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0-group cod *(Gadus morhua)* in captivity: Differential survival of certain genotypes*

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ABSTRACT: Genotypes at *LDH-3* (lactate dehydrogenase) and *PGI-1* (phosphoglucoisomerase) in 263 0-group specimens of Atlantic cod *(Gadus morhua)* caught in Trondheimsfjorden, Norway, in fall 1983 showed significantly different survival rates during 72 days in captivity. The heterozygote was nominally superior at both loci, and there was a significant accumulation of double heterozygotes among the survivors. Apparently, *LDH-3* and *PGI-1* are not selectively neutral, and allele frequency differences at these two loci between groups of cod in nature should not be interpreted as markers of reproductive isolation. The results are related to current concepts of cod population structure.

INTRODUCTION

Since the method of protein electrophoresis became available there has been a number of population genetic studies in Atlantic cod *(Gadus morhua)* (e.g. Frydenberg et al., 1965; Sick, 1965a, b; Møller, 1968, 1969; Jamieson, 1975; Cross & Payne, 1978). The following loci have shown geographic variation in allele frequencies: *Hbl* (haemoglobin), *Tf* (serum transferrin), *Est* (esterase), *LDH-3* (lactate dehydrogenase), and *PGI-1* (phosphoglucoisomerase). A considerably larger number of loci were either monomorphic, or displayed homogeneous allele frequencies in samples from throughout the distribution range of the species (Mork et al.; in prep.).

The assumption of selective neutrality at electrophoretic loci is a basic requirement when using allele frequency data in the delineation of genetic population structure. The conclusions made in most early, and some recent, population genetic studies on cod are necessarily, explicite or implicite, based on this assumption (e.g. Møller, 1969; Cross & Payne, 1978; Jørstad, 1984).

However, there is strong evidence that cod haemoglobins *(Hbl)* are affected by environmental selection (Mork et al., 1983, 1984a, b; Mork & Sundnes, 1984, 1985), and a recent study suggests that the same applies to cod serum transferrins (Gauldie, 1984). Esterases are regarded as notoriously unreliable population markers; most cases of monogenic selection in fishes concern these proteins (Kirpichnikov, 1981; p. 219 ff.). Jamieson (1975) reported selection at cod *LDH-3,* and Jørstad et al. (1981) listed selection among possible causes for their cod *PGI-1* observations in an experiment the design of which, however, did not allow conclusions on this matter.

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In the hitherto most extensive study on the amount and distribution of genetic variation in cod, only two (*LDH-3* and *PGI-1*) out of ten variable tissue enzyme loci displayed statistically significant heterogeneity of allele frequencies between samples from most of the current distribution range of the species (Mork et al.; in prep.). Hence the status of these two loci with respect to selection appears to be an important key to the understanding of the genetic population structure of cod.

The present report deals with observations on an apparent environmental selection at both these loci during an aquarium experiment with 0-group Norwegian coastal cod in 1983.

MATERIALS AND METHODS

263 0-group specimens of Atlantic cod *(Gadus morhua)* were caught with a beach seine on one location in the inner part of Trondheimsfjorden, Norway, on Oct. 12. 1983. The fish were transferred alive to a 3 m^3 running sea-water (7 °C, 33.5 ‰ S) aquarium at Trondhjem Biological Station. They were predetermined for growth studies, but unfortunately for that purpose they refused to accept dry fodder (salmon start fodder; various grain sizes). Thus despite continuous feeding efforts over several weeks the fish seemed to be starving. However, no mortality was observed in the first 35 days after transfer to the aquarium. Thereafter, dead specimens found on the aquarium floor were collected (usually every third day) and frozen batch-wise at −80 °C. On the 72nd day after transfer, 10 batches (batch size 11–28 individuals) totalling 177 dead specimens had been collected. At this point it was decided to terminate the feeding efforts, and the 86 surviving individuals were collected, killed, and frozen.

While being inadequate for growth studies, this material was subsequently found to be suitable for studying the potential effects of enzyme genotype on survival during starvation. Thus tissue extracts from all of the 263 specimens were subsequently subjected to isoelectric focusing in polyacrylamide gel (IFPAG) according to Mork & Haug (1983). The methods for specific detection of enzymes also followed Mork & Haug (1983) while, for ease of comparison with previous works in the field, we used the locus and allele designation of Mork et al. (1982), based on starch gel electrophoresis, for describing results. The correspondence of nomenclatures was obtained by analyzing starch gel genotyped specimens by IFPAG.

