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Multiple stock structure of Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador determined from genetic variation

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We examined variation at seven microsatellite loci (*Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35, *Gmo*36, and *Gmo*37) and PanI in some 5230 Atlantic cod (*Gadus morhua)* from 19 inshore and offshore locations around Newfoundland and Labrador. The mean F_{ST} estimate over all loci was 0.0080. Overall, the cod populations surveyed conformed to an isolation-by-distance structure, cod from more distant locations tending to be more genetically distinct. Among offshore sites, the Flemish Cap population (NAFO Division 3M) was the most distinctive, and among inshore sites, the Gilbert Bay population in southern Labrador (2J) was the most distinctive. In NAFO Divisions 3KL, no significant genetic differentiation was observed among inshore northern cod sampled in four bays (Notre Dame, Bonavista, Trinity, and Conception) along the northeast coast of Newfoundland, and the data do not support the hypothesis of separate "bay stocks". Annual variation within sampling sites was as large, on average, as the differentiation among sampling sites. The inshore northern cod were distinct from the population in Gilbert Bay and from most offshore northern cod populations. On average, over all populations, regional differences in allele frequencies were seven times larger than annual variation. The offshore samples were more heterogeneous, and there may be at least three distinct offshore spawning populations of northern cod. In Subdivision 3Ps, no consistent differentiation was observed among sampling sites, two in inshore bays (Placentia, Fortune), and two offshore (Burgeo Bank, Halibut Channel). The southern Grand Bank (Divisions 3NO) may have a separate spawning population from those in other offshore sites, but additional sampling is required to confirm population distinctiveness.

Keywords: cod, genetic variation, microsatellites, northwest Atlantic Ocean, population structure.

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Introduction

Determining population structure is a key component in assessing and managing marine fishery resources. In Newfoundland and Labrador, substantial effort has been directed towards determining population structure of Atlantic cod (*Gadus morhua*), because this species has historically supported substantial fisheries in the Northwest Atlantic (Halliday and Pinhorn, 1996). However, declining abundance in the 1980s and early 1990s, attributed largely to overexploitation (Myers *et al.*, 1997) and to some effect of environmental change (Rose *et al.*, 1994), led to closure of Canadian cod fisheries in 1992. The decline of greatest magnitude was in the northern cod stock complex off Labrador, the east coast of Newfoundland, and the northern half of the Grand Bank (NAFO Divisions 2J+3KL). Some limited cod fisheries have since reopened, but fishing activity has been restricted to waters off the southern coast of Newfoundland (NAFO Subdivision 3Ps) and inshore in Divisions 2J+3KL. Off northeastern Newfoundland, aggregations of adult cod have been found in some

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inshore areas, but not offshore in areas formerly occupied by the northern cod stock complex (Brattey, 1997; Rice, 1997; Lilly *et al*., 2001). An issue of current concern is whether exploitation of inshore populations will inhibit rebuilding of offshore populations. If inshore and offshore spawning groups of northern cod are components of a single stock, then it is more likely that cod from inshore locales could contribute recruits to offshore sites. If inshore and offshore populations constitute separate stocks, with independent population dynamics, then recruitment to offshore spawning areas by inshore cod is less likely.

In NAFO Subdivision 3Ps off the south coast of Newfoundland, spawning cod have been found in several inshore areas, including Fortune Bay and Placentia Bay, as well as offshore on Burgeo Bank, St Pierre Bank, and Halibut Channel (Figure 1; Hutchings *et al*., 1993; Brattey *et al*., 2000). Tagging studies suggest limited mixing between these spawning groups (Taggart *et al*., 1995; Brattey *et al*., 2000), with some indication of multiyear homing to local spawning grounds (Robichaud and Rose, 2001). Therefore, there is a potential for genetically discrete spawning populations within the 3Ps management unit.

