Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.)*

DANY GARANT, JULIAN J. DODSON and LOUIS BERNATCHEZ
Université Laval, Département de biologie, Ste-Foy, Québec, Canada, G1K 7P4

Abstract

A gene diversity analysis was performed using microsatellite loci in order to (i) describe the extent and pattern of population structure in Atlantic salmon (*Salmo salar* L.) within a river system; (ii) establish the importance of quantifying the signal:noise ratio in accurately estimating population structure; and (iii) assess the potential usefulness of two evolutionary models in explaining within-river population structure from the ecological and habitat characteristics of Atlantic salmon. We found weak, yet highly significant microscale spatial patterning after accounting for variance among temporal replicates within sites. Lower genetic distances were observed among temporal samples at four sampling sites whereas no evidence for temporal stability was observed at the other three locations. The component of genetic variance attributable to either temporal instability and/or random sampling errors was almost three times more important than the pure spatial component. This indicates that not considering signal:noise ratio may lead to an important overestimation of genetic substructuring in situations of weak genetic differentiation. This study also illustrates the usefulness of the member–vagrant hypothesis to generate a priori predictions regarding the number of subpopulations that should compose a species, given its life-history characteristics and habitat structure. On the other hand, a metapopulation model appears better suited to explain the extent of genetic divergence among subpopulations, as well as its temporal persistence, given the reality of habitat patchiness and environment instability. We thus conclude that the combined use of both models may offer a promising avenue for studies aiming to understand the dynamics of genetic structure of species found in unstable environments.

Keywords: member–vagrant, metapopulation, microsatellites, population structure, *Salmo salar*

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Introduction

Theoretical population genetics predicts the role of the evolutionary forces of selection, migration, mutation and genetic drift in shaping genetic diversity as well as their consequences on the fitness of organisms. Most often, empirical studies are designed to describe genetic population structure and make a posteriori inferences about the potential roles of these processes. Less consideration, however, is generally given to the ecological mechanisms that may drive the evolution of differing population genetic architecture in various species (but see Loveless & Hamrick (1984) and Viard *et al*. (1997)). This is partly explained by the paucity of evolutionary models that can predict population structure from both the biological characteristics and ecological requirements of species.

Atlantic salmon have a well-known homing ability (Scheer 1939; Banks 1969; Stabell 1984) enhancing its propensity to generate considerable population subdivision. Consequently, empirical support for a river by river population structure is abundant (Ståhl 1981; Jordan *et al*. 1992; Moran *et al*. 1994; Nielsen *et al*. 1996; O'Reilly *et al*. 1996; Sanchez *et al*. 1996; Fontaine *et al*. 1997; McConnell *et al*.

Correspondence: L. Bernatchez. Fax: 418-656-2043; E-mail: Louis.Bernatchez@bio.ulaval.ca

1997; Tessier *et al.* 1997). Within-river genetic structuring has also been documented, indicating that the population unit of this species may be smaller than the river (Ståhl 1983; Heggberget *et al.* 1986; Ståhl 1987; Crozier & Moffett 1989; Koljonen 1989; Verspoor & Jordan 1989; McElligott & Cross 1991; Verspoor *et al.* 1991; Hurrell & Price 1993). Because none of these studies has been systematically designed to test for potential causes of within-river genetic heterogeneity, it remains unclear whether the magnitude of apparent population subdivision within rivers is more related to the number of tributaries, the distribution of spawning beds within tributaries, and/or isolation-by-distance processes.

Also, most studies have not quantified the amount of apparent spatial genetic variance due to factors other than population subdivision. Waples (1998) recently pointed out that intralocus sampling error can be expected to introduce noise of magnitude $1/2S$ (S = sample size) on estimates of population divergence. Another potential source of error in such studies is related to the so-called 'Allendorf–Phelps effect' (Waples 1998). Allendorf & Phelps (1981) showed that the analysis of progeny produced by a relatively small effective number of breeders (Nb) at different spawning sites may result in apparent genetic differences (of expected magnitude $1/2Nb$) among samples, even though the entire adult population in the river system is panmictic. This is because such sampling does not conform to the assumption implicit to the null hypothesis that the individuals sampled have been drawn randomly from the global population. As pointed out by Waples (1998), the single most effective strategy for dealing with the signal:noise ratio problem introduced by such factors is to replicate samples over time. Thus, patterns of genetic relatedness or genetic differentiation that are consistent across time are unlikely to be caused by sampling artefacts. In this context, the first objective of this study was to describe the extent and pattern of population structure in Atlantic salmon (*Salmo salar* L.) within a river system by using microsatellite DNA loci. A second objective was to establish the importance of quantifying the signal:noise ratio in accurately estimating population structure. To do so, we assessed the temporal stability of population structure, by replicating samples over time.

A third objective was to assess the potential usefulness of two evolutionary models in explaining within-river population structure from the ecological and habitat characteristics of Atlantic salmon. The member–vagrant hypothesis is a model that has been used frequently in the ecological literature for predicting the number of distinct populations that should be found in a particular aquatic species, given its life cycle and habitat characteristics (Iles & Sinclair 1982; Sinclair & Iles 1988; Sinclair 1988). This hypothesis proposes that the number of populations of a given species and the location of their respective spawning grounds are determined by the number and position of habitats that possess physical and biotic characteristics that ensure the retention and maximize the survival of the young following hatching. Retention of young fish in such population-specific nursery areas may be achieved by active behaviour and/or passive mechanisms dictated by the hydrological structure of the habitat. This implies that natural selection will favour individuals that are adapted at young life-history stages to remain in population-specific nursery areas and at the reproductive stage to spawn in areas that ensure progeny have access to these same nursery areas in spite of extensive feeding migrations far from the nursery grounds. Surviving fish that do so are considered 'members' of the population whereas those that do not complete this cycle are considered as 'vagrants' who do not contribute to the population's locally adapted gene pool. Thus, population structure may evolve as a consequence of selective forces that maximize the survival of the young and the probability of encounter among sexually mature individuals of the same gene pool homing to appropriate spawning areas. As proposed by Sinclair (1988), this process should result in reproductive isolation, and strong genetic structure among populations.

Alternatively, McQuinn (1997) proposed that while population substructuring may develop within a given species, the environment may often be too unstable in time to allow for population persistence and isolation leading to the development of locally adapted gene pools. Instead, populations may be in a nonequilibrium state where the degree of genetic structuring is directly linked to the temporal stability of habitats. This is the basis of the metapopulation model which holds that species might exist as sets of local populations largely independent yet interconnected by migration, and possibly submitted to intermittent extinction–recolonization processes (Harrison & Taylor 1997). According to this framework, vagrants are not only an integral part of the population structure but are also necessary for the persistence of the metapopulation.

