

The genetic structure of Atlantic cod (*Gadus morhua*) around Iceland: insight from microsatellites, the *Pan I* locus, and tagging experiments

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Abstract: Allelic variation at nine microsatellite loci and the *Pan I* locus provides evidence that Atlantic cod (*Gadus morhua*) around Iceland is genetically structured ($F_{ST} = 0.003$ and $F_{ST} = 0.261$, respectively). A total of 2534 cod were sampled at 22 spawning locations. For both types of markers, most of the significant pairwise F_{ST} resulted from northeastern–southwestern comparisons. A multidimensional scaling analysis based on F_{ST} , a spatial hierarchical analysis of molecular variance (SAMOVA) and a hierarchical analysis of molecular variance (AMOVA), conducted on both types of markers confirmed a genetic differentiation between cod from the northeastern and southwestern regions. Genetic discontinuities were revealed across two main current fronts in the southeast and northwest, where the cold and warm water masses meet. The AMOVA also detected genetic differences with depth. Expected and observed heterozygosity of microsatellite loci significantly decreased with depth, whereas the B allele frequency at the *Pan I* locus increased. A tagging experiment of spawning fish conducted during the same years as the genetic work revealed that tagged individuals released in the southwestern region seldom migrated to the northeastern region and vice versa, suggesting that the southwestern and northeastern populations of Atlantic cod around Iceland represent two distinct spawning components.

Résumé : La variation allélique de neuf microsatellites et du locus *Pan I* met en évidence une structure génétique chez la morue Atlantique (*Gadus morhua*) dans les eaux islandaises (F_{ST} respectifs, $F_{ST} = 0,003$ et $F_{ST} = 0,261$). Un total de 2534 morues a été échantillonné sur 22 sites de ponte. Pour les deux marqueurs génétiques utilisés, la majorité des valeurs significatives de F_{ST} par paires de populations est due à des comparaisons nord-est et sud-ouest. Une analyse multidimensionnelle basée sur les F_{ST} par paires de populations, une analyse spatiale hiérarchique de variance moléculaire (SAMOVA) et une analyse hiérarchique de variance moléculaire (AMOVA), réalisées sur les deux types de marqueurs génétiques, révèlent une différenciation significative entre les échantillons du nord-est et du sud-ouest. La recherche de discontinuités génétiques indique la présence de deux barrières au flux génique correspondant aux principaux fronts de courants océaniques localisés dans le nord-ouest et le sud-est du pays aux points où les masses d'eaux chaudes et froides entrent en contact. L'analyse AMOVA détecte aussi des différences génétiques en fonction de la profondeur. Une analyse approfondie montre que les hétérozygoties observées et attendues des microsatellites diminuent significativement avec la profondeur, alors que la fréquence de l'allèle B du locus *Pan I* augmente. Une expérience de « capture–recapture » réalisée sur les sites de pontes en même temps que les études génétiques révèle que des individus marqués et relâchés dans la région du sud-ouest migrent rarement vers la région du nord-est et vice versa, ce qui confirme que les populations du sud-ouest et du nord-est de morues Atlantique dans les eaux islandaises représentent probablement deux unités de reproduction distinctes.

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Introduction

Marine fisheries are often managed under the assumption of panmixia without consideration of often suspected or known life history or genetic differences among components of a stock or population complex. In this respect, fishery management is primarily area-based and conducted under the assumption that neglecting the existence of life history or genetic differences among population components is not likely to have detrimental effects on population persistence and stock structure. Failure to recognize the link between management and stock structure can, however, have detrimental effects on genetic and, thus, stock diversity by disproportionately affecting the smaller or less productive population components (Begg et al. 1999; Ward 2000; Swain et al. 2001). In turn, proper management of exploited fish requires full knowledge of stock affiliation across the distribution of the targeted species. Information on stock structure of commercially exploited fish stocks is thus of paramount importance and the ability to discriminate among units that differ in life histories and (or) genetic composition, some of which may migrate seasonally and may at times overlap spatially, is a challenge for conservation biologists, population geneticists, and fishery biologists alike (Ruzzante et al. 2006).

Atlantic cod (*Gadus morhua*) has historically been one of the most valuable commercial fish species inhabiting the continental shelves of the western, central, and eastern North Atlantic. During the last century, however, cod stocks declined dramatically in some regions owing to drastic over-exploitation (see Christensen et al. (2003) for a review). The depletion and collapse of cod stocks, as well as other commercial marine fishes, has now been widely interpreted as an example of the failure of sustainable fisheries management (Myers et al. 1996; Cook et al. 1997). It has often been suggested that this failure results in part from the fact that management units do not necessarily reflect biological units as such (Stephenson and Kenchington 2000). Indeed, recent genetic studies using different types of markers have demonstrated the existence of often subtle but biologically meaningful genetic differences among population components within management regions (Ruzzante et al. 1996; Knutsen et al. 2003; Sarvas and Fevolden 2005). Oceanic currents, oceanic topology (inshore and offshore), and geographical distances were shown to be the main factors restricting passive or active exchange of migrants among these subunits. These studies indicate that genetic markers combined with ecological and life history information, as well as with environmental data (e.g., topography, current patterns, salinity, and (or) temperature), are likely to lead to improved insight on the stock composition of exploited marine fishes.

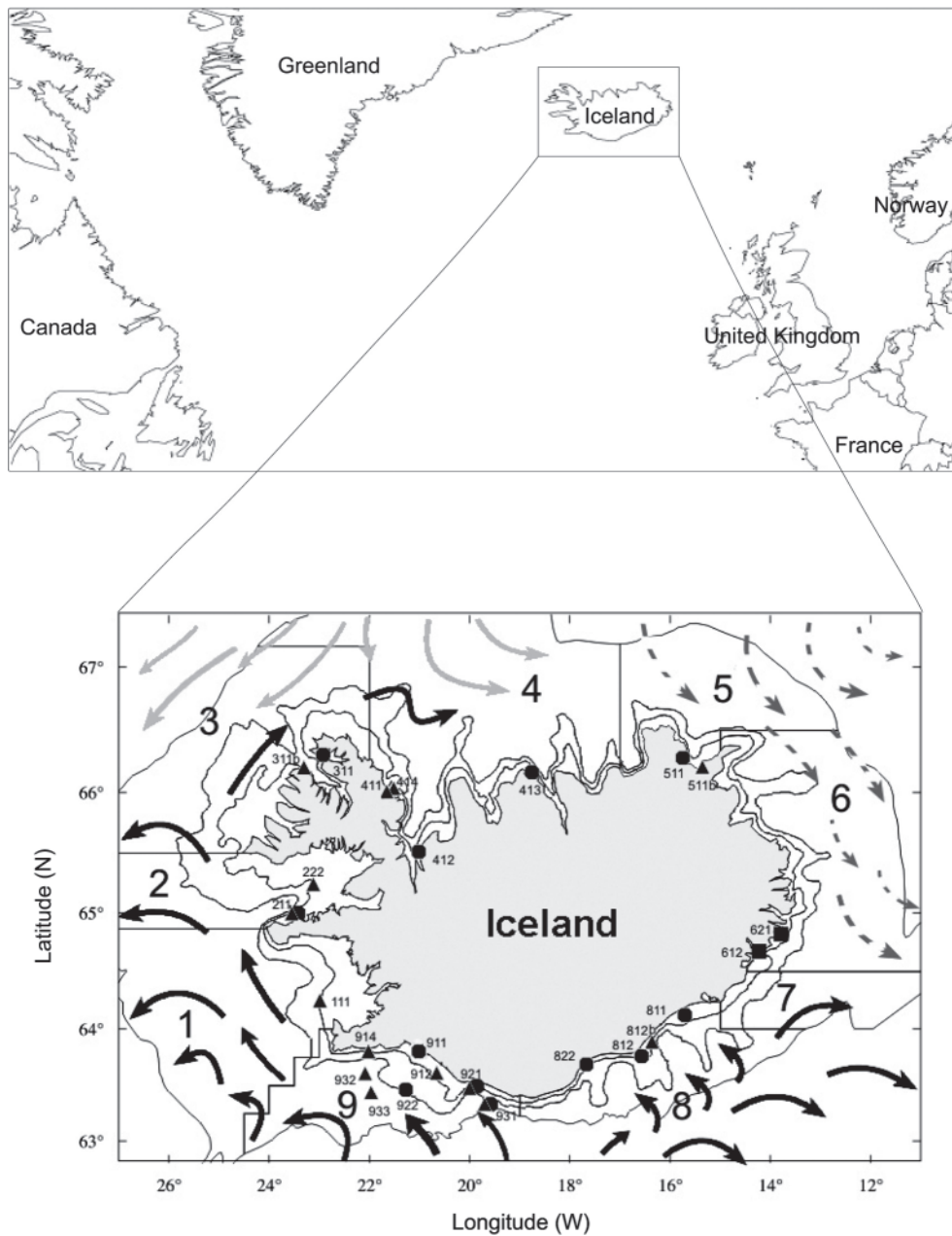
Atlantic cod off Iceland (also referred to as Icelandic cod), though historically exposed to high exploitation rates, has remained relatively stable and is one of only two or three stocks not rated outside safe biological limits by ICES (International Council for the Exploration of the Sea) (Christensen et al. 2003; Marteinsdóttir et al. 2005). Although assessed as a unique stock for management expediency, Icelandic cod have recently been suggested to comprise a multitude of spawning units distributed on offshore and inshore locations around the country, with a major

