Distinct genetic divergence between cod (*Gadus morhua*) in fjords and cod in offshore waters in northern Norway

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Since 1993 and up until today Atlantic cod sampled in various fjords and in offshore waters in northern Norway has been analysed for frequencies of alleles at the scnDNA pantophysin locus (identical to the synaptophysin locus in earlier papers). The locus is di-allelic and whereas in fjords there is an all-over dominance of the *Pan* I^A allele, in some samples up to 80-90 percent, the offshore cod (Barents Sea included) is dominated by the *Pan* I^B allele, in most samples more than 90 percent. This striking difference between two groups of cod that have the possibility to intermingle both at spawning and feeding sites has been upheld in all years since the investigation started. Moreover, the profound split in two groups is seen both in larval cod (YOY) and in adults of various ages. The allele frequencies in fjords may vary between years, but the most plausible reason for this seems to be various influxes of offshore cod into the fjords. Although we do admit that the pantophysin locus is subject to some weak selection, selection forces acting upon one common breeding unit cannot explain a consistent difference of this magnitude. We believe that breeding structure is the main causative agent of the differences found.

**INTRODUCTION**

The question about the existence of separated stocks of cod in the NE Atlantic is still considered a controversy. In particular the question of whether the coastal cod and NE arctic cod in northern Norway represent genetically diverse populations, is a matter of ongoing debate (Árnason & Pálsson 1996, Fevolden & Pogson 1997, Borisov et al. 1999, Árnason et al. 2000, Pogson & Fevolden in subm.). Numerous attempts have been made to separate the two stocks by examining meristic characters such as otoliths and vertebral numbers (Schmidt 1930; Rollefson 1934; Løken et al. 1994), tabulating frequencies of polymorphic alleles at blood protein and enzyme-coding loci (Sick 1965; Møller 1966a 1966b, 1968, 1969; Jørstad 1984; Jørstad & Nævdal 1989; Dahle & Jørstad 1993; Nordeide & Pettersen, 1998), or by comparing mtDNA haplotype frequency distributions (Dahle 1991; Árnason & Pálsson 1996). Arctic and coastal groups, however, exhibit overlap in otolith characters and vertebral numbers and the majority of polymorphic loci examined have failed to distinguish the two stocks. One notable exception is the haemoglobin-1 (*Hb*-1) locus that exhibits significant allele frequency variation between arctic and coastal populations. It has been suggested, however, that the *Hb*-1 locus may be affected by natural selection, thus limiting its usefulness as stock identification marker (e.g. Mork et al. 1984, 1985; Mork & Sundnes 1985, Mork & Giæver 1999). Recent acknowledgements intimate, on the other hand, that loci subject to weak or moderate selection may in fact be able
to detect differences among populations that would not be revealed by neutral markers (e.g. Chevillon et al. 1995; Taylor et al. 1995; Carvalho et al. 1999).

Since 1993 and onwards we have been studying the genetic differentiation between NE Arctic cod and coastal cod in northern Norway by looking at the variation at the Pan I locus. This seemingly non-neutral single copy nuclear (scn) DNA locus, codes for pantophysin, a membrane protein of synaptic vesicles. This is the same locus that in earlier papers (Fevolden et al. 1995, Fevolden & Pogson 1997) was denoted Syp I. Despite the fact that NE Arctic cod and coastal cod may intermingle both at spawning sites (e.g. Lofoten), and also at inshore feeding grounds, a deep genetic divergence has been revealed between the two groups (Fevolden & Pogson 1997, Fevolden et al.1998; papers in prep.). After having sampled cod over a series of nine years now, there is convincing evidence that the profound allele frequency difference between the two groups is stable over years.