Based on the inference of both the member–vagrant and metapopulation models, we make the following predictions regarding the within-river population structure of Atlantic salmon. A strong and temporally stable genetic structuring associated with the spawning/nursery habitats (see detailed description in Materials and methods) would support the hypothesis that these habitats are important in determining the evolution of population structure, as proposed by the member–vagrant framework. This would be further supported if the amount of genetic variance explained by the structuring of these habitats is more pronounced than that explained by other physical characteristics, namely different branches within the river, and if genetic distances are correlated to geographical distances as migration is likely to occur between the closest breeding areas. Reciprocally, evidence of nonpanmictic genetic

structure, but absence of temporal stability related to spawning/nursery habitats would indicate that the within-river population structure is better explained by the metapopulation model. This would be further supported if the extent of genetic divergence among subpopulations is weak (but statistically significant), implying straying and high gene flow, and if there is no correlation between genetic and geographical distances. Finally, the absence of genetic structuring would be suggestive of panmixia, and consequently implies that none of the two models applies to salmon at this geographical scale.

Materials and methods

Study site

The Sainte-Marguerite River (48°20′N, 70°00′W) is located approximately 250 km northeast of Quebec City, Canada (Fig. 1). The river has an annual mean discharge of 58 m³/s and is subdivided into two main branches. The 'principal' branch is 101.4 km long, has a catchment area of 1000 km² and is accessible to spawning salmon over its entire length. The 'northeast' branch is 85 km long, has a 1114 km² catchment area and access is limited to the lower 35 km by waterfalls. Spawning activities are also known to occur on the 'northwest branch', the main tributary of the principal branch, and in Xavier creek, one

of the tributaries of the upper part of the northeast branch (Fig. 1). The river is rich in salmon spawning areas that are distributed over both branches and their tributaries (see below).

Salmon life cycle in the Sainte-Marguerite River

Anadromous salmon migrate into the Sainte-Marguerite River in the middle of summer (July and August) to spawn in autumn (October and November). Females dig their nest in the river bed in areas where the substrate is appropriate for spawning (e.g. small gravel particles of diameter ranging from 20 to 30 mm and dispersed small boulders). This kind of substrate allows the retention of the fertilized eggs for the winter period, provides a well-oxygenated environment and protection from potential predators. These spawning grounds, mainly located at the tails of pools and near the beginning of riffles, are characterized by shallow (10–40 cm deep) and moderately fast running (50 cm/s) water (reviewed in Stanley & Trial (1995)). They are most often separated from other similar environmental settings by sandy beaches and other unsuitable substrates for spawning activities. Salmon fry (young of the year) emerge at the end of spring or at the beginning of summer, depending on the water temperature, and subsequently move to nursery grounds that are adjacent to spawning grounds. These nursery
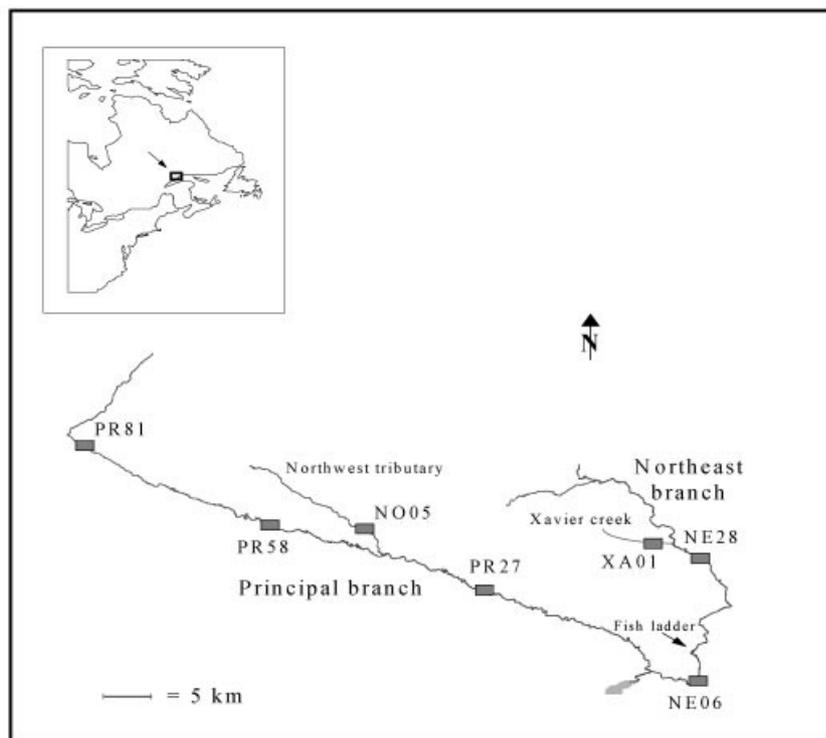
Fig. 1 Localization of sampling sites on the Sainte-Marguerite River in Québec, Canada.

grounds are located in slower running water (end of riffles) where food is abundant and well distributed in the water column. These assemblages of spawning/nursery grounds are hypothesized to be the habitats that determine the within-river population structure of Atlantic salmon within the framework of the member–vagrant hypothesis.

Sampling strategy

Sampling was conducted at a small geographical scale, but spread over the major spawning/nursery grounds of the entire river system. As sampling adults on spawning grounds was logistically difficult and ethically undesirable, we chose to sample salmon fry because this early life-history stage is directly related to reproduction and early development habitats. Fry sampled within spawning/nursery habitats should also reflect the distribution of adults returning to their natal spawning sites. Older developmental stages, such as parr, are likely to disperse within the river system and consequently may not reflect adequately the underlying population structure. On the other hand, sampling emergent fry may inflate the probability of finding statistically significant tests on genetic differentiation among sites (the Allendorf–Phelps effect). Two approaches were used to minimize this effect. First, sampling was conducted over large river stretches (> 200 m²), 1–2 weeks after fry emergence to allow their dispersal within the areas. Second, temporal replicates were analysed to quantify the extent of spatial variation once temporal variation had been accounted for.

All major spawning/nursery habitats of the principal and the northeast branches, as well as those of the northwest branch and Xavier creek were sampled (Fig. 1). One site of the northeast branch differed in location between 1996 (kilometre 28) and 1997 (kilometre 29) because the spawning ground at kilometre 28 was displaced by a summer flood in 1996. Samples consisted of 46–50 Atlantic salmon fry captured at the end of June and early July during two consecutive years (1996 and 1997) at each site (seven sites × 50 individuals × 2 years = 700 fish). Fish were sacrificed and preserved in 95% ethanol until genetic analysis.

Genetic analysis

Total DNA extraction was performed from approximately 30 mg of fin tissue according to Bernatchez et al. (1992) for 1996 samples, and using the simplified cell lysis protocol of Olsen et al. (1996) with slight modifications for 1997 samples. In the latter case, ≈ 2 mm² of caudal fin tissue was digested in 100 µL of lysis buffer (40 mm Tris-HCl [pH 9.0], 50 mm KCl, 0.5% Tween 20, 1 µL of proteinase K at 20 mg/mL) for 12 h at 37 °C with constant mixing movement. Digested samples were then held at 95 °C for

15 min and centrifuged at $16\,250\,g$ for 12 min. Samples were kept frozen at − 20 °C until polymerase chain reaction (PCR) amplification.