spawning component in an area off the southwestern coast (Fig. 1, area 9) (Marteinsdóttir et al. 2000a, 2000b; Begg and Marteinsdóttir 2002a). The spawning season lasts from mid-March to May, with older individuals spawning earlier and over a longer period than the younger and smaller cod (Marteinsdóttir and Björnsson 1999). Pelagic eggs and larvae drift from the main spawning ground off the southwestern coast with the coastal and offshore currents to the main nursery ground off the northern coast (Begg and Marteinsdóttir 2000, 2002b; Marteinsdóttir et al. 2000a). After the breeding season, individual cod tend to migrate to the main feeding regions located off the northwestern and northeastern coasts of Iceland (Jónsson 1996), but results from a recent tagging study on the main spawning ground suggest that some individuals stay in relatively shallow, warm waters in regions off the southern coast (Pálsson and Thorsteinsson 2003).

Tag recapture data from studies in the Northwest Atlantic (Taggart et al. 1995; Robichaud and Rose 2004) together with direct observations provide evidence that cod can, and probably most do, exhibit spawning site fidelity potentially promoting genetic divergence among different spawning components. Icelandic cod have also been shown to display high fidelity to local spawning regions (Thorsteinsson and Marteinsdóttir 1992, 1993). Furthermore, as juvenile cod appear to be stationary during the first 2–3 years of their life (Sæmundsson 2005), the majority of dispersal is likely to happen before the juvenile years. Thus oceanic currents promoting dispersal of eggs and larvae are probably the main factors influencing dispersal of young cod around Iceland.

Considerable information exists on the oceanography around Iceland. The region is influenced by the southward flow of cold, low-saline Arctic waters from the north (East Greenland and East Iceland currents; <0 to 2 °C) and the northward flow of warm, saline waters of the North Atlantic Current from the south (6 to 8 °C; Valdimarsson and Malmberg 1999). A branch of the North Atlantic Current, the Irminger Current, flows along the southern and western coasts of Iceland. To the west and northwest of the country, in the Denmark Strait, the Irminger Current meets the East Greenland Current; it then bifurcates, with the main branch turning west towards Greenland and a small branch continuing northwards and flowing into the North Icelandic shelf area as the North Icelandic Irminger Current. Closer to shore is the coastal current, which originates along the southeastern coast of the island and flows in a clockwise direction around it. East of the country, oceanographic conditions are shaped by the southward-flowing Arctic waters of the East Iceland Current and the frontal zone in the area southeast of the country, on the Iceland–Faroe ridge, where the East Iceland Current meets the warm Atlantic waters flowing into the areas from the south and west (Valdimarsson and Malmberg 1999). Therefore, the oceanographic conditions around Iceland are shaped by the two frontal zones located west–northwest and east–southeast of the country where the cold and warm water masses meet. The precise location of these frontal zones naturally varies between years, as well as over longer periods, resulting in large variation in hydrographical conditions (Malmberg et al. 1996).

Fig. 1. Geographical regions, main oceanic currents (modified from Gunnarsson et al. 1998), and sampling locations of Atlantic cod (*Gadus morhua*) around Iceland. Samples were collected in 2002 (●), 2003 (▲), and 2004 (■). Shaded arrows, East Greenland Current; broken shaded arrows, East Icelandic Current; black arrows, warm North Atlantic Current. The geographical areas are delimited by lines; the names of the areas are indicated by numbers. The map at the top illustrates the region from a broader geographical perspective.



The strength and direction of the Irminger Current, as well as the coastal current, have been shown to influence the transport of cod offspring from the southern spawning grounds onto the northern nursery grounds (Begg and Marteinsdóttir 2002a). Little information exists on the potential influence of the oceanographic conditions on dispersal and migration of juvenile and adult cod. Tagging results by Jónsson (1996) have been used to support the general notion that postspawning cod display feeding migrations into the northwestern and northern regions. Re-examination of Jónsson's (1996) results indicate, however, that the majority of tag returns come from the large feeding areas in waters

west of the country (located in the vicinity of the frontal zone), whereas few returns come from the more northern regions (Jónsdóttir et al. 2006). Similarly, few cod tagged in the northern region were recaptured in the vicinity of the southern spawning areas (Jónsson 1996; Jónsdóttir et al. 2006).

In this paper, we describe for the first time the genetic structure of Atlantic cod around Iceland. We examine the genetic composition (nine microsatellite DNA loci and the *Pan I* locus) among more than 2500 adult cod collected in spawning condition over a period of two years (2002 and 2003) from 22 spawning locations around the country. Our first ob-

jective was to test the null hypothesis that spawning cod around Iceland exhibit no stable genetic differences. This hypothesis was rejected as we describe genetic differences among two major spawning aggregations, one southwest and the other northeast of Iceland. We then examined the influence of geographic, oceanographic, and depth features as potential barriers to gene flow. Lastly, we interpreted our results in light of contemporary tagging experiments conducted on spawning cod.

Materials and methods

Sampling

A total of 2534 individual cod were collected from 22 spawning locations around Iceland during the spring of 2002 and 2003 (Table 1, locations 612 and 621 sampled in 2004). Six of these locations (211, 311, 511, 812, 921, 931; see Fig. 1) were sampled in 2002 and again in 2003 to assess temporal stability; thus in total there were 28 samples. Temporal replicates 311b and 511b (Fig. 1) were collected from different locations in the same fjords within a few kilometres of the corresponding 311 and 511 samples (Fig. 1). Total length (TL, mm) and eviscerated carcass wet weight (W , mg) of each individual were recorded, and otoliths (sagittae) were collected for age determination. Temperature data were collected based on a data storage tags (DST) attached to the gillnets that we used to sample spawning cod at each location in 2003 (average temperature during 24 h). Temperature data at the eastern location were based on measurements performed during the sampling.

The nine spawning regions around Iceland were classified (Fig. 1), and our samples were identified with a three-digit number, the first digit indicating spawning region (1–9), the second indicating depth (1, 0–75 m; 2, 75–125 m; 3, ≥ 125 m), and the third indicating the sampling station (see locations in Fig. 1).

DNA methods

Gill filaments or fin clips were preserved in 1 mL of 96% ethanol, and DNA was extracted using a Chelex (Bio-Rad 10%; Bio-Rad Laboratories, Hercules, California) extraction protocol (Walsh et al. 1991). Samples were genotyped at nine polymorphic microsatellite loci: Gmo2 (Brooker et al. 1994), Gmo8, Gmo19, Gmo34, and Gmo37 (Miller et al. 2000), and Tch5, Tch11, Tch14, and Tch22 (O'Reilly et al. 2000). Polymerase chain reactions (PCR) were performed in a 10 μ L volume containing 2 μ L of DNA product, 1 μ L of 10 \times buffer (10 mmol·L⁻¹ Tris-HCl, 50 mmol·L⁻¹ KCl, 1.5 mmol·L⁻¹ MgCl₂ and 0.1% Triton X-100), 1 μ L of 2.5 mmol·L⁻¹ DNTP, 0.2–0.4 units of DyNAzyme™ DNA polymerase (Finnzymes Oy, Espoo, Finland), and various concentrations of primers. PCR cycles were performed on a multiplex basis in a GeneAmp®2700 thermal block and preceded by an initial denaturation step of 4 min at 94 °C followed by 32 cycles of 40 s at 94 °C, 40 s at annealing temperature (Tch11, Gmo19, Gmo34 at 52 °C; Gmo2 at 50 °C; Tch14 at 56 °C; Gmo8 at 50 °C; Gmo37 at 50 °C; Tch5 at 50 °C; Tch22 at 49 °C), and 40 s at 72 °C. A final elongation step of 4 min at 72 °C was included. PCR products were diluted with sterile distilled water (1:3) and were electrophoresed on 36 cm 6% polyacrylamide gels and de-

tected on an automatic sequencer (ABI 377; Applied Biosystems, Foster City, California) using the software GeneScan (version 3.1.2; Applied Biosystems 2000). Products were scored using the software GeneMapper (version 3.7; Applied Biosystems 2004).

