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Mitochondrial cytochrome *b* DNA sequence variation of Atlantic cod, *Gadus morhua*, from Greenland and Ísafjarðardjúp, Iceland

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Contents

1 **Introduction** **1**

2 **Materials and methods** **2**

2.1 Sample collections 2

2.2 DNA isolation, amplification and sequencing 5

2.3 Statistical methods 8

3 **Results** **8**

3.1 Molecular nature of variation 8

3.2 Population diversity, geography and gene flow 10

3.2.1 Haplotype analysis from Iceland 10

3.2.2 Haplotype analysis from Greenland 11

4 **Discussion** **14**

5 **Acknowledgements** **16**

List of Figures

1 A map of Ísafjarðardjúp with sampling localities. 3

2 A southern map of Greenland with sampling localities. 4

3 Phylogenetic maximum parsimony network of 14 haplotypes of cytochrome *b* found among 113 cod sampled from Iceland and Greenland. The names of the haplotypes (*A, C, D, E, G, S, GJ, NI, ZI, EM, RI, BI, ED* and *HJ*) are given along with the localities (ID, between Melgraseyri and Reykjanes; SF,Skötufjörður; BN, between Vigur and Æðey; GB, Ameralik Kangerlua; GA, Uumannap Sullua N; GC, Uumannap Sullua S; GE, Amerloq Fjord). On the lines connecting the boxes are given the site and the base change relative to the *A* haplotype. 9

List of Tables

I Sampling localities, latitudes and longitudes 3

II Haplotype distribution in Ísafjarðardjúp 10

III Haplotype diversities ($\hat{h}$), associated standard errors ($SE_{\hat{h}}$) by localities intrapopulation nucleotide diversities ($\hat{\pi}$) and N is the number of individuals in Ísafjarðardjúp. 11

IV Estimates of interpopulation (d_{XY}; lower triangular), intrapopulation (d_X or $d_Y = \hat{\pi}$; boldface on diagonal) and net nucleotide differences among localities (d_A; italic on upper triangular) in Ísafjarðardjúp. All figures multiplied by 100. 11

V Haplotype distribution in Greenland 12

VI Haplotype diversities ($\hat{h}$), associated standard errors ($SE_{\hat{h}}$) by localities, intrapopulation nucleotide diversities ($d_X = \hat{\pi}$) and N is the number of individuals in Greenland 13

VII Estimates of interpopulation (d_{XY}; lower triangular), intrapopulation (d_X or d_Y; diagonal $= \hat{\pi}$) and net nucleotide differences among localities (d_A; upper triangular) in Greenland. All figures multiplied by 100. 13

VIII Estimates of interpopulation (d_{XY}; lower triangular), intrapopulation (d_X or d_Y; diagonal $= \hat{\pi}$) and net nucleotide differences among overall areas (d_A; upper triangular) in Greenland. All figures multiplied by 100. 14

IX Estimates of intercountry (d_{XY}; lower triangular), intracountry (d_X or d_Y; diagonal $= \hat{\pi}$) and net nucleotide differences between (d_A; upper triangular) Ísafjarðardjúp, Iceland and Greenland. All figures multiplied by 100. 16

Abstract

We studied sequence variation of a 269-nucleotide region of the mitochondrial cytochrome *b* gene using polymerase chain reaction (PCR) and direct sequencing among 72 Icelandic Atlantic cod, *Gadus morhua*, from three localities in the fjords in the North-West of Iceland, Ísafjarðardjúp, and among 41 cod from the South-West of Greenland, from six localities representing two coastal areas. Twelve base changes are found among 113 sequences defining 14 haplotypes which differ from each other by one to five mutations. Two sites have been hit twice. All but one mutation are at third position silent sites. In Ísafjarðardjúp one mutation altered the amino acid sequence. Intralocality nucleotide diversity is higher in Ísafjarðardjúp than in Greenland, and the interlocality nucleotide divergence is such that the net interlocality nucleotide divergence in Ísafjarðardjúp was nil. Similarly the net interarea nucleotide divergence in Greenland was nil. A comparison with Norwegian cod (Árnason and Pálsson 1996) shows that intracountry diversities are similar in Iceland and Norway but lower in Greenland. However, the net nucleotide divergence between Iceland and Greenland is nil.

1 Introduction

Atlantic cod, *Gadus morhua* L., supports a major commercial fishery in the North Atlantic. It is also a major component of the ecosystem of this ocean. Cod is the most fecund vertebrate known with an average female producing millions of eggs. So it can support a major commercial fishery. Under a stable population size an enormous number of cod eggs, larvae and fish must fall prey to a variety of predators including man. As it develops, the cod itself becomes an important predator of a variety of prey (Jónsson 1983). Cod are also widely distributed in the North Atlantic ranging from Svalbard in the north to the Bay of Biscay in the south and from the Baltic Sea in the east, north along the Norwegian coast, and through Iceland and Greenland to the eastern seaboard of North America.
Several studies have been done to detect variation within the mtDNA of cod. Møller (1968, 1969) used variation of haemoglobin electromorphs for studying historical population differentiation of Arcto-Norwegian and coastal cod. Apparent natural selection of haemoglobin