Stock structure of cod adjacent to Newfoundland and Labrador has been investigated with several techniques. Early work (reviewed by Halliday and Pinhorn, 1990) centred on age, growth, sexual maturity (Fleming, 1960), movements from tagging, parasite counts, and vertebral counts (Templeman, 1974, 1981; Templeman *et al*., 1976). These characters, influenced by both environmental and genetic factors, were used to delineate the major northern cod stock complex, and other stocks located farther south on the Grand Bank, on the Flemish Cap, and along the southern and western shores of Newfoundland. Tagging has indicated that the northern cod stock may have a number of partially isolated subcomponents (Lear, 1984), possibly at the geographic scale of coastal bays (Taggart *et al*., 1995). The level of reproductive isolation, if any, among these subcomponents or local populations, is uncertain. Trace element composition of otoliths has also been used to assess cod population structure in the Gulf of St Lawrence off southern Newfoundland, with the indication that there was little mixing of populations in overwintering habitats (Campana *et al*., 1999). Similar results were also observed in a survey of vertebral number variation in spawning and overwintering cod in the area (Swain *et al*., 2001).

Delineation of population structure is fundamental to the assessment, conservation, and management of Atlantic cod. Genetic differentiation at neutral genetic loci among spawning groups, indicative of restricted gene flow and independent population dynamics among the groups, is a good indicator of population structure (Ferguson *et al*., 1995; Waples, 1998). Moreover, if there is sufficient genetic differentiation among populations, the genetic markers can be used to provide estimates of population or stock composition in areas of population mixing. This permits determination of catch by population, with subsequent estimation of exploitation rates, allowing managers to recognize overexploitation of less productive populations in regions of mixing.

Fidelity of spawning fish to specific areas, with little exchange of spawners among areas, is a basic requirement in the designation of a "stock". Restriction of gene flow among spawning groups that results from this fidelity permits the development over time of genetic differentiation. For a marine fish such as Atlantic cod, a stock may consist of a single large, randomly breeding aggregate, or it may be subdivided into smaller groups within which mating is random, but among which there is more limited exchange of individuals. These local populations within a stock are more similar to each other than to populations in another stock complex. Analysis of genetic variation in Atlantic cod has not revealed an entirely consistent pattern of stock structure in the northwest Atlantic Ocean. Given that there are sizeable aggregations of adult cod in some inshore areas off northeastern Newfoundland, but apparently none offshore, the issue of inshore and offshore stock structure is of considerable practical significance in formulation of exploitation strategies. Surveys of variation at allozyme loci indicated the existence of three major cod stocks off North America (Cross and Payne, 1978). There seems to be little variation in mitochondrial DNA of North American cod (Carr and Marshall, 1991; Pepin and Carr, 1993), and the mitochondrial DNA results have been used to suggest that inshore and offshore cod populations constitute a single stock (Carr *et al*., 1995; Carr and Crutcher, 1998). However, higher levels of genetic variation have been observed at microsatellite loci (Bentzen *et al*., 1996; Ruzzante *et al*., 1998), and a comparison of northern cod from inshore bays with those from offshore locations suggested that separate stocks exist (Ruzzante *et al*., 1996, 1997). There have been no comprehensive genetic studies of cod off the south coast of Newfoundland, but Ruzzante *et al*. (1998, 2000a) found weak evidence of genetic heterogeneity on the basis of analyses of microsatellites in cod sampled at three sites in NAFO Subdivision 3Ps. The genetic structure and particularly the relationship between inshore and offshore spawning cod around Newfoundland and Labrador clearly needs to be resolved.

The primary objective of this study was to use microsatellite variation to investigate population structure of cod off Newfoundland and Labrador. This study differs from previous genetic work on cod by Ruzzante *et al*. (1996, 1997) in that it is based on (1) seven new microsatellites and the pantophysin (PanI) locus, (2) larger sample sizes and more extensive geographic coverage of inshore sites around insular Newfoundland,

