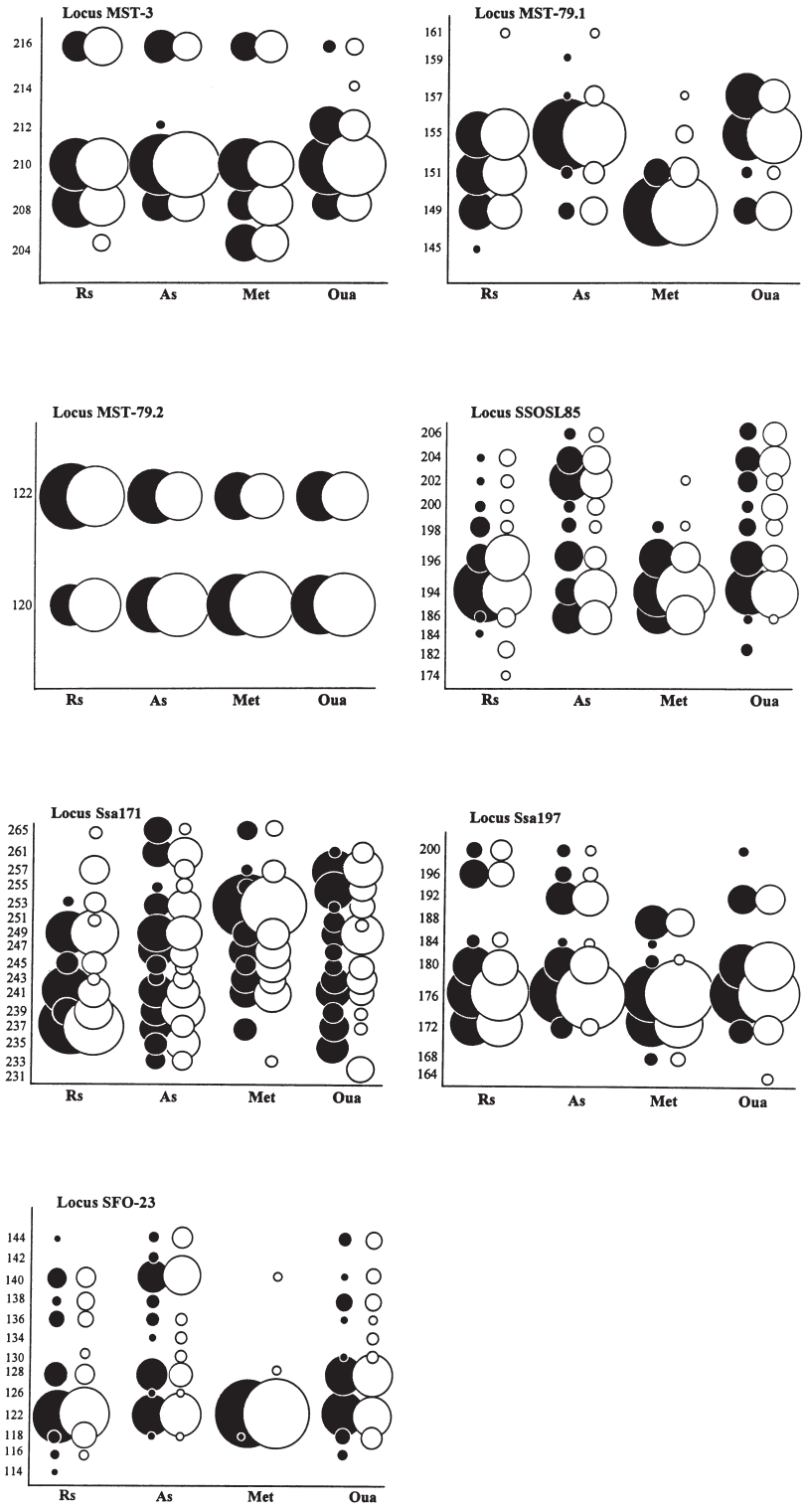

Fig. 2 Schematic illustration of relative allelic frequencies at seven loci for past (in black) and contemporary samples (in white) from River aux Saumons (Rs), Ashuapmushuan (As), Métabetchouane (Met) and Ouasiemsca (Oua) populations. Circles represent distinct alleles, and their surface areas are directly proportional to their relative frequencies. Complete allele frequency data are available upon request.