Samples were also genotyped at the pantophysin locus (*Pan I*). PCR were performed in a 15 μ L volume containing 2 μ L of DNA product, 1.7 μ L of 10 \times buffer (10 mmol·L⁻¹ Tris-HCl, 50 mmol·L⁻¹ KCl, 1.5 mmol·L⁻¹ MgCl₂ and 0.1% Triton X-100), 0.5 μ L of 50 mmol·L⁻¹ MgCl₂, 1.7 μ L of 2.5 mmol·L⁻¹ DNTP, 1 unit of DyNAzyme™ DNA polymerase (Finnzymes), and 10 mmol·L⁻¹ of primers (F: TTGGTCTCTATCTGGGCTTC; R: CGTAGCAGAAGAGTGACACAT). PCR cycles were performed in a GeneAmp®2700 thermal block and preceded by an initial denaturation step of 5 min at 95 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C. A final elongation step of 7 min at 72 °C was performed. PCR products were then cut with *DraI* restriction enzyme (Fermentas, Hanover, Maryland) using 15 μ L of PCR products and 5 μ L of *DraI* mix containing 18 units of enzyme and 2 μ L of B+ buffer per samples. Digested PCR products were visualized in 2% agarose gel stained with ethidium bromide using GELDOC™ 2000 reader (Bio-Rad Laboratories).

Tagging experiments

In April 2002 and 2003, 6536 cod (3624 and 2912 in 2002 and 2003, respectively) were tagged on spawning grounds during peak spawning time at 10 locations around Iceland in 2002 and 12 in 2003 distributed among all nine regions depicted in Fig. 1. Of all individuals tagged in 2002, 159 were recaptured in the spawning seasons (March to May) of 2003 and 2004, and of all individuals tagged in 2003, 81 were recaptured in the 2004 spawning season. For both tagging years, individuals were recaptured from all nine tagging locations in subsequent spawning seasons. These recaptures included only those with sufficient information on position of recapture. Recaptures were assessed by tagging location, with the number of returns by region tabulated. As catch rates differed among locations, the number of recaptures was scaled by the catch in the recapture region using data from fishermen's logbooks and landings to estimate total catch per region, year, and corresponding time period.

Statistical methods

Genetic data

Allele frequencies and observed (H_O) and unbiased expected (H_E) heterozygosity were calculated in GENETIX (version 4.03; Belkhir et al. 1999). Tests of Hardy-Weinberg equilibrium (HWE) were performed using GENEPOP (version 3.1; Raymond and Rousset 1995) using exact tests.

Geographical genetics

Wright's single-locus F statistics (Wright 1969) were calculated from allele frequencies at all loci examined for each population according to Weir and Cockerham's (1984) method using GENETIX. Significance of F_{ST} estimates was assessed with exact tests implemented in GENEPOP (multi-locus and pairwise estimates). When appropriate, significance levels were adjusted with a sequential Bonferroni test (Rice 1989). To accurately compare the level of genetic di-

Table 1. Sample codes, year of sampling, depth (m), mean total length (TL, cm), mean age (years), percentage of spawning individuals (Spawning), sex ratio (SR, ratio of the number of females to males), number of individuals scored (*n*), observed heterozygosity (*H_O*), expected heterozygosity (*H_E*), and mean number of alleles (\bar{n}_A) from 28 samples of cod from the Icelandic waters.

Name	Year	Depth (m)	TL (cm)	Age (years)	Spawning (%)	SR	Microsatellite loci				Pan I locus			
							<i>n</i>	<i>H_O</i>	<i>H_E</i>	\bar{n}_A	<i>n</i>	<i>H_O</i>	<i>H_E</i>	\bar{n}_A
111	2003	78	86.69	6.64	100.00	0.88	94	0.8026	0.8147	19.44	89	0.5169	0.4984	
211	2002	64	90.79	7.77	100.00	0.92	94	0.8189	0.8149	18.56	87	0.4368	0.4888	
211b	2003	48	78.25	6.25	100.00	1.35	94	0.7931	0.8104	18.44	89	0.4382	0.4270	
222	2003	73	79.31	6.38	98.94	0.88	94	0.8381	0.8278	19.33	89	0.5618	0.4820	
311	2002	19	70.18	6.84	100.00	0.96	94	0.8227	0.8402	19.56	90	0.4000	0.3310	
311b	2003	41	70.76	6.81	95.74	1.35	94	0.8582	0.8455	18.44	91	0.4286	0.4303	
411	2003	28	79.42	6.70	98.94	0.74	94	0.8499	0.8403	18.44	87	0.3218	0.3890	
412	2002	60	64.64	7.11	92.55	0.54	94	0.8150	0.8358	19.11	93	0.1720	0.1572	
413	2002	18	66.43	6.08	82.98	0.54	100	0.8133	0.8356	18.22	97	0.2268	0.2470	
414	2003	30	83.47	8.56	100.00	0.88	94	0.8286	0.8251	17.33	94	0.2553	0.2825	
511	2002	12	71.05	6.40	90.43	0.92	94	0.8168	0.8323	18.89	90	0.3222	0.3636	
511b	2003	16	79.55	8.06	77.00	1.32	94	0.8197	0.8304	18.22	87	0.5172	0.4046	
612	2004	50	65.19	—	100.00	1.16	84	0.8343	0.8343	18.00	84	0.0182	0.0180	
621	2004	57	70.53	—	100.00	2.34	77	0.8307	0.8526	18.44	77	0.3000	0.2550	
811	2002	50	93.89	7.87	98.94	0.47	94	0.8251	0.8351	19.11	85	0.5059	0.4200	
812	2002	65	101.77	8.52	98.94	1.13	94	0.7754	0.8115	18.78	82	0.4634	0.4997	
812b	2003	83	98.27	8.69	96.80	1.09	94	0.7835	0.8455	18.44	92	0.5652	0.4355	
822	2002	80	101.14	8.82	97.87	1.09	94	0.7778	0.8135	18.78	88	0.5682	0.4907	
911	2002	54	85.88	6.18	98.94	0.77	78	0.8561	0.8450	18.55	76	0.1486	0.1822	
912	2003	61	97.31	7.95	81.00	0.57	58	0.8123	0.8044	16.33	59	0.4483	0.4976	
914	2003	72	77.23	6.61	97.70	1.02	90	0.8136	0.8196	18.22	88	0.5682	0.3129	
921	2002	92	100.46	8.39	100.00	1.02	94	0.8100	0.8076	19.11	77	0.4545	0.4946	
921b	2003	88	93.73	8.11	100.00	1.14	94	0.7939	0.8080	17.89	93	0.5054	0.4917	
922	2002	92	95.48	7.37	100.00	0.42	94	0.7754	0.8132	18.33	78	0.4744	0.4861	
931	2002	200	84.45	7.12	84.00	1.04	94	0.7719	0.7934	19.33	85	0.3412	0.3129	
931b	2003	454	78.73	6.73	93.62	0.88	94	0.7352	0.7791	18.56	91	0.2308	0.2041	
932	2003	131	77.53	6.32	97.78	0.14	94	0.7766	0.7918	17.44	84	0.3452	0.2856	
933	2003	152	77.87	6.24	86.17	0.38	73	0.7884	0.8038	17.68	73	0.3699	0.3015	

Note: Bold values: *F_{IS}* values deviating from Hardy–Weinberg expectation (HWE) after corrections for multiple test (*P* = 0.000 198).

vergence detected with highly polymorphic microsatellite loci and the bi-allelic *Pan* I locus, a new standardized genetic differentiation measure was used. This new measure is expected to allow comparisons between loci with different level of genetic variation (G'_{ST} ; Hedrick 2005). G'_{ST} represents the proportion of the maximum differentiation possible for the level of subpopulation homozygosity observed and is defined as

$$G'_{ST} = \frac{G_{ST}(1 + H_S)}{(1 - H_S)}$$

Pairwise comparisons of temporal samples were carried out using the software Chifish (Ryman 2006), which tests the hypothesis of no difference at any locus by both chi-square and Fisher's method of combining P values obtained by Fisher's exact test (Ryman 2006). This approach has been shown to give better estimation of genetic differentiation than the Fisher's exact test, especially when comparing two populations scored for few loci (Ryman 2006).

We carried out a multidimensional scaling (MDS) approach on the pairwise F_{ST} values for both microsatellite loci and the *Pan* I locus using Statistica 6.0 (StatSoft Inc. 2001). Analysis of genetic linkage disequilibrium between locus pairs was estimated according to Cockerham and Weir (1979) and tested on contingency tables under the null hypothesis of independence.

