FINAL REPORT

Genetic diversity in Tasmanian Atlantic salmon.

PROJECT NUMBER 92/152

RESEARCH ORGANISATION

Salmon Enterprises of Tasmania Pty.Ltd.

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FUNDING SOURCE

Fisheries Research and Development Corporation

SUMMARY

Genetic analysis was undertaken of Atlantic salmon parr from Tasmania and from the ancestral population in Canada (River Philip, Nova Scotia). Allozyme analysis of seven variable loci showed that the two samples were very similar to each other, although some minor differences were apparent. One of 18 restriction enzymes used in mitochondrial DNA analysis revealed variation, with small but significant differences in frequencies different between the Tasmanian and Canadian samples. There was therefore little evidence of significant inbreeding in the Tasmanian stock when compared to its ancestral stock.

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BACKGROUND

Poor stocking or breeding programs can lead to inbreeding and the loss of genetic variation in hatchery reared fish. The effects of inbreeding may be seen, for example, in reduced fry survival or an increased proportion of abnormal fry (Aulstad and Kittlesen, 1971; Gjerde *et al.*, 1983). The loss of genetic variation in hatchery stocks of salmonids is a common although not universal phenomenon (see Allendorf and Ryman, 1987), and has been recorded in many Atlantic salmon hatcheries (Cross and King, 1983; Stahl, 1983).

In recent years, a jaw deformity has become apparent in some farmed Tasmanian Atlantic salmon (almost exclusively in triploids) which renders them less tolerant of normal husbandry practises and reduces their value. While it is uncertain whether this deformity has a genetic or environmental basis, one possibility is that it has been caused by inbreeding in the Tasmanian stock. All Australian Atlantic salmon are descendants of the River Philip population (Nova Scotia, Canada), ova of which were imported into Australia in 1963, 1964 and 1965. These gave rise to a landlocked population in Gaden, New South Wales, from which salmon were introduced to Tasmania in 1983, 1984 and 1985.

OBJECTIVES

This project aimed to assay levels of genetic variation in the Tasmanian stock and compare these levels with those of the ancestral population in River Philip. The purpose of this comparison was to provide some estimate of the amount of inbreeding that has occurred in the Tasmanian stock and to provide an assessment of the genetic "health" of this stock.

INTRODUCTORY INFORMATION

In order to carry out the comparison, two methods of assessing genetic variability were used. The first is the *allozyme* method. Here, proteins are extracted from the fish and subjected to electrophoresis in a porous matrix. This process causes the proteins to migrate under the influence of an electric field and the locations of specific proteins at the end of the experiment are revealed by histochemical stains specific to the protein under study. Variation among animals in the position of these stained bands is under genetic control, and is determined by the nucleotide base sequence of the nuclear gene coding for that enzyme. This type of variation is rather analogous to variation in the ABO blood group of humans.

The second method employs the more recent approach of *mitochondrial DNA* analysis. Here, mitochondrial DNA (a relatively small molecule quite separate from normal chromosomal or nuclear DNA) is extracted from the fish and analysed for variation directly, rather than via its protein products. Mitochondrial DNA is inherited only from females, and evolves faster than nuclear DNA. For these reasons mitochondrial DNA variation is generally considered to be more sensitive to the effects of small population sizes ("bottlenecks") than allozyme variation revealed by electrophoresis.

RESEARCH METHODOLOGY

Tasmanian samples

Liver and white muscle samples from 100 parr were taken on January 7 1993 from on-growing tanks C2 and C4 at Saltas Freshwater Operations, Wayatinah, Tasmania. Tissues were stored at CSIRO Fisheries at -80°C. The parr represented a random sample of the progeny of 177 female and 95 male fish from the 1989 year class. This gave a biologically effective parental population of 247, assuming that all these adults had an equal probability of being the parent of any of the 100 sampled parr. The effective population size is estimated from (4mf)/(m+f), where m and f are the numbers of males and females respectively.

Canadian samples

Liver and white muscle samples were extracted from 100 parr taken on November 19, 1992 from the Canadian Department of Fisheries and Oceans Mersey Federal Fish Hatchery, Milton, Queen's County, Nova Scotia. Tissues were placed in 2ml plastic vials and transported frozen in a liquid nitrogen dry shipper to CSIRO Fisheries, where they were stored at -80°C. The 100 parr represented a random sample from about 15,000 parr themselves derived from 16 female and 14 male wild returns to the River Philip in 1991. This gave a biologically effective parental population of just under 30 (29.9).