Microsatellite polymorphism was analysed using either radioactive or fluorescent detection methods. We performed PCR amplifications at five loci using primers specifically developed for *Salmo salar* (*SSOSL85*, *Ssa85*, *Ssa171*, *Ssa197* and *Ssa202*; Slettan et al. 1995; O'Reilly et al. 1996), and known to be highly polymorphic (O'Reilly et al. 1996; McConnell et al. 1997; Tessier et al. 1997). For the 1996 samples, radioactive PCR was performed in 15 µL reaction volumes containing 1 unit of *Taq* DNA polymerase, 1.56 µL of reaction buffer (10 mm Tris-HCl [pH 9.0], 1.5 mm MgCl$_2$, 0.1% Triton X-100, 50 mm KCl), 1.33 µm of each primer, 75 µm of each dGTP, dCTP, dTTP, 5 µm dATP, 0.15 µL of α[^{35}S]-dATP and 1 µL of total DNA. The following PCR profile was used: one initial denaturing step of 5 min at 95 °C; 35 cycles of 20 s at 94 °C, 20 s at annealing temperature 55 °C (for *SSOSL85*) or 58 °C (for others) and 20 s at 72 °C. Multiplexed PCRs for *Ssa85* and *Ssa197* and for *Ssa171* and *Ssa202* were performed in a Perkin-Elmer 480 DNA thermal cycler. Electrophoresis, fixation, drying and autoradiography followed standard procedures (Sambrook et al. 1989). A M13-mp18 (USB Inc.) sequencing ladder, as well as a subset of standard samples run on all gels were used to estimate allelic size at each locus.

Samples from the 1997 cohort were analysed using fluorescent dyes. One of the primers for each locus was 5′-labelled with two different colours: yellow (HEX) for *Ssa85*, *Ssa197* and *Ssa202* loci and green (TET) for *SSOSL85* and *Ssa 171* loci. PCR was carried out in a 10 µL reaction volume containing 1 unit of *Taq* DNA polymerase, 1.0 µL of reaction buffer described above, 750 µmol of dNTPs, 1.0 µL of total DNA (obtained from the cell lysis method) and primer concentrations ranging from 0.04 to 0.28 pmol/µL (Table 1). Multiplexed PCR was used for *Ssa85*, *Ssa197* and *SSOSL85* and for *Ssa171* and *Ssa202*, and performed in a Perkin-Elmer 9600 thermocycler (version 2.01), with the following profile: an initial denaturing step of 3 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C (same annealing temperature for both multiplexes) and 30 s at 72 °C. The samples were heated to 95 °C for 5 min and chilled on ice prior to gel loading. Electrophoresis procedures were conducted on a denaturing 5% poly-acrylamide gel with an ABI 377 automated sequencer/genescanner and analysis software. Loading product consisted of 0.2 µL of internal sizing standard (red colour TAMRA 350 bp) and 2 µL of deionized formamide that were combined to 1 µL for each PCR reaction. Gels were run for 2.25 h at 3000 V. Analysis of each sample was made automatically and allelic size was determined (using the GENESCAN software, version 2.1; ABI 1996a) by reference to the internal sizing standard and by

Multiplexed amplification sets	Annealing temperature (°C)	Primer concentration (μM)		
Radioactive labelling				
Ssa 85-Ssa197	58	1.33		
Ssa171-Ssa202	58	1.33		
SSOSL85	55	1.33		
Fluorescent labelling		HEX (yellow)		TET (green)
Ssa85-Ssa197-SSOSL85	56	Ssa85 0.04		SSOSL85 0.25
		Ssa197 0.15		
Ssa171-Ssa202	56	Ssa202 0.20		Ssa171 0.28

Table 1 Polymerase chain reaction (PCR) amplification set composition for both radioactive and fluorescent labelling. The PCR annealing temperature and primer concentration at each locus are given for each amplification procedure

comparison with the same standard sample of known allelic size that was run on each gel. The final scoring of allelic size and tabulation of data for each locus were conducted with the GENOTYPER software, version 2.0 (ABI 1996b). Similarity of the allelic identification methods in both radioactive and fluorescent cases was ensured by comparing 30 individuals with the two detection methods. No differences were found between the allele scoring results of these individuals.

Data analysis

Genetic diversity was quantified over all and for each sample by the number of alleles per locus, and observed and expected heterozygosity. Conformity with Hardy–Weinberg equilibrium at each locus and in each sample was tested using the score test (*U*-test) described in Rousset & Raymond (1995). This test was performed using GENEPOP software, version 3.0 (Raymond & Rousset 1995a).

We also used GENEPOP to compute unbiased estimates of the *P*-value of the homogeneity test (Raymond & Rousset 1995b), among all pairs of samples for all loci to test for genic differentiation under the null hypothesis that allelic distribution is identical among samples. We then quantified the extent of genetic differentiation among samples from different sites and years by computing pairwise F_{ST} values according to Weir & Cockerham (1984) and using ARLEQUIN version 1.1 (Schneider *et al*. 1997). Significance values were obtained by a permutation procedure (10 000 permutations) and adjusted for multiple simultaneous tests using the sequential Bonferroni correction (Holm 1979; Rice 1989), with an initial α-value of $0.05/k$, *k* being the number of pairwise comparisons (number of pairwise comparisons = 91 in this case). Isolation by distance was tested by Mantel tests (Mantel 1967) performed between pairwise estimates of $[F_{ST}(1 - F_{ST})]$ and geographical distance for all samples of both years separated (Rousset 1997).

We further estimated the extent of differentiation among samples using the chord distance (D_{CE}) of Cavalli-Sforza & Edwards (1967) using a program written by J.-M. Cornuet (INRA, Laboratoire de Neurobiologie Comparée des Invertébrés, Bures-sur-Yvettes, France). We chose this measure because it requires no assumptions regarding mode of mutation among loci, and leads to a higher probability of depicting the correct tree topology among closely related populations (Takezaki & Nei 1996; Angers & Bernatchez 1998). The resulting distance matrix was used to build a neighbour-joining (NJ) phenogram for which confidence statements on branching patterns were generated by bootstrapping over loci (10 000 replicates).

The significance of the spatial variation in gene diversity among sites independently of that of temporal variation within sites was estimated in two ways. We first performed a hierarchical analysis of gene diversity using the analysis of molecular variance model (AMOVA), as described in Michalakis & Excoffier (1996) and available in ARLEQUIN, in order to assess the component of genetic diversity (based on allelic frequency) attributable to (i) variance among river branches; (ii) variance among sampling sites (geographical component); (iii) variance between temporal samples within sites (temporal component); and (iv) variance among individuals within samples. The significance of the variance components associated with the different possible levels of genetic structure was also tested using nonparametric permutation procedures (Excoffier *et al*. 1992). Finally, we statistically assessed differences in temporal and spatial comparisons for (i) the number of loci showing significant differences in allelic frequencies; (ii) pairwise F_{ST}; and (iii) D_{CE} values, averaged for both interannual and intersite comparisons, by performing a parametric *t*-test on dependent variables using STATISTICA (version 4.3) software (Statistica 1994).

