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Screening for genetic markers to assess potential genetic impact from salmon ranching on wild stocks

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Abstract

Genetic markers are needed to assess the potential genetic impact on wild salmon populations from ocean ranching. In 1990 spawners from four Norwegian rivers: Lone, Vosso, Dale and Onarheim were screened for genetic variability in isozymes and genomic DNA. Variability was detected in LDH, MDH, MEP, IDDH, and with single locus probes (SLP's) in DNA fingerprint analysis. Sibling groups with the observed variability in isozymes are kept separate for further studies on the suitability of the observed variants as genetic markers. Tests of growth rates and survival on different genotypes are being conducted. Groups of genetically marked salmon will be released to study the potential impact on wild populations from ocean ranching and enhancement activities.

Introduction

Several nations have developed ocean ranching to a commercial level. In the Pacific ocean Canada, USA, USSR and Japan release billions of juvenile salmon annually. The historical reason for this is apparently similar in the different geographical areas: [1]over harvesting of the wild populations and [2]losses of freshwater habitats for salmonid reproduction. As a compensation hatcheries were built and large release programmes were initiated, like FRED in Alaska and SEP in Canada. The release of juvenile salmonids has reached a very high level in some regions, and in some areas the abundance of ranched salmon now exceeds that of wild salmon. There is now concern about the potential ecological and genetic interactions of wild and ranched/enhanced stocks.

Lately there has also been an increased interest in ocean ranching of Atlantic salmon. After the opening of a research station in Iceland in the early 1960's several commercial farms have been established, releasing millions of smolt annually. Extensive research on salmon ranching is also going on in Ireland. In Sweden and other countries bordering the Baltic sea a major part of the landed salmon now results from release of hatchery fish.

In Norway a large programme, PUSH, has been initiated to evaluate the potential and limitations for ranching of four different species, Atlantic salmon, Arctic char (*Salvelinus alpinus*), cod (*Gadus morhua*) and lobster (*Homarus gammarus*). Based on recommendations from a board of scientist, the Norwegian parliament decided that the potential ecological and genetic impact on wild populations should be assessed before final decisions about developing commercial ranching is taken. This is also in agreement with the recommendations of sustainable yield made by the World Commission of Environment and Development, and also with the position of Norway, being responsible for managing the major part of the remaining stocks of large salmon.

The aim of this study, was to detect potential genetic markers which could be used in controlled experiments, to quantify the genetic impact on wild salmon populations from ocean ranching.

Materials and methods

Spawners were captured from wild populations of Atlantic salmon in the four rivers Lone, Vosso, Dale and Onarheim on the west coast of Norway, near Bergen (Fig 1). The spawners were captured alive during October and November 1990 by gillnetting and electrofishing, and transported to a hatchery for stripping and incubation of eggs. All family groups were tested for BKD, IPN and furunculosis, and eggbatches found to be infested were discarded. The broodstock was characterized genetically by two methods, a) allozyme electrophoresis and b) single locus probe DNA analyses.

Allozyme electrophoresis

Samples of muscle and liver tissue were taken from all individuals and stored at -20 ^{0}C until electrophoresis was carried out. Two buffer systems were applied: A) a TCB buffer pH 8.6, and B) a citric acid buffer, pH 6.1 (Skaala and Jørstad 1987). The following enzymes were stained for:LDH (E.C. 1.1.1.27), MDH (E.C. 1.1.1.37), MEP (E.C. 1.1.1.40), GPI (E.C. 5.3.1.9), PGM (E.C. 5.4.2.2), and IDDH (E.C. 1.1.1.14).

Single locus probe DNA analyses

Genomic DNA was isolated from 70 mg of muscle tissue according to Taggart and Ferguson (1990a). DNA samples were subjected to restriction endonuclease digestion (*HaeIII*) over night and restricted DNA samples were separated by horizontal agarose gel electrophoresis for 24 hours at 2.0 V/cm. The DNA was transferred from the gel onto a nylon hybridization membrane.

