Biological Journal of the Linnean Society, 2008, **94**, 315–329. With 6 figures

Recolonization history and large-scale dispersal in the open sea: the case study of the North Atlantic cod, *Gadus morhua* L.

CHRISTOPHE PAMPOULIE[1]*, MAGNÚS ÖRN STEFÁNSSON[1], THÓRA DÖGG JÖRUNDSDÓTTIR[1], BRET S. DANILOWICZ[2] and ANNA KRISTÍN DANÍELSDÓTTIR[1]

[1]*Marine Research Institute, Skúlagata 4, 101 Reykjavík, Iceland*
[2]*University College Dublin, Department of Zoology, Belfield, Dublin 4, Ireland*

Received 20 February 2007; accepted for publication 24 August 2007

Most studies of the genetic structure of Atlantic cod have focused on small geographical scales. In the present study, the genetic structure of cod sampled on spawning grounds in the North Atlantic was examined using eight microsatellite loci and the *Pan* I locus. A total of 954 cod was collected from nine different regions: the Baltic Sea, the North Sea, the Celtic Sea, the Irish Sea and Icelandic waters during spring 2002 and spring 2003, from Norwegian waters and the Faroe Islands (North and West spawning grounds) in spring 2003, and from Canadian waters in 1998. Temporal stability among spawning grounds was observed in Icelandic waters and the Celtic Sea, and no significant difference was observed between the samples from the Baltic Sea and between the samples from Faroese waters. *F*-statistics showed significant differences between most populations and a pattern of isolation-by-distance was described with microsatellite loci. The *Pan* I locus revealed the presence of two genetically distinguishable basins, the North-west Atlantic composed of the Icelandic and Canadian samples and the North-east Atlantic composed of all other samples. Permutation of allele sizes at each microsatellite locus among allelic states supported a mutational component to the genetic differentiation, indicating a historical origin of the observed variation. Estimation of the time of divergence was approximately 3000 generations, which places the origin of current genetic pattern of cod in the North Atlantic in the late Weichselian (Wisconsinian period), at last glacial maximum. *Biological Journal of the Linnean Society*, 2008, **94**, 315–329.

ADDITIONAL KEYWORDS: genetic structure – microsatellite – *Pan* I locus – postglacial expansion.

INTRODUCTION

For decades, the genetic structure of marine organisms has been thought to be homogeneous due to their extended egg/larval dispersal capabilities, active migration of adults, and the lack of obvious barriers to gene flow in the marine environment. Yet this view has been challenged now that complex genetic structure has been described for several marine species. Indeed, oceanic features have promoted genetic differentiation among populations on large or small geographical scales (Pérrin, Wing & Roy, 2004; Shaw, Arkhipin & Al-Khairulla, 2004). Eggs and/or larval dispersal can be restricted by physical barriers such as frontal systems (Shaw *et al*., 2004), oceanic currents (Ruzzante, Taggart & Cook, 1998), and estuarine circulation (Pérrin *et al*., 2004). In addition, genetic differentiation among populations might be due to restricted gene flow of adults following isolation-by-distance, especially on large geographical scales. However, there is growing evidence that historical events such as the isolation of populations in glacial refugia might have also played a role in the origin of marine population structure (Gysels *et al*., 2004; Hoarau *et al*., 2007). A key assumption is that present-day populations of a species inhabiting past refugium show a higher level of genetic diversity than those inhabiting formerly glaciated regions due to

*Corresponding author. E-mail: chrisp@hafro.is

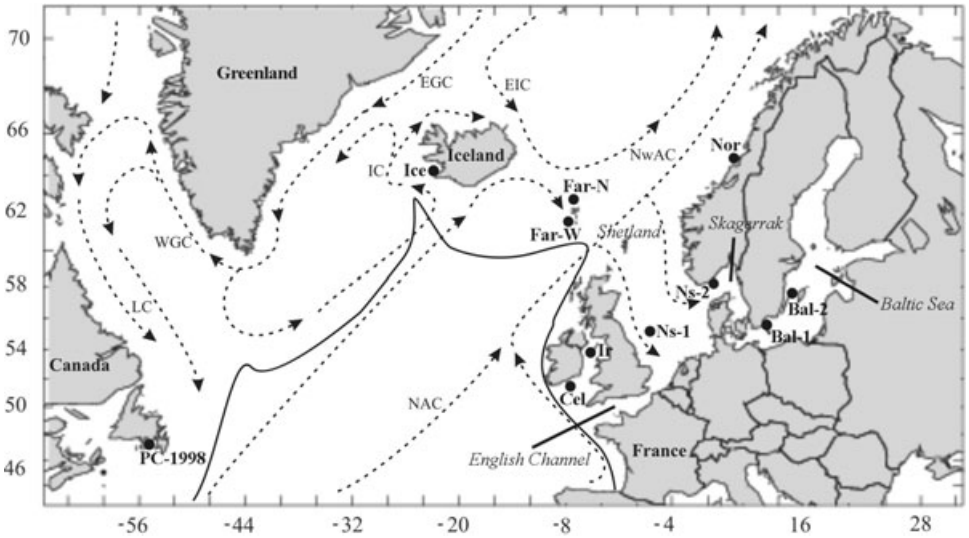

Figure 1. Location of cod samples (closed circles), main oceanic currents and –2 °C isotherm (———— sea ice limit) during the last glacial maximum 20 Kya (according to Siegert & Dowdeswell, 2004). NAC, North Atlantic current; NwAC, Norwegian Atlantic current; IC, Irminger current; EIC, East Icelandic current; EGC, East Greenland current; WGC, West Greenland current; LC, Labrador current. For sample codes, see Table 1.

range expansion and genetic drift following deglaciation (Hewitt, 2000).

The Atlantic cod (*Gadus morhua* L.) is one of the most valuable commercial species occurring on continental shelves and banks on both sides of the North Atlantic Ocean. Its distribution is mainly controlled by environmental parameters such as temperature and salinity (O'Brien *et al*., 2000), although other factors such as interspecific competition might be important. Cod occurs over a wide range of temperatures (0–20 °C) but has a preference for the temperature range 3–7 °C and usually avoids temperatures lower than 2 °C (D'Amours, 1993). It is also tolerant to a wide range of salinity but shows an optimum around 30 ppt (Smith & Page, 1996). Although tagging experiments tend to demonstrate that spawning site fidelity is stock dependent (Robichaud & Rose, 2004), some studies have shown that cod do exhibit spawning site fidelity (Jónsson, 1996; Pampoulie *et al*., 2006; Wright *et al*., 2006), potentially promoting genetic differentiation among spawning areas. Eggs and larvae are pelagic for several months and subjected to passive dispersal. In the North-east Atlantic, the most important oceanic currents potentially promoting or restricting dispersal are the North Atlantic current and the Norwegian Atlantic current (NwAC; Fig. 1) flowing from the British Isles to the North of Norway. Two branches flow into the North Sea: one south and one north of the coast of Shetland Islands. The former flows southward along the east coast of the British Isles whereas the latter flows off the coast of south Norway. Both currents flow eastward towards the Skagerrak (Turrell, 1992). Atlantic waters also flow into the North Sea via the English Channel. Thus, if the genetic structure of cod in the North-east Atlantic is mainly due to passive dispersal of eggs and larvae, a genetic difference is expected between Icelandic and European populations due to the NwAC (Fig. 1). Furthermore, a low genetic differentiation among European populations is expected due to the complex hydrography of the North Sea and British Isles, which should facilitate egg and larval dispersal. However, several oceanic features, such as the seasonal Flamborough front and cyclonic pattern of water circulation in the central and northern North Sea, have been suggested to prevent larval dispersal in the North Sea (Brown *et al*., 1999).

