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Genetic history of the population of Atlantic salmon, *Salmo salar* L., under restoration in the Connecticut River, USA

Fernando Ayllon, Jose L. Martinez, Francis Juanes, Stephen Gephard, and Eva Garcia-Vazquez

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The Connecticut River lost its Atlantic salmon population as a result of human activity 200 years ago. Cultured stocks, derived mainly from the Penobscot River, were employed to restore the population, and an annual run of salmon has been successfully re-established, although the population is not yet self-sustaining. We examined variation at microsatellite loci in historical scale and modern tissue samples to evaluate the degree and direction of any genetic changes that have occurred in the introduced population. The current genetic pattern of the Connecticut River population is very similar to that of its Penobscot River donor population. We found no differences in heterozygosity, mean number of alleles per locus, number of migrants, or F_{ST} values between the two populations, suggesting that no genetic bottlenecks had occurred during the restoration programme.

Keywords: Atlantic salmon, genetic changes, microsatellite loci, population restoration, stock transfer.

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F. Ayllon, J. L. Martinez, and E. Garcia-Vazquez: Departamento de Biologia Funcional, Facultad de Medicina, Universidad de Oviedo, 33006 Oviedo, Spain. F. Juanes: University of Massachusetts, Department of Natural Resources Conservation, Amherst, MA 01003-4210, USA. S. Gephard: Connecticut Department of Environmental Protection, Inland Fisheries Division, PO Box 719, Old Lyme, CT 06371, USA. Correspondence to E. Garcia-Vazquez: tel: +34 985 102726; fax: +34 985 103534; e-mail: egv@fq.uniovi.es.

Introduction

The Connecticut River (mouth at 41°S 72°W; Figure 1) is located in the USA at the southernmost limit of the North American range of the Atlantic salmon (MacCrimmon and Gots, 1979). The species was lost from this river nearly 200 years ago as a result of dam construction, which blocked adult access to upstream spawning habitats. An extensive restoration effort has been in operation for 30 years, based mainly on stock transfers from the Penobscot River, located in the State of Maine, and eastern Canada (Rideout and Stolte, 1988). Since 1995, all salmon stocked in the Connecticut River have come from eggs obtained from adult salmon trapped at fishways in the river and artificially spawned to produce fry (Gephard and McMenemy, 2004). The restoration programme has succeeded in re-establishing an annual run of salmon (Letcher and King, 2001), and the number of returning adults has increased significantly during the past 30 years. However, some significant changes in both genetic (isozyme and microsatellite loci) and life history traits (age at maturity) have been reported for the stock adapted to the Connecticut River compared with the donor Penobscot River population (Martinez *et al.*, 2001; Spidle *et al.*, 2004). The study of variation at hypervariable microsatellite loci, considered neutral and without adaptive value (Schlötterer, 2000), can help to determine if gene drift has occurred. The aim of this study was to identify possible genetic bottlenecks or gene drift in the Connecticut River salmon population during the restoration programme. This information will improve understanding of the mechanisms underlying adaptation of Atlantic salmon to a new environment.

Material and methods

Samples

Historical samples from the 1960s and 1970s were obtained from the scale collection of the Maine Atlantic Salmon Commission (Bangor, ME). This collection derived from

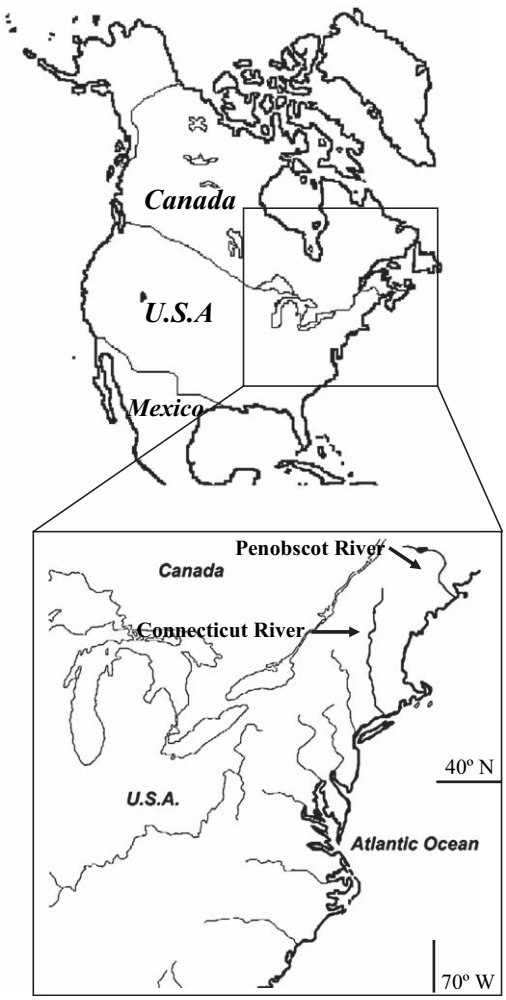

Figure 1. Map showing the location of the two study rivers.

adult fish captured in traps in the Penobscot River and bred in a hatchery to produce progeny for stocking into the Connecticut River. We analysed Penobscot River samples from the 1968, 1970, and 1975 cohorts (PN-68, PN-70, and PN-75, respectively).