Single locus probes (*SLP*'s) 3.15.34, 3.15.45 and 3.15.60 isolated by Taggart and Ferguson (1990b), were radiolabeled with ^{32}P dCTP by random priming and hybridized to HaeIII digested DNA samples. The filters can be washed and reprobed a number of times. Autoradigraphy was carried out at - 70^{0}C for 2-4 days with one intensifying screen.

Results and discussion

The biological characteristics of the four populations are somewhat different. The population in R. Lone is very distinct in producing almost exclusively small grilse, staying only one winter in sea. R. Vosso contrasts with this as the salmon here consists of larger 2 and 3 seawinter fish.The R.s Dale and Onarheim appears to have intermediate sized salmon, staying one and two winters in seawater. The occurrence of dwarf males differs among the populations, with a maximum of about 40% (mean about 20%) being found in some familygroups from Lone, while in families of the other stocks the percentage ranges from 4 to 7.

Genetic variability was observed at the LDH-4* locus exposed in liver tissue where a slow allele (Fig 2, Tab 1), termed *80 was found in heterozygous form in several individuals from Rivers Lone and Dale. To our knowledge, variability has not been described in this locus previously. The observed allele will be studied in detail and evaluated as a genetic marker. Variability was also detected at MEP-2*, where a fast allele, termed *125, was found. The variation at this locus is a matter of discussion (Verspoor and Jordan 1989). Correlations have been found between this variability and environmental conditions. If this reflects a strong selective action on this specific locus, or on other loci associated with MEP-2*, is not known.

In MDH-3/4*, most clearly expressed in muscle, a slow allele , MDH-3/4*80, was also found. As this is a duplicated locus, it is not possible to decide which of the two loci that is variable. Further, it is only possible to identify two genotypes, the single banded fast homozygote, and a double banded type. The double banded type is either a heterozygote or a homozygote for the slow allele in one of the two loci. Irrespective of the limitations connected to this locus, it could still be an interesting marker on an experimental scale. Sibling groups with a high frequency of this allele, where 50% or 100% of the individuals are marked are now available for experimental releases in selected locations. The variability observed in IDDH and PGM is omitted due to inconsistent staining.

Both *SLP*'s 3.15.34 and 3.15.45 identified a large number of alleles (4-6) (Fig 4 and 5). The very large fragments identified by 3.15.60 (approx 20 kb) could not be unambiguously distinguished under the standard electrophoretic conditions, but three alleles were identified. The preliminary results based on few individuals from each population (8-13 individuals) revealed very high heterozygosity values (69 - 100%) which should suggest potential value as chromosomal markers for family identification using one,

or a combination, of probes. The shearing of presumed identical allelic fragments among individuals from different locations also suggest a useful role for these probes at the population level.

The present study is closly related to th Norwegian research programme on sea ranching (Holm et al. 1991)

References

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Table 1. Mean weight of the collected spawners from the four populations. Number of individuals in parenthesis.

	Lone	Vosso	Dale	Onarheim
Males	1.4 (13)	3.9 (5)	2.5 (9)	3.1 (8)
Females	1.3 (27)	6.3 (10)	4.2 (17)	4.6 (13)

Table 2. Genetic characteristics (frequency of 100 allele) of sampled spawners.

Population	N	LDH-4*	MDH-3/4*	MEP-2*
Lone	45	0.956	0.978	-
Vosso	15	1.000	0.931	0.509
Dale	27	0.926	0.981	-
Onarheim	12	-	0.913	0.700