Results

Microsatellite polymorphism

All loci were highly polymorphic within each of the 14 samples analysed. The number of alleles per locus varied

Table 2 Sample size (N), number of total alleles per locus (A), F_{IS}, observed (H_O) and expected (H_E) heterozygosity for each locus and sample and estimated number of breeders (Nb) with associated standard error (SE)

Locus	A		1997							1996						
			PR27	PR58	PR81	NO05	NE06	NE29	XA01	PR27	PR58	PR81	NO05	NE06	NE28	XA01
Ssa85	14	A	9	8	8	5	7	6	6	7	8	10	6	8	6	8
		F_{IS}	+0.042	−0.085	−0.064	−0.040	−0.029	−0.102	+0.067	+0.007	+0.048	+0.085	−0.087	−0.072	+0.049	+0.017
		H_O	0.68	0.82	0.61	0.77	0.74	0.65	0.64	0.66	0.74	0.66	0.73	0.80	0.73	0.67
		H_E	0.71	0.76	0.58	0.74	0.72	0.59	0.69	0.66	0.78	0.72	0.67	0.74	0.77	0.68
Ssa171	33	A	17	18	14	16	16	18	11	14	16	14	15	18	17	18
		F_{IS}	−0.014	−0.096	−0.115	−0.074	+0.030	−0.059	−0.041	+0.015	+0.019	−0.007	+0.033	+0.049	+0.036	−0.019
		H_O	0.90	0.96	0.94	0.88	0.82	0.93	0.88	0.84	0.83	0.88	0.81	0.86	0.88	0.92
		H_E	0.89	0.88	0.84	0.82	0.84	0.88	0.85	0.85	0.84	0.87	0.84	0.90	0.91	0.90
Ssa197	19	A	11	11	10	11	13	13	13	11	11	11	13	14	13	12
		F_{IS}	+0.016	−0.070	−0.067	−0.105	+0.017	+0.029	−0.029	−0.118	−0.037	−0.022	−0.129	−0.079	−0.041	−0.115
		H_O	0.82	0.94	0.94	0.96	0.86	0.85	0.92	0.96	0.91	0.88	0.96	0.92	0.92	0.94
		H_E	0.83	0.88	0.88	0.87	0.87	0.87	0.89	0.86	0.88	0.86	0.85	0.85	0.88	0.84
Ssa202	16	A	12	12	8	10	13	11	9	12	10	10	6	9	9	10
		F_{IS}	+0.026	−0.015	+0.112	−0.014	−0.033	−0.077	+0.017	−0.060	−0.118	−0.023	+0.026	−0.137	−0.038	−0.089
		H_O	0.82	0.88	0.71	0.73	0.88	0.91	0.76	0.92	0.89	0.84	0.73	0.92	0.84	0.94
		H_E	0.84	0.87	0.80	0.72	0.85	0.85	0.77	0.87	0.80	0.82	0.75	0.81	0.81	0.86
SSOSL85	15	A	10	9	9	11	13	10	10	12	11	11	11	12	12	12
		F_{IS}	−0.104	−0.009	−0.189	−0.015	−0.005	−0.029	−0.085	+0.012	−0.120	−0.023	+0.070	+0.088	+0.048	−0.006
		H_O	0.96	0.88	0.92	0.90	0.86	0.83	0.74	0.84	0.91	0.86	0.79	0.76	0.84	0.75
		H_E	0.87	0.87	0.77	0.88	0.86	0.80	0.81	0.85	0.82	0.84	0.85	0.83	0.88	0.75
		N	50	50	49	48	50	46	50	50	46	50	48	49	49	48
	H_E		0.83	0.85	0.77	0.81	0.83	0.80	0.80	0.82	0.82	0.82	0.79	0.83	0.85	0.81
	A		12	12	10	11	12	12	10	11	11	11	10	12	11	12
Nb			86.7	26.0	19.3	30.1	17.2	38.8	43.6	41.1	62.8	31.1	41.8	44.6	76.4	35.3
SE			139.6	20.7	13.7	26.2	11.7	39.4	45.0	41.0	85.7	27.0	43.2	47.2	114.2	33.2

from 14 at *Ssa85* to 33 at *Ssa171* and expected heterozygosity ranged from 0.58 to 0.91 depending on locus and population (Table 2). The per-sample number of alleles and expected heterozygosity averaged over loci varied between 10 and 12 and 0.77 and 0.85, respectively. Details of allele frequency distribution are presented in Appendix I. No significant departures from Hardy–Weinberg equilibrium were detected (Table 2) within samples using the Markov chain method for estimating P-values (initial $\alpha = 0.0007$ with Bonferroni sequential correction for 70 comparisons). These results suggested that sampling effects related to small numbers of families were limited.

Genetic differentiation among samples

The number of loci showing significant differences in allele frequency distribution varied between one and five depending on comparisons. This also translated into significant F_{ST} values and non-null D_{CE} values between all pairwise spatial or temporal comparisons, except three involving NE28–96 (Table 3). The overall F_{ST} was

0.034. Globally, these results provided a first indication of significant allelic variance on both spatial and temporal axes.

Spatial vs. temporal components of genetic variance

No significant genetic variance ($P = 0.1673$) was attributable to the groupings of sampling sites by river branches (Table 4). Most of the intersample genetic variance (2.5%) was attributable to interannual sample-within-site differentiation. Nevertheless, a significant component of variance remained attributable to spatial variation (0.9%, $P = 0.001$). This indicated that temporal changes in allelic frequency distribution was important but did not override the persistence of spatial structuring associated with spawning/ nursery habitats.