variants cast doubt on the utility of haemoglobin variation for this study. Cod stocks all over the North Atlantic have been investigated using both restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR), based direct sequencing of mtDNA (e.g., Árnason et al. 1992; Carr et al. 1995). These studies strongly suggest gene flow and lack of population subdivision. The complete sequence of an Atlantic cod (*Gadus morhua*) mitochondrial genome has been determined by Johansen and Bakke (1996). The 16,696-bp genome contains the same 37 mitochondrial structural genes found in all other vertebrates analyzed, in an organisation similar to that of the placental mammals. Moreover Johansen and Bakke (1996) have found in comparisons among the major noncoding region in mtDNAs from different codfishes, as well as different stock samples of Atlantic cod that sequence features are conserved. Extensive studies of mitochondrial cytochrome *b* coding sequence variation of Newfoundland cod (Carr and Marshall 1991; Pepin and Carr 1993)(Carr et al. 1995) have found no evidence for population substructuring. In this paper we present results of a study of sequence variation of a 269-bp region of the same cytochrome *b* (Árnason and Pálsson 1996) fragment among cod from Iceland and Greenland.

2 Materials and methods

2.1 Sample collections

Samples of cod from Iceland (figure 1)(Table I) were obtained from three localities in the westfjords Ísafjarðardjúp. Samples from Skötufjörður, from coastal area between the towns Melgraseyri and Reykjanes and samples situated between the two islands Æðey and Vigur. In 1995 these samples were obtained using a shrimp trawl from Hjalti Karlsson.

Samples from the South-West of Greenland (figure 2)(Table I), were situated in Ikertooq Fjords (Sisimiut area) and the neighbouring fjord (about 66° 70'latitude, corresponding in the next tables at the locality Ikertooq Fjord S), in the Uumannap Sullua and the Ameralik Kangerlua (Lysefjord) (about 64° latitude, Nuuk area). The samples were collected on the annual inshore young cod survey, in 1995, of the Greenland Institute of the Natural Ressources by Dr. Jens Jacob Engelstoft.

Table I: Sampling localities, latitudes and longitudes

Locality		latitude	longitude
Iceland	Vigur-Æðey(BN)	66°07'	22°75'
	Melgraseyri-Reykjanes(ID)	65°98'	22°40'
	Skötufjörður(SF)	65°95'	22°81'
Greenland	IkertooqFjord(GE5-11)	66°84'	52°90'
	IkertooqFjord(GE13-15)	66°70'	52°68'
	IkertooqFjord(GE16-23)	66°85'	52°35'
	Uumannap Sullua(GA)	64°30'	51°21'
	Uumannap Sullua(GC)	64°25'	50°86'
	Ameralik Kangerlua(GB)	63°96'	51°36'

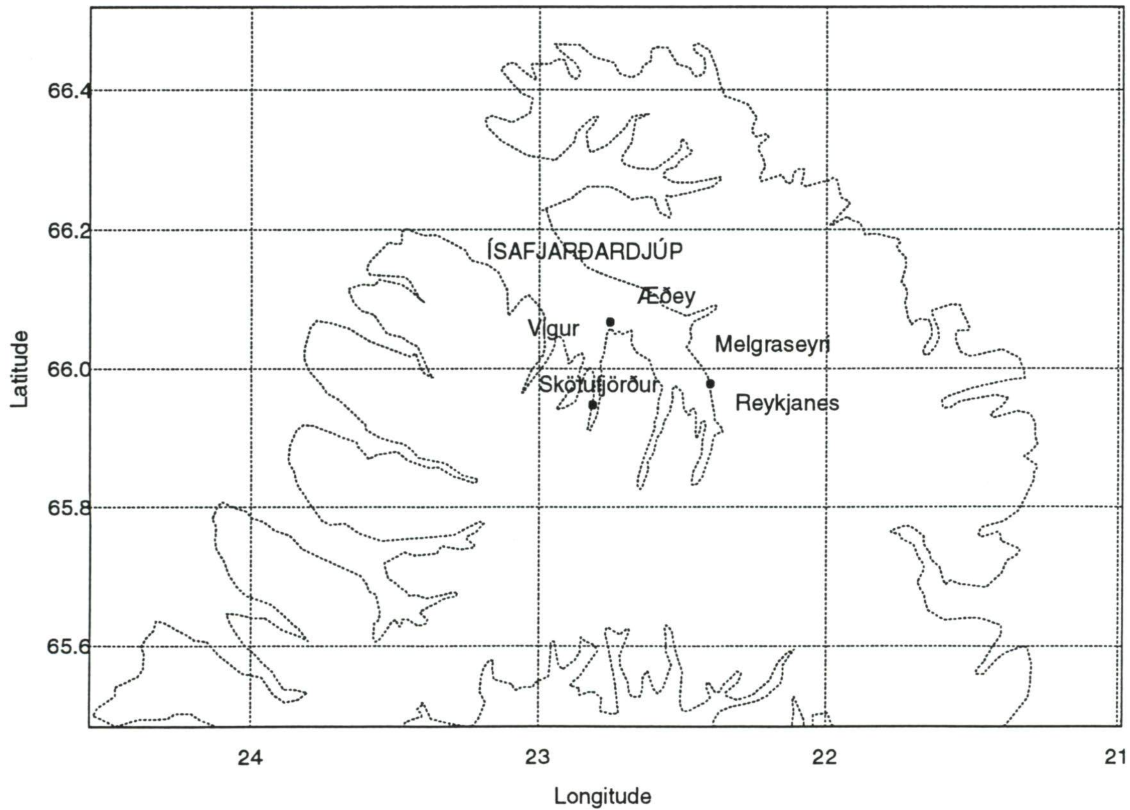

Figure 1: A map of Ísafjarðardjúp with sampling localities.