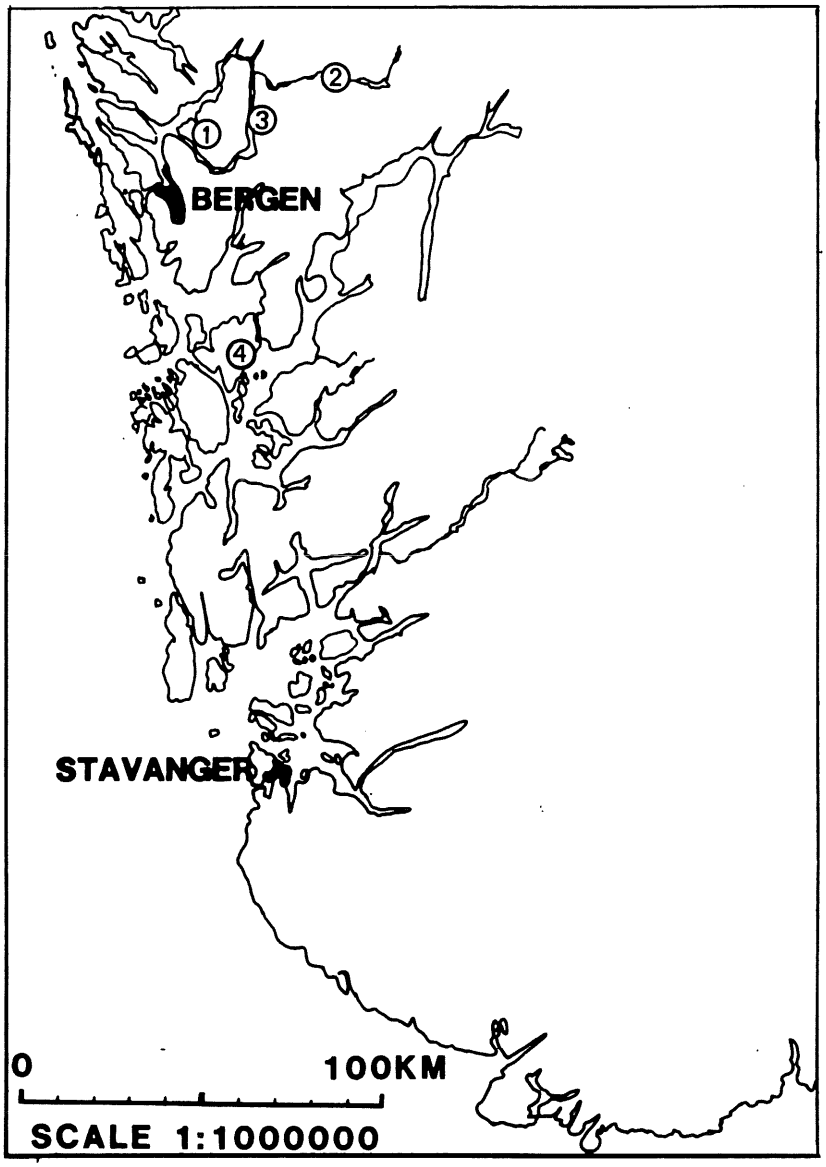

Fig. 1. Map with sampling localities indicated. 1: Lone, 2: Vosso, 3: Dale, 4: Onarheim.

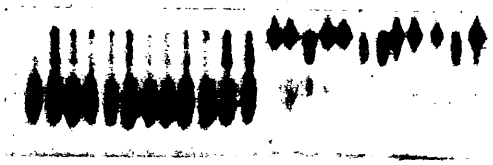

Fig. 2. The described variability in LDH-4* from liver tissue.

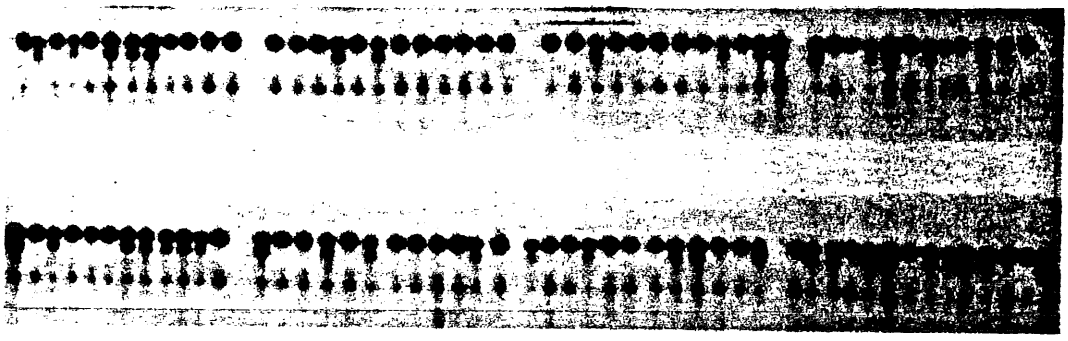

Fig. 3. The described variability in MDH-3/4* from muscle tissue.