Further evidence for the temporal persistence of spatial genetic structuring was provided by the comparisons of the number of loci showing significant differences in allele frequency distribution, pairwise F_{ST} and D_{CE} values. In all instances, the mean values of these parameters were statistically higher for comparisons involving spatial

Table 3 Pairwise F_{ST} values (above diagonal) and Cavalli-Sforza and Edwards' chord distance (D_{CE}) values (below diagonal); bold characters: pairwise comparisons with nonsignificant allele frequency differences. The initial α level for Bonferroni sequential correction for 91 comparisons is 0.0006

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) PR27–97		0.0261	0.0476	0.0338	0.0214	0.0288	0.0339	0.0129	0.0194	0.0232	0.0363	0.0187	**0.0077**	0.0296
(2) PR58–97	0.0252		0.0490	0.0433	0.0273	0.0413	0.0294	0.0175	0.0260	0.0184	0.0477	0.0174	**0.0128**	0.0409
(3) PR81–97	0.0395	0.0314		0.0874	0.0488	0.0431	0.0513	0.0305	0.0369	0.0380	0.0857	0.0392	0.0417	0.0445
(4) NO05–97	0.0260	0.0365	0.0436		0.0418	0.0452	0.0565	0.0432	0.0483	0.0411	0.0396	0.0423	0.0269	0.0549
(5) NE06–97	0.0311	0.0365	0.0461	0.0375		0.0282	0.0363	0.0192	0.0255	0.0257	0.0582	0.0169	0.0140	0.0290
(6) NE29–97	0.0275	0.0347	0.0424	0.0310	0.0337		0.0338	0.0201	0.0323	0.0380	0.0464	0.0296	0.0192	0.0291
(7) XA01–97	0.0354	0.0300	0.0397	0.0410	0.0389	0.0358		0.0305	0.0222	0.0296	0.0728	0.0270	0.0193	0.0352
(8) PR27–96	0.0187	0.0212	0.0307	0.0271	0.0323	0.0276	0.0327		0.0198	0.0215	0.0480	0.0196	**0.0113***	0.0280
(9) PR81–96	0.0270	0.0231	0.0283	0.0371	0.0365	0.0370	0.0268	0.0223		0.0148	0.0498	0.0229	0.0143	0.0353
(10) PR58–96	0.0229	0.0230	0.0332	0.0308	0.0327	0.0319	0.0281	0.0246	0.0232		0.0457	0.0160	0.0142	0.0305
(11) NO05–96	0.0316	0.0420	0.0548	0.0299	0.0539	0.0371	0.0537	0.0388	0.0433	0.0338		0.0427	0.0313	0.0540
(12) NE06–96	0.0265	0.0257	0.0376	0.0333	0.0298	0.0275	0.0300	0.0272	0.0292	0.0221	0.0346		**0.0091**	0.0232
(13) NE28–96	0.0224	0.0172	0.0305	0.0291	0.0259	0.0243	0.0213	0.0234	0.0232	0.0178	0.0308	0.0157		0.0241
(14) XA01–96	0.0336	0.0369	0.0428	0.0356	0.0369	0.0336	0.0348	0.0312	0.0372	0.0306	0.0384	0.0293	0.0285	

*Pairwise comparison of NE28–96 vs. PR27–96 has a P-value = 0.001.

Variance component	d.f.	% total variance	F-statistic	P
Among river branches	1	0.1	0.0014	0.167
Among sampling sites	6	0.9	0.0085	0.001
Among years within sampling sites	7	2.5	0.0255	< 0.0001
Within samples	1352	96.6	0.0337	< 0.0001

Table 4 Hierarchical analysis of molecular variance (AMOVA) of microsatellite loci allele frequencies among samples of anadromous Atlantic salmon within the Sainte-Marguerite River

| Comparison components | Mean | | d.f. | P-value |
	Temporal	Spatial		
No. of significantly different loci	2.29 ± 1.11	3.54 ± 0.55	6	0.0036
F_{ST}	0.0256 ± 0.0112	0.0370 ± 0.0086	6	0.0010
Chord distance	0.0270 ± 0.0053	0.0339 ± 0.0036	6	0.0036

Table 5 t-test results for dependent variables for comparisons of pairwise number of loci showing significant heterogeneity in allelic distribution, F_{ST} values and chord distances between temporal samples of same locations vs. spatial samples within the same year of sampling

samples within years than for those involving temporal samples within sites (Table 5).

No significant relationships between genetic and geographical distances were detected within each cohort (Mantel test: P = 0.55 for 1996 and P = 0.06 for 1997). The overall lack of correspondence of genetic and geographical distances is also illustrated in the D_{CE} phenogram (Fig. 2). Namely, salmon from the northwest branch, a tributary of the principal branch, were the most distinct of all, independent of geographical proximity. In contrast, salmon from sampling site NE28–96 were the most similar to salmon from any other sites independent of geographical distance (Table 3). It is noteworthy, however, that the NJ phenogram illustrates a tendency to group temporal samples by site of origin, providing additional evidence for the existence of partial temporal stability in population structuring within the river system. Namely, a grouping of temporal samples of the northwest branch was supported by a very high (90%) bootstrap value. Although less important, the grouping of temporal samples for sites PR81 and PR27 (located 54 km apart within the same river branch) was supported by bootstrap values exceeding the majority-rule criterion of 50%. Finally, although NE06 samples did not cluster together, the branch length between them was smaller than with any other sample. The other three sampling sites, however, did not show any tendency of temporal persistence of genetic composition.
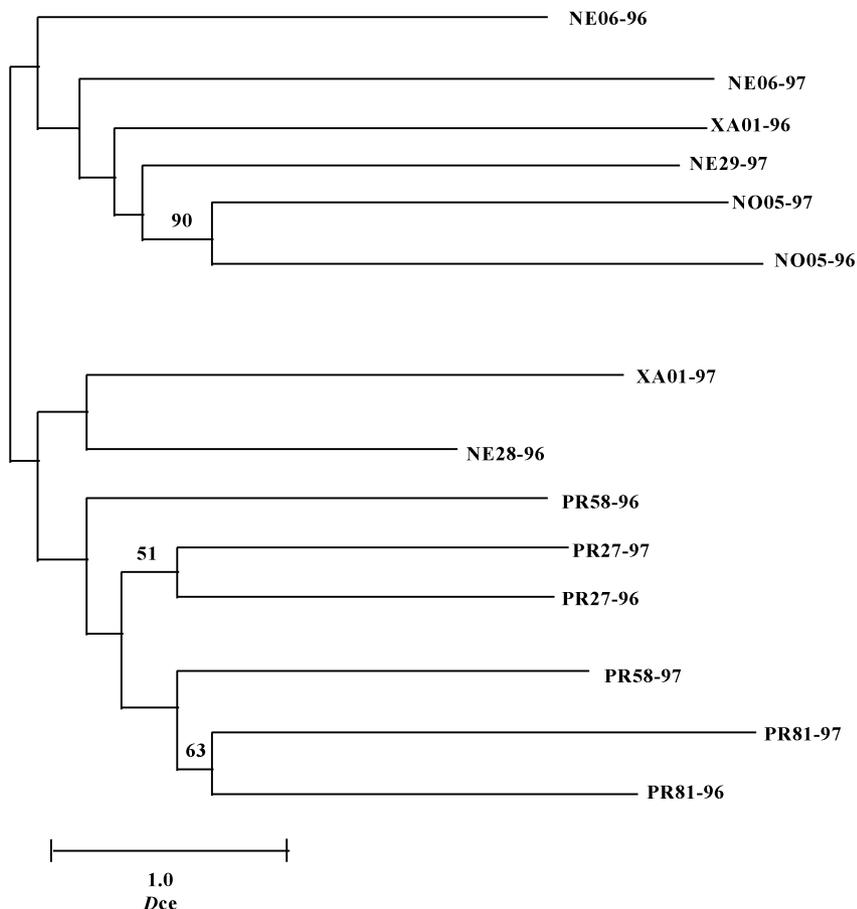
Discussion

Spatiotemporal variation of within-river genetic structuring

As previously reported, this study confirmed the much higher polymorphism of microsatellite loci in Atlantic salmon compared with traditional markers. This variation provided sufficient resolution to refute the hypothesis of homogeneity in allele frequency (and related F_{ST} and D_{CE} values) within a river on a scale of a few kilometres, in some cases. A highly significant, although weak, spatial patterning associated with the number of spawning/ nursery units was observed after accounting for variance among temporal replicates within sites. This resulted in lower genetic distances between temporal samples at four sampling sites than with any other sites for the same cohort. In contrast, no evidence for temporal stability of genetic composition was observed for the three other sites.