Another major factor that may be responsible for genetic structure in cod is the past geological and climatological history of the North Atlantic Ocean. During the last glacial maximum (LGM; 15–25 Kya), in the late Weichselian period (Wisconsinian period), an ice sheet covered most of the North Atlantic from the Barents Sea, the Scandinavian peninsula to the British Isles (Siegert & Dowdeswell, 2004; Svendsen *et al*., 2004). A numerical model shows a sea ice limit (isotherm of –2 °C) located south of Iceland and west of the British Isles (Fig. 1; Siegert & Dowdeswell, 2004). The Southern Bight of the North Sea was suggested to be dry during the LGM (van der Molen & de Swart, 2001) and a glacial lake potentially served as a refugium in the southern North Sea (Balson *et al*., 1991). The ocean invaded the Southern Bight of the North Sea again through the Strait of

Table 1. Number of individuals sampled (*N*), name and code of populations of cod investigated at spawning grounds of the North Atlantic

Sampling site	Period	Latitude	Longitude	Code	*N*
Baltic Sea	Spring 2002	55°41′N	14°30′E	Bal-1	94
Celtic Sea	Spring 2002	51°22′N	07°30′W	Cel-1	64
Iceland	Spring 2002	64°20′N	22°45′W	Ice-1	70
Irish Sea	Spring 2002	53°51′N	05°05′W	Ir-1	68
North Sea	Spring 2002	55°22′N	01°09′E	Ns-1	29
Baltic Sea	Spring 2003	57°28′N	16°33′E	Bal-2	60
Celtic Sea	Spring 2003	51°46′N	07°30′W	Cel-2	60
Iceland	Spring 2003	64°15′N	22°15′W	Ice-2	94
Irish Sea	Spring 2003	53°52′N	04°42′W	Ir-2	64
North Sea	Spring 2003	58°04′N	09°04′E	Ns-2	59
Norway	Spring 2003	63°45′N	11°22′E	Nor	60
Faroe North	Spring 2003	62°27′N	07°05′W	Far-N	85
Faroe West	Spring 2003	61°52′N	07°16′W	Far-W	55
Pool Cove	Spring 1998	47°42′N	55°22′W	PC-1998	92

Dover when the sea level rose following deglaciation (van der Molen & de Swart, 2001). The contemporary oceanic connection between the southern North Sea and the English Channel appeared approximately 7.5 Kya. Additional refugia have been suggested at the boundaries of the ice sheets in the Baltic region during LGM (Koljonen *et al*., 1999; Verspoor *et al*., 1999). Although the Baltic Sea formed millions of years ago, the present-day connection to the North Sea was established when the salty Lake Ancylus formed 8–9 Kya became a sea again. Although Ruzzante *et al*. (1996) suggested that cod may have been subjected to local adaptations to temperatures below 0 °C, resulting in genetic differentiation among inshore and offshore cod populations, Atlantic cod that existed in the North Atlantic prior to the LGM probably had to migrate southward to avoid temperature below 3 °C (lowest preference; D'Amours, 1993). This migration could have lead the cod into one of several refugia within the Atlantic Ocean and later to other refugia, such as the North Sea glacial lake and the Ancylus Lake (8–9 Kya). When the ice retreated, cod populations probably recolonized the newly opened environment such as the northern part of Europe (North Sea and Irish Sea), the Icelandic waters and the Baltic Sea. Based on the different reconstruction models of the LGM (Siegert & Dowdeswell, 2004; Svendsen *et al*., 2004), Atlantic cod should exhibit high genetic diversity in regions close to potential refugia (Icelandic, Celtic and North Sea waters) and low genetic diversity in more distant areas.

Regarding the potential history of North Atlantic cod (i.e. potential postglacial expansion) or the effect of oceanographic currents, the genetic structure of cod has received little attention on a macro-geographical scale. On such a large scale, oceanic features as well as the geology and climatology of the North Atlantic could have influenced the genetic structure of the species. In the present study, we aimed to assess the present-day genetic differentiation of North Atlantic cod on a large geographical scale using eight microsatellite and the *Pan* I locus. We hypothesized that the Atlantic cod is genetically structured in the North Atlantic and that: (1) the geographical distances and oceanic currents are barriers to contemporary gene flow and (2) historical events such as the isolation of cod populations in glacial refugia and postglacial expansion might be the source of the contemporary genetic differentiation.

MATERIAL AND METHODS

A total of 954 mature cod was collected from nine different spawning grounds within the North Atlantic: the Baltic Sea, the North Sea, the Celtic Sea, the Irish Sea, and Icelandic waters (Fig. 1; Table 1) in spring 2002 and spring 2003, Norway and Faroe Plateau (Faroe North and West spawning grounds) in spring 2003, and from Canada (Pool Cove) in 1998. Gill filaments or fin clips were preserved in 96% alcohol. Samples were genotyped at eight microsatellite loci, namely Gmo2 (Brooker *et al*., 1994), Gmo8, Gmo19, Gmo34 (Miller, Le & Beacham, 2000), Tch5, Tch11, Tch14 and Tch22 (O'Reilly *et al*., 2000), and at the *Pan* I locus (initially known as cDNA clone GM798; Pogson, Mesa & Boutilier, 1995). DNA extraction, polymerase chain reaction and genotyping were performed as described previously (Pampoulie *et al*., 2006).

Hardy–Weinberg expectations (HWE) and population diversity

For both type of genetic markers, the allele frequencies, total number of alleles (N_A), mean number of alleles (A), observed (H_o), unbiased expected heterozygosity (H_e), and tests for HWE were calculated in GENEPOP, version 3.1 (Raymond & Rousset, 1995). For the microsatellite loci, the allelic richness (A_R) was computed in FSTAT, version 1.2 (Goudet, 1995) based on the smallest sample size.

Genetic differentiation

Wright's single-locus F-statistics (Wright, 1969) were calculated from allele frequencies at all loci examined for each population according to the method of Weir & Cockerham (1984) using GENEPOP. Significance of pairwise and multilocus F_{ST} values was assessed in GENEPOP (5000 replicates). Levels of significance were adjusted with a sequential Bonferroni test (Rice, 1989). A multidimensional scaling analysis (MDS) was conducted on F_{ST} values using STATISTICA, version 6.0 (Statsoft Inc., 2001) for both types of markers.

Gene flow and potential genetic discontinuities

To assess whether geographical distances had an effect on the observed genetic differences, genetic ($\log[F_{ST}/(1 - F_{ST})]$) versus geographical distances were plotted for each pairwise microsatellite sample comparisons. Significance was assessed using a Mantel test (5000 permutations) in GENETIX, version 4.03 (Belkhir *et al*., 1999).

Potential genetic discontinuities were investigated using the microsatellite data in BARRIER, version 2.2 (Manni, Guérard & Heyer, 2004). Geographical coordinates for each sampling location were connected by Delaunay triangulation associated with genetic distances (F_{ST}). The algorithm sets the edge with the largest distance in the triangulation network as the starting edge and extends barriers across the adjacent edge associated with the largest genetic distance. Additional sections are added to the barrier until it reaches the outer edge of the network or meets another barrier (Manni *et al*., 2004). The analysis was conducted as described by Pampoulie *et al*. (2006). The impact of the detected barriers on the genetic structure was tested using a hierarchical analysis of molecular variance (AMOVA; ARLEQUIN, version 2.0; Schneider, Roessli & Excoffier, 2000). Testing was carried out among post-hoc defined regions isolated by BARRIER analysis for both types of genetic markers. Gene flow among populations was estimated using MIGRATE, version 2.1 (Beerli & Felsenstein, 2001), which calculates maximum likelihood estimates for migration rates and effective population sizes (N_e). The program allows for asymmetric migration rates and different subpopulation sizes. Parameter values were ten short chains with 500 steps and 10 000 sampled genealogies, and three long chains with 5000 steps and 100 000 sampled genealogies. The number of immigrants per generation (N_m) was calculated as $\theta M/4$ where M is the ratio of the immigration (m) and the mutation rate (μ), and (N_e) as $\theta/4\mu$ with $\mu = 10^{-4}$–10^{-5}.

Historical origin and time of divergence

Potential historical signatures in the genetic data were assessed by permutating allele sizes at each microsatellite locus among allelic states (2000 replicates) to simulate distribution of R_{ST} values (ρR_{ST}) with 95% confidence intervals (CI) using SPAGEDI, version 1.1 (Hardy & Vekemans, 2002). Differentiation is likely to originate from drift if $R_{ST} \approx \theta$ and if the observed R_{ST} do not exceed the 95% CI of ρR_{ST} values. By contrast, mutation will be the origin of the differentiation if $R_{ST} > \theta$ and if R_{ST} exceeds the 95% CI of ρR_{ST} (Hardy *et al*., 2003).

We predicted the multilocus F_{ST} under complete isolation (absence of gene flow) as a function of time (generations), the effective population size N_e, the number of subpopulation and the heterozygosity (H_o averaged across subpopulations) as described by Reusch, Wegner & Kalbe (2001), using the sampling equation developed by Jin & Chakraborty (1995). N_e was calculated under the Infinite Allele Model (IAM) according to the equation $N_e = (H_o/1 - H_o)/4\mu$ (Crow & Kimura, 1970). Assuming a mutation rate $\mu = 10^{-5}$, overall N_e was in the range 94 699–247 057. Time of divergence was then estimated by comparing the evolution of the predicted value of F_{ST} to $2tN_e$, where t is the number of generations (Reusch *et al*., 2001) (i.e. by assessing the value of t required to reach equilibrium F_{ST} in the absence of gene flow).

RESULTS

HWE and population diversity

The eight microsatellite loci studied varied in allelic diversity (see Appendix, Table A1), ranging from ten alleles at locus Tch22 to 53 at locus Gmo8. H_o varied from 0.552 (Gmo34) to 0.901 (Tch14) whereas H_e varied from 0.583 (Gmo34) to 0.952 (Tch14). A ranged from 5.9 (Tch22) to 26.6 (Tch14). Although, levels of genetic diversity were similar within the range of the study area (see Appendix, Table A2), the Icelandic samples exhibited a slightly higher allelic richness (A_R) and total number of alleles (N_A) per locus than other samples. Genotype proportions were out of

HWE in only three out of 112 exact tests after Bonferroni correction (less than by chance alone) and were not due to any specific loci or samples. Permutation tests for linkage disequilibrium yielded several significant values ($0.01 < P < 0.05$) involving different pairs of loci in different populations, thus suggesting that the results were not due to physical linkage of the loci, and allowing allelic variation at all loci to be treated as independent.