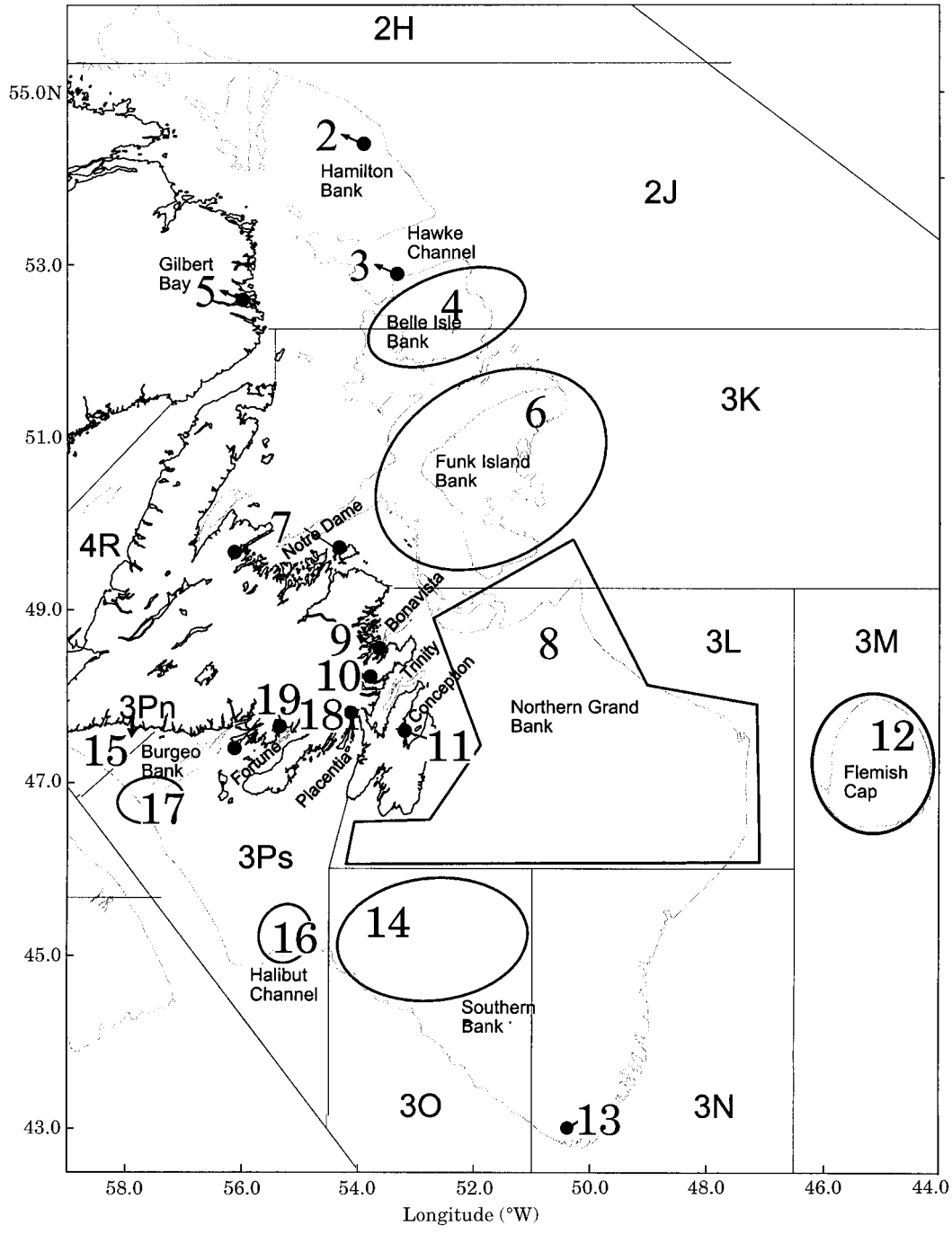

Figure 1. Approximate locations where cod DNA samples were obtained. Numbers indicate reference locations indicated in Table 1, which gives further details of sampling: 1, offshore 2G (not shown); 2, Hamilton Bank; 3, Hawke Channel; 4, Belle Isle Bank; 5, Gilbert Bay; 6, Funk Island Bank; 7, Notre Dame Bay; 8, northern Grand Bank; 9, Bonavista Bay; 10, Trinity Bay; 11, Conception Bay; 12, Flemish Cap; 13, southern Grand Bank (3N); 14, southern Grand Bank (3O); 15, Rose Blanche Bank; 16, Halibut Channel; 17, Burgeo Bank; 18, Placentia Bay; 19, Fortune Bay. Boundaries of NAFO Divisions and the 300 m depth contour are also indicated.