other three populations for the two temporal periods. The genetic divergence between the Ashuapmushuan population and salmon from its tributary the River aux Saumons was also more important for both periods than between the Ashuapmushuan and the neighbouring Ouasiemsca population (Table 3). Consequently, population relationships as depicted in the NJ phenogram constructed from D_{CE} distances were completely congruent with high bootstrap values grouping the Ashuapmushuan and Ouasiemsca populations together,

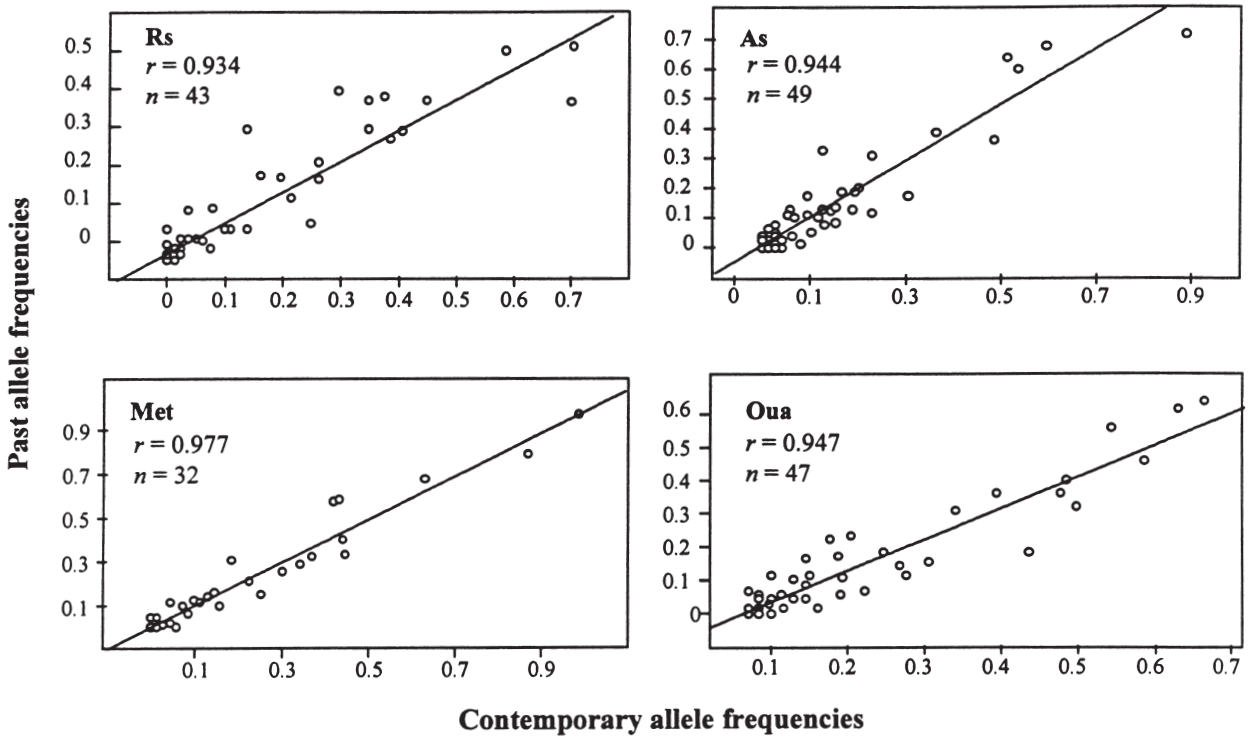

Fig. 3 Pearson correlation coefficient in individual allele frequencies between the two temporal periods for River aux Saumons (Rs), Ashuapmushuan (As), Métabetchouane (Met) and Ouasiemsca (Oua) populations. Each graph includes pooled information of all alleles across loci. The line illustrates the one-to-one relationships in allele frequency over time. Illustrated numbers of observations may be less than the actual values due to overlap in frequencies.

Table 3 Pairwise θ, standardized R_{ST} estimates for 95% confidence interval (CI), and effective number of migrants per generation (*Nm*) among River aux Saumons (Rs), Ashuapmushuan (As), Métabetchouane (Met) and Ouasiemsca (Oua) in past and contemporary samples and between the two periods for each sample of landlocked salmon originating from the same river.