To estimate the number of genetically distinguishable populations, we used the spatial (geographical) analysis of molecular variance (SAMOVA; Dupanloup et al. 2002) on the microsatellite loci. The SAMOVA method employs a simulated procedure and uses allele frequency data along with geographical coordinates of the sampled populations for identifying groups of populations that exhibit close genetic relationships. The grouping of populations is based on conducting a hierarchical analysis of ρ_{ST} (genetic variation within samples) and maximising the proportion of total genetic variance among groups (ρ_{CT}). We conducted the analysis for two to 15 populations.

We additionally assessed potential barriers to current gene flow events with the software BARRIER (version 2.2; Manni et al. 2004) using the Monmonier's maximum distance algorithm. BARRIER has recently been used to highlight geographical areas with current pronounced genetic discontinuity (Bekkevold et al. 2005; Jørgensen et al. 2005). Basically, geographical coordinates are supplied for each sampling location and connected by Delaunay triangulation associated with genetic distances such as F_{ST} . The algorithm will assess the edge with the largest associated distance in the triangulation network as its starting edge and then extends the barriers across the directly 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 (see Manni et al. (2004) for additional information). We conducted the analysis using the F_{ST} values for each of the nine microsatellite loci separately and the overall values based on all microsatellites to ensure that barriers would not be identified by strong differentiation to one or few loci. The number of barriers is set up by the user, and based on the number of loci supporting each bar-

rier, we found that only two barriers were strongly supported by the data (see Results).

Because geographical coordinates between samples might not reflect connection on such a small geographical scale, an analysis of molecular variance (AMOVA) was carried out in ARLEQUIN (version 2.0; Schneider et al. 2000) to assess the hierarchical partitioning of genetic variability among post-hoc defined regions using the observed structure in the MDS analysis for both types of genetic markers (divergence among northeastern and southwestern regions).

Depth pattern

An AMOVA was also used to assess the hierarchical partitioning of genetic variability among post-hoc defined regions using the observed structure in the MDS analysis (divergence among shallow, intermediate, and deep water) for both types of genetic markers.

In addition, we examined whether the effect of depth on genetic diversity as measured by heterozygosity and number and frequency of alleles differed between the genetic markers.

To ensure that the observed genetic divergence between northeastern and southwestern samples was not due to the presence of deep-water samples only available for area 9, we calculated the overall genetic divergence with and without the deep and intermediate depth samples (only shallow water samples). A hierarchical partitioning of genetic variability among post-hoc defined regions (divergence among northeastern and southwestern regions) using only the shallow water samples was also carried out.

Results

Sampled cod

Most of the cod sampled were identified as spawning, i.e., females containing hydrated oocytes in their ovaries and males having running sperm (Table 1).

Genetic variability

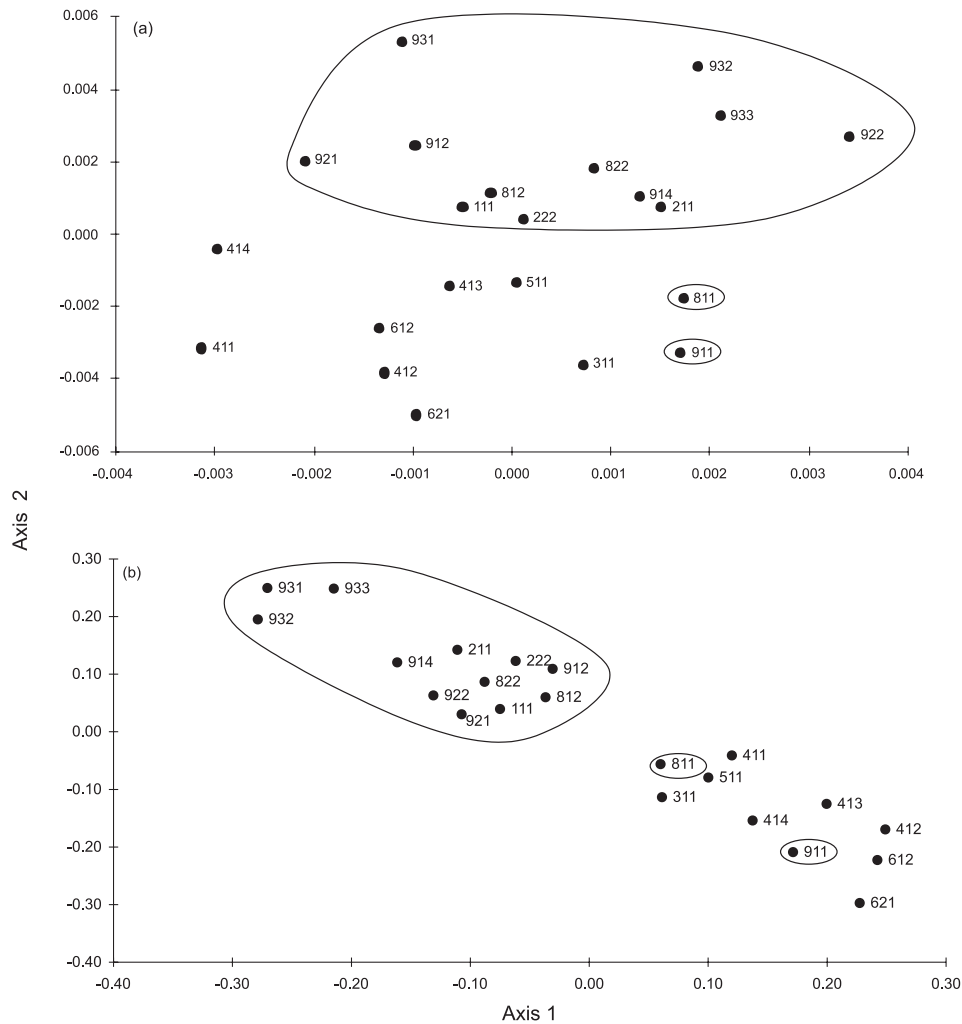
All studied microsatellite loci were highly polymorphic. The number of alleles per locus across all samples ranged from 11 (Gmo34) to 52 (Gmo8). Per-sample H_O averaged over all loci ranged from 0.7352 to 0.8582, whereas the per-sample H_E ranged from 0.7791 to 0.8526 (Table 1). Genotypic proportions were out of HWE in only five out of 232 exact tests after Bonferroni correction for multiple tests and were not due to any specific loci or samples. This is less than expected by chance alone. All samples but two (111, 811) exhibited an overall HWE (Table 1). Exact test for linkage disequilibrium did not yield significant values.

H_O per sample at the *Pan* I locus ranged from 0.0182 to 0.5682, whereas H_E ranged from 0.0180 to 0.4997 (Table 1). There was no evidence for departure from HWE for any of the samples (Table 1).

Geographical genetics

The partitioning of genetic variance based on microsatellite loci among and within the 28 samples as estimated by F statistics showed a mean significant F_{ST} value of 0.003 and an F_{IS} of 0.017. After Bonferroni correction ($P = 0.000132$), pairwise differentiation between populations

Fig. 2. Multidimensional scaling analysis (MDS) on 22 spawning areas of Atlantic cod (*Gadus morhua*) based on F_{ST} values computed for (a) nine microsatellite loci and (b) the *Pan I* locus. Southwestern samples are encircled.



yielded 114 significant comparisons out of 378, among which 36 were due to the samples 931 and 931b (data not shown). Out of those 114 significant pairwise comparisons, 62 were observed between samples from the northeast (regions 3, 4, and 5) and southwest of Iceland (regions 8 and 9). A pairwise comparison of the temporal samples (2002 and 2003) from the same spawning location were genetically indistinguishable (211, $F_{ST} = 0.0002$, $P = 0.3973$; 311, $F_{ST} = 0.0038$, $P = 0.0501$; 511, $F_{ST} = 0.0017$, $P = 0.0899$; 812, $F_{ST} = -0.0004$, $P = 0.3664$; 921 $F_{ST} = 0.0070$, $P = 0.0967$; 931 $F_{ST} = 0.0019$, $P = 0.0614$). Additionally, overall within-year spatial genetic divergence was similar in the two years (2002, $F_{ST} = 0.0038$; 2003, $F_{ST} = 0.0029$). Therefore, for further analysis, temporal samples from the same location were clustered into one single group for each location (22 spawning locations).

Based on pairwise F_{ST} values, MDS clearly clustered samples from the northeastern region separate from those from the southwestern region (Fig. 2a; stress values on axes 1 and 2, 0.1411). Only two samples from the southwestern region clustered with the northeastern region samples, namely 811 and 911. SAMOVA revealed that genetic differentiation among groups was highest ($\rho_{CT} = 0.00354$) when

samples were pooled into two groups, one comprising the samples from the northeast, and the second, those from the southwest of Iceland (Table 2). Samples 811 and 911, collected from the southwestern region, clustered in the northeastern region, thus confirming the results observed with the MDS analysis. A subsequent AMOVA based on these two sample pools confirmed that among-group differentiation was significant between samples collected from northeastern and southwestern regions (Table 3).