Table 1. Samples collected and analysed from cod populations off Newfoundland. N is the sample size in each year. Locations sampled during the study are shown in Figure 1.

Area	Location	Local area	Depth range (m)	Date	Year	Latitude (dec.)	Longitude (dec.)	N	Total N
2G Offshore	(1) Offshore	—	313	6 October	1996	60.25	61.25	20	20
2J Offshore	(2) Hamilton Bank	—	230–277	9–13 November	1999	—[1]		16	16
	(3) Hawke Channel	—	377–465	13–16 June	1998	52.75	53.00	236	236
	(4) Belle Isle Bank	—	251–602	17–25 November	1999	—[2]		10	10
Inshore	(5) Gilbert Bay	—	15	29 May	1997	52.58	55.83	86	132
			15	25 May	1998	52.58	55.83	46	
3K Offshore	(6) Funk Island Bank	—	204–1364	11–21 November	1996	—[3]	—	97	188
			225–782	23 Nov.–10 Dec.	1999	—[4]	—	91	
Inshore	(7) Notre Dame Bay	Miles Cove	20–21	18 June–3 July	1997	49.54	55.77	126	739
		Fogo	10	18 June	1998	49.65	54.05	213	
		Jacksons Cove	7–22	22–24 June	1999	49.70	55.98	200	
		Fogo	5–10	1 June	1999	49.73	54.25	200	
3L Offshore	(8) Northern Grand Bank	—	324–410	25–27 June	1998	—[5]	—	113	218
		—	41–407	13–20 June	1999	—[6]	—	50	
			66–397	23 Nov.–10 Dec.	1999	—[7]	—	55	
Inshore	(9) Bonavista Bay	Open Hall	13	29 May–11 June	1997	48.51	53.51	147	503
		Plate Cove	15	4 June	1998	48.52	53.52	162	
		Sandy Cove	15–24	10 June	1999	48.64	53.71	5	
		Rachet Cove	20–48	10 June	1999	48.61	53.73	12	
		Shag Islands	21	12 June	1999	48.71	53.63	32	
		Rachet Cove	26	13 June	1999	48.60	53.77	24	
		Swale Island	25	13 June	1999	48.62	53.69	19	
		Plate Cove	24–51	28 June	1999	48.52	53.52	18	
		Plate Cove	22–26	7 July	1999	48.51	53.51	51	
	(10) Trinity Bay	West Trinity Bay	17–279	23–26 April	1997	48.18	53.61	155	
		Petley	40	28 May	1999	48.21	53.56	44	
			110–256	23 June	1999	48.18	53.79	156	355
	(11) Conception Bay		99	5 November	1996	47.67	53.02	20	
		Brigus	33–135	1 June–7 July	1999	47.56	53.18	200	220

Table 1. *Continued*

Area	Location	Local area	Depth range (m)	Date	Year	Latitude (dec.)	Longitude (dec.)	N	Total N
3M	(12) Flemish Cap	—	127–707	25 Sep.–12 Oct.	1996	—[8]	—	103	103
3	(13) Southern Grand Bank	—	220	30 May	1999	42.89	49.95	13	13
3	(14) Southern Grand Bank	—	69–113	11–14 May	1999	—[9]	—	100	100
3Pn	(15) Rose Blanche Bank	—	206–217	18 April	1999	47.55	58.25	17	17
3Ps Offshore	(16) Halibut Channel	—	181–307	2–4 April	1998	45.10	55.20	214	413
			157–213	1–3 April	1999	45.30	55.40	199	
3Ps	(17) Burgeo Bank	—	264–309	6–7 April	1998	46.75	57.65	250	500
			265–326	4–18 April	1999	46.80	57.65	250	
Inshore	(18) Placentia Bay	Bar Haven	21–50	22–26 April	1998	47.73	54.19	223	902
		Warehams Rock	41–53	1–3 May	1998	47.70	54.15	205	
		Paradise Sound	151–188	27–29 April	1998	47.51	54.49	95	
		Inner Placentia Bay	15–70	30 April–3 May	1999	47.70	54.20	200	
		Arnold's Cove	42–73	7 June	2000	47.45	54.00	179	
	(19) Fortune Bay	Pools Cove	69	21–28 May	1998	47.70	55.38	264	565
			33	18–20 May	1999	47.70	55.38	106	
		Pass Island	212–217	8 April	1999	47.40	56.20	195	