MATERIAL AND METHODS

Fish used in the still ongoing survey have been sampled annually since 1993. The major sampling areas have been fjords and coastal areas in northern Norway (Troms and Finnmark county), the Barents Sea north of Norway, and waters around Spitsbergen. Some localities have been sampled every year, whereas others more sporadically. In recent years limited sampling have also been done off the Kola Peninsula (with assistance from Murmansk Marine Biology Institute), and along the Norwegian coast south of Lofoten. Our southernmost sampling site is from the North Sea (near Helgoland). 0-group cod (YOY = young of the year) have been sampled in shallow water by a shore seine, pelagically and at the bottom by trawls. Older cod were sampled by trawling. The main sampling activity has been in late August/early September with the purpose of being able to sample newly settled 0-group specimens. Over the last three years, selected fjords have also been sampled in the late winter spawning season. The majority of the samples were taken on cruises with research vessels of the University of Tromsø, whereas some sampling has been under the regime of Fiskeriforskning (Norwegian Institute of Fisheries and Aquaculture). Detailed information about the samples will be published in pertinent papers. As yet, a total of some 200 subsamples comprising more than 8000 fish have been analysed for Pan I variation.

The pantophysin locus is being analysed by the PCR based assay described in details by Fevolden & Pogson (1997). Methods for DNA-extraction are also in that paper. The locus is two-allelic, thus only three genotypes are scored: the two homozygotes $\text{Pan I}^{AA}$ and $\text{Pan I}^{BB}$, and the heterozygote $\text{Pan I}^{AB}$.

For statistical analyses the programs BIOSYS I, Genepop, and Phylips are those normally applied. Detailed statistical analyses of the data will be published elsewhere.

RESULTS

Detailed results from this study will be presented in various papers that are in preparation. Some preliminary results were presented in Fevolden & Pogson (1997), Fevolden et al. (1998), and Pogson & Fevolden (1998; and in subm.). In the following
some of the more contrasting genetic divergence that we have observed will be visualised and commented.

**Overall variation**

By simply estimating genetic distances between samples, the 200 subsamples and approximately 8000 specimens that we have analysed for *Pan I* variation, form two clearly distinct groups. One consists of:

1) adult fish caught at known spawning sites for NE arctic cod,
2) adults and YOY caught in the open Barents Sea, plus
3) adults and YOY caught at deep water in outer parts of fjords with more or less direct access to open Barents Sea water.

Thus, these are all samples that we believe are NE Arctic cod. All samples in this group exhibit high frequencies of the *Syp I*\(^B\) allele (mean for offshore samples \(\approx 0.9\)).

The other group consists almost entirely of:

1) YOY caught at shallow inshore water,
2) adults caught well within fjords, and
3) cod from more southern parts of Norway and from the North Sea.

In contrast to the former group these coastal cod group possess significantly lower frequencies of the *Syp I*\(^B\) allele (mean \(\approx 0.2\)) and a correspondingly high frequency of the *Syp I*\(^A\) allele (\(\approx 0.8\)).

**North-south cline?**

When allele frequencies from north to south are compared in post-juvenile cod an appearance of a latitudinal cline is revealed (Fig. 1). Subsamples from the northern Barents Sea, including samples taken in the vicinity of the Bear Island, and around Spitsbergen, all have *Syp I*\(^B\) frequencies > 0.9. No single *Syp I*\(^{AA}\) homozygote, the predominant coastal *Syp I* genotype, is observed in those regions. In sharp contrast to this, in the southernmost areas the *Syp I*\(^A\) allele is totally dominating and there is an absolute absence of the *Syp I*\(^{BB}\) genotype (Fig. 1). The appearance of a gradual shift in allele frequencies from north to south, however, is falsified by the existence of fjords and coastal areas in northern Norway, particularly in Finnmark, where both coastal cod and NE Arctic cod exist together. The pooled samples of NE Arctic and coastal cod will consequently exhibit intermediate allele frequencies, thus, the appearance of a gradual shift from north to south is created.

As to Fig.1 it should be pointed out that the apparent dominance of coastal cod (the *Pan I*\(^A\) allele is dominating) in Lofoten, which is a main spawning ground for NE Arctic cod, is due to the fact that these cod were sampled in the fall, outside the spawning season. We have other data from the spawning season in Lofoten, which show a dominance of NE Arctic cod, although even then with coastal cod present (at shallower depths; unpublished data).