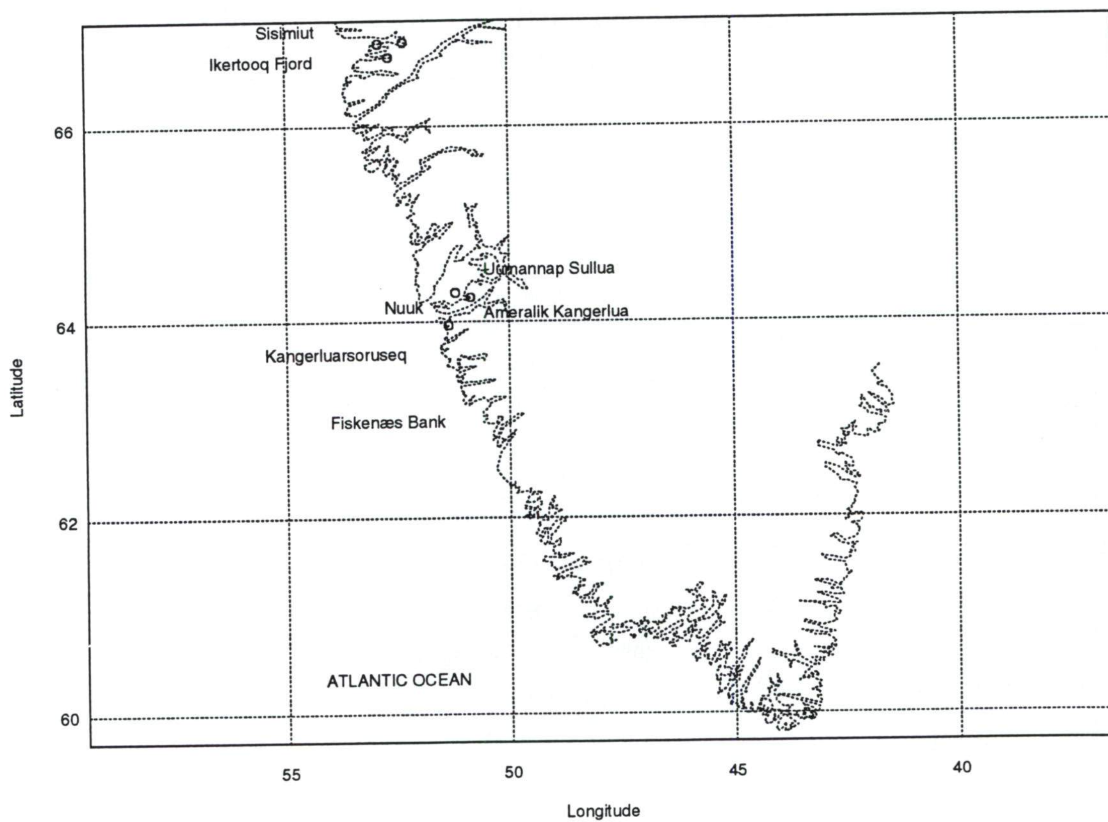

Figure 2: A southern map of Greenland with sampling localities.

2.2 DNA isolation, amplification and sequencing

Mitochondrial DNA was isolated in accordance with the protocol of Ruzzante et al. (1996). Primers for PCR amplification and sequencing were the following oligonucleotides, which correspond to highly conserved cytochrome *b* sequences :

5′-atttggctctcttctaggc-3′ (L14861-bio)

5′-ccctcctgtattggatgc-3′ (H15129 complement)

These primers were used to amplify a 269-base-pair region, approximately one-quarter of the cytochrome *b* gene and about 1.6% of the mt-DNA molecule.

```
          ->
14341 ttaatggcca gccttcggaa aacccatcca atcctaaaaa ttgctaatag cgcattagtt
                                                   |5'-L primer        ->
14401 gatctccccg ccccctccaa tatctcagta tgatgaaatt ttggctctct tctaggcctt
14461 tgcttaatta ctcaacttct aacaggacta tttctagcca tacactatac ctcagacatc
14521 gagacagcct tctcatccgt agtccacatc tgtcgtgatg taaactacgg ctgactaatt
14581 cggaatatac atgctaatgg tgcctctttc tttttcattt gtctttatat gcacattgcc
14641 cgaggtctct attatggttc ctatcttttt gtagagacat gaaacatcgg ggttgtcctt
14701 ttccttttag taataataac ctctttcgta ggttatgtcc tcccctgagg acaaatatca
                                         <-     5'-> H primer|
14761 ttctgaggag ctaccgtaat tacgaattta atatctactg ttccttatgt aggtgatgcc
14821 ttagttcaat ggatctgagg aggtttctca gtagataatg ctaccctaac tcggtttttt
14881 gcattccatt tcttattccc ctttgttgtt gctgctttta caatactcca cctacttttt
14941 ctccatgaaa caggctcaaa taatcccaca ggaatcaatt caaatgcaga caaaattcca
15001 ttccacccat atttcaccta caaagacctg cttggctttg ctgtgatgct tctgggctta
15061 accgccctcg ccctcttcgc acctaattta ctcggagatc cagataattt cacccctgct
15121 aaccccatcg ttaccccacc tcatgttaag cccgaatgat atttcttgtt tgcctatgcc
15181 atcttacgct ctattccaaa taagctaggt ggcgtacttg cactcctatt ctcgattcta
15241 gtcctcatgg ttgtaccctt tctccatacg tcaaaacaac gaggtttaac attccgccct
15301 cttacccaaa tactattctg agtcctcgtt gcagatatac tagttcttac atgaattgga
```

```
15361 ggcgtacctg tagaacaccc cttcattatc atcggacaag tggcatcagt actatatttc
15421 tccctcttcc tagttttatt ccccttgca ggaataactg aaaataaggc ccttgaatga
15481 aact
       <-
```