[1]Collected at various locations on or adjacent to Hamilton Bank during FV "Teleost" trip 86.
[2]Collected at various locations on or adjacent to Belle Isle Bank during FV "Teleost" trip 86 and 87.
[3]Collected at various locations on or adjacent to Funk Island Bank during FV "Teleost" trip 40 and FV "Wilfred Templeman" trip 198.
[4]Collected at various locations on or adjacent to Funk Island Bank during FV "Teleost" trip 87 and 88.
[5]Collected at various locations in offshore northern 3L north of 47.6°N during FV "Wilfred Templeman" trip 224.
[6]Collected at various locations in offshore northern 3L north of 46.0°N during FV "Wilfred Templeman" trips 240 and 241.
[7]Collected at various locations in offshore northern 3L north of 46.5°N during FV "Wilfred Templeman" trips 247 and 248.
[8]Collected at various locations on the Flemish Cap between 44 and 46°W and 46 and 48°N during FV "Wilfred Templeman" trip 195 and part of trip 196.
[9]Collected at various locations in 3O south of 45.9°N during FV "Wilfred Templeman" trip 238.

and (3) only mature adult fish collected mainly during the spawning period. We examined whether there are distinct "bay stocks" of cod, i.e. is there genetic differentiation among cod populations in neighbouring bays along the coast of northeastern Newfoundland? We also examined the degree of genetic differentiation between inshore- and offshore-spawning populations to determine whether they constitute separate stocks. In addition, we surveyed microsatellite variation in cod from different spawning sites in Subdivision 3Ps to investigate whether there was evidence of genetically discrete multiple-spawning populations in the management unit. Finally, we compared differentiation among 19 samples of cod from the Northwest Atlantic. To define a group of fish as genetically distinct and thus a separate stock, we compare annual variation in allele frequencies relative to differentiation among samples and regions, where appropriate. Differentiation among putative populations or stocks must be greater than annual variation within populations if they are genetically distinct, because it is necessary to separate genetic differentiation from sampling or annual variation (Waples, 1998).

Materials and methods

Collection of DNA samples and PCR

Blood, heart, or muscle samples were collected from prespawning, spawning, or postspawning cod from some 5230 fish in several locations around Newfoundland (Table 1, Figure 1). All inshore samples were collected during the spring spawning season, but most offshore northern cod samples were collected in autumn. We stress that autumn-collected samples do not necessarily represent spawning groups in our analyses; such samples were divided into broad geographic regions that correspond with offshore banks and general bathymetry. For all samples, the gonads of individual fish were examined to determine maturation stages, and samples were only collected from fish with ripe, running, or partly spent gonads; immature and spent fish were not sampled. By excluding spent fish, particularly for inshore samples collected in late spring, we excluded the possibility that our samples would include offshore spawners that had migrated inshore. For autumn offshore samples, fish with ripening gonads were sampled; the precise location of spawning offshore of these fish is not known. For the tissue samples, approximately 0.3 g of tissue was placed in each well of a 96-well plate containing 0.2 m of 5% chelex in TE buffer (10 mM Tris pH 7.4, 1 mM EDTA pH 8.0, 0.10 mg m^{-1} proteinase K, and 0.1% SDS) and incubated for 15 min at 50°C, then incubated for an additional 15 min at 95°C. The supernatant from each well was collected and placed in a fresh 96-well plate and stored at −20°C. About 1 µ of this extract was required for each amplification of the sample by the polymerase chain reaction (PCR).