H_o and H_e at the *Pan* I locus varied from 0.028 to 0.644 and from 0.027 to 0.496, respectively, for the samples where variability was detected (Iceland, Faroe Islands, Norway, and Canada). The *Pan* I locus was fixed for the allele A in all other collected samples. There was no evidence for departure from HWE for any of the samples in which variability was detected (see Appendix, Table A2).

Genetic differentiation

The overall genetic estimates based on microsatellite loci revealed highly significant F_{ST} and nonsignificant F_{IS} values of 0.013 and 0.008, respectively. This genetic pattern was reflected among pairwise comparisons of the values of F_{ST} because 73 of 91 comparisons were significant after Bonferroni correction (Table 2). Temporal stability was observed in Iceland and Celtic Seas (Table 2). No significant differentiation was observed among the Baltic Sea and among Faroe Islands samples. On the other hand, a significant differentiation was observed among the Irish Sea samples ($F_{ST} = 0.018$; Table 2). Based on the pairwise F_{ST} values, the MDS analysis clustered samples in four groups, the Canadian, the Baltic Sea, the Irish sample collected in 2002 and the rest of the samples (Fig. 2, stress value = 0.0980).

Variation at the *Pan* I locus showed highly significant F_{ST} and nonsignificant F_{IS} values of 0.304 and –0.015, respectively. Out of 91 pairwise F_{ST} comparisons, 33 were significant after Bonferroni correction (Table 2). Most significant comparisons were observed between either the Icelandic samples or the Canadian sample and all other samples. Comparisons between the Icelandic and the Canadian samples were not significant. Temporal stability was observed in all replicates. Using chi-square tests, genotype frequencies were significantly different among populations ($\chi^2 = 9.60$, d.f. = 2, $P = 0.0019$; Fig. 3). BB genotypes were present in Icelandic and Canadian samples but absent in the North-east Atlantic samples. Based on pairwise F_{ST} values, MDS clearly separated the Icelandic and Canadian samples from the other samples (data not shown, stress value = 0.0545).

Gene flow and genetic discontinuities

Analysis using the program MIGRATE suggested a high level of gene flow per generation across the

Table 2. Estimates of F_{ST} among pairs of samples of *Gadus morhua* using eight microsatellite loci (above diagonal) and the *Pan* I locus (below diagonal)

	Bal-1	Cel-1	Ice-1	Ir-1	Ns-1	Bal-2	Cel-2	Ice-2	Ir-2	Ns-2	Nor	Far-N	Far-W	PC-1998
Bal-1	0	0.056*	0.045*	0.071*	0.057*	0.002	0.055*	0.048*	0.055*	0.039*	0.048*	0.042*	0.048*	0.063*
Cel-1	0	0	0.009*	0.021*	0.007	0.047*	–0.006	0.006*	0.002*	0.006*	0.007*	0.005*	0.004*	0.023*
Ice-1	0.435*	0.385*	0	0.024*	0.013*	0.036*	0.008*	0.002	0.008*	0.004	0.008*	0.003*	0.005*	0.011*
Ir-1	0	0	0.392*	0	0.028*	0.062*	0.021*	0.021*	0.018*	0.023*	0.025*	0.022*	0.020*	0.038*
Ns-1	0	0	0.312*	0	0	0.047	0.007*	0.009*	0.004*	0.007*	0.012*	0.006*	0.007*	0.020*
Bal-2	0	0	0.378*	0	0	0	0.043*	0.036*	0.044*	0.028*	0.037*	0.031*	0.039*	0.053*
Cel-2	0	0	0.378*	0	0	0	0	0.004*	–0.0006	0.005	0.003*	0.002*	0.002*	0.024*
Ice-2	0.341*	0.302*	–0.001	0.307*	0.247*	0.296*	0.296*	0	0.003*	0.001	0.003*	–0.001	0.002	0.014*
Ir-2	0	0	0.385*	0	0	0	0	0.302*	0	0.001	0.005*	0.002	0.001	0.019*
Ns-2	0.015	0.010	0.342*	0.010	0	0.009	0.009	0.265*	0.010	0	0.001	0.001	0.002	0.014*
Nor	0.057	0.044	0.283*	0.046	0.026	0.042	0.042	0.212*	0.044	0.009	0	0.005*	0.004*	0.015*
Far-N2	0.098	0.076	0.233*	0.079	0.047	0.073	0.073	0.169*	0.076	0.029	–0.004	0	–0.001	0.016*
Far-W2	0.010	0.006	0.369*	0.006	–0.002	0.005	0.005	0.286*	0.006	–0.007	0.015	0.039	0	0.016*
PC-1998	0.459*	0.414*	0.001	0.421*	0.351*	0.408*	0.408*	0.020	0.414*	0.378*	0.325*	0.279*	0.401*	0

Significance was assessed in GENEPOP using exact tests for F_{ST} (5000 permutations); Significant values after sequential Bonferroni correction are indicated (*$P < 0.0001$). For sample codes, see Table 1.

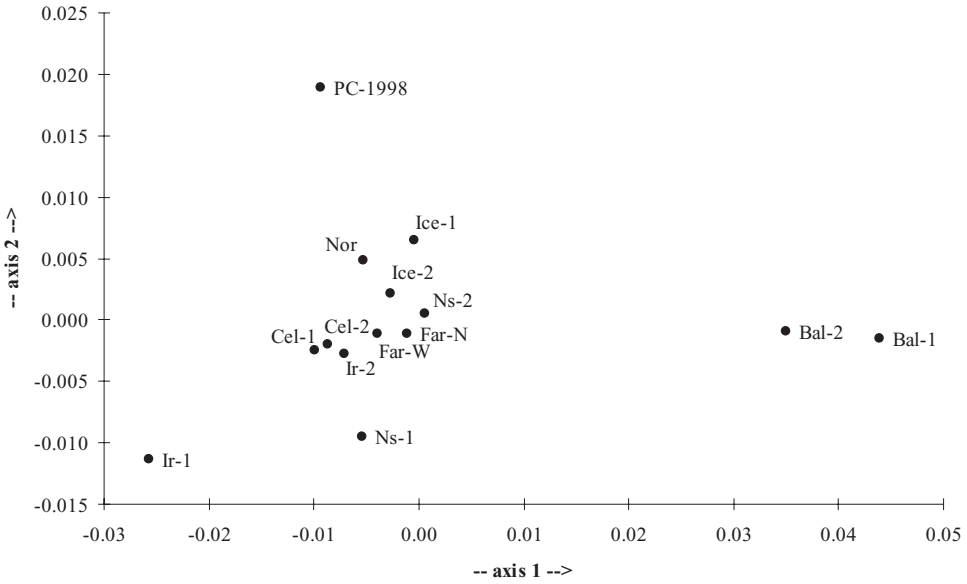

Figure 2. Multidimensional scaling analysis based on F_{ST} values among 14 samples of North-east Atlantic cod computed for eight microsatellite loci. For sample codes, see Table 1. The stress value was 0.0980.

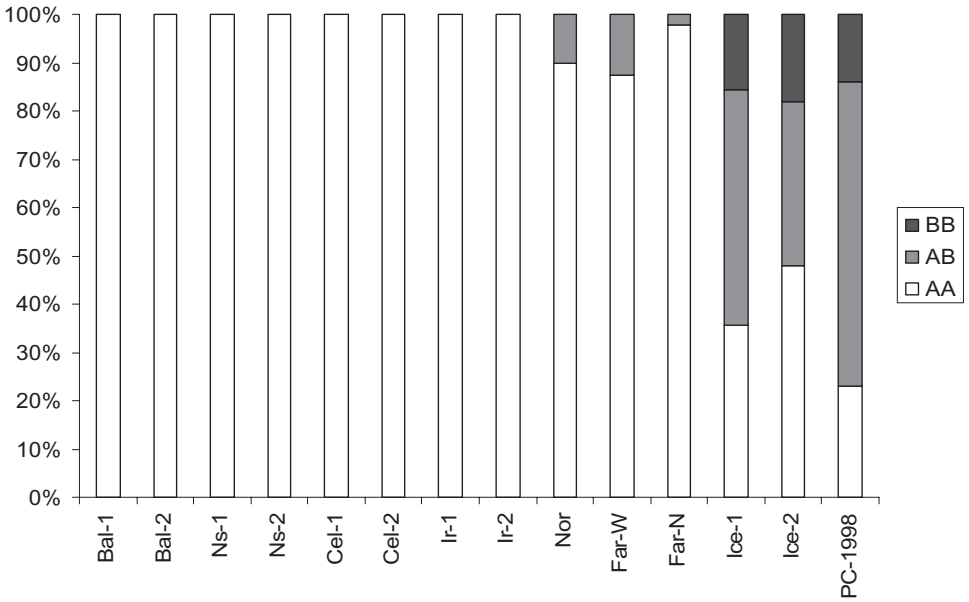

Figure 3. Genotype distribution of the *Pan* I locus among 14 samples of North-east Atlantic cod. For sample codes, see Table 1. Black bars, *Pan* I^{BB} genotypes; grey bars, *Pan* I^{AB} genotypes; white bars, *Pan* I^{AA} genotypes.