Two enzyme loci, *LDH-3* (Lactate dehydrogenase; E.C. No. 1.1.1.27) and *PGI-1* (Phosphoglucoisomerase; E.C. No. 5.3.1.9), both previously described by Mork et al. (1982) for Norwegian coastal cod, were scored. For tabular and computational simplicity, only the three most common genotypes were included in the present analyses. Hence, the omission of 1 individual with a rare *LDH-3,* and 13 with rare *PGI-1* genotypes (3 different) will result in different sample sizes for the two loci in the following.

Accurate knowledge of milieu parameters in the aquarium is restricted to temperature and salinity records. Some factors, which in retrospect may seem to be of potential significance to the present observations, were observed but not formally recorded. For instance, since the cod refused to accept the fodder, there were problems created by bacterial degradation of undigested fodder on the aquarium floor. H_2S development was observed, indicating at least periodically anoxic conditions near the bottom. In combination with general aquarium stress this could be a factor with a potentially discriminating effect on *LDH-3* genotypes. Another observation of potential significance was that newly

dead specimens appeared to have extravasations in the eye- and brain region. This effect was also observed in apparently very weak individuals, seemingly increasing in magnitude prior to death. We do not know, however, if these effects are symptoms of a particular death cause. At least initially, the aquarium was relatively crowded. Such conditions may increase the general stress level and make the fish more vulnerable to diseases. The size distribution was presumably too homogeneous for cannibalism to take place, but aggressive behaviour was frequently noticed and might have caused some mortality. Finally, it may be mentioned that the temperature in the experiment aquarium was 5–6 °C lower than at the actual sampling habitat.

RESULTS

Both *LDH-3* and *PGI-1* genotype had a significant effect on survival under the present experimental conditions. At *LDH-3* the genotypic composition was very different from the initial values among the survivors after about two and a half months in the aquarium (Table 1). At this point in time, there was a marked excess of *LDH-3* heterozygotes among the survivors (Table 1). At *PGI-1*, the test of heterogeneity in genotypic composition in dead individuals and survivors did not indicate changes in the genotypic composition during the experiment (Table 2). Such changes were, however, revealed by more detailed analyses (below).

Table 1. *Gadus morhua*. *LDH-3* genotypic composition in dead individuals and survivors in aquarium experiment, together with result from a 2×3 contingency table test (G-statistic) for heterogeneity in composition in the two groups. Expected values under the null hypothesis of no difference are shown in parentheses

Group	*LDH-3 (100/100)*	*LDH-3 (100/70)*	*LDH-3 (70/70)*	Total
Deads	74 (65.8)	70 (82.0)	32 (28.2)	176
Survivors	24 (32.2)	52 (40.0)	10 (13.8)	86

$G = 9.981$, d.f. $= 2$, $P = 0.007$

Since the genotypic composition was known for each of the 10 batches of dead specimens, we were able to calculate the average survival time (number of days from incubation to death in the aquarium) for each genotype at the two loci. In order to utilize information from the group of surviving specimens, this group was assigned a value of 75

Table 2. *Gadus morhua*. *PGI-1* genotypic composition in dead individuals and survivors in aquarium experiment. Result of test for heterogeneity (cf. legend of Table 1) is shown

Group	*PGI-1 (100/100)*	*PGI-1 (100/135)*	*PGI-1 (135/135)*	Total
Deads	78 (77.6)	74 (76.2)	16 (14.2)	168
Survivors	37 (37.4)	39 (36.8)	5 (6.8)	81

$G = 0.976$, d.f. $= 2$, $P = 0.614$

days. (The 75th day would have been the next sampling day if the experiment had been continued.) The results from these calculations are shown in Table 3. Prior to a 2-factor analysis of variance of these data, the variances were tested for heteroscedasticity (Bartletts B-test), and the distribution of individual measures was tested for skewness. Neither effect was found significant, but it was found that square-root transformation of

Table 3. *Gadus morhua.* Mean survival time ($\bar{x}$; in days) for genotypes at *LDH-3* and *PGI-1* in aquarium experiment. Number of specimens in groups (*N*), and standard deviations (*SD*) of mean values also shown

Genotypes		*PGI-1 (100/100)*	*PGI-1 (100/135)*	*PGI-1 (135/135)*
LDH-3 (100/100)	N	42	48	6
	$\bar{x}$	51.4	58.3	46.3
	SD	15.5	14.7	14.6
LDH-3 (100/70)	N	60	47	9
	$\bar{x}$	59.7	61.8	58.4
	SD	14.9	14.2	17.1
LDH-3 (70/70)	N	12	18	6
	$\bar{x}$	60.3	56.7	45.3
	SD	15.5	13.5	6.2

the raw data made variances more homoscedastic. After this transformation, Bartletts test showed B = 4.682, d.f. = 8, P = 0.791; the coefficient of skewness was −0.161, P > 0.20.