We first compare our results with previous studies in order to infer the importance of quantifying the signal: noise ratio for more accurately estimating the extent of spatial population structure. None of the previous studies of within-river genetic structure in Atlantic salmon was based on microsatellite analysis and none comprised samples collected within short geographical distances (20–55 km) along the same river branch over the major spawning beds. Despite these analytical differences, the extent of overall genetic differentiation observed ($F_{ST} = 0.034$ without accounting for variation between temporal replicates) is similar to values reported previously, which ranged from 0.7 to 3.6% (Ståhl 1987; Verspoor & Jordan 1989; McElligott & Cross 1991; Jordan *et al.* 1992; Galvin *et al.* 1996; Sanchez *et al.* 1996). With few exceptions, however, none of these studies has systematically assessed the extent of temporal genetic variation within putative subpopulations. It is therefore impossible in such cases to firmly refute the hypothesis that genetic heterogeneity among samples was more related to sampling variance and/or temporal stochasticity of allelic composition rather than to partial reproductive isolation among subpopulations. In contrast, the systematic analysis of replicate cohorts for all sampling sites, coupled with a hierarchical analysis of gene diversity (AMOVA), allowed quantification of the influence of temporal and/or sampling variance, thus confirming the temporal persistence

of population substructuring within the Sainte-Marguerite River for four of the seven sampling sites. This analysis, however, also revealed that the component of genetic variance attributable to other factors was nearly three times more important (2.5% vs. 0.9%) than the pure spatial component. This suggests that not considering such an effect may have led to an important overestimation of genetic substructuring in most previous studies. Clearly, an important component of genetic variance is associated with either sampling variance or changes in allelic composition due to the nonstability of genetic structure in time. Although these two effects cannot easily be separated with our sampling design, both imply an overestimation of population structuring if not taken into account. The extent of such overestimation, however, cannot easily be generalized. For instance, Tessier & Bernatchez (1999) found no significant component of genetic variance among temporal samples over a period of three to five generations. In contrast, in one of the only few other studies on Atlantic salmon in which temporal stability was assessed systematically, Jordan *et al.* (1992) estimated that components of genetic variance attributable to variation within cohorts, among sites within year, and among cohorts within river were congruent with those we observed (Table 4).

It could be argued that the apparent existence or absence of temporal stability depending on sites may be related to factors other than habitat stability. Namely, differences among sites could be related to the differential Allendorf–Phelps effect, whereby sites with lower effective numbers of breeders (*Nb*) would be more prone to temporal stochasticity in genetic composition (Waples 1998). To test for this possibility, we quantified the effective numbers of breeders (*Nb*) for each site, using the method of Hill (1981), and as recommended by Waples (1998). This was performed by programming the equations of Hill (1981) using the algebraic computer system MAPLE V. Globally, *Nb* estimates averaged 43, and varied between 17 and 87, depending on sites (Table 2). There was no association between the absence of temporal stability in genetic composition and *Nb* estimates (Pearson's correlation coefficient between *Nb* and F_{ST} estimates: $r^2 = 0.22$, $P = 0.288$). Furthermore, estimates for the most unstable sites NE28-NE29 and XA01 ranked among the highest estimates, whereas the lowest values were observed in temporally stable sites, such as PR81. Also, estimates varied between both years of sampling in an unpredictable manner. Although not ruling out the possibility of the Allendorf–Phelps effect in the system, the absence of correspondence between *Nb* and temporal stability is indicative that other factors, namely habitat instability and intralocus sampling error, must also be taken into consideration to explain the variation in the temporal stability of genetic composition at the different sites.

Predicting population structure from evolutionary models

Our results partly support the prediction that assemblages of spawning/nursery grounds determine the within-river population structure of Atlantic salmon within the framework of the member–vagrant hypothesis. Thus, we found a significant genetic differentiation among all sampling sites of the river system, with smaller genetic differentiation between temporal samples than among geographical samples for four sampling sites. These results therefore suggest that the member–vagrant model is useful in making general predictions regarding the expected number of subpopulations within a river system for Atlantic salmon. On the other hand, the weak and temporally unstable amount of genetic divergence among some of the sampling sites did not provide evidence for strong and persistent reproductive isolation, as inferred by the member–vagrant hypothesis. While potentially useful to predict the number of genetically distinct populations, the inference of strong reproductive isolation may render the member–vagrant hypothesis too rigid to realistically predict the extent of genetic divergence among Atlantic salmon populations, especially in the face of unstable environments. A case in point to illustrate the instability of genetic structure which could result from unpredictable environmental changes is the upper northeast branch spawning/nursery site (NE28) which was displaced by a major summer flood in 1996, becoming NE29 in 1997. The relatively high genetic differentiation between samples collected at NE28 and NE29 suggested that the new spawning grounds were not mainly recolonized by salmon associated with site NE28 but instead, by salmon born in other sections of the river.

Consequently, Atlantic salmon is neither panmictic nor very strongly genetically subdivided within the river. This pattern is thus more compatible with a metapopulation model operating on a small geographical scale. Given the evolutionary benefits of homing behaviour, namely that of increasing the probability of both finding mates to reproduce and suitable habitats for early life-history survival (reviewed in Dodson (1997)), selective forces will tend to favour the development of many subpopulations associated with favourable environmental structures. On the other hand, the temporal persistence of such subpopulations will be a direct function of that of suitable habitats. In situations of habitat instability, more benefits may be gained from vagrancy because of the potentially adaptive importance for the colonization of new habitats (Olivieri *et al.* 1990; Quinn & Dittman 1990). In this view, species such as Atlantic salmon may oscillate between vagrancy during periods of habitat instability and precise homing once a population is established in a more stable environment.

In summary, there is clear evidence of within-river population substructuring in Atlantic salmon, although its importance has probably been overestimated in the majority of previous studies. This emphasizes the benefits than can be obtained by performing the analysis of temporal replicates, in order to more precisely estimate the pattern and magnitude of population differentiation, especially in situations of low divergence. This study also illustrates the usefulness of the member–vagrant hypothesis to generate a priori predictions regarding the number of subpopulations that should compose a species, given its life-history characteristics and habitat structure. On the other hand, a metapopulation model appears better suited to explain the extent of genetic divergence among subpopulations, as well as its temporal persistence, given the reality of habitat patchiness and environmental instability. We thus conclude that the combined use of both models may offer a promising avenue for studies aiming to understand the dynamics of the genetic structure of species found in unstable environments.