North Atlantic (Fig. 4). Most of the studied populations received more than eight immigrants per generation. The highest total number of emigrants and immigrants per generation was detected for Iceland (17 and 20, respectively) whereas the lowest was detected for the Faroe West population (8 and 13, respectively). Figure 4 also suggests that dispersal was asymmetrical between pairs of populations.

An overall significant positive correlation was found between geographical and microsatellite genetic distances (Fig. 5). This result was confirmed by a Mantel test using F_{ST} values ($Z = 4274$, $R = 0.473$, $P = 0.0209$ with the Canadian sample; $Z = 5799$, $R = 0.391$, $P = 0.0405$ without the Canadian sample). A significant negative correlation was found between longitude and A ($R = 0.58$, $N = 14$, $P < 0.0283$; Fig. 6) and A_R

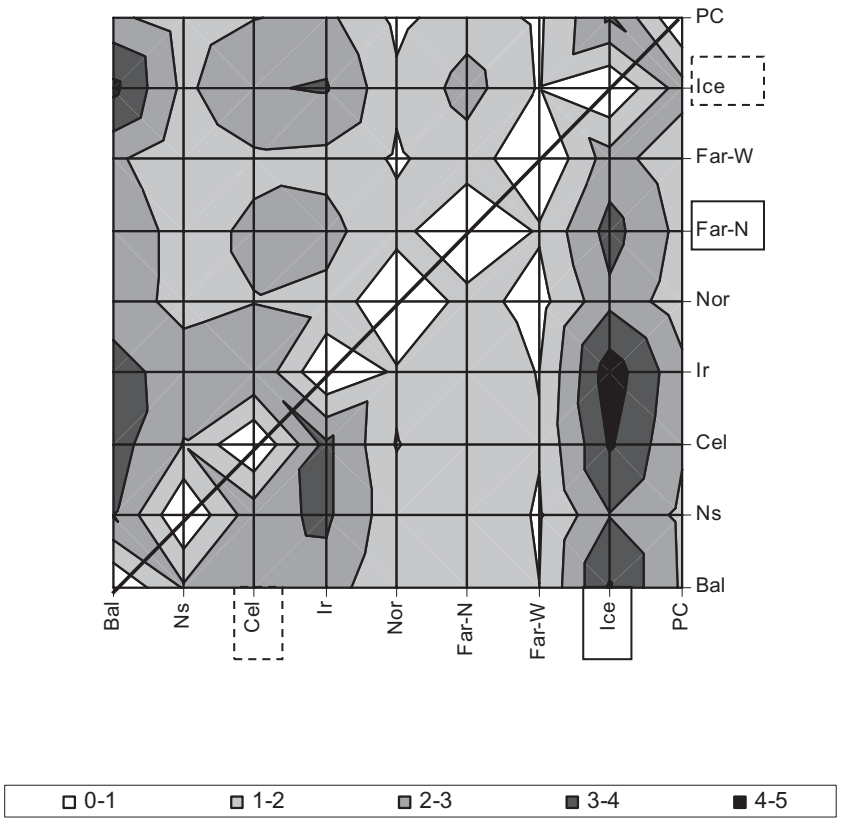

Figure 4. Dispersal per generation among Atlantic cod populations in the studied area, estimated through variability at eight microsatellite loci with the program MIGRATE. Above the diagonal, number of emigrants; below the diagonal, number of immigrants. The solid square represents the number of immigrants from the Far-N to the Icelandic waters (3–4), whereas the opened square represents the number of emigrants from Icelandic waters to the Celtic Sea (2–3). For sample codes, see Table 1.

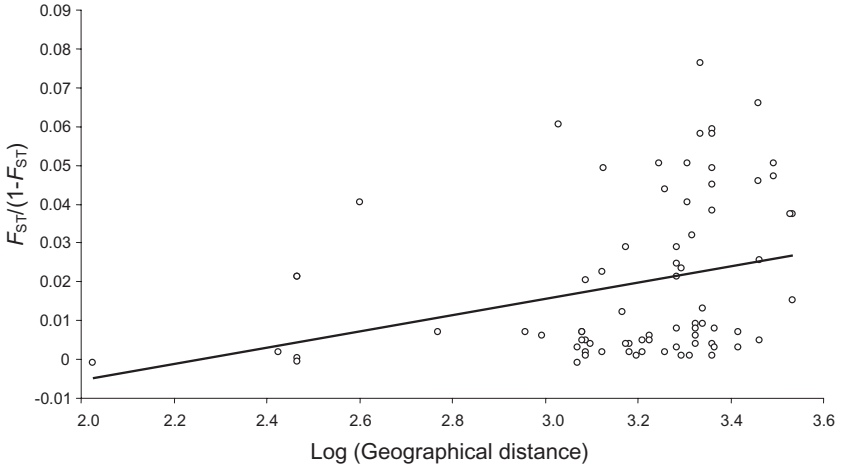

Figure 5. Overall correlation between geographical distances in km (x-axis) and genetic distances given as $\log([F_{ST}/(1 - F_{ST})])$ (y-axis) of 14 samples of Atlantic cod using eight microsatellite loci. The line represents the linear regression ($P = 0.001$).

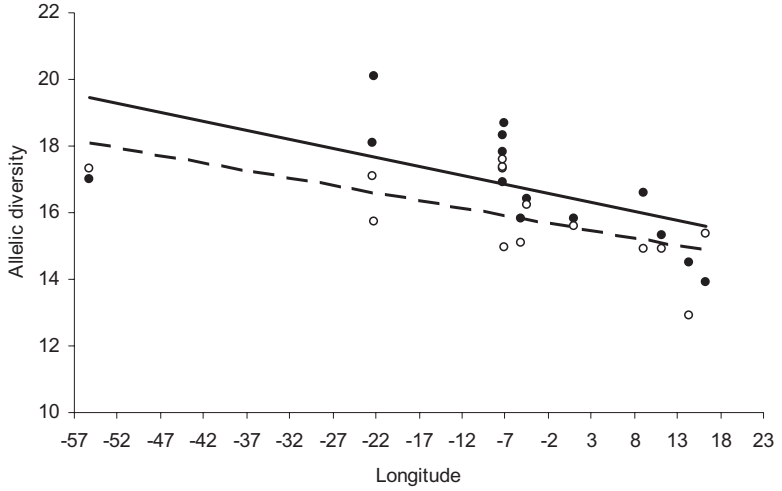

Figure 6. Allelic diversity measured as mean number of alleles (black dots and plain line) and allelic richness (white dots and dashed line) correlated with longitude.

Table 3. Hierarchical analysis of molecular variance among samples of *Gadus morhua* grouped according to BARRIER analysis for the microsatellite and *Pan* I locus (number of iterations 5000)

Loci	Source of variation	d.f.	Variance components	% variation	Fixation indices	*P*-values
Pan I	Among groups	3	0.0266	23.66	CT = 0.2366	< 0.0135
	Among samples within groups	10	0.0119	10.59	SC = 0.1387	< 0.00001
	Within samples	1850	0.0740	65.76	ST = 0.3424	< 0.00001
	Total	1863	0.1125	100		
Microsatellites	Among groups	3	0.0364	1.09	CT = 0.0109	< 0.00139
	Among samples within groups	10	0.0427	1.27	SC = 0.0129	< 0.00001
	Within samples	1896	3.274	97.64	ST = 0.0236	< 0.00001
	Total	1909	3.352	100		

Genetic partition was tested among groups. Group 1, Iceland and Faroese samples; Group 2, Canadian sample; Group 3, Baltic Sea, Norway, and North Sea 2003 (Ns-2) samples; Group 4, Irish Sea, Celtic Sea, and North Sea 2002 (Ns-1) samples.
d.f., degrees of freedom.

($R = 0.62$, $N = 14$, $P = 0.0183$; Fig. 6). A relationship was also found between longitude and H_o ($R = 0.62$, $N = 13$, $P = 0.0251$, the Canadian sample not included) and H_e ($R = 0.62$, $N = 13$, $P = 0.0240$; Canadian sample not included).

Several genetic discontinuities (barriers to gene flow) were identified using BARRIER. The first barrier supported by eight loci separated the Baltic Sea from all other samples (data not shown). The second barrier, also supported by eight loci, separated Ns-2, Nor, and the Baltic Sea from all other samples. The third barrier, supported by six loci, separated the Canadian sample from all other samples. Finally, the fourth barrier was supported by five loci, and separated the Celtic and Irish Sea samples from Iceland and Faroese samples. AMOVA among post-hoc defined regions using the structure obtained in the BARRIER analysis (for both types of genetic markers, see Table 3) confirmed that a small but significant portion of the variation was due to among groups component (Table 3).

