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A GENETIC MARKER FOR ARTIFICIALLY REARED COD (GADUS MORHUA L.)

by

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ABSTRACT

Artificially reared cod at the Aquaculture Station Austevoll on the west coast of Norway, were screened for genetic markers by using biopsies of white muscle, individual tags and enzyme genotype as determined by starch gel electrophoresis. In February 1986, 70 parental fish, all heterozygote for a rare

allele in phosphoglucose isomerase (PGI-1(30)), were selected and placed in a spawning tank. The fish spawned naturally, and in all samples of offspring the PGI-1(30/30) homozygote accounts for about 25% of total offspring. At present 3 500 cod are kept in net pens, where 847 individuals are positively identified as PGI-1(30/30) homozygotes and raised as broodstock. Offspring from this genetically tagged cod will be used in farming and/or stock enhancement programmes in the future.

INTRODUCTION

During the last decades, electrophoretic method have been extensively used to study genetic variation in natural populations of different fish species. The need for knowing the population structure to properly manage of the fishery resources, has been discussed by several authors (Allendorf and Utter, 1979; Schacklee, 1983). These workers have also proposed the possibilities of application of genetic markers in studies of natural fish stock as well as in controlled breeding studies (Moav et al., 1976) and aquaculture (Moav, 1979; Wilkins 1981).

Individuals possessing rare alleles in a population of reared fish can be identified and selected for breeding and give offspring with unique genetic markers. The introduction of such genetic markers in artificially produced fish and mass releases of these into the natural environment have been suggested by Moav et al. (1978). Taggert and Ferguson (1984) used this approach to breed a brown trout variant with a genetic marker in a tissue enzyme, proposing that this trout should be used in stock enhancement programmes in Ireland.

The breakthrough of artificially rearing of cod fry in ponds occurred in 1983 (Øiestad et al., 1985) mainly aimed at mass releasing of fry for coastal cod stock enhancement. Such release experiments were initiated in 1983 (Svåsand et al., 1985)

and genetic studies were incorporated in the programme carried out (Svåsand et al., 1987). In addition, artificially reared fish from the 1983 yearclass, were used in an intensive cod farming experiment at the Aquaculture Station Austevoll (Kvenseth et al., 1985). These fish were mature in 1986-87 and were used in more controlled breeding studies aimed on producing a specific genetic marker in the offspring. In this contribution, we describe the details of that study and the results obtained so far.

MATERIALS AND METHODS

In 1986, about 1 000 artificially produced mature cod were kept at the Aquaculture Station Austevoll, as potential parental fish for producing offspring with a specific genetic marker.

In January 1986, these cod were individually tagged and a small amount of white muscle tissue were sampled by biopsy (Jørstad et al., 1981). These samples were analysed by starch gel electrophoresis and stained for phosphoglucose isomerase. Each individual was classified to PGI-1 genotype, and all individuals possessing the rare allele PGI-1(30) were removed and transferred to a separate spawning tank. This selected broodstock, were all heterozygotes for the 30-allele (either PGI-1(30/100) or PGI-1(30/150). They were allowed to spawn naturally in the spawning tank (Huse and Jensen, 1983) and eggs were collected through a surface outlet before transferred to the hatchery.

After hatching, selected groups of yolksac larvae were analysed for PGI-1 genotypes, while the main part of larvae were released 5 days after hatching into Svartatjønn, a saltwater basin near the Aquaculture Station.

Cod larvae and fry were taken from the basin at various times for PGI-1 genotyping. Length and weight were also recorded.

In June-July 1986, most of the fish in Svartatjønn was captured and transferred to feeding tanks at the Aquaculture Station. The fish were fed dry pellets. In March 1987 all fish (totally 3 633 individuals) were tagged, length and weight were recorded, and white muscle samples for PGI-1 genotyping were taken by biopsy.

All samples of white muscle were analysed by starch gel electrophoresis either using histidin gel (pH=7.0) or the buffer system (pH=6.1) described by Clayton and Tretiak (1977), the last one being superior for PGI-1 allozyme separation.