To complement this analysis and detect whether a potential genetic discontinuity (a boundary reflecting relatively low gene flow) could be detected between northeastern and southwestern samples, we carried out an analysis using the software BARRIER. Two relevant genetic discontinuities (barriers to gene flow) were identified and these were located between the northeastern and southwestern samples. The northwestern barrier was supported by four out of nine loci used, whereas the southeastern barrier was supported by five out of nine loci used (Fig. 3). Further genetic discontinuities were detected between samples from different depths collected from area 9 (data not shown). These barriers were supported by four (areas 91 and 92) to six loci (areas 92 and 93). Further barriers identified were supported by only one to three loci and were thus not considered.

Table 2. Results of the spatial analysis of molecular variance (SAMOVA) analyses carried out on microsatellite loci (Dupanloup et al. 2002).

No. of groups	Group composition	ρ_{CT}	ρ_{SC}
2	(111, 211, 222, 812, 822, 912, 914, 921, 922, 931, 932, 933) (311, 411, 412, 413, 414, 511, 612, 621, 811, 911)	0.00354***	0.00132***
3	(931) (912, 932, 933) (other populations)	0.00304***	0.00187***
4	(411) (931) (922, 932, 933) (other populations)	0.00338***	0.00174***
5	(411) (414) (911) (311, 412, 413, 511, 612, 621, 811) (111, 211, 222, 812, 822, 912, 914, 921, 922, 931, 932, 933)	0.00319***	0.00114***
6	(411) (414) (911) (931) (311, 412, 413, 511, 612, 621, 811) (111, 211, 222, 812, 822, 912, 914, 921, 922, 932, 933)	0.00322***	0.00087***
7	(411, 414) (911) (922) (931) (932, 933) (311, 412, 511, 612, 621) (other populations)	0.00300***	0.00083***
10	(311, 411, 511, 612) (412) (413) (414) (621, 811) (911) (912) (111, 211, 222, 413, 812, 822, 914, 921) (922) (931) (932, 933)	0.00300***	0.00063***
15	(111, 211, 222) (311) (411) (413) (414) (511, 612) (811) (931) (921) (922) (911) (411, 914) (812, 822) (932, 933) (912, 914) (412, 621)	0.00328***	0.00013***

Note: The analysis was carried out considering 22 spawning locations. Significance was assessed by 1000 permutation: ***, $P < 0.001$. Groups are presented between parentheses. ρ_{CT} and ρ_{SC} are the genetic variation among groups and among samples within groups, respectively.

Table 3. Hierarchical analysis of molecular variance (AMOVA) among samples of *Gadus morhua* clustered into two groups (depth (shallow, intermediate, and deep waters) and region (northern vs southern components)) for the microsatellite and *Pan I* locus.

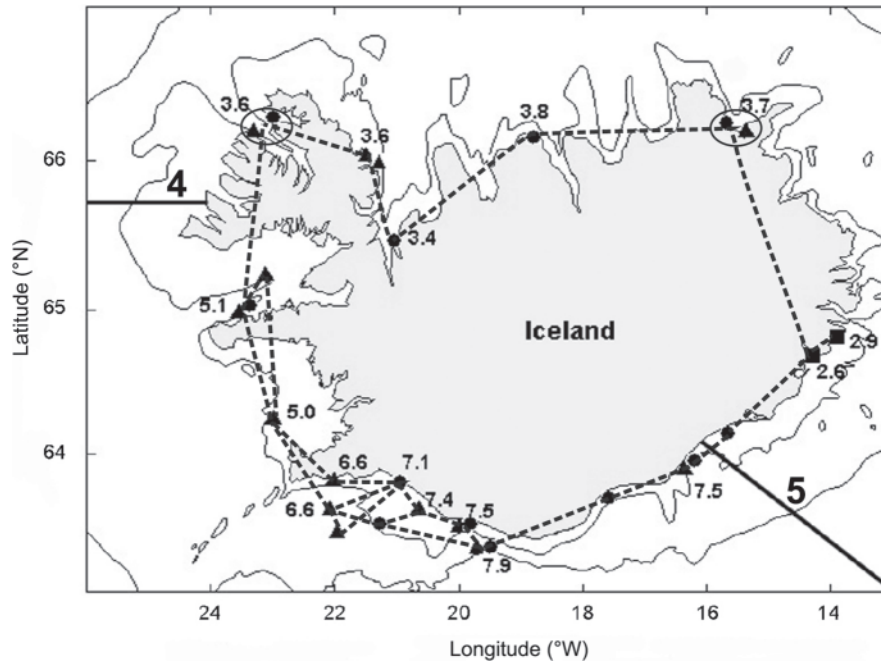
Loci	Source of variation	df	Variance components	Variation (%)	Fixation indices	p values
Shallow, intermediate, and deep waters						
<i>Pan I</i>	Among groups	2	0.05	17.36	CT = 0.1736	<0.0001
	Among samples within groups	19	0.04	14.66	SC = 0.1774	<0.0001
	Within samples	4766	0.18	67.98	ST = 0.3202	<0.0019
	Total	4787	0.27	100		
Microsatellites	Among groups	2	0.0064	0.17	CT = 0.0017	<0.01
	Among samples within groups	19	0.0086	0.23	SC = 0.0023	<0.0001
	Within samples	5044	3.7158	99.60	ST = 0.0040	<0.0001
	Total	5065	3.7308	100		
Northern vs southern components						
<i>Pan I</i>	Among groups	1	0.08	28.29	CT = 0.2829	<0.0001
	Among samples within groups	20	0.02	8.43	SC = 0.1176	<0.0001
	Within samples	4766	0.18	63.28	ST = 0.3672	<0.0001
	Total	4787	0.28	100		
Microsatellites	Among groups	1	0.010	0.27	CT = 0.0027	<0.0001
	Among samples within groups	20	0.0071	0.19	SC = 0.0019	<0.0001
	Within samples	5044	3.7158	99.54	ST = 0.0046	<0.0001
	Total	5065	3.7331	100		

A comparison of the level of genetic diversity among northeastern and southwestern components using the software FSTAT (Goudet 1995) revealed significant differences at the level of expected and observed heterozygosity (northeastern component, $H_O = 0.825$, $H_E = 0.841$; southwestern component, $H_O = 0.793$, $H_E = 0.813$; $P = 0.009$ and 0.0004 , respectively) but not at the level of allelic richness (northeastern component, 16.865; southwestern component, 16.874; $P = 0.965$).

The partitioning of the *Pan I* locus genetic variance among and within the 28 samples as estimated by F statistics showed a mean significant F_{ST} value of 0.261 and an F_{IS} of -0.042 . The standardized genetic differentiation measured as G'_{ST} ($G'_{ST} = 0.568$) was tenfold higher than the value observed for microsatellite loci ($G'_{ST} = 0.032$). After Bonferroni correction, pairwise differentiation between populations yielded

261 significant comparisons out of 378, among which 123 were observed between samples from the northeast (areas 3, 4, and 5) and southwest (regions 8 and 9) and 70 of these involved samples from area 9 (sample 931b, 26 comparisons; 931, 23; 911, 21, data not shown). Using χ^2 tests, allele frequencies were significantly different among population samples ($\chi^2_{[23]} = 690.80$, $P = 0.0001$). A pairwise comparison of the temporal samples, conducted with the software Chifish, showed that temporal samples (2002 and 2003) from the same spawning location were genetically indistinguishable (211, $F_{ST} = 0.0104$, $P = 0.0954$; 311, $F_{ST} = 0.0187$, $P = 0.0537$; 511, $F_{ST} = -0.0005$, $P = 0.3596$; 812, $F_{ST} = 0.0033$, $P = 0.2376$; 921, $F_{ST} = -0.0050$, $P = 0.6622$; 931, $F_{ST} = 0.0207$, $P = 0.0524$). Additionally, overall levels of genetic divergence were similar for the years 2002 ($F_{ST} = 0.2289$) and 2003 ($F_{ST} = 0.1970$). Therefore, for further analysis,

Fig. 3. Areas of genetic discontinuity identified by BARRIER using the Monmonier algorithm (Manni et al. 2004): broken lines, Delaunay triangulation; solid lines, barriers to gene flow. Larger numbers at the barriers represent the number of loci supporting the barrier. Samples were collected in 2002 (●), 2003 (▲), and 2004 (■). Circled locations indicate the two pairs of temporal samples that were pooled (they were genetically indistinguishable) despite not being from exactly the same location. Average temperature data of the data storage tags (DST) are given. Temperature of the eastern coast (samples 612 and 621) was recorded during the sampling process.



temporal samples from the same location were clustered into one single group for each location (total of 22 spawning locations).