Double-stranded PCR amplifications were carried out in 52.55μl reactions containing ddH2O, 5μl of 10$\times$ Taq DNA polymerase buffer (500mM KCl, 15mM $MgCl_2$, and 100mM Tris-HCl), 166μM of dNTP (50μl of each deoxyribonucleotide in 800μl of ddH2O), one and half unit of Taq polymerase (Pharmacia Biotech) and 0.32μM of each heavy and light strand primer. Five μl of the extracted DNA was added to this mixture. The DNA was amplified in a Techne PHC-3 thermal cycler on the following step cycle profile : strand denaturation at 93°C for 20 secondes, primer annealing at 50°C for one minute and primer extension at 72°C for 2 minutes, repeated for 35 cycles. It produced multiple copies of a specific DNA sequence , in our study a 269-base-pair region in the cytochrome *b* gene.

Electrophoresis of a 5–10μl portion of the amplification product was done for 10-15 minutes in a 1% Agarose gel (1g Agarose powder, 100ml 0.5 $\times$TBE, 8μl ethidium bromide) in 0.5$\times$TBE buffer (the amounts of each component for one liter of 5$\times$TBE were 54 g of Tris base, 27.5 g of Boric acid, 20 ml of EDTA 0.5M ph 8.0). The DNA fragments were examined under UV-illumination. The single-stranded DNA was obtained with dynabeads. Moreover we tried for four samples a new method for preparing the products of polymerase chain reaction (PCR) for sequencing with the Sequenase PCR Product Sequencing Kit (Amersham, USB). All gel purifications, sedimentations, filtrations and magnetic separations (dynabead) are eliminated by the use of two enzymes, Exonuclease I and Shrimp Alkaline Phosphatase, which effectively remove the excess deoxyribonucleic acid (DNA) produced by PCR amplification from the PCR mixture. The Exonuclease I removes residual single-standed primers and any extraneous single-stranded DNA produced by PCR. The Shrimp Alkaline Phosphatase removes the remaining dNTPs from the PCR mixture which would interfere with the labeling step of the sequencing process. Previously we precipated 35μl of the PCR product with ethanol. We added it 5μl of 1$\times$ Taq polymerase buffer in each sample and added 1μl of each enzyme. We treated this solution in the Techne PHC-3 thermal cycler with the

following step cycle: incubation at 37°C for 15 minutes and inactivation of the enzymes by heating to 80°C for 15 minutes. We got good DNA sequence on gels but with a lot of extra bases. We tried again this enzymatic pre-treatment according to the Amersham protocol with the automatic sequencer Alfexpress which we obtained recently. We mixed 5μl of PCR amplification mixture (without precipitation with ethanol), 1μl Exonuclease I (10 units/μl) 1μl of Shrimp Alkaline Phosphatase and we increased the time of inactivation to 20 minutes. The results were very satisfactory. Single-stranded DNA sequencing reactions were prepared with sequenase kit (version 2.0 T7 DNA polymerase, USB). A single annealing reaction was used for each template combining : 0.175 μl H primer, 0.825 μl ddH2O (3.5:16.5) and 2 μl sequenase reaction buffer. The capped tubes were warmed to 65°C for 5 minutes then the mixture cooled slowly to 30°C over a period of 30 minutes. The samples were chilled on ice after spinning. To the annealed template-primer we added the following mix: 2μl labelling nucleotide mix diluated 1:50, this dilution permits the sequence to be read immediately after the primer, 1μl DTT (0.1M), 0.29μl T7 sequenase, 1.71 μl enzyme dilution buffer and 0.5μl [α^{-35}S] (Amersham). We labeled microtitre plate wells and added 2.5μl of the appropriate dideoxy termination mixture (ddGTP, ddATP, ddTTP, ddCTP). We pre-warmed these plates to 37°C. When the labeling reaction was complete, we transfered 3.5μl of it to the first well labeled G (pre-warmed to 37°C). Similarly we transfered 3.5μl of the labeling reaction (containing the annealed DNA) to each of the other three wells (A,T and C). After 3–5 minutes incubation at 37°C, we added 4μl of stop solution (1150μl of 95% Formamide, 4.8μl of 0.5M EDTA, 24μl of 2.5% Bromophenol blue, 24μl of 2.5% Xylene cyanol FF) to each termination reaction in one minute, we mixed (by pumping), transfered in tubes and stored in ice. Sequencing products were run on 6% Long Ranger gel (AT Biochem). We preran the gel for 30 minutes to one hour to heat the glass plates to between 45 and 50°C and before loading the samples we denatured them for 2 to 5 minutes at 75°C. The gels were usually run for 2h30' at constant power of 48W. The Long Ranger gels dried onto Whatman 3MM paper in a model 583 gel dryer, for 2 hours at 80°C, and autoradiographied with an X-ray film (Cronex, Dupont).