Allozyme analysis

There are as many as one hundred enzymes and proteins that could be analysed for genetic variation, but past surveys showed that the majority of these were invariant in Atlantic salmon (Cross and Ward, 1980; Stahl, 1987) and therefore do not give useful information in comparative studies. Ten enzymes were sampled (Table 1), eight of which had been shown earlier to be variable in one or more North American populations (Stahl, 1987; Verspoor, 1988). Liver extracts were used for *Aat-3, Idh-4, Sdh-1,2,* and *Ada,* and muscle extracts for *Mhd-3,4, Me-2, Ldh-4, Pgi-1, aGpdh-2,* and *Pgm-2*. Variation for a phosphoglucomutase regulatory gene (*Pgm-reg*) which had been shown to be variable in liver tissue of North American salmon (Verspoor, 1988) was also examined. Cellulose acetate (Helena Labs) gel electrophoresis was employed, essentially following the methods of Hebert and Beaton (1989).

Mitochondrial DNA analysis

Total DNA was isolated from white muscle using a modified CTAB (hexadecyltrimethylammonium bromide) isolation procedure (Grewe, 1991). This DNA was then cut with one of eighteen restriction enzymes which cleave the DNA at specific nucleotide sequences. These enzymes included those that had previously

revealed variation in North American and European populations (Davidson *et al.*, 1989; Bermingham *et al.*, 1991; Birt *et al.*, 1991; King *et al.*, 1993). The resulting DNA fragments were separated in horizontal 1.0% agarose gels submerged in a tris-borate-EDTA (TBE) buffer system (Maniatis *et al.*, 1982). DNA was transferred to a nylon membrane filter (Amersham's Hybond^{TM}) by southern transfer (Maniatis *et al.*, 1982). These filters were probed with P^{32} labelled lake trout or blue-eye trevalla mtDNA purified by CsCl ultracentrifugation. Filters were exposed to Kodak X-ray film for 12-48 hours and the resulting mtDNA fragment patterns examined.

Restriction fragments were sized using the program DNAGEL (Keiser, 1984; modified by P. Grewe) run on an Apple II computer in conjunction witha GRAFBAR digitizing Pallette (model GP-7, Science Accessories Corporation).

DISCUSSION

In 1963, 1964 and 1965, approximately 100,000 Atlantic salmon ova per year were imported from River Philip (Nova Scotia, Canada) into Australia and used to found the Gaden, New South Wales, population. Assuming that a single female contributes 6,000 eyed ova, these represented the progeny of a total of about 45 female fish. Standard spawning procedures in the Nova Scotian hatchery at the time included the fertilisation of the eggs of three females with the milt from two males, thus the total number of male parents contributing genes would have been around 30. The effective initial population size (N_e) is thus estimated at around 72, assuming that all males and females contributed equally to the following generation. The expected proportion of the original genetic variability remaining after a bottleneck of size 72 for one generation is 0.993 (derived from 1-(1/2 N_e)). If some females contributed substantially more progeny (eggs) than others, then this proportion would have to be reduced. However, it is likely that the initial imports included a good sampling of the genetic variation present in the original population.

These imported ova allowed the establishment of a landlocked population in Gaden, which was maintained through natural spawnings supplemented with hatchery stockings or plantings. Historical data of the early years of the Gaden hatchery population are not available, but records from the late 70s and early 80s indicate that then several hundred brood stock were used each year in the hatchery. During these years, losses of genetic variation should have been minimal. In 1984, 1985 and 1986, approximately 100,000 ova per year were imported into Tasmania to initiate the Tasmanian stock. The first Tasmanian broodstock were available in 1987, and for that and each subsequent year, several hundred males and females were used as broodstock. Thus changes in the genetic composition of the Tasmanian stock *vis-a-vis* the Gaden stock should again have been very limited.

This brief consideration of the broodstock history of the Tasmanian salmon suggests that, with the possible exception of the early Gaden period for which records are not available, a sufficient sampling of the genetic diversity should have occurred throughout the history of the Tasmanian stock to prevent any substantial genetic changes from the River Philip stock occurring through genetic drift.