RESULTS

The PGI-1 polymorphism in cod from Norwegian waters consists of two common alleles (called 100 and 150) and one rare allele called 30. The frequency distribution of the genotypes in cod from different areas are summerized in Table 1. As seen, the frequency of PGI-1(30) is nearly constant in the areas given and occur at frequency of about 2-5% in the populations sampled. The table also gives the genotype frequencies for the different samples, and in the material shown only one of 6130 individuals have been classified as (PGI-1(30/30) homozygote for the rare allele. Even if there could be a few mistyped specemens in the total material, the occurrence of this phenotype in nature must be very low.

As the selected parental fish all were heterozygotes for the 30 allele, the expected frequency of PGI-1(30/30) homozygote should be 25% of the individuals in the offspring population. This is based on simple Mendelian inheritance which has been proven for tissue enzyme polymorphism in cod (Jørstad et al. 1981) as well as in other fish species (King, 1983; Taggert and Ferguson, 1984; Kornfield et al. 1983).

In two samples of yolksac larvae hatched from eggs collected from the spawning tank, the frequency on PGI-1(30/30) homozygote was 0.22 and 0.28 (Table 2) which agree with the expected value. Similar results were obtained for samples of yolksac larvae taken in Svartatjønn a few days after release.

In the samples drawn from Svartatjønn after metamorphosis (Table 3), the frequency of PGI-1(30) homozygote were from 22 to 27%, the last value obtained when the fish were transferred feeding tanks at the Aquaculture Station.

The frequency the homozygote in the population of juvenile cod in March 1987 was 26%. At this time 3 263 of a total population of 3 633 individuals were classified and 847 fish were PGI-1(30/30) homozygotes. As seen in Fig. 1 the homozygote individuals are easily characterized by an anodially moving isozyme with only one band in the PGI-1 locus position. Clearly, the gel buffer used (pH=6.1) give a nice separation of PGI allozymes and easy classification of the PGI-1(30) allele possessing individuals. Due to technical reasons (too small biopsy samples obtained), about 10% of the total population of juveniles have not been classified. This means that the actual number of PGI-1(30/30) homozygotes are larger than estimated so far (847 fish).

When comparing the frequency of the homozygote PGI-1(30/30) at different ages, (Table 3) no changes have occurred during the age period investigated so far. The frequency is approximately constant and the value estimated is the one expected from simple Mendelian inheritance.

Length distribution and mean length of the homozygote was also compared to the total sample (including all PGI-1 genotypes) of cod fry (Fig. 2).

The mean length of homozygotes is not different from the mean length of the total population in June 86 at an age of about 90 days. Similar, no differencies in length distribution were detected.

No differences in length distribution and mean length were detected between the homozygotes and the total offspring population when measured in March 1987 (Fig. 3), at one year of age.

DISCUSSION

Taggart and Ferguson (1984) also used the PGI polymorphism to produce homozygotes for a rare allele in trout. The offspring in their different crossings fit well to the expected values from a simple Mendelian inheritance. Growth studies of the fish of different PGI-1 genotype show no differences in survival and growth characteristics. The data reported here for cod, is very similar to the trout experiment. The cod study, hovewer, differ with respect to the method of producing eggs. For the trout, the fish were stripped and artifically fertilized, in the cod experiment the fish were allowed to spawn naturally in a tank.

The results from the cod experiment show no differences in genotypic survival and growth during any of the periods investigated. No changes in genotype frequencies or allele frequencies were observed after metamorphosis. There were no differences in length distribution and mean length of the PGI-1 genotypes compared. For this reason, the low frequency of the PGI-1(30) allele in nature is possibly due to other mechanisms than directional selection against individuals possessing this gene. The existance of rare alleles in natural populations are more likely explained by being neutral genes, at least under normal conditions. Survival and growth of the PGI-1(30/30) homozygote of cod must, of course, be tested under various environmental conditions to reach a more firm conclusion.

In order to obtain further information, the population of cod juveniles produced in Svartatjønn 1986 is kept under controlled conditions at the Aquaculture Station Austevoll. This population, where about 25 % are genetically marked in the PGI-1, are now being raised under farming conditions. The results obtained from these studies must be carefully evaluated before application of the fish as broodstock for production of genetically tagged offspring in the future.