Thus the data seemed to meet the assumptions for analysis of variance, and were processed by the BMDP2V program for 2-factor analysis of variance (BMDP Statistical Software, Inc.) which allows for unequal sample sizes. The results are shown in Table 4. Both *LDH-3* and *PGI-1* genotype had a significant effect on mean survival time, but there was no detectable interaction effect. Among genotypic mean values within loci, the heterozygote was superior at both *LDH-3* and *PGI-1.* The significance of the differences between genotypic means were tested by Newman-Keuls test procedures. At *LDH-3* the test showed the heterozygote to have significantly (P < 0.05) higher mean value than the

Table 4. *Gadus morhua.* Summary of 2-factor analysis of variance of mean survival time (days) of genotypes at *LDH-3* and *PGI-1* in aquarium experiment. The analysis was performed on square-root transformed values (cf. section "Results")

Source of variation	SS	d.f.	MS	F-value
A *(LDH-3)*	7.95183	2	3.97591	4.08
B *(PGI-1)*	6.14202	2	3.07101	3.15
A × B interaction	4.52740	4	1.13185	1.16
Within	233.06188	239	.97515	
Total	251.68313			

Significance: A *(LDH-3):* P = 0.018, B *(PGI-1):* P = 0.044, A × B: P = 0.329

(100/100) genotype, but not than the *(70/70)* genotype. The two latter were not significantly different. At *PGI-1* the heterozygote had significantly higher mean than the *(135/135)* genotype but not than the *(100/100)* genotype, while the difference between the two latter was unsignificant.

The fact that the heterozygotes appeared superior at both loci (although no significant interaction effect in Table 4) called for a closer inspection of the performance of the double heterozygotes. The accumulation of *LDH-3* among the survivors indicated in Table 1 was confirmed by testing (Kendall's test of rank correlation) the increase in the proportion of *LDH-3* heterozygotes among the survivors after each sampling of dead specimens (Kendall's $S = 53$, $n = 11$, $P = 0.00004$). Within *LDH-3* heterozygotes, the proportion of the *PGI-1* heterozygotes increased significantly during the experiment (Kendall's $S = 27$, $n = 11$, $P = 0.036$). This increase occurred at the expense of the *PGI-1 (100/100)* homozygotes which showed a significant trend of decreasing proportion (Kendall's $S = -32$, $n = 11$, $P = 0.013$). The *PGI-1 (135/135)* proportion (still within *LDH-3* heterozygotes) showed no significant trend (Kendall's $S = 5$, $n = 11$, $P = 0.697$).

The genetic composition at *LDH-3* and *PGI-1* was in accordance with Hardy-Weinberg expectations at the time of sampling of the 263 0-group cod specimens. The goodness of fit statistics (all genotypes included) computed for *LDH-3* and *PGI-1* were $G = 0.185$, d.f. $= 1$, $P = 0.667$, and $G = 0.605$, d.f. $= 1$, $P = 0.437$, respectively. There was no detectable linkage disequilibrium (Hill, 1974) at the loci under study (tested for the three most common genotypes at each locus); the largest G (d.f. $= 1$) in 6 comparisons was 3.757 in the initial group, 1.999 in the group of survivors.

DISCUSSION

The unplanned nature of the experiment which provided the present observations restricts conclusions on the causal relationships behind the genotypical differences in survival rates. The potential causes, and their interactions, may be very complex. However, from previous work in this field (reviewed by Kirpichnikov, 1981) it appears that some factors are frequently observed to act as discriminators, e.g. temperature, temperature-changes, water oxygenation, salinity, general level of activity, and various bacterial and viral diseases. Induced stress, e.g. by crowding and starvation, may act to reinforce the effects. None of these factors can be excluded as potential causes in the present experiment. On the other hand one cannot exclude the possibility that the differential survival rates were caused by environmental factors prior to catch, e.g. the genotypes may have differed with respect to the size of the energy resources they brought with them to the aquarium.