The genetic data are generally consistent with this expectation. While the allozyme analysis shows evidence of small but significant changes in the genetic structure of the Tasmanian Atlantic salmon sample as compared with the River Philip sample, the overall allozyme variability of the Tasmanian stock (estimated from the proportion of heterozygous individuals) is very similar to that of River Philip. Mitochondrial DNA variation in both samples is low, but for the one restriction enzyme revealing variation, relatively small but nevertheless statistically significant frequency differences were found between the two samples. It is perhaps worth pointing out that the genetic sampling of the Tasmanian stock was better than that of the River Philip stock. With

respect to the allozymes, the parr analysed came from effective parental population sizes of 247 Tasmanian fish but only 30 River Philip fish. With respect to the mitochondrial DNA, which is solely maternally inherited, the parr came from 177 Tasmanian females but only 16 River Philip females. It is difficult to be certain whether the small degree of differentiation between the two samples arises from an artefact of limited sampling (especially of the River Philip stock) or from small genetic drift effects in the Tasmanian population. Examination of additional fish from the River Phillip stock would help to resolve this issue.

RESULTS

Allozymes

Allozyme gene frequencies are given in Table 1. All polymorphic loci were diallelic. Allele notations follow Cross and Ward (1980) and Verspoor (1988). Few valid tests of Hardy-Weinberg equilibrium of the present samples could be performed, both because few had expected numbers of all three genotypes greater than one, and because for *Pgm-1-t* it is not possible to unequivocally distinguish the heterozygotes from one of the homozygotes. Four tests were performed (Tasmania *Mdh-3,4*, χ^2=5.716, P=0.017; Canada *Mdh-3,4*, χ^2=8.466, P=0.004; Canada *Aat-3*, χ^2=0.384, P=0.535; Canada *Idh-4*, χ^2=0.540, P=0.462). The two deviant results, both from *Mdh-3,4*, were characterised by a small but significant deficiency of heterozygotes. They could represent random sampling errors, errors introduced by sampling the progeny of a relatively small number of parents (especially for the River Philip sample), errors ascribable to the difficulties in scoring products of duplicated loci, or real deviations from equilibrium.

The first point to note is that all genes present in the River Philip sample are still present in the Tasmanian sample (Table 1). With respect to the enzymes we examined, there was no evidence of gene loss in Tasmania. The second point to note is that for most of the enzymes, there were no significant differences in gene frequencies at the 5% probability level between the two sets of samples. Fisher's exact test was applied to allele numbers for all loci except *Sdh-1,2* and *Pgm-reg*, where numbers of the two distinguishable phenotypes were used. Not only were all genes still present in Tasmania, most of them had not changed significantly in frequency. Two exceptions were the enzyme *Mdh-3,4* and *Pgm-reg*, where in both cases there had been a significant increase in the frequency of the rarer allele in the Tasmanian sample (P=0.008 and P=0.033 respectively). The *Pgm-reg*, result was only just significant at the 0.05 level, and considering that seven locus comparisons were carried out, could be ascribed to chance.

Combining probabilities (Sokal and Rohlf, 1981) across all seven enzymes, provides evidence of a small but statistically significant difference in the genetic structure of the two populations (P=0.030).

Another measure of genetic diversity is heterozygosity. This measure estimates the proportion of individuals which are heterozygous for each enzyme, i.e. the proportion of individuals which receive non-identical genes from the two parents. If there are two genes, say A and B, present in a population for a particular character, then in that population both heterozygous (AB) and homozygous (AA and BB) genotypes will be found. Where the heterozygotes can be clearly identified from both homozygous classes, the observed number and therefore proportion of heterozygotes could be directly estimated for each character. In our case, for two of the characters (sorbitol

dehydrogenase-1,2 and phosphoglucomutase regulatory gene) the heterozygotes could not be distinguished from one of the homozygotes, but an expected proportion of heterozygotes could still be estimated assuming that the population was mating at random with respect to genotype (i.e. accords with the Hardy-Weinberg equilibrium). Across all seven variable enzymes, the average expected heterozygosity was 0.207 in the Tasmanian sample, a figure very close to the corresponding figure for the River Philip sample of 0.182. So this analysis also showed no evidence of any loss in overall genetic diversity in the Tasmanian sample.