Based on pairwise F_{ST} values, MDS clearly clustered samples of the northeastern region and southwestern region (Fig. 2b; stress values on axes 1 and 2, 0.0644). The AMOVA analysis confirmed those results and revealed that among-group differentiation was significant between samples collected in northeastern and southwestern regions (Table 3).

A comparison of the level of genetic diversity among northeastern and southwestern components, using the software FSTAT (Goudet 1995), revealed significant differences at the level of expected and observed heterozygosity (northeastern component, $H_O = 0.283$, $H_E = 0.282$; southwestern component, $H_O = 0.445$, $H_E = 0.421$; $P = 0.007$ and 0.014 , respectively).

After Bonferroni correction, pairwise genotypic differentiation between populations yielded 298 significant genotype comparisons out of 378 (data not shown). Using χ^2 tests, genotype frequencies were also significantly different among populations ($\chi^2_{[2]} = 1165.92$, $P = 0.0001$; Fig. 4). BB genotypes were present in relatively high proportion in the southwestern but not in the northeastern samples (Fig. 4).

Depth pattern

An AMOVA based on a clustering related to the depth of the collected samples (shallow, intermediate, and deep waters) confirmed that part of the among-group microsatellite differentiation could be explained by depth (Table 3). Depth of sample collection was negatively correlated with microsatellite heterozygosity (see Fig. 5) but not with allelic rich-

ness ($r^2 = 0.0293$, $P = 0.4459$, $n = 22$, data not shown). When significant relationships (H_O , H_E) were tested within the two main components separately, they were significant in the southwestern region (H_O , $r^2 = 0.5232$, $P = 0.0035$, $n = 14$; H_E , $r^2 = 0.6006$, $P = 0.0011$, $n = 14$) but not in the northeastern region (H_O , $r^2 = 0.1340$, $P = 0.3721$, $n = 8$; H_E , $r^2 = 0.1146$, $P = 0.4120$, $n = 8$).

Because of this depth pattern and to ensure that the observed genetic divergence at microsatellite loci between northeastern and southwestern samples was not due to the presence of deep-water samples in area 9 and not elsewhere, we calculated the overall genetic divergence without the deep-sea and intermediate water samples (only shallow water samples). The F -statistics estimates also showed a mean significant F_{ST} value of 0.002 and an F_{IS} of 0.012, similar to the values obtained when all samples, including those obtained in intermediate and deep waters, were included in the analysis. An AMOVA based on shallow water samples only clearly confirmed that among-group differentiation was significant between samples collected in northeastern and southwestern regions (fixation index: CT = 0.0008, $P = 0.0323$; SC = 0.004, $P = 0.0001$).

An AMOVA based on a clustering related to the depth of the collected samples (shallow, intermediate, and deep waters) confirmed that among-group *Pan I* locus differentiation could be explained by depth (Table 3). Additionally, the frequency of allele B in the *Pan I* locus was found to be positively correlated with depth (Fig. 6), and this correlation persisted in both regions when analysed separately (southwest, $r^2 = 0.6982$, $P = 0.0002$, $n = 14$; northeast, $r^2 = 0.6543$, $P = 0.0150$, $n = 8$). Depth of sample collection was not correlated with *Pan I* locus heterozygosity (data not

Fig. 4. Genotypes distribution of the *Pan* I locus in the different spawning locations: BB genotypes, solid bars; AB genotypes, shaded bars; AA genotypes, open bars.

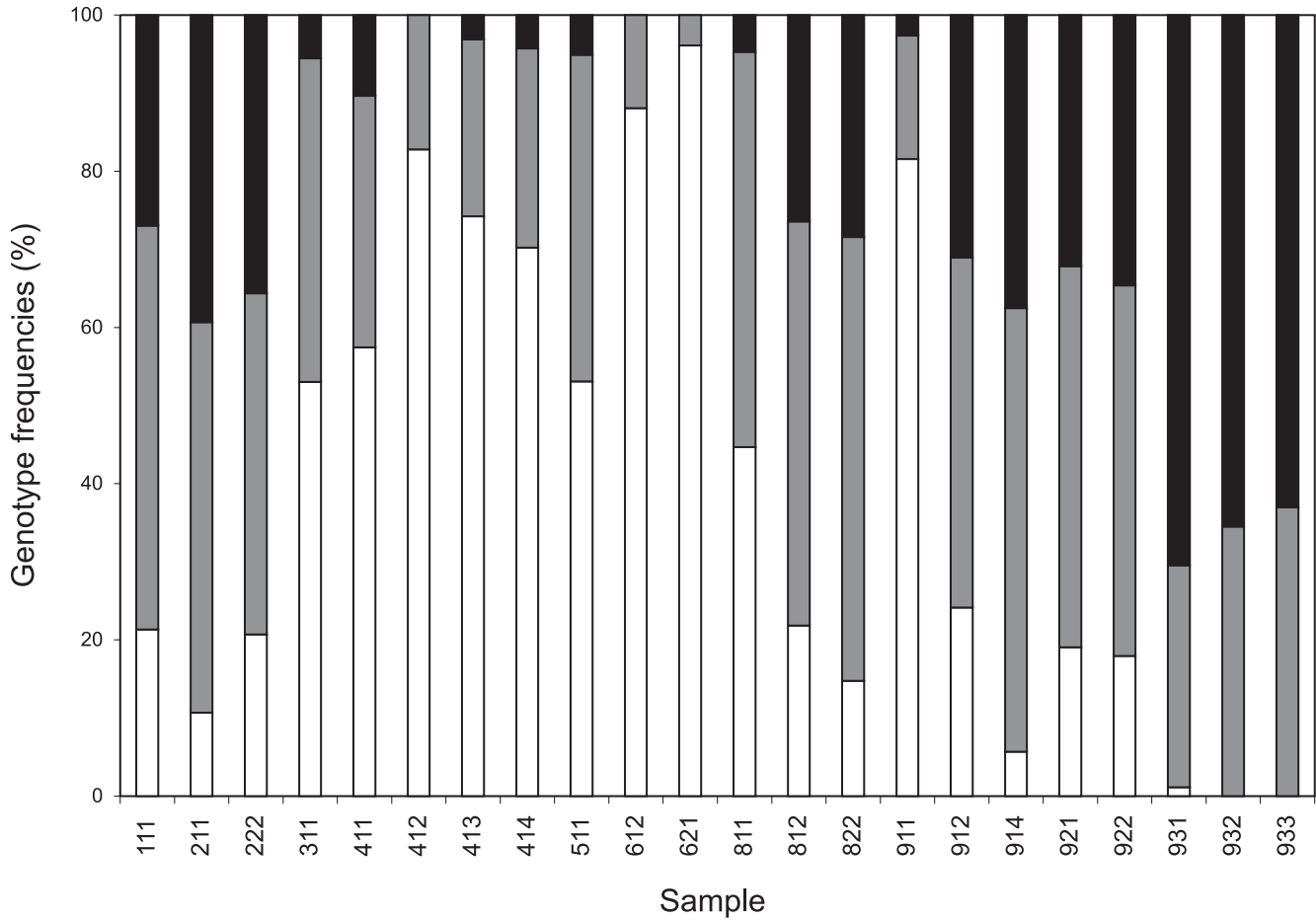


Fig. 5. Microsatellite overall observed heterozygosity (H_O) according to depth (m) ($r^2 = 0.3765$, $P = 0.0030$, $n = 22$). The relationship was also significant for the H_E ($r^2 = 0.3196$, $P = 0.0060$, $n = 22$; data not shown).

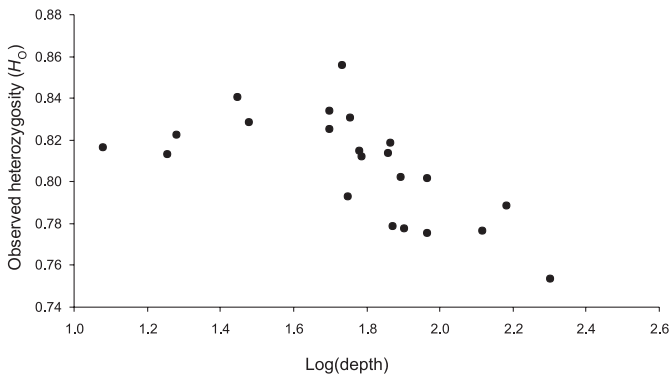
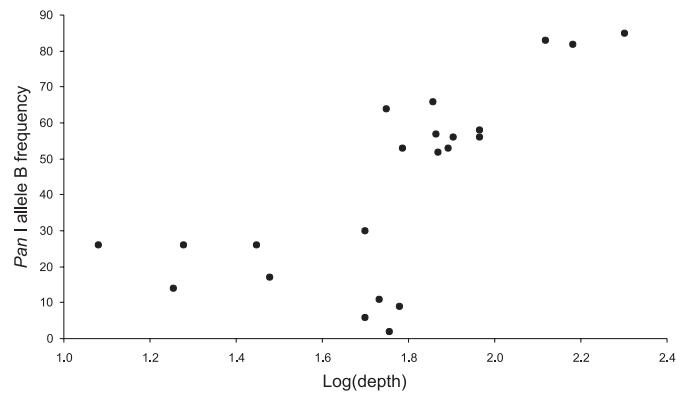


Fig. 6. Frequencies of the B allele of the *Pan* I locus according to depth (m) ($r^2 = 0.5195$, $P = 0.0002$, $n = 22$).