Acknowledgements

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Appendix I Table of allelic frequencies at each locus and population

	Alleles													
Sites	111	113	115	125	127	129	131	133	135	137	139	145	147	151
Locus: *Ssa85*														
PR27–97	0.140	0.000	0.040	0.010	0.490	0.000	0.040	0.110	0.150	0.000	0.010	0.000	0.000	0.010
PR58–97	0.350	0.000	0.010	0.020	0.310	0.000	0.130	0.070	0.100	0.000	0.000	0.010	0.000	0.000
PR81–97	0.082	0.010	0.041	0.000	0.633	0.000	0.133	0.020	0.041	0.000	0.000	0.000	0.041	0.000
NO05–97	0.240	0.000	0.000	0.000	0.302	0.000	0.021	0.323	0.115	0.000	0.000	0.000	0.000	0.000
NE06–97	0.210	0.000	0.000	0.020	0.420	0.000	0.030	0.060	0.250	0.000	0.010	0.000	0.000	0.000
NE29–97	0.163	0.000	0.000	0.022	0.609	0.000	0.033	0.076	0.098	0.000	0.000	0.000	0.000	0.000
XA01–97	0.080	0.000	0.000	0.040	0.520	0.000	0.120	0.120	0.120	0.000	0.000	0.000	0.000	0.000
PR27–96	0.190	0.010	0.010	0.000	0.520	0.000	0.010	0.100	0.160	0.000	0.000	0.000	0.000	0.000
PR81–96	0.100	0.000	0.000	0.010	0.480	0.000	0.040	0.070	0.190	0.040	0.010	0.000	0.030	0.030
PR58–96	0.196	0.022	0.000	0.054	0.370	0.011	0.207	0.098	0.043	0.000	0.000	0.000	0.000	0.000
NO05–96	0.313	0.000	0.000	0.000	0.469	0.010	0.094	0.021	0.094	0.000	0.000	0.000	0.000	0.000
NE06–96	0.265	0.000	0.000	0.031	0.408	0.010	0.092	0.071	0.112	0.010	0.000	0.000	0.000	0.000
NE28–96	0.163	0.000	0.000	0.061	0.388	0.000	0.122	0.082	0.184	0.000	0.000	0.000	0.000	0.000
XA01–96	0.219	0.000	0.000	0.021	0.510	0.000	0.031	0.073	0.115	0.021	0.000	0.010	0.000	0.000

	178	182	184	186	188	190	192	194	196	198	200	202	204	206	208
Locus: *SSOSL-85*															
PR27–97	0.000	0.210	0.210	0.120	0.050	0.090	0.000	0.080	0.010	0.060	0.070	0.000	0.100	0.000	0.000
PR58–97	0.000	0.070	0.210	0.170	0.040	0.090	0.000	0.070	0.000	0.080	0.160	0.000	0.110	0.000	0.000
PR81–97	0.000	0.082	0.429	0.051	0.092	0.020	0.000	0.000	0.143	0.051	0.082	0.000	0.051	0.000	0.000
NO05–97	0.000	0.115	0.094	0.167	0.083	0.063	0.000	0.000	0.125	0.010	0.177	0.000	0.135	0.010	0.021
NE06–97	0.000	0.130	0.300	0.100	0.030	0.080	0.010	0.010	0.010	0.070	0.110	0.020	0.030	0.100	0.000
NE29–97	0.000	0.174	0.304	0.065	0.011	0.087	0.000	0.022	0.000	0.022	0.261	0.000	0.033	0.022	0.000
XA01–97	0.000	0.050	0.310	0.150	0.060	0.020	0.000	0.020	0.010	0.000	0.260	0.000	0.060	0.060	0.000
PR27–96	0.000	0.130	0.280	0.100	0.030	0.130	0.010	0.010	0.020	0.110	0.150	0.010	0.020	0.000	0.000
PR81–96	0.000	0.160	0.250	0.230	0.120	0.030	0.000	0.020	0.010	0.030	0.080	0.000	0.060	0.010	0.000
PR58–96	0.000	0.065	0.293	0.283	0.022	0.054	0.011	0.043	0.000	0.011	0.098	0.000	0.065	0.054	0.000
NO05–96	0.010	0.146	0.052	0.167	0.125	0.021	0.000	0.052	0.052	0.073	0.021	0.000	0.281	0.000	0.000
NE06–96	0.000	0.061	0.367	0.051	0.010	0.071	0.031	0.020	0.051	0.092	0.092	0.000	0.112	0.041	0.000
NE28–96	0.000	0.122	0.224	0.112	0.041	0.061	0.010	0.071	0.020	0.041	0.184	0.000	0.061	0.051	0.000
XA01–96	0.010	0.010	0.438	0.094	0.031	0.021	0.000	0.010	0.073	0.021	0.229	0.000	0.042	0.021	0.000

	152	156	160	164	168	172	176	180	184	188	192	196	200	204	208	212	216	220	232
Locus: *Ssa197*																			
PR27–97	0.000	0.000	0.060	0.000	0.030	0.330	0.100	0.060	0.190	0.080	0.030	0.000	0.000	0.040	0.050	0.030	0.000	0.000	0.000
PR58–97	0.000	0.020	0.130	0.000	0.090	0.100	0.220	0.100	0.140	0.020	0.000	0.010	0.110	0.060	0.000	0.000	0.000	0.000	0.000
PR81–97	0.000	0.000	0.061	0.020	0.102	0.092	0.224	0.082	0.071	0.143	0.000	0.000	0.071	0.133	0.000	0.000	0.000	0.000	0.000
NO05–97	0.000	0.000	0.104	0.021	0.042	0.156	0.177	0.031	0.198	0.135	0.000	0.010	0.010	0.115	0.000	0.000	0.000	0.000	0.000
NE06–97	0.020	0.000	0.170	0.000	0.040	0.160	0.220	0.000	0.080	0.100	0.010	0.010	0.030	0.010	0.080	0.000	0.070	0.000	0.109
NE29–97	0.000	0.054	0.043	0.000	0.250	0.120	0.174	0.022	0.076	0.065	0.011	0.011	0.000	0.043	0.022	0.000	0.000	0.030	0.109
XA01–97	0.000	0.160	0.200	0.030	0.130	0.050	0.080	0.080	0.080	0.080	0.020	0.000	0.000	0.030	0.030	0.000	0.000	0.030	0.000
PR27–96	0.000	0.020	0.070	0.030	0.090	0.170	0.280	0.030	0.100	0.080	0.000	0.000	0.000	0.040	0.000	0.090	0.000	0.000	0.000
PR81–96	0.000	0.010	0.130	0.020	0.140	0.080	0.150	0.230	0.010	0.160	0.000	0.000	0.050	0.020	0.000	0.000	0.000	0.000	0.000
PR58–96	0.000	0.000	0.152	0.000	0.065	0.120	0.196	0.120	0.087	0.152	0.022	0.000	0.022	0.022	0.043	0.000	0.000	0.000	0.000
NO05–96	0.000	0.000	0.010	0.010	0.156	0.198	0.188	0.021	0.135	0.198	0.042	0.010	0.010	0.010	0.010	0.000	0.000	0.000	0.000
NE06–96	0.000	0.010	0.224	0.010	0.051	0.184	0.235	0.071	0.051	0.031	0.071	0.020	0.010	0.000	0.010	0.000	0.000	0.000	0.020
NE28–96	0.000	0.020	0.051	0.000	0.112	0.224	0.184	0.031	0.102	0.061	0.041	0.020	0.082	0.041	0.031	0.000	0.000	0.000	0.000
XA01–96	0.000	0.042	0.073	0.135	0.021	0.260	0.083	0.031	0.042	0.250	0.021	0.000	0.000	0.021	0.021	0.000	0.000	0.000	0.000