Of specially interest is the use of genetically tagged cod fry in connection with stock enhancement programmes. With a sufficently large number of parental fish, extremely large numbers of offspring can be produced which all can be identified by their specific enzyme marker. No additional mechanical tagging is necessary. In large release programmes, where several hundreds of thousands of fry are released, this may be the only possible method of identification. Most interesting is, hovewer, to use genetically tagged cod to study the genetic impact of mass releases on the native fish stocks. The genetic tag lasts the entire life of the fish and, in addition, is transferred to the next generation. In this way, the reproductive success of released fish can be studied as well as introgressing with the native gene pool. Releases of genetically tagged cod in a geographically limited area over 2-3 years, permit longterm studies of the genetic impact from artifically produced fish on the natural fish population. Proper evaluation of the genetic effects can only be studied by using unique genetic markers, and broodstock, such as the one described here, should preferentially be used in such experiments.

We also want to emphasize that the use of an unique genetic marker in cod should only be used in research project to provide data which can illuminate important questions mentioned above. Broodstock, which have the genetic characteristics of the local stock, should be used for enhancement programs, large scale releases and searanching.

At present, there is a lot of interest in cod farming in Norway. The fish used are mainly wild cod that are caught in the sea and fed in net pens until market size. Until recently, only a few farmers have had the opportunity to buy artificially produced cod fry. In the future, cod farming must be based on a domesticated cod produced through selective breeding programmes. With increasing cod farming, problems caused by escapement of domesticated cod and potensial impact on native stock must be considered. Such problems have received a lot attention during the last years, especially regarding salmonids. The benefits of introducing a specific genetic marker in domesticated fish stock have been discussed by several authors (Wilkins, 1981; Taggert and Ferguson, 1984). The PGI-1(30/30) homozygotes reported here, could be used as a broodstock in a breeding programme for domesticated cod. It must be pointed out, hovewer, that inbreeding must be prevented and for that reason a homozygote population should be assembled from other populations of cod than the single one applied in the Austevoll experiment. Furthermore, single pair crossings and methods for single family groups production should be established.

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Table 1. Genotype frequencies of phosphoglucose isomerase (PGI-1) expressed in white muscle in Arctic cod samples from different areas (data pooled from several years). For comparison a sample of artificially produced cod from Hyltropollen (1983).

Area	N	Genotype frequencies						Frequency of PGI-1(30)
		30/30	30/100	30/150	100/100	100/150	150/150	
Lofoten	511	0	14	1	251	204	4	0.015
Møre	4193	0	136	79	1855	1736	387	0.026
Spitsbergen	502	0	13	2	213	220	54	0.015
Barents Sea	466	1	21	5	402	370	119	0.015
Total	6124	1	184	87	2721	2530	601	0.022
Hyltro-pollen, 1983	466	0	20	20	240	156	30	0.043

Table 2. PGI-1 genotypes and frequency of PGI-1(30) in different samples of yolksac larvae hatched from eggs collected from the spawning tank. Sample no. 3 and 4 were from Svartatjønn a few days after release at five days of age.

Sample	N	Genotype frequencies						Frequency of PGI-1(30)
		30/30	30/100	30/150	100/100	100/150	150/150	
Broodstock	70		0.59	0.41				0.500
1. Hatchery	94	0.22	0.12	0.26	0.07	0.16	0.17	0.410
2. Hatchery	96	0.28	0.20	0.19	0.16	0.11	0.06	0.474
3. Svartatjønn	84	0.21	0.17	0.24	0.23	0.13	0.02	0.417
4. Svartatjønn	38	0.24	0.26	0.21	0.11	0.08	0.11	0.474
Total yolksac larvae	312	0.24	0.17	0.22	0.14	0.14	0.09	0.434

Table 3. PGI-1 genotype frequency and frequency of PGI-1(30) in cod fry and juveniles from Svartatjønn.