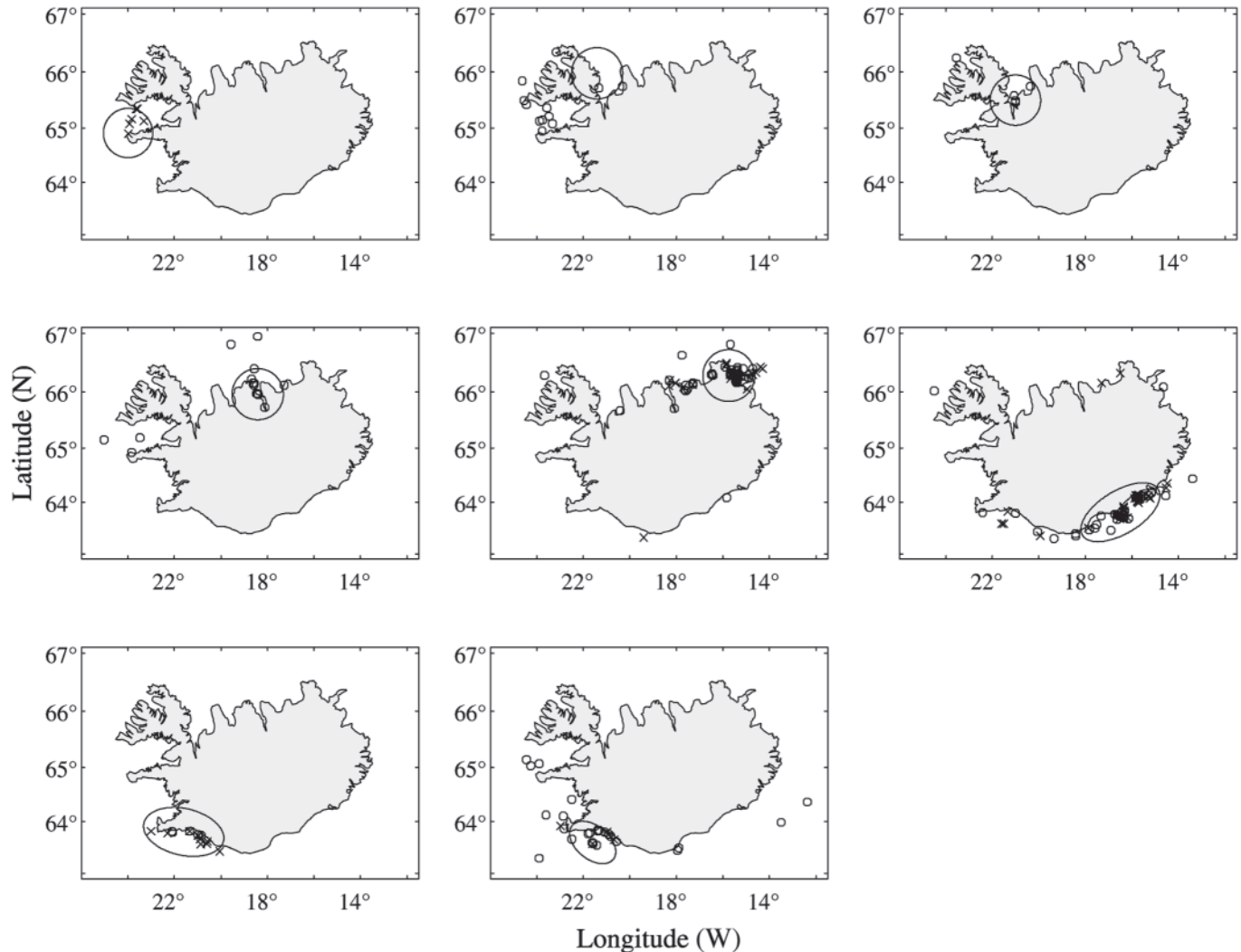
shown) (H_O , $r^2 = 0.0400$, $P = 0.2720$, $n = 22$; H_E , $r^2 = 0.0123$, $P = 0.6332$, $n = 22$).

At the *Pan* I locus, the genetic differentiation between the northeastern and southwestern regions persisted even when only shallow water cod samples were included in the analysis ($F_{ST} = 0.213$, $F_{IS} = -0.016$; AMOVA fixation index: $CT = 0.1742$, $P = 0.005$; $SC = 0.0940$, $P = 0.001$).

Tagging

Cod tagged on the different spawning sites demonstrated high fidelity to their tagging sites (Fig. 7). Most of the tagged cod captured the year after tagging were captured during the spawning season within the region in which they were tagged, indicating that they had returned to spawn in the same region in which they had first been captured and tagged. Out of the recaptures for each region of tagging, the

Fig. 7. Icelandic tagging experiment carried out during the spawning peak of 2002 and 2003. The large circle or ellipse represents the area in which spawning Atlantic cod (*Gadus morhua*) were tagged. ○, the position of recapture of each individual (tagged in 2002) during the spawning season of 2003; ×, the position of recapture of each individual (tagged in 2003) during the spawning season of 2004.



percentage recaptured in the same region was 100% for region 2, 86% for region 4, 72% for region 5, 84% for region 8, and 64% for region 9. The percentage returning to the same or an adjacent region was 100% for region 2, 90% for region 4, 100% for region 5, 91% for region 8, and 87% for region 9.

Discussion

All three of the techniques that we employed to examine spatial structure in Atlantic cod (*Gadus morhua*) around Iceland (microsatellite DNA, the *Pan I* locus, and tagging experiments) clearly reject the hypothesis of panmixia for cod in this region. Although the tagging experiments, which were conducted on adults, provided evidence for homing to the regions where fish had been tagged one or two years before recapture, they naturally do not provide evidence for natal homing. The analysis based on a suite of nine presumed neutral microsatellite DNA loci provided evidence for at least two spatially well defined spawning components of Icelandic cod, one in waters off northeastern Iceland involv-

ing regions 3, 4, 5, and 6 and the other in waters off southwestern Iceland involving regions 1, 2, 8, and 9. The spatial structure identified was stable in all the regions for which we had temporal replication. That the evidence for spatial structure identifying two major spawning components was consistent across two types of molecular markers (a suite of neutral markers and a marker known to be under selection) provides strong support for the hypothesis that the structure may be driven by two distinct but nonexclusive processes: (i) one of larval–juvenile retention driven by oceanographic mechanisms involving currents, fronts, and eddies, as well as the topography of the ocean floor; and (ii) one of adaptive changes driven by natural selection and facilitated perhaps by the differential characteristics of the water masses (e.g., temperature) northeast and southwest of Iceland. Below we discuss each of these issues in detail.

Spatial structure as determined by microsatellites and the *Pan I* locus

The genetic diversity of the nine microsatellite loci assessed as H_E , H_O , and mean number of alleles exhibited a

wide range of variation and was generally comparable with what has been observed in marine fishes (DeWoody and Avise 2000). However, with respect to other cod studies, the Icelandic cod samples exhibited a generally higher level of genetic diversity at microsatellite loci, measured either as heterozygosity or allelic richness, than any other European cod populations (Hutchinson et al. 2001; Knutsen et al. 2003; Nielsen et al. 2003) but were, however, comparable with Canadian samples (Ruzzante et al. 1996, 1998; Beacham et al. 2002).

The level of genetic differentiation that we observed with the microsatellite loci is in accordance with earlier findings in cod from coastal regions (Ruzzante et al. 2001, $F_{ST} = 0.0039$ to 0.0053 ; Beacham et al. 2002, $F_{ST} = 0.0080$; Knutsen et al. 2003, $F_{ST} = 0.0023$). The persistence of the overall genetic signal detected in consecutive years ($F_{ST} = 0.0038$ for 2002 and $F_{ST} = 0.0029$ for 2003) suggests that the structure identified is real (Waples 1998). Additionally, temporal stability was observed for all of the repeated samples across years.