Effective population size, historical origin, and time of divergence

Assuming a mutation rate (μ) of 10^{-5} per locus per generation, N_e estimates using the program MIGRATE suggested the largest effective population size in Iceland ($N_e = 21\,647$) and the lowest in the Faroe West population ($N_e = 6661$; Table 4).

The random permutation of different allele sizes among allelic states at each locus revealed that esti-

Table 4. Estimates of effective population sizes (N_e) using the coalescence-based method implemented in the program MIGRATE (Beerli & Felsenstein, 2001)

	Bal	Cel	Ice	Ir	Ns	Nor	Far-W	Far-N	PC-1998
MIGRATE									
N_e	10 935	16 157	21 467	17 117	10 755	7 532	6 661	11 226	11 156
25% quartile	3 994	4 311	5 324	4 154	2 781	1 592	1 782	2 939	2 699
Median	8 827	6 984	9 050	7 781	4 820	3 433	3 189	5 007	4 979
95% quartile	18 323	23 742	32 315	25 313	16 484	11 154	10 250	17 449	18 648

For sample codes, see Table 1.

Table 5. Mean single locus and multilocus pairwise estimates of R_{ST}, θ_{ST} and ρR_{ST} (95% distribution of central values in parentheses) between 14 sampling areas of North-east Atlantic cod following 2000 allele permutations (Hardy *et al*., 2003)

	θ_{ST}	R_{ST}	ρR_{ST} (95% range)
Gmo2	0.009	0.005	0.009 (–0.002–0.032)
Gmo8	0.073	0.195*	0.068 (0.007–0.166)
Gmo19	0.018	0.036	0.018 (–0.001–0.065)
Gmo34	0.020	0.022	0.018 (0.001–0.041)
Tch5	0.001	0.004	0.002 (–0.004–0.011)
Tch11	0.015	0.028	0.015 (–0.002–0.048)
Tch14	0.014	0.022*	0.014 (–0.003–0.19)
Tch22	0.013	0.010	0.010 (0.003–0.018)
Multilocus	0.013	0.097*	0.035 (0.008–0.078)

*Statistically significant ($P = 0.01$).

mates of R_{ST} were significantly larger than the 95% CI range of the ρR_{ST} values at two microsatellite loci (Gmo8 and Tch14; Table 5), suggesting a mutational component to genetic differentiation. A general trend could be observed at a total of five out of eight loci as R_{ST} were also larger than the ρR_{ST} values at Gmo19, Gmo34, and Tch11, but not significantly. The overall R_{ST} was also significantly larger than the 95% CI range of the ρR_{ST} values. A comparison of the expected F_{ST} as a function of generations ($2tN_e$) shows that 3000 generations would be sufficient to reach the observed F_{ST} of 0.013. Based on a generation time of 4–7 years for Atlantic cod (Myers, Mertz & Fowlow, 1997), this result suggests that the origin of the observed genetic differentiation could have been in the Late Weichselian during LGM (12–21 Kya).

DISCUSSION

GENETIC DIFFERENTIATION

Most previously reported studies on the genetic structure of Atlantic cod based on microsatellite loci did not include Icelandic samples and were consequently confined to a relatively small geographical scales (Ruzzante *et al*., 1996, 1997; Nielsen *et al*., 2003; Hardie, Gillett & Hutchings, 2006) compared with the present study (but see Bentzen *et al*., 1996; Hutchinson, Carvalho & Rogers, 2001; O'Leary *et al*., 2007). During our study, temporal stability was found in Icelandic waters and the Celtic Sea, and nonsignificant differentiation was found among the Baltic Sea samples and among the Faroese samples. Current findings also show that genetic differences among Iceland, the Faroe Islands, the Celtic Sea, and the Baltic Sea persisted over time, which may indicate that the appropriate population structure has been elucidated for these locations (Waples, 1998). However, the results obtained in the Irish Sea suggest that large movements of individuals might occur from year to year, or that the genetic structure of the population is more complex. In the North Sea, the samples collected were genetically different as expected according to Nielsen *et al*. (2003) and Hutchinson *et al*. (2001), who, respectively, described a hybrid zone near the sample Ns-2 and several stocks among which gene flow seemed sufficiently limited to establish differentiation within the North Sea. The observed overall F_{ST} (0.013) was similar or lower than the values obtained in the study of Bentzen *et al*. (1996) comparing Newfoundland and Barents Sea samples ($F_{ST} = 0.037$), the study of Hutchinson *et al*. (2001) comparing north-west and north-east Atlantic samples ($0.02 < F_{ST} < 0.07$) as well as Barents Sea and other samples collected around the Norwegian and UK coasts ($0.03 < F_{ST} < 0.08$), and the study of O'Leary *et al*. (2007; $F_{ST} = 0.030$) of the genetic structure across the geographical range of the species. Most of the pairwise F_{ST} comparisons were significant but the MDS analysis only revealed the presence of four groups. Two of those were composed of samples from geographical extremes of the sampling area (namely the Canada and the Baltic Sea), one was composed of the Irish sample collected in 2002, and the last one was composed of all other samples, therefore confirming previous studies. These results

corroborate the findings of Hutchinson *et al*. (2001), which suggested that, on a trans-oceanic scale, European cod is likely to be divergent to the Canadian cod because of geographical distances. Although the brackish waters of the Baltic Sea and the near oceanic salinities of the North Sea are in geographical proximity, the sharp salinity gradient across the Skagerrak may act as a barrier to gene flow. On the other hand, the temporal instability observed in the Irish Sea was surprising with respect to the results of Hutchinson *et al*. (2001). It is likely that these results reflect the diversity of migratory patterns found in Scottish waters (Wright *et al*., 2006).

The *Pan* I locus revealed a stronger genetic differentiation than the microsatellite loci, as expected for a genetic marker under directional selection (Pogson & Mesa, 2004). The observed F_{ST} (0.304) was similar to previous studies (Jónsdóttir, Daníelsdóttir & Nædval, 2001; Karlsson & Mork, 2003; Sarvas & Fevolden, 2005). Temporal stability at the *Pan* I locus was found in all replicated samples, which is comparable for other regions in the North Atlantic (Jónsdóttir *et al*., 2001; Sarvas & Fevolden, 2005; Pampoulie *et al*., 2006). In the present study, most of the observed differentiations were due to the Icelandic and Canadian samples which exhibited a high genetic variability compared with the North-east Atlantic samples. Indeed, the *Pan* I locus was monomorphic in most of the samples collected in the North-east Atlantic, except the Norwegian and Faroe samples, in which some *Pan* I^{AB} genotypes were found (less than 10%). As a consequence, the genetic analyses carried out (such as the MDS) on this locus revealed the presence of two genetically distinguishable basins, the North-west Atlantic composed of the Icelandic and Canadian samples and the North-east Atlantic composed of all other samples.

Gene flow and genetic discontinuities

Understanding species distributions and connectivity of their populations remains a challenge and a necessity for biodiversity conservation (Alleaume-Benharira, Pen & Ronce, 2006). Although exceptions have been documented (Luttikhuizen *et al*., 2003), a common consensus is that gene flow preserves both genetic and phenotypic diversity within a species, and counteracts differentiation caused by drift or differential selection, hence preventing adaptation to local environments. Although the isolation-by-distance model could explain the genetic differentiation found at the microsatellite loci in the present study, the genetic discontinuities statistic revealed the importance of the Labrador Current, the North Atlantic Current and the Norwegian Atlantic Current, as probable barriers to contemporary gene flow. Although genetic methods are increasingly used to assess potential connectivity among populations, estimates of gene flow based on genetic methods should be considered with caution. In Atlantic cod, for example, large populations might have been connected in the past but are now functionally isolated (as shown with tagging experiments). These contemporary isolated populations could therefore be fixed for different alleles at equilibrium, but the time it would take to reach this equilibrium after perturbation of gene flow might be greater than the age of the studied species (Neigel, 2002). Therefore, the calculated estimates of gene flow suggesting a relatively high migration rate among North Atlantic populations of cod might not reflect the real connectivity of the present-day populations because loci that are not at mutation–drift–migration equilibrium can distort these genetic estimates (Nichols & Freeman, 2004). Tagging experiments performed at spawning grounds in Greenland waters, Iceland waters, Faroese waters, Irish Sea, Celtic Sea, and North Sea did not show extensive migration, even at small geographical scales (Storr-Paulsen *et al*., 2004; Joensen *et al*., 2005; Pampoulie *et al*., 2006; Wright *et al*., 2006). A 40-year long tagging study found that only five out of 10 969 recaptured Icelandic cod were caught in Faroese waters (Jónsson, 1996). Likewise, one out of 1043 recaptured individuals marked in Faroese waters from 1952 to 1965 was found in Icelandic waters (Joensen *et al*., 2005). The tagging experiments contradict genetic estimates, which suggest a large number of migrants per generation from Icelandic to Faroese waters and vice-versa. This discrepancy could be explained by two different but non-exclusive theories: the 'adopted migrant' hypothesis (McQuinn, 1997) and the presence of historical signals in the genetic data (Hewitt, 1996). The 'adopted migrant' hypothesis proposes that local populations 'which are founded through dispersion from existing populations, are perpetuated in geographical space through the social transmission of migration and homing patterns from the adults to the recruiting juveniles in the year preceding first spawning. Local populations are therefore maintained through the behavioural isolation of adults, which exhibit repeat homing to spawning grounds (McQuinn, 1997). Indeed, the limited dispersal of adults observed via tagging suggests fidelity to spawning grounds which can be learned by individuals with a social transmission from older spawning fish to the new recruits. Therefore, gene flow might occur during eggs and larval stages, and populations might be more differentiated in areas where oceanic features prevent the dispersal of eggs and larvae (see the results of BARRIER and AMOVA).