Modern tissue samples were obtained from the adipose fins of adults that returned to the Connecticut River during 1997 (CT-97), and from alevins that were the progeny of adult salmon trapped in the Penobscot River during 1996 (PN-96). A sample of these alevins was collected at random from the progeny of all artificially mated adults in the breeding programme and, therefore, can be considered representative of the 1996 adult run. These samples were provided by the US Fish and Wildlife Service (Sunderland, MA).

Genetic analyses

One or two scales per fish were used for the genetic analysis. Total DNA extraction was based on Chelex methodology (Estoup *et al.*, 1996). We studied variation at five microsatellite loci: *SSOSL85* and *SSOSL417* (Slettan *et al.*, 1995), *SSA202* and *SSA197* (O'Reilly *et al.*, 1996), and *BRFO002* (Sušnik *et al.*, 1997). Polymerase Chain Reaction (PCR) amplifications were performed on reaction mixtures containing approximately 50 ng of extracted Atlantic salmon DNA template, 10 nM Tris−HCl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 1.5 μM of each primer, 0.5 Units of DNA Taq Polymerase (Promega, Madison, WI), and 250 μM of dNTPs in a final volume of 20 μl. The PCR products were separated electrophoretically in denaturing polyacrylamide sequencing gels. Allele sizes were established through comparison with a pUC sequence. Gel staining was carried out using the DNA silver staining system (Promega).

Data analysis

Exact *p*-values for testing conformation of genotypes to Hardy−Weinberg (HW) proportions and exact *p*-values for testing homogeneity of allele frequencies between rivers were estimated by the Markov Chain Method (1000 dememorization steps, 1000 batches, 1000 iterations per batch). Global tests across populations were performed using Fisher's method. For all tests we employed the GENEPOP (version 3.3) program (Raymond and Rousset, 1995). Global *F* analyses (estimates of F_{ST}, F_{IS}, and F_{IT}) were performed with the GENEPOP program. F_{ST} and F_{IS} values are indicators of the degree of genetic differentiation between populations and of inbreeding within populations, respectively. Sequential Bonferroni corrections (Rice, 1989) for multiple tests were applied when relevant. Comparisons between groups of samples for different parameters of genetic variability (e.g. old vs. modern samples) were carried out using the program FSTAT version V2.9.3 (Goudet, 2001), with 10 000 iterations. The parameters considered were allele richness for comparison of allele numbers, relatedness as an estimate of how closely individuals are related to each other in a population, and expected and observed mean heterozygosities (H_e and H_o, respectively). Genetic differences between paired samples and their statistical significance were estimated using the program ARLEQUIN version 2000 (Schneider *et al.*, 2000) with the following settings: 10 000 permutations for significance, 10 000 steps in Markov chain.

Finally, effective numbers of breeders were estimated, based on temporal variance of allele frequencies using a likelihood-based estimation (Berthier *et al.*, 2002).

Results and discussion

Five microsatellite loci were considered in this study. All loci except *BRFO002* (4−6 alleles) showed high variation of up to 13 alleles. When assessment of the levels of diversity and heterozygosity between closely related samples is the primary objective, the use of a large number of markers is important. Although the number of loci analysed in this

study was modest, they are codominant, each locus having more than two alleles and with several alleles in modest frequency. Therefore, the number of loci used was acceptable for the purposes of this study (Smouse and Chevillon, 1998).

Observed heterozygosities at the five loci in all samples ranged from 0.5357 (for *BRFO002* in the 1975 Penobscot sample) to 0.9259 (for *SSA202* in the 1970 Penobscot sample). These values are typical of values described at microsatellite loci in Atlantic salmon (McConnell *et al.*, 1995; Reilly *et al.*, 1999) and similar to those found in the Connecticut and Penobscot Rivers by other authors (King *et al.*, 2001; Martinez *et al.*, 2001; Spidle *et al.*, 2004). The mean observed heterozygosities per locus were not statistically different from those expected in any of the samples analysed. The salmon population in the Connecticut River did not show any evidence of genetic erosion compared with the samples from the donor population of the Penobscot River. Similar values of heterozygosity (observed and expected) and mean number of alleles per locus were obtained for the Connecticut and the Penobscot samples (Table 1). Similar F_{IS} values for all samples, including PN-96 (alevins), indicated similar levels of inbreeding (level of kinship). Significant deviations from Hardy–Weinberg equilibrium were found at different loci for some Penobscot River samples; however, all samples from both rivers were in conformity with Hardy–Weinberg equilibrium when all loci were combined (Table 1).