2.3 Statistical methods

Haplotype diversity $\hat{h}$ and its variance (V) were estimated by the equations of Nei (1987) :

$$\hat{h} = \frac{2n(1 - \sum \hat{x}_i^2)}{2n - 1} \quad (1)$$

Where n is the number of individuals sampled and x_i is the population frequency of the ith haplotype at a locality.

$$V(h) = \frac{2}{2n(2n-1)} 2(2n-2) \sum x_i^3 - (\sum x_i^2)^2 + \sum x_i^2 - (\sum x_i^2)^2 \quad (2)$$

The intrapopulation nucleotide diversity $\hat{d}_X$ or $\hat{d}_Y$ $(=\hat{\pi})$, the gross $\hat{d}_{XY}$ and the net $\hat{d}_A$ interpopulation nucleotide divergence (see Stephens & Nei 1985) were estimated by:

$$\hat{d}_X = \frac{n_x}{n_{x-1}} \sum_{ij} x_i x_j d_{ij}$$

$$\hat{d}_{XY} = \sum_{ij} \hat{x}_i \hat{y}_j d_{ij}$$

$$\hat{d}_A = \hat{d}_{XY} - \frac{(\hat{d}_X + \hat{d}_Y)}{2}$$

Where $\hat{x}_i$ and $\hat{y}_i$ are the samples frequencies of the ith haplotype for populations X and Y, respectively. n_X is the number of sequences sampled and d_{ij} is the number of nucleotide substitutions per site between the ith and jth haplotypes. The average number of nucleotide substitutions (d_Y) for Y can be estimated in the same way.

3 Results

3.1 Molecular nature of variation

Twelve base changes were found at eleven sites among the 113 sequences examined defining 14 haplotypes, five of which have not been found before. Five base changes were a ↔g purine transitions, six were c ↔t pyrimidine transitions, and one was a g ↔t transversion. All changes but one were at third position silent sites. The t ↔c transition in second position