An alternative hypothesis would be the presence of a historical signal in the genetic data. Recently, mic-

rosatellite studies have established that the genetic structure of populations can be embedded in their history (Gysels *et al*., 2004; Hoarau *et al*., 2007). Studies carried out with other genetic markers proposed a recent origin of the cod populations and as cod populations are large (Árnason & Pálsson, 1996; present study), they may still not have reached migration/drift equilibrium (Pogson *et al*., 1995). Therefore, if a recent population expansion signal exists in the genetic data, it could explain the low F_{ST} values and the high number of migrants per generation estimated among North Atlantic populations of cod.

Effective population size, historical origin, and time of divergence

Recent discussions about the weak genetic structure of cod populations and its origin have been flourishing (Pogson *et al*., 1995; Árnason & Pálsson, 1996; Hardie *et al*., 2006) and weak gene genealogy (Carr *et al*., 1995; Árnason, 2004) and recent population origins (Pogson *et al*., 1995) have been suggested as the underlying causes. In the present study, the presence of a spatial pattern to genetic differentiation and of genetic discontinuities indicates a mechanism of contemporary gene flow that is mainly dependent upon geographical distances and oceanic currents. In addition, genetic estimates of the effective population size suggest relatively high population size in the studied areas (6 661–21 467; Table 4), approaching previous estimates found by Árnason & Pálsson (1996). Estimates of gene flow and effective population size should nevertheless be interpreted with caution as they assume equilibrium, constant migration rate, and constant population size through recolonization events, respectively, premises that are likely to be violated in the system under study (Waples, 1991). However, the discrepancy in the estimation of migration rate between tagging experiments and genetic estimates, provides a reason to believe that historical events such as postglacial expansion may have contributed to the genetic pattern observed. Postglacial expansion of a species frequently results in a lower genetic diversity in populations inhabiting formerly glaciated areas (Hewitt, 1996, 2000). The clear west–east gradient (with longitude) of the microsatellite genetic diversity and the distribution of the *Pan* I genotypes found during this study may reflect repeated founder events apparent in previously glaciated areas. Analyses on the *Pan* I locus may reflect the effect of positive selection of the *Pan* I^{AA} genotypes in Northern Europe after the postglacial expansion. Although a general trend could be observed at five out of eight microsatellite loci, the random permutation test of different allele sizes conducted during our study only detected a significant mutation effect to the genetic difference at the two most variable microsatellite loci (Gmo8 and Tch14) in which rare alleles were detected at the extreme range of the allelic distribution. It is unlikely that these extreme allele sizes followed a strict Stepwise Mutation Model. Instead, the signal may reflect historical mutations that accumulated over time when isolation barriers were more pronounced between the actual populations. Postglacial expansion has been suggested for cod in the Canadian Arctic (Hardie *et al*., 2006) as the calculated time of divergence (approximately 8 Kya) coincides with last glacial retreat. Several mtDNA analyses reported a weak gene genealogy of cod populations characterized by a typical star-like phylogeny (Carr *et al*., 1995; Árnason, 2004), which suggests a recent population increase/expansion (Avise *et al*., 1987). During LGM (Wisconsinian period, 15–25 Kya), the northern part of the North Atlantic Ocean, the North Sea and the Baltic Sea were covered with ice (Svendsen *et al*., 2004), and the average reconstructed temperature in Irish Sea and in North Sea was around –4 °C (Siegert & Dowdeswell, 2004). Therefore, present-day North Atlantic Ocean, North Sea, Irish Sea, Celtic Sea, and Baltic Sea were uninhabitable for cod throughout LGM, and probably later due to low water temperatures. The comparison of the predicted F_{ST} to the number of generations ($2tN_e$) revealed that 3000 generations would be sufficient to reach the observed F_{ST}. This would correspond to the climate change of the Late Weichselian. Although refugia areas have been suggested in North Sea (Balson *et al*., 1991) and Baltic Sea (Ancylus lake, 8–9 Kya; Verspoor *et al*., 1999), there was no clear evidence to support this in our data. It would be difficult to elucidate the migration route of Atlantic cod following deglaciation as the ancestral population was probably located in a region neighbouring the Rockall Plateau (around the Ireland refugia, see Jolly *et al*., 2006 and Hoarau *et al*., 2007) and/or the Irminger Sea (Cross & Payne, 1978; Hardie *et al*., 2006). At present, no cod populations inhabit these areas, so they could not be included in our study. Nevertheless, the low observed genetic differentiation could not be explained by gene flow alone, and might find its origin in a step-by-step postglacial expansion of the species following the retreat of glacial coverage during the late Weichselian.

Conclusion

The present study yields two important findings for the Atlantic cod. First, microsatellite loci revealed a weak but significant genetic differentiation of cod in the North Atlantic. This differentiation could find its origin in large current gene flow and/or a recent

origin of cod populations. The former possibility is precluded because the identified barriers to gene flow corresponded to known oceanic currents, and because of a lack of active migration of cod in the North Atlantic. The drastic reduction in cod population sizes due to fishing might hamper the detection of postglacial colonization patterns. Nevertheless, estimation of divergence times showed that historical isolation of cod populations in glacial refugia during LGM may have resulted in genetic differentiation across the North Atlantic. The *Pan* I locus, which probably reflects a positive selection of the allele A in Europe after recolonization of ice-free environments, corroborates these findings. Therefore, as suggested by previous studies (Pogson *et al*., 1995; Hardie *et al*., 2006), we believe that the weak genetic differentiation of Atlantic cod detected with microsatellite loci is due to a rapid expansion of the species after the late Weichselian.

ACKNOWLEDGEMENTS

This research was carried out under the CODTRACE EU-project (Q5RS-2000-01697). We would like to thank all partners of the project who kindly collected samples for our study. Special thanks to D. E. Ruzzante for providing Canadian samples, to P. Steingrund for providing Faroe samples, to P. Beerli for his help with the MIGRATE runs, to D. Falush, G. E. Maes and J. A. M. Raeymaekers for their valuable comments, and to two anonymous referees who greatly improved the quality of the manuscript.

REFERENCES

Alleaume-Benharira M, Pen IR, Ronce O. 2006. Geographical patterns of adaptation within a species' range: interaction between drift and gene flow. *Journal of Evolutionary Biology* **19:** 203–215.

Árnason E. 2004. Mitochondrial cytochrome *b* DNA variation in the high fecundity Atlantic cod: trans-Atlantic clines and shallow gene genealogy. *Genetics* **166:** 1871–1885.

Árnason E, Pálsson S. 1996. Mitochondrial cytochrome b sequence variation of Atlantic cod *Gadus morhua*, from Norway. *Molecular Ecology* **5:** 715–724.

Avise JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18:** 489–522.

Balson PS, Laban C, Frantsen PJ, Parker N, Henriet JP, de Batist M, Mostaert F. 1991. *Ostend, sheet 52°N/02°E. Solid Geology, 1:250 000 Series British Geological Survey, Geological Survey of The Netherlands & Belgian Geological Survey.* Southampton: Ordnance Survey for the British Geological Survey.

Beerli P, Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* populations using a coalescent approach. *Proceedings of the National Academy of Sciences of the United States of America* **98:** 4563–4568.

Belkhir K, Borsa P, Goudet J, Bonhomme F. 1999. *GENETIX: logiciel sous Windows pour la génétique des populations*, Version 3.0. Montpellier: Laboratoire Génome & Population, CNRS-UPR, Université de Montpellier II.

Bentzen P, Taggart CT, Ruzzante DE, Cook D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the Northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* **53:** 2706–2721.

Brooker AL, Cook D, Bentzen P, Wright JM, Doyle RW. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **51:** 1959–1966.

Brown J, Hill AE, Fernand L, Horsburgh KJ. 1999. Observations of a seasonal jet-like circulation at the central North Sea cold pool margin. *Estuarine, Coastal and Shelf Science* **48:** 343–355.

Carr SM, Snellen AJ, Howse KA, Wroblewski JS. 1995. Mitochondrial DNA sequence variation and genetic stock structure of Atlantic cod (Gadus morhua) from bay and offshore locations on the Newfoundland continental shelf. *Molecular Ecology* **4:** 79–88.