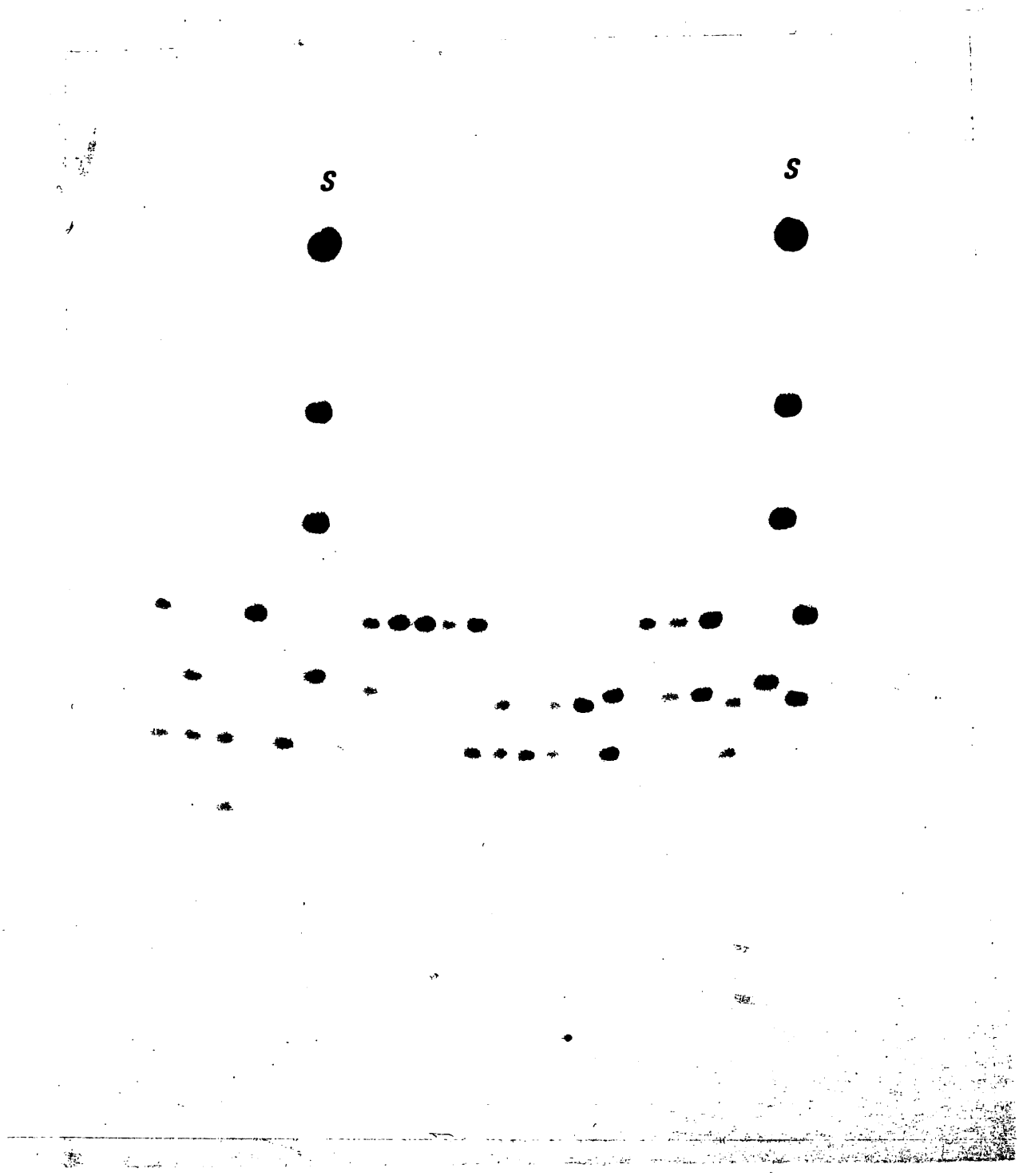

Fig. 4. DNA fingerprints of broodstock salmon using *SLP* 3.15.34. (S = fragment size standard).