Sample	Date	N	Genotype frequencies						Frequency of PGI-1(30)
			30/30	30/100	30/150	100/100	100/150	150/150	
Broodstock		70		0.59	0.41				0.500
1 Larvae	210486	207	0.22	0.29	0.16	0.10	0.15	0.08	0.444
2 Cod fry	180686	128	0.27	0.30	0.22	0.08	0.10	0.03	0.531
3 Juveniles	0387	3263	0.26	0.32	0.19	0.10	0.09	0.04	0.516
Total offspring		3598	0.26	0.32	0.19	0.10	0.10	0.04	0.513

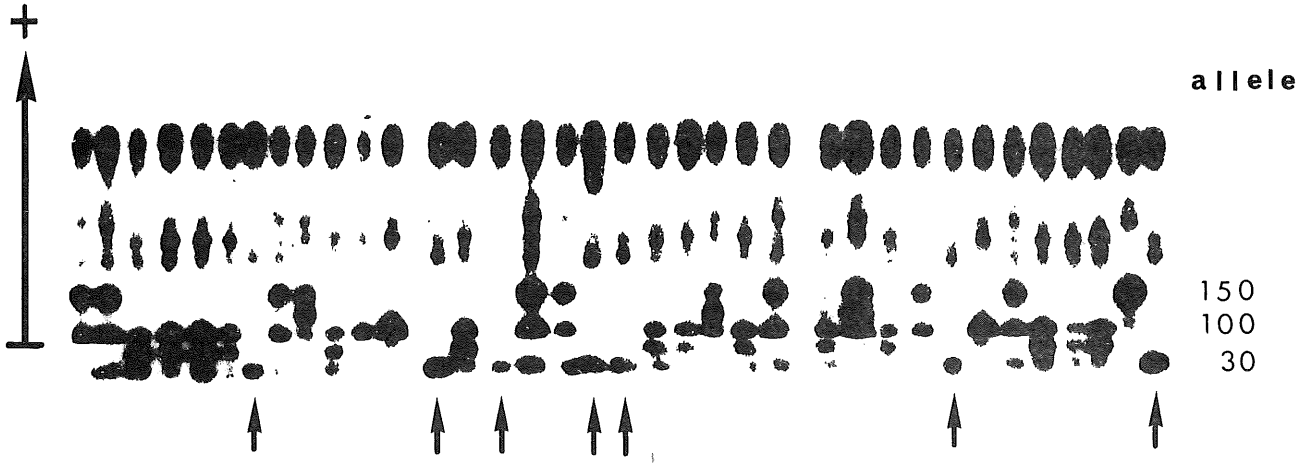

Fig 1 Starch gel electrophoresis (gel buffer pH=6.1) and selective staining of phosphoglucose isomerase (PGI). White muscle samples of cod fry from Svartatjønn June 86. The different alleles of the PGI-1 locus are given and the PGI-1(30/30) homozygotes are indicated.