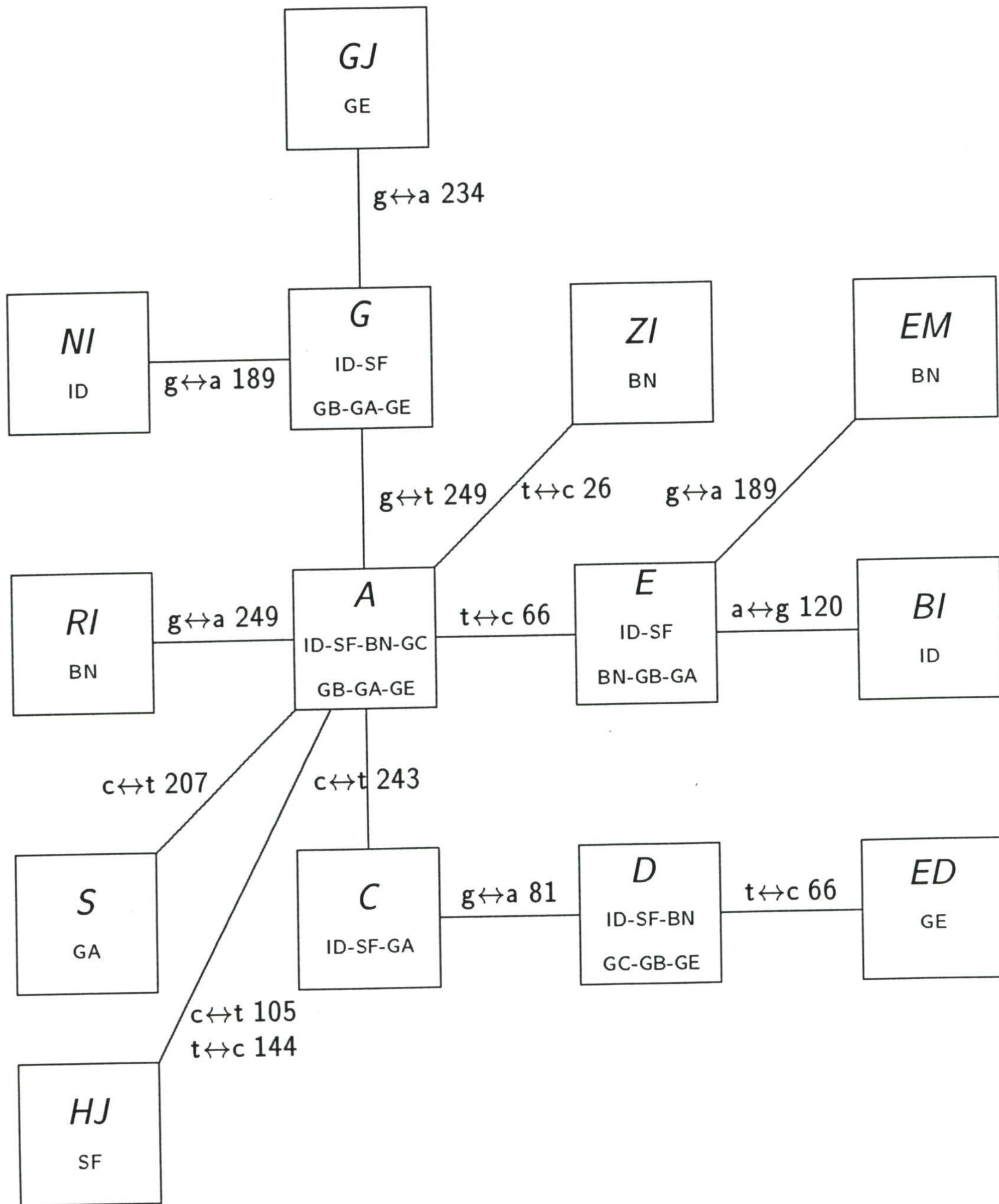

Figure 3: Phylogenetic maximum parsimony network of 14 haplotypes of cytochrome *b* found among 113 cod sampled from Iceland and Greenland. The names of the haplotypes (*A, C, D, E, G, S, GJ, NI, ZI, EM, RI, BI, ED* and *HJ*) are given along with the localities (ID, between Melgraseyri and Reykjanes; SF,Skötufjörður; BN, between Vigur and Æðey; GB, Ameralik Kangerlua; GA, Uumannap Sullua N; GC, Uumannap Sullua S; GE, Amerloq Fjord). On the lines connecting the boxes are given the site and the base change relative to the *A* haplotype.

at site 26 replaces an isoleucine by a threonine in the protein of haplotype *ZI*. Site number 249 has been hit twice with a g ↔a transition in haplotype *RI* and a g ↔t transversion in haplotype *G*. Two of 12 mutations occured twice. The change at site 189 occured as changes from haplotypes that already are one step away from A. A double mutation must be cited to account for haplotypes *E* and *ED* : either a c ↔t loss at site 243 and a g ↔a loss at site 81, in haplotype *E* or an independent t ↔c gain at site 66 in haplotype *ED*. The latter is more parsimonius and the network is drawn based on this view. Most of the variants were three or four mutational steps away from the *A* haplotype and there were at most five steps between haplotypes. This last way represented about the sixth of the network.

3.2 Population diversity, geography and gene flow

3.2.1 Haplotype analysis from Iceland

Two changes, c ↔t at site 105 and t ↔c at site 144, in haplotype *HJ* suggested a migration of this cod from another area. Because we did not sample cod with the intermediate haplotype *H* or *GI*, with respectively c ↔t 105 and t ↔c 144 changes. Four haplotypes *A*, *E* and *D* were found in each locality in Iceland. Haplotype *C* and *G* were absent in the area between islands Vigur and Æðey, moreover haplotype *C* was represented only one time in the two other areas. However the rare haplotype *RI* and *ZI* (only one individual each), with respectively g ↔a transition and g ↔t transversion, both at site 249, were sampled in the region between Vigur and Æðey and only in this one. Furthermore we found in the same area the new type *EM* seemingly derived from haplotype *E*. The rare haplotype *NI* and *BI* were both found only in the coastal area near of the town Melgraseyri in Ísafjarðardjúp.

Table II: Haplotype distribution in Ísafjarðardjúp

Locality	*A*	*D*	*E*	*G*	*NI*	*C*	*ZI*	*RI*	*HJ*	*EM*	*BI*	Sum
Skötufjörður	13	2	2	2	0	1	0	0	1	0	0	21
Vigur-Æðey	12	6	4	0	1	0	1	1	0	1	0	26
Melgraseyri-Reykjanes	11	5	2	4	1	1	0	0	0	0	1	25
Sum	36	13	8	6	2	2	1	1	1	1	1	72

Genetic variability was high in Ísafjarðardjúp whether measured as haplotype diversity $\hat{h} = 0.71 \pm 0.034$ (SE)(Table III) or as nucleotide diversity $\hat{\pi} = 0.0044$ (Table III). But we can note a lower haplotype and nucleotide diversity in Skötufjörður respectively, $\hat{h} = 0.61 \pm 0.081$ and $\hat{\pi} = 0.0033$, where the haplotype A dominated (Table III). So the standard error $SE_{\hat{h}}$ in this area was very high compared with both other localities. The two diversity indices differ in that haplotype diversity only takes into account the frequency and number of haplotypes, whereas nucleotide diversity is also based on the mutational differences between haplotypes.

Table III: Haplotype diversities ($\hat{h}$), associated standard errors ($SE_{\hat{h}}$) by localities intrapopulation nucleotide diversities ($\hat{\pi}$) and N is the number of individuals in Ísafjarðardjúp.

Locality	N	$\hat{h}$	$SE_{\hat{h}}$	$\hat{\pi}$
Skötufjörður	21	0.61	0.081	0.33
Vigur-Æðey	26	0.73	0.047	0.48
Melgraseyri-Reykjanes	25	0.76	0.046	0.48
Total	72	0.71	0.034	0.44

The interpopulation diversities d_{XY} (Table IV) were relatively similar. The net nucleotide differences between localities d_A were nil or close to zero which means that we don't have more genetic differences between localities than between haplotypes within populations.

Table IV: Estimates of interpopulation (d_{XY}; lower triangular), intrapopulation (d_X or $d_Y = \hat{\pi}$; boldface on diagonal) and net nucleotide differences among localities (d_A; italic on upper triangular) in Ísafjarðardjúp. All figures multiplied by 100.