Primers for microsatellite loci developed at the Pacific Biological Station were: *Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35, *Gmo*36, and *Gmo*37 (Miller *et al*., 2000); in addition, variation at the pantophysin locus (PanI; Fevolden and Pogson, 1997) was surveyed. For all microsatellite primer sets, PCR was conducted in 12.5-µ reactions containing 15 pmol (0.60 µM) of each primer, 0.3 µ DNA polymerase, 80 µM of each nucleotide, 20 mM Tris-pH 8.8, 2 mM $MgSO_4$, 10 mM KCl, 0.1% Triton X-100, 10 mM $(NH_4)SO_4$, and 0.1 mg m^{-1} nuclease-free Bovine Serum Albumin. All microsatellite PCR in this study was preceded by an initial denaturation step of 3 min at 94°C. All cycle extension steps (30 cycles for all loci) were for 60 s at 72°C, and all cycle denaturation steps were for 20 s at 94°C. PCR amplification of loci *Gmo*3, *Gmo*8, *Gmo*19, *Gmo*34, *Gmo*35, *Gmo*36, *Gmo*37, and PanI was accomplished with annealing temperatures of 46, 50, 50, 50, 55, 50, 46, and 55°C respectively. Annealing times were 60 s for all loci. For *Gmo*19 and *Gmo*34 together, PCR amplification was conveniently multiplexed in the same 12.5-µ reactions. PanI PCR products were digested with DraI (New England Biolabs, Ontario, Canada) for 2 h at 37°C.

Gel electrophoresis and band analysis

Microsatellite PCR products were size-fractionated on 16 × 17 cm non-denaturing polyacrylamide gels and visualized by staining with 0.5 mg m^{-1} ethidium bromide in water and ultraviolet light illumination. Nelson *et al*. (1998) provide a complete description of gel electrophoretic conditions. All microsatellite gels were run for 14–18 h at 65–70 V, using 8% acrylamide for analysis of *Gmo*3, *Gmo*36, and *Gmo*37, and 10% acrylamide for analysis of *Gmo*8, *Gmo*19, *Gmo*34, and *Gmo*35. In all, 29 lanes per gel were loaded, one outside lane containing one-kb ladder (Gibco BRL), three lanes containing 20 base pair (bp) ladders (Gensura Labs Inc., Del Mar, California) evenly spaced across the gel, one lane containing DNA from a standard fish to determine precision of estimation of allele size, and 24 lanes of DNA amplified from individual fish for analysis.

Gels were scanned at a pixel density of 1024 × 1024 with a Kodak charge-coupled-device camera with low light capability and a yellow filter. Images were analysed using BioImage Whole Band software (Millipore Corp. Imaging Systems, Ann Arbor, Michigan), with the size of the amplified microsatellite alleles reported to the nearest bp based upon the molecular size grid created with the 20-bp markers. As there was some uncertainty in estimating microsatellite allele size from the 20-bp grid, we identified alleles on the basis of a binning procedure (Gill *et al*., 1990). Peaks in the estimated allele size frequency distribution by base pair were used to

Table 2. Precision of estimates of allele size (in base pairs) at each microsatellite locus for standard fish run only once per electrophoretic gel. N is the number of gels on which allele sizes for a standard fish were estimated. Standard deviation is in parenthesis. The number of alleles observed at each locus over all fish surveyed is also indicated.

Locus	N	Allele size	Range	Allele size	Range	Observed alleles
*Gmo*3	90	180.0 (0.69)	179–181	187.4 (0.83)	186–189	9
*Gmo*8	83	126.4 (0.54)	125–128	164.9 (0.73)	163–166	24
	25	126.2 (0.50)	125–127	164.5 (0.59)	163–165	
*Gmo*19	37	148.6 (0.65)	147–150	183.2 (0.60)	182–184	24
	32	148.5 (0.57)	148–150	183.1 (0.75)	182–185	
*Gmo*34	75	91.7 (0.50)	91–92	107.0 (0.50)	106–108	9
*Gmo*35	101	120.2 (0.43)	120–121	134.7 (0.55)	134–136	13
*Gmo*36	95	185.0 (0.67)	184–186	202.4 (0.61)	201–204	13
*Gmo*37	96	237.3 (0.84)	235–239	284.9 (0.92)	283–287	18

identify alleles empirically, and bin widths generally corresponding to a repeat unit were set with the peak in the middle of the bin. Precision of estimation of allele size was evaluated with the standard fish analysed for each locus. Allele frequencies for all location samples surveyed in this study are available at http://www.sci.pac.dfo-mpo.gc.ca/aqua/pages/bgsid.htm.