Mitochondrial DNA

Initially, a survey of mitochondrial DNA variation among 72 fish from each of the two salmon populations was conducted using the 10 restriction enzymes (*Bam*HI, *Bcl*I, *Bgl*II, *Bsp*DI, *Bst*EII, *Dra*I, *Eco*RI, *Pst*I, *Pvu*II and *Xba*I. However, no variation among fish was revealed for any of these enzymes and an additional eight enzymes (*Ava*I, *Ban*I, *Dpn*II, *Eco*O109, *Hin*fI, *Hin*PI, *Sty*I and *Taq*I) were used to examine 29 fish from the River Philip sample. One of these showed variation. This was *Hin*PI which revealed two mitochondrial DNA variants. This enzyme was then used to characterise 72 fish from each of the two samples. The two variants were found in both samples, but with significantly different frequencies (0.81 and 0.19 in Tasmania versus 0.94 and 0.06 in River Philip, P=0.021).

Table 1. Gene frequencies in those enzymes screened by electrophoresis.

Enzyme	Gene	allele	Tasmania	Canada	[a]*P*
malate dehydrogenase-3,4	*Mdh-3,4*	f	0.323	0.181	0.008
		s	0.677	0.819	
		N	65	72	
aspartate aminotransferase-3	*Aat-3*	f	0.077	0.153	ns
		s	0.923	0.847	
		N	65	72	
malic enzyme-2	*Me-2*	f	0.977	0.958	ns
		s	0.023	0.042	
		N	65	72	
isocitrate dehydrogenase-4	*Idh-4*	f	0.117	0.160	ns
		s	0.883	0.840	
		N	64	72	
[b]sorbitol dehydrogenase-1	*Sdh-1,2*	f	0.080	0.075	ns
		s	0.919	0.924	
		N	65	69	
adenosine deaminase	*Ada*	f	0.984	0.986	ns
		s	0.016	0.014	
		N	63	71	
[b]phosphoglucomutase regulatory gene	*Pgm-reg*	plus	0.669	0.882	0.033
		minus	0.331	0.118	
		N	71	72	

N is the number of fish sampled.

The following four enzymes showed no variation in Tasmanian and Canadian samples (n=65 and 72 respectively): lactate dehydrogenase-4 (*Ldh-4*), phosphoglucose isomerase-1 (*Pgi-1*), a-glycerophosphate dehydrogenase-2 (*aGpdh-2*) and phosphoglucomutase-1 (*Pgm-1*).

Liver extracts were used for *Aat-3*, *Idh-4*, *Sdh-1,2*, *Ada* and *Pgm-reg*, and muscle extracts for *Mdh-3,4*, *Me-2*, *Ldh-4*, *Pgi-1*, *aGpdh-2*, and *Pgm-1*.

[a] Probabilities of no significant genetic differentiation between the two samples, based on Fisher's exact test. Except for *Sdh-1,2* and *Pgm-reg*, allele numbers are compared. For *Sdh-1,2* and *Pgm-reg*, it was not possible to distinguish all genotypes and here the distinguishable phenotypes (two in each case) rather than allele frequencies are compared.

[b] Allele frequencies assume Hardy-Weinberg equilibrium

IMPLICATIONS AND RECOMMENDATIONS

The apparent genetic health of the population suggests that the deformity is unlikely to be the result of inbreeding. High levels of inbreeding would have led to an erosion of genetic variability, and such erosion was not observed. However, the possibility that the deformity has an inherited basis cannot be eliminated at this stage, although other explanations based on environmental or triploidy-induced physiological effects seem more plausible.

The genetic similarities of the Tasmanian and River Philip samples, indicate that the genetic health of the Tasmanian stock is likely to be very similar to that of the River Philip stock. Providing current broodstock practises are adhered to, the long-term genetic outlook should remain favourable. However, it might be worth establishing a monitoring program, so that periodically, say once every five years, levels of variation in the Tasmanian stock are re-assessed and compared to these 1993 results.

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INTELECTUAL PROPERTY

N/A

TECHNICAL SUMMARY

N/A

ACKNOWLEDGEMENTS

Authorship of the present report should be credited to Drs. Ward and Grewe since it has been directly adapted from the text which they presented to Saltas upon completion of the genetic analysis in April 1993.