Locality	Skötufjörður	Vigur-Æðey	Melgraseyri-Reykjanes
Skötufjörður	**0.33**	*0.00*	*0.00*
Vigur-Æðey	0.41	**0.48**	*−0.01*
Melgraseyri-Reykjanes	0.40	0.47	**0.48**

3.2.2 Haplotype analysis from Greenland

Haplotype *A* was found in the four areas (GA, GB, GC, GE; Figure) in Greenland with a high frequency (50% or more). Haplotype *D* was absent in the north of Uumannap Sullua

whereas both haplotypes C and S were only found in this locality in Greenland. Moreover the haplotype D was absent in Ikertooq Fjord South and north, found only in the mouth of this fjord (M). But it is may be because of the low number of individuals sampled in the neighbour fjord of Ikertooq (S) (three cod). The haplotype G was absent in Uumannap Sullua S which locality correspond to a recess of this fjord whereas this haplotype was found in the main part of the fjord (Uumannap N = GA; Figure 2, Table V). Similarly the haplotype E was absent in the recess of Uumannap Sullua, and in any part of the Ikertooq fjord whereas the rare new haplotypes ED and GJ were found only in the latitude between 66°70' and 66°85',i.e, respectively near and in the Ikertooq fjord, the most northern localities studied in this paper.

Table V: Haplotype distribution in Greenland

locality	A	G	E	D	S	GJ	ED	C	Sum
Ameralik Kangerlua	2	1	3	1	0	0	0	0	7
Uumannap Sullua N	7	1	1	0	1	0	0	1	11
Uumannap Sullua S	4	0	0	1	0	0	0	0	5
Ikertooq Fjords S	1	1	0	0	0	0	1	0	3
Ikertooq Fjords M	5	1	0	1	0	0	0	0	7
Ikertooq Fjords N	6	1	0	0	0	1	0	0	8
Sum	25	5	4	3	1	1	1	1	41

Genetic variability was not very high in the south-west of Greenland. The haplotype diversity ($\hat{h}$) and the nucleotide diversity measures respectively, 0.61 ± 0.057 and 0.0033 (Table VI can seem high, it is still due to a low number of individuals sampled in the neighbour fjord of Ikertooq Fjord where only three cod were found having three differents haplotypes (Table V, Table VI). So $\hat{h}$ was equal to 1 ± 0.122 and $\hat{\pi} = 0.90$ in this locality. If we don't take into account this locality the genetic diversity will be lower. The nucleotide diversity was relatively low in Uumannap Sullua and Ikertook Fjords. It was highest in the fjord Ameralik Kangerlua, which one is very close of Uumannap Sullua (Table VII) (I don't take into account the very high diversity in Ikertooq Fjord S), the same than in two localities among three in Iceland. Furthermore the haplotype diversity is higher in the area of Nuuk compared with the area of Sisimiut (figure 1). The interpopulation nucleotide divergences

d_{XY} which are the interpopulation equivalents of d_X ($=\hat{\pi}$) were on average equal to 0.37% (Table VII). The intrapopulation diversity was on average lower (mean of diagonal 0.33%; Table VII). It will be appear that the mean of the net nucleotide differences will be positive (+0.04% on average). Yet we do not have to take into account the very high values of Ikertooq Fjord S as we mentioned previously. The estimates of interpopulation, intrapopulation and net nucleotide differences among overall areas looks like more correct (Table VIII). The net interpopulation nucleotide divergence was negative (= −0.0035; Table VIII, upper triangular) indicating no net divergence among populations in Greenland.

Table VI: Haplotype diversities ($\hat{h}$), associated standard errors ($SE_{\hat{h}}$) by localities, intrapopulation nucleotide diversities ($d_X = \hat{\pi}$) and N is the number of individuals in Greenland

Locality/Area	N	$\hat{h}$	$SE_{\hat{h}}$	$\hat{\pi}$
Ameralik Kangerlua	7	0.81	0.078	0.48
Uumannap Sullua N	11	0.62	0.114	0.24
Uumannap Sullua S	5	0.40	0.159	0.27
Ikertook Fjords S	3	1.00	0.122	0.90
Ikertook Fjords M	7	0.52	0.142	0.29
Ikertook Fjords N	8	0.46	0.138	0.23
Nuuk	23	0.66	0.068	0.32
Sisimiut	18	0.55	0.089	0.33
Total	41	0.61	0.057	0.33

Table VII: Estimates of interpopulation (d_{XY}; lower triangular), intrapopulation (d_X or d_Y; diagonal = $\hat{\pi}$) and net nucleotide differences among localities (d_A; upper triangular) in Greenland. All figures multiplied by 100.

Locality	1	2	3	4	5	6
1 Ameralik kangerlua	0.480	0.009	0.004	−0.145	0.007	0.036
2 Uumannap Sullua S	0.390	0.270	−0.012	−0.091	−0.039	0.012
3 Uumannap Sullua N	0.370	0.245	0.245	−0.062	−0.017	−0.003
4 Ikertooq Fjord S	0.550	0.495	0.511	0.901	−0.097	−0.044
5 Ikertooq Fjord M	0.390	0.241	0.249	0.498	0.289	−0.012
6 Ikertooq Fjord N	0.390	0.261	0.233	0.521	0.247	0.229

Table VIII: Estimates of interpopulation (d_{XY}; lower triangular), intrapopulation (d_X or d_Y; diagonal = $\hat{\pi}$) and net nucleotide differences among overall areas (d_A; upper triangular) in Greenland. All figures multiplied by 100.