Data analysis

As offshore cod samples were very few from Division 2J, samples from Hamilton Bank (16 fish) and Belle Isle Bank (10 fish) were combined into a single sample and subsequently referred to as the Belle Isle Bank sample. Annual variation in allele frequencies within populations was tested with GENEPOP version 3.1 with the Markov–Chain approach, using χ^2 probability values (Raymond and Rousset, 1995). The dememorization number was set at 1000, and 50 batches were run for each test with 1000 iterations per batch. Each population in each year (31 total comparisons) at each locus was tested for departure from Hardy–Weinberg equilibrium using GENEPOP. Tests of genetic differentiation utilizing pairwise comparisons among the populations were also conducted using GENEPOP with the Markov–Chain approach and χ^2 probability values. Critical significance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice, 1989). Cavalli-Sforza and Edwards (1967) chord distance (CSE) was used to estimate distance among samples, only samples of at least 20 fish being included in the analysis. An unrooted unweighted pair-group mean (UPGMA) dendrogram was generated with PHYLIP (Felsenstein, 1993). F_{ST} estimates for each locus were calculated with GENEPOP. Estimation of variance components of stock differences, variation among populations within stock groups, and annual variation within populations was determined with BIOSYS (Swofford and Selander, 1981). Negative variance components were set to zero.

Results

Precision of estimation of allele size

Standard deviations of the estimate of allele size for the heterozygous standard fish analysed at each locus ranged from 0.43 to 0.92 bp, the larger alleles being estimated with the least precision (Table 2). For the trinucleotide-repeat *Gmo*35 locus, all estimated sizes of a particular standard allele were within a 3 bp interval. For the trinucleotide repeat *Gmo*36 locus, all estimated sizes of the smaller (185 bp) allele were within a 3 bp interval, and 97% (91/94) of the estimated sizes of the larger allele were within a 3 bp interval. Estimated sizes for the alleles of the standard fish at the tetranucleotide-repeat *Gmo*3, *Gmo*8, *Gmo*19, and *Gmo*34 loci were all within a 4 bp interval. At the *Gmo*37 locus, estimated allele sizes for the 237 bp allele were within a 4 bp interval for 99% (95/96) of occurrences, and those of the 285 bp allele were within a 4 bp interval for 98% (94/96) of occurrences. Precision of estimation of allele size was well within the range required for consistent determination of size.

Variation within populations

Observed heterozygosities of the loci examined over all samples were: *Gmo*3, 0.31 (sample range 0.23–0.44); *Gmo*8, 0.84 (0.73–1.00); *Gmo*19, 0.87 (0.60–1.00); *Gmo*34, 0.45 (0.00–0.48); *Gmo*35, 0.73 (0.56–0.82); *Gmo*36, 0.57 (0.38–0.73); *Gmo*37, 0.79 (0.45–0.82); and PanI, 0.53 (0.18–0.87). Genotypic frequencies observed at the seven microsatellite loci surveyed in our study were in accordance with Hardy–Weinberg proportions, with a few exceptions. Of 31 tests conducted at each locus (217 total tests for microsatellites), three samples

Table 3. Probability of homogeneity of annual allele frequencies estimated from probability tests derived from GENEPOP version 3.1 with the Markov–Chain approach using χ^2 probability values (Raymond and Rousset, 1995). Values considered statistically significant are emboldened.