Cross TF, Payne RH. 1978. Geographic variation in Atlantic cod, *Gadus morhua*, off Eastern North America: a biochemical systematics approach. *Journal of the Fisheries Research Board of Canada* **35:** 111–123.

Crow JF, Kimura M. 1970. *An introduction to population genetics theory*. New York, NY: Harper & Row.

D'Amours D. 1993. The distribution of cod (*Gadus morhua*) in relation to temperature and oxygen level in the Gulf of St. Lawrence. *Fisheries Oceanography* **2:** 24–29.

Goudet J. 1995. FSTAT (vers. 1.2): a computer program to calculate F-statistics. *Journal of Heredity* **86:** 485–486.

Gysels ES, Hellemans B, Pampoulie C, Volckaert FAM. 2004. Phylogeography of the common goby, *Pomatoschistus microps*, with particular emphasis on the colonisation of the Mediterranean and the North Sea. *Molecular Ecology* **13:** 403–417.

Hardie DC, Gillett RM, Hutchings JA. 2006. The effects of isolation and colonization history on the genetic structure of marine-relict populations of Atlantic cod (*Gadus morhua*) in the Canadian Arctic. *Canadian Journal of Fisheries and Aquatic Sciences* **63:** 1830–1839.

Hardy OJ, Charbonnel N, Freville H, Heuertz M. 2003. Microsatellite allele sizes: a simple test to assess their significance on genetic differentiation. *Genetics* **163:** 1467–1482.

Hardy OJ, Vekemans X. 2002. SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology* **2:** 618–620.

Hewitt GM. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58:** 247–276.

Hewitt GM. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907–913.

Hoarau G, Coyer JA, Veldsink JH, Stam WT, Olsen JL. 2007. Glacial refugia and recolonization pathways in the brown seaweed *Fucus serratus*. *Molecular Ecology* **16:** 3606–3616.

Hutchinson WF, Carvalho GR, Rogers SI. 2001. Marked genetic structuring in localised spawning populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. *Marine Ecology Progress Series* **223:** 251–260.

Jin L, Chakraborty R. 1995. Population structure, stepwise mutation, and heterozygote deficiency and their implications in DNA forensics. *Heredity* **74:** 247–285.

Joensen JS, Steingrund P, Henriksen A, Mouritsen R. 2005. Migration of cod (*Gadus morhua*): tagging experiments at the Faroes 1952–1965. *Fróðskaparrit* **53:** 100–135.

Jolly MT, Viard F, Gentil F, Thiébault E, Jollivet D. 2006. Comparative phylogeography of two coastal polychaete tubeworms in the Northeast Atlantic supports shared history and vicariant events. *Molecular Ecology* **15:** 1840–1855.

Jónsdóttir ÓDB, Daníelsdóttir AK, Nædval G. 2001. Genetic differentiation among Atlantic cod (*Gadus morhua* L.) in Icelandic waters: temporal stability. *ICES Journal of Marine Science* **58:** 114–122.

Jónsson J. 1996. Tagging of cod (*Gadus morhua*) in Icelandic waters 1948–1986. *Rit Fiskideildar* **14:** 1–82.

Karlsson S, Mork J. 2003. Selection-induced variation at the pantophysin locus (*Pan* I) in a Norwegian fjord population of cod (*Gadus morhua*). *Molecular Ecology* **12:** 3265–3274.

Koljonen MJ, Jansson H, Paaver T, Vasin O, Koskiniemi J. 1999. Phylogeographic lineages and differentiation pattern of Atlantic salmon (*Salmo salar*) in the Baltic Sea with management implications. *Canadian Journal of Fisheries and Aquatic Sciences* **56:** 1766–1780.

Luttikhuizen PC, Drent J, Van Delden W, Piersma T. 2003. Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. *Journal of Evolutionary Biology* **16:** 260–272.

Manni F, Guérard E, Heyer E. 2004. Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by 'Monmonier's algorithm'. *Human Biology* **76:** 173–190.

McQuinn IH. 1997. Metapopulations and the Atlantic herring. *Reviews in Fish Biology and Fisheries* **7:** 297–329.

Miller KM, Le KD, Beacham TD. 2000. Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). *Molecular Ecology* **9:** 238–239.

Myers RA, Mertz G, Fowlow S. 1997. The maximum population growth rates and recovery times of Atlantic cod (*Gadus morhua*). *Fishery Bulletin* **95:** 762–772.

Neigel JE. 2002. Is F_{ST} obsolete? *Conservation Genetics* **3:** 167–173.

Nichols RA, Freeman KLM. 2004. Using molecular markers with high mutation rates to obtain estimates of relative population size and distinguish the effects of gene flow and mutation: a demonstration using data from endemic Mauritarian skinks. *Molecular Ecology* **13:** 775–787.

Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Gronkjaer P. 2003. Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Molecular Ecology* **12:** 1497–1508.

O'Brien CM, Fox CJ, Planque B, Casey J. 2000. Climate variation and North Sea cod. *Nature* **404:** 142–142.

O'Leary DB, Coughlan J, Dillane E, McCarthy TV, Cross TF. 2007. Microsatellite variation in cod *Gadus morhua* throughout its geographic range. *Journal of Fish Biology* **70:** 310–335.

O'Reilly PT, Canino MF, Bailey KM, Bentzen P. 2000. Isolation of twenty low stutter di- and tetranucleotide microsatellites for population analyses of walleye pollock and other gadoids. *Journal of Fish Biology* **56:** 1074–1086.

Pampoulie C, Ruzzante DE, Chosson V, Jörundsdóttir ThD, Taylor L, Thorsteinsson V, Daníelsdóttir AK, Marteinsdóttir G. 2006. The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the *Pan* I locus, and tagging experiments. *Canadian Journal of Fisheries and Aquatic Sciences* **63:** 2660–2674.

Pérrin C, Wing SR, Roy MS. 2004. Effect of hydrographic barriers on population genetic structure of the sea star *Coscinasterias mircata* (Echinodermata, Asteroida) in the New Zealand fiords. *Molecular Ecology* **13:** 2183–2195.

Pogson GH, Mesa KA. 2004. Positive Darwinian selection at the pantophysin (*Pan* I) locus in marine gadid fishes. *Molecular Biology and Evolution* **21:** 65–75.

Pogson GH, Mesa KA, Boutilier RG. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. *Genetics* **139:** 375–385.

Raymond R, Rousset F. 1995. *GENEPOP* (Version 1.2): population genetic software for exact tests and ecumenicism. *Journal of Heredity* **86:** 248–249.

Reusch TBH, Wegner KM, Kalbe M. 2001. Rapid divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographical proximity. *Molecular Ecology* **10:** 2435–2445.

Rice WR. 1989. Analyzing table of statistical tests. *Evolution* **43:** 223–225.

Robichaud D, Rose GA. 2004. Migratory behaviour and range in Atlantic cod: inference from a century of tagging. *Fish and Fisheries* **5:** 185–214.

Ruzzante DE, Taggart CT, Cook D. 1998. A nuclear DNA basis for shelf- and bank-scale population structure in northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. *Molecular Ecology* **7:** 1663–1680.

Ruzzante DE, Taggart CT, Cook D, Goddard SV. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: microsatellite DNA variation and antifreeze level. *Canadian Journal of Fisheries and Aquatic Sciences* **53:** 634–645.

Ruzzante DE, Taggart CT, Cook D, Goddard SV. 1997. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: a test and evidence of temporal stability. *Canadian Journal of Fisheries and Aquatic Sciences* **54:** 2700–2708.

Sarvas TH, Fevolden SE. 2005. Pantophysin (*Pan* I) locus divergence between inshore v. offshore and northern v. southern populations of Atlantic cod in the north-east Atlantic. *Journal of Fish Biology* **67:** 444–469.

Schneider S, Roessli D, Excoffier L. 2000. *ARLEQUIN, version 2.000: a software for population genetic analysis.* Genova: Genetics and Biometry Laboratory, University of Genova.

Shaw IW, Arkhipin AI, Al-Khairulla H. 2004. Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep–water troughs as barrier to genetic exchange. *Molecular Ecology* **13:** 3293–3303.

Siegert MJ, Dowdeswell JA. 2004. Numerical reconstruction of the Eurasian ice sheet and climate during the late Weichselian. *Quaternary Science Reviews* **23:** 1273–1283.

Smith SJ, Page FH. 1996. Associations between Atlantic cod (*Gadus morhua*) and hydrographic variables: implication for the management of the 4VsW cod stock. *ICES Journal of Marine Science* **53:** 15–24.

Statsoft Inc. 2001. *STATISTICA (data analysis software system)*, Version 6.0. Tulsa, OK: Statsoft Inc. Available at http://www.statsoft.com.

Storr-Paulsen M, Wieland K, Hovgard H, Ratz HJ. 2004. Stock structure of Atlantic cod (*Gadus morhua*) in West Greenland waters: implications of transport and migration. *ICES Journal of Marine Science* **61:** 972–982.