Locality	Nuuk	Sisimiut
Nuuk	0.322	−0.0035
Sisimiut	0.327	0.339

4 Discussion

The material used in our study, mitochondrial DNA, is very advantageous for determining the evolutionary events. Indeed in vertebrates ranging from fish to mammals the rapidly evolving mtDNA has proved a most useful tool for studying intraspecific phylogeography (Bermingham and Avise 1986; Avise et al. 1987; Moritz and Dowling 1987). In most instances considerable genetic differentiation has been found in the mtDNA of conspecific but allopatric populations (Árnason et al. 1992). Important exceptions –category 4 of Avise et al. (1987)– occur possibly in humans , in red-winged black birds, and in several marine organism (Avise et al. 1987), organism whose life histories are highly conducive to dispersal, perhaps even over very long distances.

In this paper we have demonstrated the lack of net variation of cod mtDNA cytochrome *b* among populations in the west fjord, Ísafjarðardjúp, in Iceland as well as on the south-west coast in Greenland. The amount of variation observed at the mitochondrial cytochrome *b* in Ísafjarðardjúp (measured as nucleotide diversity of 0.0044) is higher than in cod in Greenland (0.0033) and is only slightly lower than in Norwegian cod (0.0048) (Árnason and Pálsson (1996)). However difference between the samples from Iceland and Norway must be due to the results of cod sampled in Skötufjörður. The lower haplotype and nucleotide diversities in this locality were caused either by the presence in the two other locality in Ísafjarðardjúp, of a higher number of different haplotypes, or because the haplotypes found in this area are closer genetically (*A, C, G, E, HJ, D*) than they are in the other localities or because the haplotype frequencies are more uneven in Skötufjörður. Excluding the data from this locality we would get exactly the same nucleotide diversity as in Norway.

In term of individual variation between cod from Ísafjarðardjúp, Greenland and Norway, the difference is significant. It is also interesting to compare our results with those studied by Árnason and Pálsson (1996) from three overall areas from Bear Island to Puddefjord in Norway, who sampled in 1992 and in 1993. A few haplotypes found all at once in the three areas : *A, C, D, E* and *G*. Moreover we found in the locality between the towns Melgraseyri and Reykjanes (ID) in Ísafjarðardjúp all the haplotypes in common with the samples from Norway (at all seven haplotypes) even the two rares haplotypes *NI* and *BI* which in our study were only found in this locality. It is also possible to have a link between cod from Norway and Ísafjarðardjúp. This hypothesis has been strengthened by the presence of the haplotype *HJ* in Skötufjörður. On the one hand two changes, c ↔t at site 105 and t ↔c at site 144 in this haplotype suggested a migration of this cod from another area. Because we did not sample cod with the intermediate haplotype *H* or *GI*, with respectively c ↔t 105 and t ↔c 144 changes, whereas the *GI* haplotype was found in Barents Sea. It would appear to be a fish from Norway having undergone a further genetic change at site 105 has migrated to Skötufjörður. On the other hand each female produces a vast number offspring that are dispersed widely. Sibs therefore can come to reside in widely separated locations but a neighbourhood receives offspring from many different females and is thus an assemblage of heterogeneous individuals. Negative genetic correlations between individuals within neighbourhood could result. This will be imply that there would exist a gene flow at least between Ísafjarðardjúp and Norway. We also note that haplotypes that are genetically most distant from each other are found in the same locality. Among others *NI* and *BI* in Ísafjarðardjúp; *EM* and *RI* in the locality between the islands Vigur and Æðey; *F* and *B* in Norway; *GJ* and *ED* in the area around Sisimiut (in two very close fjords), the latter being the most distant separated by five mutation steps. Whereas haplotypes that are not distant genetically are found in separate localities (for example, *RI*; *S*; *HJ* respectively between Vigur and Æðey; in Uumannap Sullua N and in Skötufjörður)

Furthermore the variation observed in this study and the study of Atlantic cod from Norway is neutral, being largely at silent third position site. A caveat is introduced by the amino acid variants in haplotype *ZI* in Ísafjarðardjúp replacing an isoleucine by a threonine, and in haplotype *F* (Carr and Marshall 1991) in Tromso in Norway replacing a glutamine by a

glycine. The mutation probability matrix, which provides the information to simulate any degree of evolutionary change in an unlimited number of proteins, classifies the amino acid change isoleucine to threonine as the fifth most conservative change and the amino acid change glutamine to glycine, found in Norway, at the second place of the most conservative (Dayhoff 1972). So in both there are not radical protein change thus supporting our claim for neutrality.

In conclusion the analysis of the Table IX permits us to say that we found a high intracountry diversity both in Ísafjarðardjúp, Iceland and Greenland, but the net nucleotide divergence between them is nil.

Table IX: Estimates of intercountry (d_{XY}; lower triangular), intracountry (d_X or d_Y; diagonal $= \hat{\pi}$) and net nucleotide differences between (d_A; upper triangular) Ísafjarðardjúp, Iceland and Greenland. All figures multiplied by 100.

Locality	Greenland	Ísafjarðardjúp
Greenland	0.328	−0.0006
Ísafjarðardjúp	0.382	0.436

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