	257	261	265	277	281	285	289	293	297	301	305	309	313	317	321	329
Locus: *Ssa202*																
PR27–97	0.000	0.000	0.030	0.020	0.040	0.010	0.000	0.040	0.080	0.150	0.260	0.240	0.030	0.080	0.020	0.000
PR58–97	0.000	0.000	0.000	0.010	0.090	0.010	0.010	0.170	0.060	0.200	0.130	0.190	0.070	0.050	0.000	0.010
PR81–97	0.000	0.000	0.000	0.000	0.316	0.000	0.051	0.000	0.092	0.041	0.224	0.061	0.194	0.020	0.000	0.000
NO05–97	0.000	0.000	0.031	0.031	0.021	0.073	0.000	0.021	0.021	0.177	0.479	0.135	0.010	0.000	0.000	
NE06–97	0.000	0.010	0.010	0.020	0.000	0.020	0.050	0.140	0.160	0.060	0.250	0.190	0.070	0.010	0.010	0.000
NE29–97	0.022	0.000	0.011	0.163	0.033	0.043	0.000	0.011	0.022	0.065	0.174	0.261	0.163	0.033	0.000	0.000
XA01–97	0.000	0.000	0.040	0.000	0.020	0.010	0.030	0.000	0.110	0.340	0.160	0.280	0.000	0.010	0.000	0.000
PR27–96	0.000	0.000	0.000	0.020	0.020	0.030	0.020	0.040	0.150	0.100	0.170	0.220	0.150	0.060	0.020	0.000
PR81–96	0.000	0.000	0.000	0.000	0.040	0.000	0.010	0.060	0.050	0.120	0.310	0.240	0.040	0.090	0.040	0.000
PR58–96	0.000	0.000	0.000	0.000	0.022	0.011	0.000	0.022	0.065	0.120	0.359	0.174	0.174	0.022	0.033	0.000
NO05–96	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.125	0.240	0.365	0.229	0.021	0.000	0.000
NE06–96	0.000	0.000	0.000	0.010	0.102	0.000	0.010	0.041	0.031	0.194	0.286	0.245	0.082	0.000	0.000	0.000
NE28–96	0.000	0.000	0.000	0.000	0.051	0.020	0.010	0.020	0.092	0.143	0.255	0.306	0.102	0.000	0.000	0.000
XA01–96	0.000	0.000	0.000	0.000	0.021	0.167	0.021	0.083	0.042	0.146	0.208	0.177	0.115	0.021	0.000	0.000

Appendix I *Continued*

	Sites													
Alleles	PR27–97	PR58–97	PR81–97	NO05–97	NE06–97	NE29–97	XA01–97	PR27–96	PR81–96	PR58–96	NO05–96	NE06–96	NE28–96	XA01–96
Locus: *Ssa171*														
217	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
219	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000
221	0.010	0.000	0.000	0.000	0.020	0.011	0.000	0.000	0.000	0.011	0.000	0.031	0.010	0.000
223	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.031	0.000	0.000	0.000
225	0.050	0.030	0.000	0.010	0.000	0.000	0.000	0.020	0.000	0.000	0.010	0.031	0.000	0.000
229	0.000	0.000	0.031	0.000	0.010	0.098	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000
231	0.000	0.100	0.031	0.000	0.110	0.010	0.010	0.000	0.000	0.000	0.000	0.010	0.051	0.021
233	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
235	0.170	0.200	0.306	0.104	0.150	0.098	0.230	0.240	0.220	0.228	0.010	0.163	0.102	0.125
237	0.010	0.000	0.000	0.000	0.010	0.065	0.010	0.000	0.000	0.000	0.031	0.031	0.000	0.042
239	0.210	0.180	0.184	0.375	0.260	0.261	0.250	0.160	0.200	0.283	0.198	0.194	0.194	0.219
241	0.130	0.190	0.143	0.042	0.000	0.033	0.100	0.240	0.100	0.152	0.031	0.020	0.102	0.010
243	0.120	0.010	0.010	0.146	0.070	0.043	0.050	0.030	0.050	0.043	0.094	0.143	0.082	0.000
245	0.050	0.020	0.020	0.010	0.020	0.022	0.030	0.040	0.070	0.022	0.021	0.020	0.051	0.094
247	0.020	0.040	0.041	0.000	0.030	0.011	0.000	0.000	0.030	0.000	0.000	0.000	0.031	0.010
249	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
251	0.000	0.010	0.010	0.010	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.010
253	0.010	0.010	0.000	0.052	0.020	0.000	0.000	0.050	0.030	0.011	0.000	0.010	0.000	0.000
255	0.040	0.010	0.071	0.104	0.230	0.130	0.030	0.040	0.040	0.022	0.021	0.031	0.051	0.042
257	0.000	0.080	0.082	0.000	0.000	0.022	0.140	0.040	0.150	0.011	0.010	0.061	0.133	0.000
259	0.060	0.050	0.000	0.052	0.000	0.109	0.000	0.070	0.040	0.033	0.260	0.082	0.041	0.125
261	0.020	0.020	0.000	0.042	0.000	0.033	0.030	0.030	0.000	0.043	0.219	0.031	0.041	0.052
263	0.010	0.020	0.041	0.010	0.010	0.011	0.020	0.010	0.010	0.065	0.021	0.051	0.061	0.104
265	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.020	0.031
267	0.070	0.010	0.020	0.010	0.010	0.022	0.000	0.000	0.000	0.000	0.010	0.000	0.010	0.021
269	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000	0.021
271	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
273	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
275	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000
277	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.030	0.000	0.000	0.000	0.000	0.000
279	0.010	0.000	0.000	0.010	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
281	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031
287	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.031