Svendsen JIS, Alexanderson H, Astakhov V, Demidov I, Dowdeswell JA, Funder S, Gataullin V, Henriksen M, Hjort C, Houmark-Nielsen M, Hubberten HW, Ingolfsson O, Jakobsson M, Kjær KH, Larsen E, Lokrantz H, Lunkka JP, Lysa A, Mangerud J, Matiouchkov A, Murray A, Moller P, Niessen F, Nikolskaya O, Polyak L, Saarnisto M, Siegert C, Siegert MJ, Spielhagen RF, Stein R. 2004. Late quaternary ice sheet history of northern Eurasia. *Quaternary Science Reviews* **23:** 1229–1271.

Turrell WR. 1992. New hypothesis concerning the circulation of the Northern North Sea and its relation to North Sea fish stock recruitment. *ICES Journal of Marine Science* **49:** 107–123.

van der Molen J, de Swart HE. 2001. Holocene tidal conditions and tide-induced sand transport in the southern North Sea. *Journal of Geophysical Research* **106:** 9339–9362.

Verspoor E, McCarthy EM, Knox D, Burke EA, Cross TF. 1999. The phylogeography of European Atlantic salmon (*Salmo salar* L.) based on RLFP analysis of the ND1/16S RNA region of the mtDNA. *Biological Journal of the Linnean Society* **68:** 129–146.

Waples RS. 1991. Genetic methods for estimating the effective size of cetacean populations. *Report for the International Whaling Commission (Special Issue)* **13:** 279–300.

Waples RS. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89:** 438–450.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* **38:** 1358–1370.

Wright S. 1969. *Evolution and the genetics of populations*, vol. 2. *The theory of gene frequencies*. Chicago, IL: University of Chicago Press.

Wright PJ, Galley E, Gibb IM, Neat FC. 2006. Fidelity of adult cod to spawning grounds in Scottish waters. *Fisheries Research* **77:** 148–158.

APPENDIX

Table A1. Mean number of alleles (A), total number of alleles (N_A), allelic size ranges (AS), and mean expected (H_e) and observed (H_o) heterozygosity across the 14 sampling areas of Atlantic cod analysed at eight microsatellite loci.

	Gmo2	Gmo8	Gmo19	Gmo34	Tch5	Tch11	Tch14	Tch22
A	12.5	21.5	21.3	7.0	18.7	18.3	26.6	5.9
N_A	22	53	28	10	28	27	38	10
AS	106–148	108–316	124–232	88–120	172–288	114–222	106–270	75–115
H_e	0.851	0.909	0.923	0.583	0.928	0.926	0.952	0.606
H_o	0.737	0.798	0.884	0.552	0.883	0.901	0.870	0.602

Table A2. Number of individuals scored (N), total number of alleles (N_A), mean number of alleles (A), allelic richness (A_R), expected heterozygosity (H_e), observed heterozygosity (H_o) and F_{IS} according to Weir & Cockerham (1984) for each microsatellite loci and the *Pan* I locus

	Bal-1	Cel-1	Ice-1	Ir-1	Ns-1	Bal-2	Cel-2	Ice-2	Ir-2	Ns-2	Nor	Far-W	Far-N	PC-1998
N	94	64	70	68	29	60	60	94	64	59	60	55	85	93
Gmo2														
N_A	12	14	15	14	13	11	11	15	13	13	10	13	13	15
H_e	0.817	0.848	0.870	0.835	0.881	0.793	0.846	0.850	0.850	0.827	0.803	0.837	0.838	0.803
H_o	0.851	0.813	0.814	0.774	0.793	0.750	0.783	0.862	0.671	0.763	0.767	0.782	0.858	0.710
F_{IS}	–0.037	0.079	0.071	**0.098**	0.117	0.063	0.083	–0.008	**0.217**	0.086	–0.042	0.075	–0.019	0.122
Gmo8														
N_A	19	30	28	19	25	17	28	31	20	22	22	28	31	17
H_e	0.598	0.928	0.922	0.733	0.939	0.632	0.930	0.926	0.915	0.894	0.913	0.924	0.938	0.910
H_o	0.564	0.969	0.942	0.650	0.966	0.533	0.933	0.851	0.898	0.932	0.833	0.927	0.824	0.781
F_{IS}	0.033	–0.036	–0.015	0.087	–0.011	0.165	0.005	0.087	0.018	–0.034	0.095	0.006	**0.116**	0.148
Gmo19														
N_A	18	22	23	22	18	19	22	27	21	23	21	21	24	22
H_e	0.880	0.917	0.922	0.919	0.822	0.903	0.897	0.914	0.879	0.924	0.921	0.898	0.914	0.928
H_o	0.851	0.969	0.900	0.838	0.724	0.867	0.933	0.904	0.906	0.848	0.933	0.887	0.918	0.871
F_{IS}	0.039	–0.048	0.031	0.095	0.136	0.049	–0.032	0.017	–0.024	0.091	–0.005	0.022	0.002	0.067
Gmo34														
N_A	6	8	7	7	7	5	9	8	7	8	7	6	7	6
H_e	0.464	0.713	0.420	0.653	0.613	0.419	0.686	0.531	0.655	0.577	0.679	0.574	0.636	0.424
H_o	0.479	0.750	0.442	0.632	0.655	0.433	0.717	0.457	0.672	0.576	0.617	0.547	0.624	0.441
F_{IS}	–0.025	–0.046	–0.047	0.040	–0.050	–0.026	–0.036	0.143	–0.017	0.010	0.100	0.057	0.025	–0.033
Tch5														
N_A	21	19	20	19	15	17	21	19	19	17	18	18	19	22
H_e	0.917	0.924	0.925	0.925	0.907	0.912	0.919	0.926	0.921	0.915	0.918	0.914	0.917	0.922
H_o	0.893	0.859	0.929	0.956	1.000	0.933	0.850	0.819	0.859	0.932	0.917	0.927	0.835	0.850
F_{IS}	0.030	0.078	0.003	0.026	–0.085	–0.015	0.083	–0.045	0.075	–0.010	0.010	–0.006	0.095	0.085
Tch11														
N_A	13	18	20	21	20	15	19	21	19	18	18	18	22	25
H_e	0.832	0.926	0.922	0.926	0.924	0.843	0.931	0.930	0.924	0.931	0.919	0.920	0.926	0.929
H_o	0.862	0.953	0.861	0.941	0.862	0.800	0.950	0.947	0.906	0.915	0.950	0.906	0.942	0.957
F_{IS}	–0.030	–0.022	0.078	–0.009	0.084	0.060	–0.012	–0.013	0.027	0.026	–0.025	0.025	–0.011	–0.024
Tch14														
N_A	22	28	25	28	23	23	26	33	27	26	21	31	31	23
H_e	0.883	0.939	0.940	0.940	0.932	0.912	0.946	0.946	0.934	0.933	0.918	0.953	0.951	0.916
H_o	0.734	0.953	0.900	0.912	0.931	0.800	0.950	0.915	0.875	0.898	0.867	0.946	0.941	0.810
F_{IS}	0.174	–0.007	0.047	0.038	0.019	0.111	0.005	0.038	0.071	0.046	0.064	0.017	0.017	0.126
Tch22														
N_A	5	7	7	6	5	4	6	7	5	6	5	5	8	6
H_e	0.579	0.657	0.657	0.640	0.491	0.582	0.627	0.622	0.585	0.576	0.581	0.607	0.622	0.416
H_o	0.606	0.641	0.614	0.677	0.483	0.600	0.683	0.628	0.500	0.627	0.600	0.546	0.635	0.387
F_{IS}	–0.042	0.033	0.072	–0.049	0.033	–0.022	–0.082	–0.022	0.153	–0.080	–0.024	0.110	–0.015	0.076
Total														
A	14.5	18.3	18.1	15.8	15.8	13.9	17.8	20.1	16.4	16.6	15.3	16.9	18.7	17.0
A_R	12.9	17.3	17.1	15.1	15.6	15.4	17.4	15.8	16.2	14.9	14.9	17.6	15.0	17.3
H_e	0.744	0.859	0.822	0.822	0.814	0.750	0.848	0.830	0.833	0.822	0.832	0.829	0.845	0.781
H_o	0.730	0.863	0.800	0.754	0.802	0.715	0.850	0.834	0.777	0.811	0.785	0.808	0.824	0.723
F_{IS}	0.024	–0.003	0.034	0.086	0.032	0.055	–0.006	–0.004	0.075	0.022	0.064	0.034	0.021	0.080
Pan I														
N_A	1	1	2	1	1	1	1	2	1	1	2	2	2	2
H_e	0	0	0.480	0	0	0	0	0.452	0	0	0.095	0.135	0.027	0.496
H_o	0	0	0.486	0	0	0	0	0.330	0	0	0.100	0.146	0.028	0.644
F_{IS}	–	–	–0.005	–	–	–	–	0.276	–	–	–0.045	–0.069	–0.007	–0.295

Bold values: F_{IS} values deviating significantly from Hardy–Weinberg expectations assessed in GENEPOP with exact test after correction for multiple tests. For sample codes, see Table 1.