Comparison	θ	95% CI	*Nm*	R_{ST}	95% CI	*Nm*
Past						
Rs vs. As	0.133**	0.053 – 0.205	1.630	0.130**	0.087 – 0.201	1.671
Rs vs. Met	0.161**	0.093 – 0.256	1.303	0.224**	0.188 – 0.280	0.867
Rs vs. Oua	0.104**	0.063 – 0.126	2.154	0.143**	0.097 – 0.220	1.504
As vs. Met	0.215**	0.069 – 0.464	0.913	0.329**	0.293 – 0.383	0.511
As vs. Oua	0.062**	0.021 – 0.123	3.782	0.015	0.001 – 0.062	16.941
Met vs. Oua	0.167**	0.071 – 0.328	1.247	0.314**	0.272 – 0.377	0.545
Contemporary						
Rs vs. As	0.071**	0.060 – 0.084	3.271	0.071**	0.036 – 0.131	3.245
Rs vs. Met	0.121**	0.058 – 0.223	1.816	0.158**	0.103 – 0.237	1.332
Rs vs. Oua	0.067**	0.050 – 0.083	3.481	0.060**	0.032 – 0.118	3.949
As vs. Met	0.157**	0.062 – 0.330	1.342	0.296**	0.235 – 0.366	0.594
As vs. Oua	0.020	0.012 – 0.050	12.250	0.021	0.006 – 0.076	11.867
Met vs. Oua	0.138**	0.076 – 0.271	1.562	0.285**	0.229 – 0.357	0.627
Past-Contemporary						
Rs(70) Rs(94)	0.017	0.000 – 0.042		0.006	0.000 – 0.050	
As(80) As(94)	0.010	0.000 – 0.021		0.000	0.000 – 0.045	
Met(81) Met(94)	0.004	0.000 – 0.010		0.000	0.000 – 0.023	
Oua(78) Oua(94)	0.003	0.000 – 0.011		0.008	0.000 – 0.059	

**Significant differences reflect adjustments for multiple tests (initial α value = 0.05/16 = 0.003).

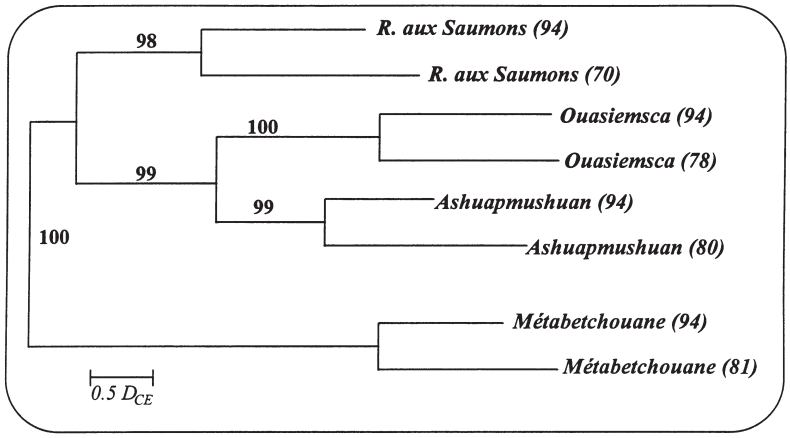

Fig. 4 Chord distance (D_{CE}) phenogram illustrating relationships among four landlocked Atlantic salmon populations of Lake St-Jean for past and contemporary asamples. Percentages of replication of the observed topology based on 1000 bootstraps on loci is given along branches.

and the Métabetchouane being the most distant population for both periods (Fig. 4). θ and R_{ST} values were generally similar for any given pairwise comparison involving River aux Saumons, Ashuapmushuan and Ouasiemsca populations (Table 3). However, in all comparisons involving the Métabetchouane, standardized R_{ST} was consistently higher than θ suggesting the contribution of a more important mutation component in the extent of differentiation of this population. Despite the overall stability in the pattern of population structure in time, it is, however, noteworthy that the extent of population differentiation, based on either θ and R_{ST} values, was generally lower between all pairwise comparisons in contemporary samples (Table 3).