**YOY inshore and offshore**

When YOY are compared from coastal and offshore areas in North Norway, a sharp divergence in allele frequencies is seen between samples taken in fjords (*Pan I*\(^A\))
DISCUSSION

The highly significant difference in Pan I allele frequencies that we observe between alleged NE arctic and coastal cod populations in northern Norway is considerably larger than previously reported for any other locus, including Hb-1 (e.g. Møller 1968; Dahle & Jørstad 1993). There is now also accumulating evidence that the divergence between the two groups is stable over years. The fact that we up until today have found no single representative of the Pan I^{AA} genotype in the Barents Sea proper, reinforces our suggestion that the NE Arctic cod is genetically distinct from the coastal cod. There are areas were both groups appear simultaneously, either as premature individuals on nursery grounds near the coast or even within fjords, or as adults on spawning grounds (Lofoten). Frequent heterozygote deficits (Wahlund effect) support the presence of genetically distinct groups in such cases [although a few cases of heterozygote excesses are also recorded (to be discussed in a separate paper)]. The dominant coastal Pan I^{AA} genotype, however, does seem unable to establish itself in open Barents Sea water, at the same time as the Pan I^{B} allele is always inferior in frequencies to the Pan I^{A} allele well within fjords sheltered from the Barents Sea.

It has been well known among fishermen for generations that the coastal or fjord cod has a variety of local spawning sites in northern Norway at which the NE Arctic cod will not spawn. It is also well known, however, that in Lofoten, which is the largest spawning site for NE Arctic cod, there is also spawning among coastal cod. The reasons why the two groups do not seem to interbreed in this area are intriguing. Various mechanisms have been suggested to prevent interbreeding, e.g. spawning at different depths, at different times, or at different temperatures. Behavioural differences have also been suggested to prevent interbreeding, e.g. mating dance or lekking (Nordeide and Folstad 2000).

Whatever causes this lack of interbreeding between the two groups, it seems to be a reality. We do not claim that interbreeding never happens because we have a few samples where intermediate allele frequencies are found without being accompanied by deficits of heterozygotes. Beside the simple explanation that those cod could belong to a separate population that displays such divergent allele frequencies, interbreeding of mixed groups can also cause it. We are convinced, however, that the overall ‘extremely’ significant difference in allele frequencies that we observe at Pan I between coastal cod and NE Arctic cod in northern Norway, cannot be caused by selecting forces acting on a common gene pool. We have in several papers acknowledge that Pan I is not neutral (Pogson et al. 1995, Fevolden & Pogson 1997, Pogson & Fevolden 1998, Pogson 2001, Pogson & Fevolden, in subm.), but the extreme differences that we observe between coastal cod and NE Arctic cod cannot be caused by selection alone. Unrealistically high selection coefficients would then be needed (e.g. Fevolden & Pogson 1997). The usefulness of Pan I as a marker to distinguish between fjord populations may, however, be questioned. We and others (Karlson & Mork 2001) have observed significant allele frequency differences at Pan I between years within one and the same fjord. In northern Norway this could be
explained by varying influx of NE Arctic cod to the fjord, in other regions selection and sampling error must be considered as contributing agents.

This study is still in progress. At present we are testing a series of microsatellite primers. The main reason for including highly variable microsatellite loci is to possibly reveal differences between populations in various fjords, but we also have in mind the criticism often raised when conclusions are drawn from single locus data. We do believe, however, that it is difficult to find alternatives to breeding structure to explain the profound divergence at the Pan I locus between NE Arctic cod and coastal cod in northern Norway.

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Figure 1. Average frequencies (over years) of the two Pan I alleles in post-juvenile cod from the northernmost (Svalbard) to the southernmost (Helgoland) sampling locality.
Figure 2. Average frequencies (over years) of the two Pan 1 alleles in 0-group cod from various fjords in North Norway and from three offshore samples (three columns to the left). Alleles as in Fig. 1.
Figure 3. Frequencies of the two Pan I alleles in 0-group samples of cod taken in consecutive years in Dønnesfjorden at Sørøya, Finnmark (lower columns) compared with three random samples of 0-group cod taken in open water just north of Sørøya (upper columns). Black filled circle indicates the position of Dønnesfjorden.