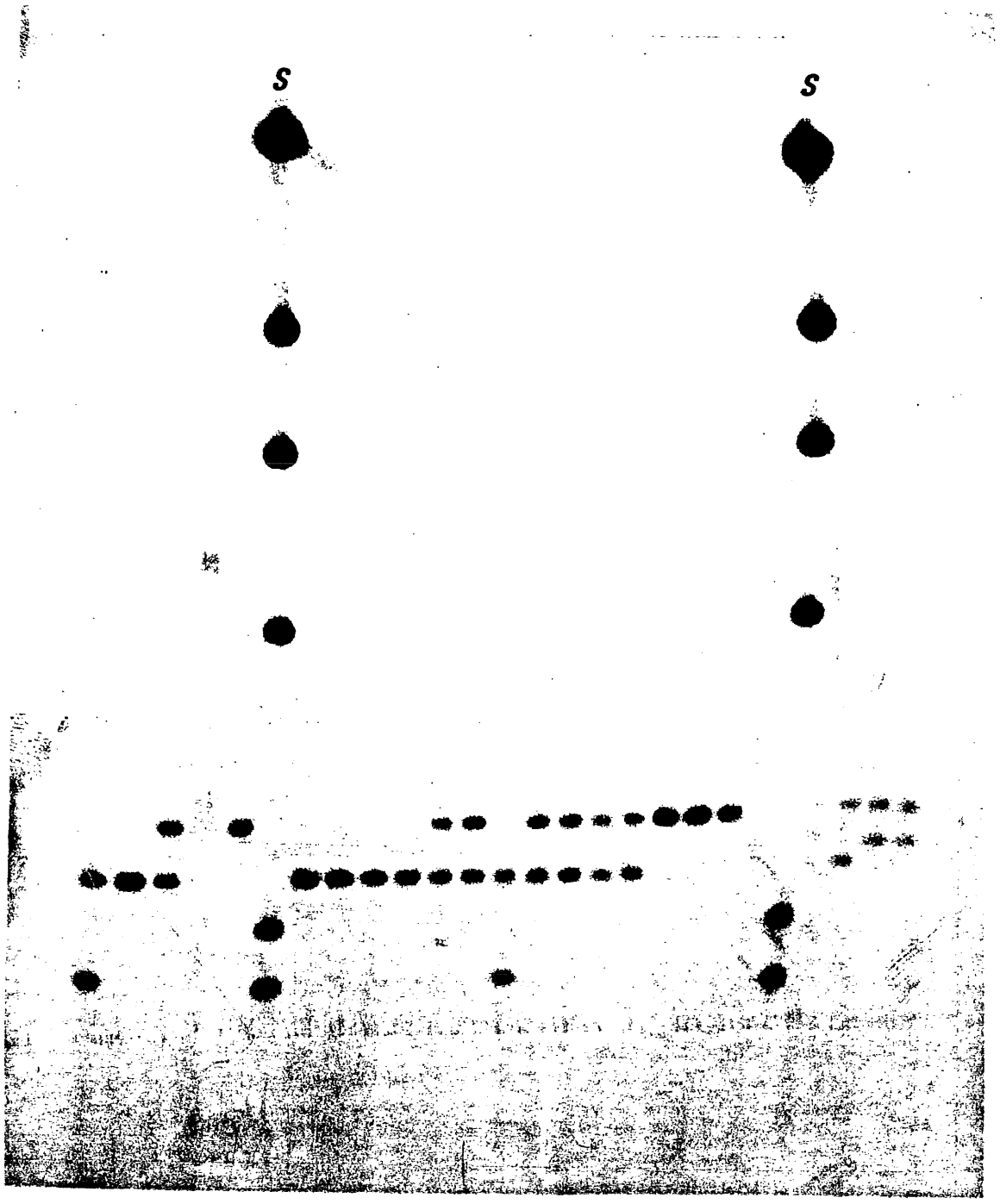

Fig. 5. DNA fingerprint of broodstock salmon using *SLP* 3.15.45. (S = fragment size standard).