Discussion

The first objective of this study was to confirm that the genetic differences among rivers that we have previously documented reflected the existence of distinct populations. This was achieved by comparing genetic diversities among contemporary samples with diversities among samples that were three to five generations older. We found highly significant allele frequency differences, θ and R_{ST} estimates between all pairwise comparisons of old samples, and between all comparisons of contemporary samples, confirming that salmon from the different tributaries represent genetically distinct and temporally stable populations. Temporal variation observed here over a period of three to five generations between samples from the same site was low in comparison to spatial differences, and this is typical of Atlantic salmon in other areas and of other salmonids (e.g. Grant *et al*. 1980; Beacham *et al*. 1987, 1989; Ståhl 1987; Crozier & Moffett 1989; Jordan *et al*. 1992). Consequently, the results of the present study support the conclusions of our previous report (Tessier *et al*. 1997) that the magnitude of genetic divergence among salmon populations within Lake St-Jean is greater than the magnitude of divergence among anadromous salmonid populations over a much greater geographical distance. Our results also confirm the existence of a more pronounced differentiation between populations associated with two tributaries within a single river system than between populations from two neighbouring river systems. Therefore, this unexpected pattern of population structure appears to be temporally stable and is not a result of recent demographic changes caused by either stocking or over-fishing. We are currently investigating the potential ecological and historical factors that may potentially explain this unusual situation in Lake St-Jean (Tessier *et al*. 1997; N. T. Tessier, L. B. Bernatchez, unpublished).

Our second objective was to assess the temporal stability of genetic diversity in the face of demographic changes. We generally observed similar allelic composition and levels of diversity between temporal samples of a given population (but see below for River aux Saumons), as indicated by the strong correlation between past and present allele frequencies and by the absence of significant differences in the number of alleles per locus and heterozygosity values. This suggests that effective population sizes were not reduced sufficiently by recent demographic changes to cause important departures from migration/drift equilibrium that would appreciably alter intrapopulation diversity within the time frame of three to five generations. Theoretical changes in heterozygosity from random genetic drift (Hartl & Clark 1989) can be used to approximate the amplitude of change in diversity that could be expected with the values of N_e commensurate with population censuses in the lake. An approximate estimate of minimal N_e values varying between 50 and 100 is probably appropriate because the number of spawners in these populations is known to have varied between several hundred to about 1000 during the period we studied and the ratio of N_e/N is about 0.1–0.2 in salmonids

(Hedrick *et al*. 1995). With this range of values and five generations, a reduction in H_E of only 2.5% is expected with $N_e = 100$, and 5% for $N_e = 50$. These values would be lower if the real N_e was higher than these hypothetical values, and if even a small amount of gene flow occurred among populations. The quantification of such small changes in genetic diversity could easily be masked by stochastic sampling errors. These observations do not indicate that human-induced perturbations such as over-fishing and stocking have had no impact on the genetic integrity of populations but, instead, reiterate the view that temporal changes in intrapopulation genetic diversity are not easily depicted unless demographic changes are extreme (Waples & Teel 1990), effective sizes are very small (Richards & Leberg 1996), or genetic diversity is monitored over a large number of generations (Miller & Kapuscinski 1997).

The stocking procedure used in Lake St-Jean prior to 1990 may also have contributed to the apparent stability of H_E and the number of alleles when considering population decline during the period that we studied. However, this should also be accompanied by shifts in allelic composition. This is exactly what was observed for the River aux Saumons population which suffered the most from both population decline and stocking. We observed a trend toward increased heterozygosity and number of alleles per locus in this population (see Table 1), which would not be expected from its recent history of severe population reduction. Increased H_E and number of alleles in River aux Saumons was also accompanied by a shift in frequencies and the occurrence of new alleles not observed during the 1970s. Similar, although milder patterns, were also observed for the Ashuapmushuan population, which was also affected by stocking, but to a lesser degree (O. Gauthier, Ministère Environnement et Faune, Québec, personal communication). Smaller θ and R_{ST} estimates observed among pairwise comparisons of contemporary samples relative to old ones are also not compatible with the overall trend of population decline in the lake during the temporal period that we studied, which should instead increase drift, and therefore accentuate divergence over time. This trend may therefore suggest increased gene flow among populations over time related to stocking practices, although the present data set does not strictly allow this hypothesis to be confirmed, because of the important overlap of confidence intervals on those estimates.

To conclude, this study illustrates the usefulness of microsatellite analysis performed on temporal samples, not only to infer changes in intrapopulation genetic diversity, but also to assess the stability of population structure over generations. We were limited in this study to a maximum time frame of five generations, but previous studies have shown that dried scale samples as old as 60 years may also be used for microsatellite analysis (Miller & Kapuscinski 1997; Nielsen *et al*. 1997. This study also revealed that limiting the temporal assessment of stocking impacts or other factors causing population decline (such as over-fishing) to intrapopulation diversity alone can be misleading, as such changes may not easily be detectable in many situations. Finally, the results of this study also suggest that the lack of temporal replicates may not always represent a major drawback in genetic studies of population structure although the generality of our findings remains to be confirmed by similar studies in other systems.

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