The estimated number of migrants among samples was 7.027 after correction for size (mean sample sizes of 48.37 individuals), emphasizing the close similarity between Penobscot and Connecticut River samples. This similarity is also supported by the low total F_{ST} value (0.0149, 20.05% of the total F_{IT}) and the very low F_{ST} estimates between population pairs based on Weir and Cockerham (1984), which were all statistically non-significant after Bonferroni correction (Table 2).

The mean number of effective spawners per generation in the Penobscot River, estimated from the temporal variation of allele frequencies in 1970–1996 (27 years, i.e. about eight generations at this latitude where most spawners are two sea-winter salmon), was 245.6 (maximum likelihood), ranging from 135 to 825. Based on temporal variation (1970–1997), the most likely mean effective number of spawners in the Connecticut River was 215.4 (range 119–447). This value is very similar to that estimated by Spidle *et al.* (2004) for the Connecticut River, also based on temporal changes in microsatellite loci variation but during a shorter period (1993–1998; estimate $N_{EV} = 193.85$). The absence of statistically significant differences between the old samples of the donor Penobscot River population and the modern samples from both the Penobscot and Connecticut Rivers for all the parameters of genetic variability (Table 3) indicates that the number of spawners employed was large enough to preserve genetic variability during more than two decades of supportive breeding. Therefore, there was no evidence of genetic bottlenecks resulting from the restoration programme in the Connecticut River.

In conclusion, using microsatellite loci variation we have demonstrated that the salmon population under restoration in the Connecticut River represents the variation present in the donor Penobscot River population. Our findings confirm the existence of a healthy level of genetic variation in the Connecticut River population (Spidle *et al.*, 2004). The estimated effective number of breeders per generation for both the Penobscot and Connecticut Rivers, more than 200 in each river, was large enough to preserve genetic variability without expecting short-term gene drift (Franklin, 1980). Large effective population sizes were estimated to

Table 2. F_{ST} values between paired samples. PN and CT = Penobscot and Connecticut Rivers, respectively.

	PN-68	PN-70	PN-75	PN-96	CT-97
PN-68					
PN-70	0.013				
PN-75	−0.068	−0.013			
PN-96	0.014	−0.023	−0.100		
CT-97	−0.002	−0.026	−0.113	0.001	

Table 1. The variation at five microsatellite loci in the samples analysed. PN and CT = Penobscot and Connecticut rivers, respectively. n = sample size; N_a = number of alleles per locus; H_o = observed heterozygosity (s.d.); H_e = expected heterozygosity (s.d.); HWE = conformity to Hardy–Weinberg equilibrium.

	Mean N_a	H_o	H_e	HWE
PN-68 ($n = 28$)	9.00	0.693 (0.088)	0.794 (0.051)	Yes
PN-70 ($n = 31$)	7.25	0.737 (0.093)	0.787 (0.046)	Yes
PN-75 ($n = 61$)	7.00	0.601 (0.100)	0.718 (0.085)	Yes
PN-96 ($n = 95$)	9.50	0.738 (0.101)	0.784 (0.037)	Yes
CT-97 ($n = 55$)	9.50	0.798 (0.065)	0.808 (0.052)	Yes

Table 3. Parameters of genetic variability in old (PN-68, PN-70, and PN-75 with sample sizes $n = 28$, 31, and 61, respectively) and modern (PN-96 and CT-97, with sample sizes $n = 95$ and 55, respectively) samples derived from the Penobscot River stock. p-values of the statistical significance of differences between the two groups of samples. H_e = expected heterozygosity; H_o = observed heterozygosity; AR = allelic richness; Rel = relatedness.

Parameter	Old samples	Modern samples	p-Values
H_e	0.784	0.800	0.616
H_o	0.695	0.762	0.193
AR	7.396	7.818	0.586
Rel	0.040	0.019	0.416

have been used for the restoration programme in the Connecticut River during the 1990s (Spidle *et al.*, 2004). The results of this study extend this conclusion to the two previous decades (1970s and 1980s), suggesting that managers have carefully followed the principle of using sufficient numbers of spawners to preserve genetic variability. The significant differences between modern Penobscot and Connecticut River samples found previously by other authors (Martinez *et al.*, 2001; Spidle *et al.*, 2004) can be considered in the context of the temporal variation of wild Atlantic salmon populations (Nielsen *et al.*, 1997).

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