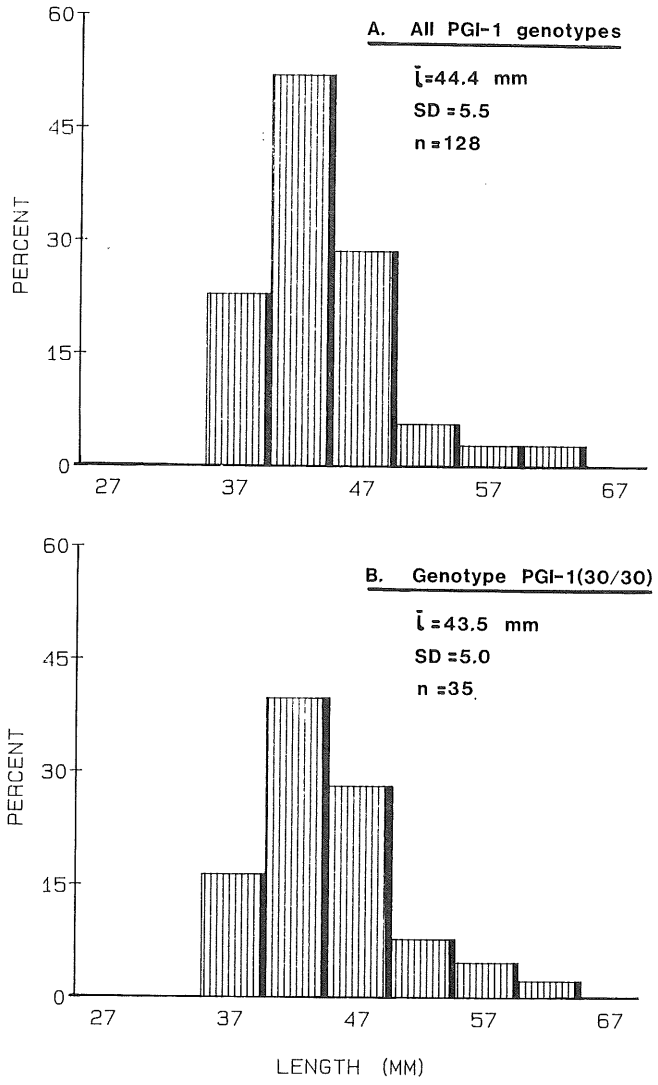

Fig 2 Length distribution and mean length of cod fry from Svartatjønn , June 1986.

A. Total sample including all PGI-1 genotypes

B. Sample of PGI-1(30/30) homozygotes

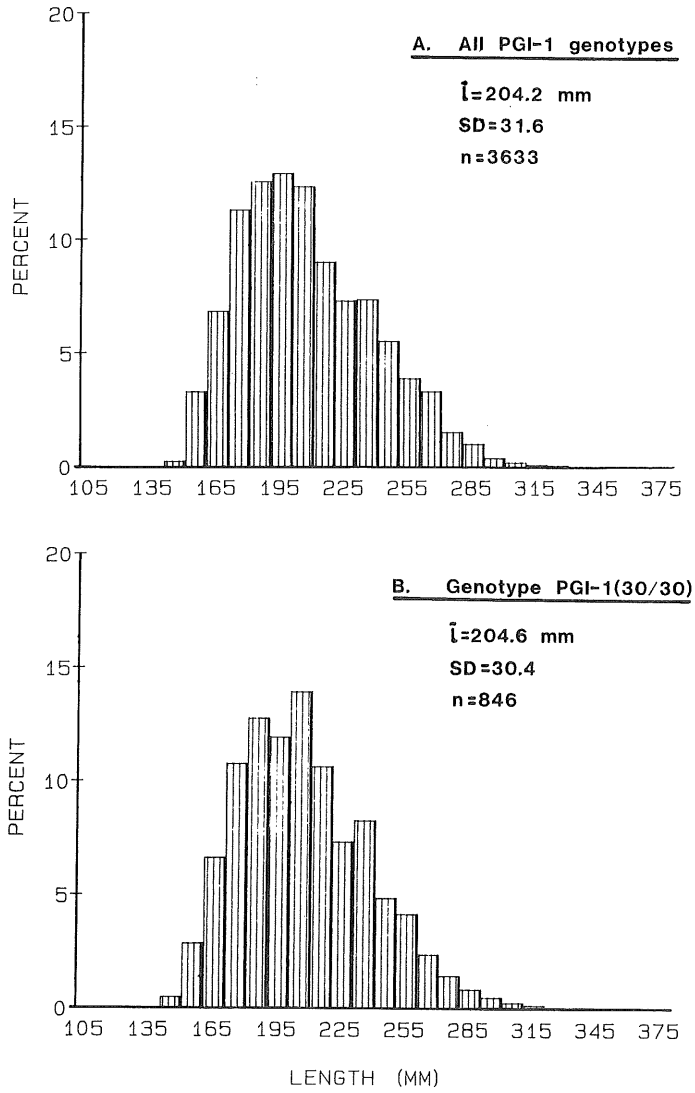

Fig 3 Length distribution and mean length of the population of cod juveniles in March 1987.

A. Total sample including all PGI-1 genotypes

B. Sample of PGI-1(30/30) homozygotes