Our genetic analyses clearly revealed the presence of two cod spawning components in the Icelandic waters. The MDS analysis clustered the samples in two different groups: the northeastern region (areas 3, 4, 5, and 6 + samples 811 and 911) and the southwestern region (area 1, 2, 8, and 9). The AMOVA conducted on northeastern–southwestern comparisons revealed that the among-group variation (northeast vs southwest) was significant. A recent biological study on fishing mortality and spawning stock biomass for Icelandic cod strongly suggests that the reproductive component is unevenly distributed in waters around the country (Begg and Marteinsdóttir 2003). An important finding of that study was that large fish were caught in the southwestern region where mean cod abundance was lowest and small fish were essentially caught in northeastern region where mean abundance was highest. These results suggest the existence of life history variation among cod from the northern and southern regions (Begg and Marteinsdóttir 2003) and thus support our genetic results. The tagging experiment reported here is also consistent with our genetic results in that we observe strong year-to-year spawning site fidelity, at least to the level of neighbouring areas.

As expected for a genetic marker supposedly under selection, the *Pan I* locus revealed a stronger genetic divergence ($F_{ST} = 0.261$) than the microsatellite loci among Icelandic populations. Previous studies with this marker revealed similar levels of genetic divergence at broadly comparable geographic scales (Fevolden and Pogson 1997; Pogson and Fevolden 2003; Sarvas and Fevolden 2005) but not at small geographic scales (Jónsdóttir et al. 1999, 2001; Karlsson and Mork 2003). In the present study, the largest difference at the *Pan I* locus occurs between the northeastern and southwestern regions ($F_{CT} = 0.283$), with B alleles and *Pan I*^{AB}/*Pan I*^{BB} genotypes mainly encountered in the southwestern regions of Iceland (areas 1, 2, 8, and 9). Fevolden and Pogson (1997) investigated the cod structure with *Pan I* locus along the Norwegian coast at a similar geographical scale and found an overall differentiation of 40%, mainly linked to the differentiation of the arctic–coastal cod populations. The Barents Sea exhibited a high *Pan I*^{BB} genotype frequency, which is in contradiction with our findings

suggesting that northeastern populations mainly comprise *Pan I*^{AA} genotypes. Recently, the *Pan I* locus has been suggested to experience positive Darwinian selection (Pogson and Fevolden 2003; Pogson and Mesa 2004), and depth, salinity, and temperature have been suggested to be the driving forces (Karlsson and Mork 2003; Case et al. 2005). Thus, the observed discrepancy between our results and those of Fevolden and Pogson (1997) might be due to differences in the selective environment. The northeastern region is affected by the East Greenland and East Icelandic currents originating from the Arctic Ocean and transporting cold water (0–2 °C). In contrast, the southwestern and western regions are affected by the highly saline (35 PSU) and relatively warm (6–8 °C) water originating from the Gulf Stream (Griffiths 1995). It is therefore likely that selection may act differently in the northeastern and southwestern regions, thus leading to the observed genetic divergence (stable over two consecutive years) at the *Pan I* locus.

Oceanography

The very low correlation between genetic differentiation and spatial coordinates detected with microsatellites indicates that gene flow is likely less related to geographic distance per se than it is to oceanic currents and environmental characteristics. Indeed, eggs and (or) larval dispersal can be restricted by physical barriers such as frontal systems (Shaw et al. 2004), oceanic currents (Ruzzante et al. 1998; Knutsen et al. 2003), and estuarine circulation (Pérrin et al. 2004) and to a lesser extent by environmental conditions, which can make habitat unsuitable for settlement (Palumbi 1994). Also, oceanic currents and temperature differences can restrict the dispersal of adults, thus facilitating adaptation to local environmental conditions.

Oceanographic conditions around Iceland are mainly shaped by the two frontal zones located west–northwest and east–southeast of the country, where cold and warm water masses meet, and by temperature differences observed between the northern and southern regions. The pattern of genetic differences observed here for cod around Iceland using neutral, as well as selected, markers suggests that both oceanic currents and environmental conditions such as temperature have a strong structuring effect on this species. The geographical genetics approach conducted with the software BARRIER on the microsatellite loci suggested two main barriers to gene flow located in the two areas that coincide with oceanic fronts, the northwestern and southeastern regions, where warm and cold waters meet.

Previous studies have clearly established the influence of oceanic currents on dispersal of eggs, larvae, and pelagic juveniles, especially those coming from the southern spawning grounds (Begg and Marteinsdóttir 2000, 2003; Marteinsdóttir et al. 2000a). In this study, we have added to this knowledge by demonstrating that oceanic currents, temperature, and oceanic fronts may also play an important role in the dispersal of adult cod.

Depth also appears to play a role in the genetic divergence of cod around Iceland as indicated by the differences observed between inshore and offshore samples. Differences between inshore and offshore cod have been shown to exist in the Northwest Atlantic in Newfoundland waters where both types intermingle in inshore areas during the

offshore–inshore summer feeding migration (Ruzzante et al. 1996). Similarly, recent tagging experiments suggested that inshore cod are more sedentary than their offshore counterparts (Robichaud and Rose 2004; Storr-Paulsen et al. 2004). Very few data exist on the presence of coastal and offshore cod forms in Iceland and on their migration pattern, but Pálsson and Thorsteinsson (2003) suggested that the deep-water fish migrate to deeper waters after the spawning season, whereas shallow-water fish follow the seasonal trend in temperature for the shelf waters. The tagging experiment conducted during this study was carried out during the breeding season to assess spawning site fidelity. It therefore suggested high spawning site fidelity of cod (64% to 100%) but does not assess the complete migration pattern of cod around Iceland. Additionally, biological data collected in previous studies suggested that a high proportion of postspawning cod migrate to the main feeding grounds located to the northwest and northeast of the country (Begg and Marteinsdóttir 2000, 2002b; Marteinsdóttir et al. 2000a) and therefore migrate over long distances. Coastal and offshore life forms of cod from other regions of the North Atlantic are known to exhibit different migration patterns, the former being more sedentary than the latter (Knutson et al. 2004; Robichaud and Rose 2004). The observed high return of Icelandic cod to spawning regions during the breeding season does not mean that coastal cod populations are more sedentary than their offshore counterparts. We therefore interpret that the observed genetic differentiation with depth is not due to the presence of different cod life forms but to the dynamics of cod around Iceland involving spawning site fidelity and postspawning migration to feeding grounds.

Our results therefore indicate that both oceanic fronts and depth have an effect on the genetic composition of cod around Iceland. Additionally, the AMOVA carried out on the shallow water samples suggested that the observed genetic divergence between the northeastern and southwestern regions was not due to the deep-water samples collected in area 9 (931, 932, 933) and therefore that depth was not the primary source of the observed genetic variation.

Gene flow vs local adaptation

The conclusion drawn from genetic data depends crucially on the assumption of selective neutrality of the genetic markers used. The expectation is that all neutral loci would be affected in a similar way by drift and migration (Slatkin 1987), whereas natural selection should affect certain loci differently. One of the recent popular approaches to assessing the potential effect of selection in the genetic structure of a species is to compare genetic patterns obtained from putative selected loci with those of neutral markers such as microsatellite loci. Indeed, the variation of fixation indices among loci and types of marker is one of the most powerful methods for assessing whether natural selection has played a key role in the observed genetic divergence (see Allendorf and Seeb 2000).

In this study, both genetic markers clearly reject the hypothesis of panmixia for cod in Icelandic waters and reveal the presence of two main spawning components. However, the microsatellite loci yielded lower mean fixation indices

than the *Pan I* locus, which has been suggested to experience positive Darwinian selection. Although it remains a challenge to compare the amount of differentiation between different loci, the standardized genetic measure (G'_{ST}), which represents the proportion of the maximum differentiation possible for the level of subpopulations homozygosity observed, suggested that the level of genetic divergence is far more pronounced with the *Pan I* locus than with the microsatellite loci. The results might indicate that balancing selection is acting on the microsatellite loci or that divergent selection is acting on the *Pan I* locus. The microsatellite loci are not regarded as being under selection (neutral expectation) unless linked to functional genes under selection, which is unlikely to be a general pattern (Schlötterer and Wiehe 1999). Consequently, divergent selection acting on the *Pan I* locus is probably the most likely explanation for the observed differences in the fixation indices (F_{ST} and G'_{ST}) of both genetic markers.

In conclusion, the present study used three techniques to identify stock components: microsatellite loci, the *Pan I* locus, and a tagging experiment. Both types of genetic markers clearly indicate that Iceland cod is structured genetically into two major components, northeastern and southwestern. The tagging experiment conducted simultaneously with the collection of samples for genetic analysis is consistent with the notion that spawning cod tagged on the southwestern spawning ground of Iceland seldom migrate to the northeastern spawning ground and vice versa. In addition, the interpretation of these results in light of potential effects of geographic, oceanographic, and depth barriers lead us to conclude that environmental conditions such as oceanic fronts and temperature gradient are likely to affect the temporally stable genetic structure we observed and might have lead to local adaptation of spawning cod around Iceland.

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