The two enzymes here studied have different types of functions in the metabolism; *LDH* catalyses the lactate<--->pyruvate reaction, and genotypic differences at this locus may be particularly manifest in the physical performance under stressed or anoxic conditions. *PGI* is a glycolytic enzyme involved in G-6-P<--->F-6-P conversion. For both these enzymes, milieu-dependent differences in the functional properties of various genotypes in fish species have been reported previously (e.g. by Champion & Whitt, 1976; Place & Powers, 1979). Also, there are a number of reports on apparent selection in natural habitats for both these loci in various fish (e.g. Johnson, 1971; Koehn & Williams, 1978; Mitton & Koehn, 1975; Powers & Place, 1978; Smith & Francis, 1984). In fact, such

observations were also reported for *LDH-3* in cod from North Atlantic waters (Jamieson, 1975). The present observation of heterozygote superiority is not unique; mono- and oligogenic heterosis have been observed in several studies on fish; char *LDH* (Wright & Atherton, 1970); *Lepomis* sp. *PGI* (Whitt et al., 1976). Thus it appears that environmental selection favouring certain genotypes at certain loci is not an uncommon phenomenon in fishes. It may seem surprising that the effect of only two out of all the loci in the genome can be traced in survival rates; it is noted, however, that these two loci are among those which from previous studies seem to be particularly susceptible for selection, and that, after all, their effect did not explain more than about 7 % of the total variation in survival rates (Table 4).

The present sample was drawn from a fairly large and presumably well established stock of cod with no dramatic size fluctuations. Cod generations overlap extensively, and population bottlenecks are probably rarely encountered except in the fringes of the species range. Against this background it seems unnecessary to make the reservations that closely linked loci, instead of *LDH-3* and *PGI-1,* were responsible for the present observations. Further, eggs and larvae from the annual spawning undergo extensive mixing in the water masses during a pelagic period of several months before settling. Therefore it is unlikely that the here observed genotypic differences reflect, for example, family effects. Finally, immigration into the cod stock in Trondheimsfjorden appears negligible (Mork & Sundnes, 1985), and population mixture can thus probably safely be excluded as a potential cause for the present observations. In conclusion, there appears to be little reason to doubt that the genotypic differences observed were de facto effects of differences in the functional properties of the various active proteins coded at *LDH-3* and *PGI-1.*

Results from aquarium experiments are not automatically valid for fish in their natural environment. In the present case, however, it seems that the potential aquarium effect would mainly be to accelerate selection processes which actually will occur under certain environmental circumstances. The aquarium conditions were not qualitatively different from those encountered by cod specimens in their natural habitats. The magnitude of the genetic changes during the aquarium experiment suggests that allele frequency changes at both *LDH-3* and *PGI-1* can be very rapid (in evolutionary terms) in a fluctuating natural environment. Thus, neither *LDH-3* nor *PGI-1* allele frequency differences between cod groups can any longer be regarded as reliable signs of reproductive isolation. This fact challenges the conclusions of genetic isolation reached in previous studies utilizing these loci, e.g. for cod stocks off eastern North America (Cross & Payne, 1978) and in northern Norwegian waters (Jørstad, 1984).

Based on analyses of a large number of tissue enzyme loci, Mork et al. (in prep.) report genetic distances between current stocks of Atlantic cod to be far below usual population level. As mentioned in the introduction, only *LDH-3* and *PGI-1* showed regional variation in allele frequencies. The present results are significant against this background; they provide a plausible cause for the maintenance of such regional allele frequency differences even in the presence of a gene flow between stocks.

Thus, out of the five protein loci (listed in 'Introduction') which currently are known to display geographical variation in allele frequencies in cod, only esterases are now without reported observations on selection effects. From studies in other fish species, however, esterases are among those proteins for which selection most frequently has

been observed (cf. review by Kirpichnikov, 1981). This fact seriously affects their reliability as markers of reproductive isolation in cod as well as in other species.

Jamieson & Turner (1978, p. 724) stated "It is possible that those polymorphic loci, which show the greatest regional variation in allele frequencies, code those proteins that are most buffeted by evolutionary forces presently moulding the genetic composition of races within species". Recent studies on the merits of haemoglobins (Mork et al., 1983, 1984a, b; Mork & Sundnes, 1984, 1985), serum transferrins (Gauldie, 1984), and the present study on *LDH-3* and *PGI-1*, seem to lend support to this view. However, with reference to the generally very low amount of genetic differentiation throughout the species range reported by Mork et al. (in prep.), there is hardly any genetic evidence for the existence of "races" of cod in the North Atlantic. The accumulated information on selection effects at those loci showing regional variability in allele frequencies makes it even more difficult to argue in favour of models postulating reproductive isolation between cod stocks or stock subunits. Current knowledge allows an alternative model to be suggested. This model would suggest that there is genetic communication between all investigated cod stocks in the North Atlantic. The gene flow has been large enough to prevent a general genetic differentiation. For some loci, local selection forces are strong enough to inhibit, or even override, the levelling effect of the gene flow. It appears that this model deserves consideration in light of available information from tagging surveys, mating experiments, genetic studies, as well as studies on the transport of pelagic eggs and larvae by ocean currents.

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