Population	*Gmo*3	*Gmo*8	*Gmo*19	*Gmo*34	*Gmo*35	*Gmo*36	*Gmo*37	PanI
Inshore populations								
Gilbert Bay	0.8279	0.4188	0.8302	0.8035	0.0902	0.1816	**<0.0001**	1.0000
Notre Dame Bay	0.0268	0.0296	0.5787	0.4031	0.0813	0.9877	**<0.0001**	0.1082
Bonavista Bay	0.7559	0.1392	0.2644	0.8293	0.3369	0.2889	0.6942	0.6471
Trinity Bay	**<0.0001**	0.5937	0.2434	0.1621	0.2256	0.8690	0.9902	0.0442
Placentia Bay	0.0702	0.2751	0.0222	0.0828	0.0506	**<0.0001**	0.2385	**<0.0001**
Fortune Bay	0.0544	0.7398	0.5278	0.2892	0.0594	0.3015	0.0535	**<0.0001**
Offshore populations								
Funk Island Bank	0.0666	0.2901	0.8001	0.8537	0.0939	0.2581	0.0760	0.0613
Northern Grand Bank	0.0195	0.2945	0.7415	0.2470	0.0629	0.8962	0.7029	0.3999
Burgeo Bank	**<0.0001**	0.6142	0.2621	0.0855	0.1430	0.0245	0.2663	**0.0003**
Halibut Channel	0.9554	0.2378	0.2621	0.0895	0.8370	0.6998	0.0179	0.6388

were not in Hardy–Weinberg equilibrium at *Gmo*3, seven samples at *Gmo*8, four samples at *Gmo*19, one each at *Gmo*34 and *Gmo*35, and five each at *Gmo*36 and *Gmo*37. There was no evidence of a consistent departure of genotypic frequencies from Hardy–Weinberg distribution at any locus, indicating that null alleles were not in significant frequency. Over all microsatellite loci, the Burgeo Bank population samples accounted for four of the 26 tests that were significant, as did the Division 3O population and northern Grand Bank population samples. Three significant tests were observed in the Halibut Channel and Bonavista Bay samples. Nonconformance to Hardy–Weinberg equilibrium was due to an excess of homozygous fish at the loci concerned. This indicates that those samples may have contained fish from two separate spawning populations (homozygote excess as a result of the Wahlund effect). At PanI, significant departures from the expected Hardy–Weinberg distribution of genotypic frequencies were observed in 11 of the 31 tests conducted. In each case, there was an excess of heterozygotes observed, indicating that balancing selection favouring heterozygotes may operate at PanI in some populations.

Population structure

Genetic differentiation at all loci was observed among the putative cod populations surveyed in our study. For example, the frequency of *Gmo*3^{186} in offshore populations (Flemish Cap, 0.59; 2J+3KL, <0.80) was generally less than in other populations (>0.75), the Gilbert Bay population having the highest observed frequency (0.88). The Gilbert Bay sample was similarly distinctive at *Gmo*8, with a frequency of *Gmo*8^{144} (0.49), substantially higher than that of other populations (<0.27). The cod population in Division 2G had higher frequencies of *Gmo*19^{144} (0.20) than did other populations (all other populations <0.10). Offshore populations in Divisions 2J+3KL and on the Flemish Cap had higher frequencies of *Gmo*34^{98} (e.g. Hawke Channel, 0.79; Funk Island Bank, 0.80; Flemish Cap, 0.91) than did inshore populations or those in 3Ps (all <0.75), the Gilbert Bay population having the lowest observed frequency (0.38). Differentiation between inshore and offshore populations in 2J+3KL was also observed at *Gmo*35 and *Gmo*36. The Hawke Channel population tended to have higher frequencies of *Gmo*37^{256} (0.22) than did other populations (usually <0.15). At PanI, offshore populations of northern cod had higher frequencies of the allele possessing the DraI restriction site than did inshore populations. F_{ST} estimates by locus were: *Gmo*3, 0.0068; *Gmo*8, 0.0047; *Gmo*19, 0.0027; *Gmo*34, 0.0162; *Gmo*35, 0.0028; *Gmo*36, 0.0069; *Gmo*37, 0.0038; and PanI, 0.0374. The mean over all loci was 0.0080.

We compared the relative levels of temporal variation in allele frequencies within samples with differentiation among samples. Ten locations were sampled in multiple years. At six, there was significant annual variation in allele frequencies for at least one locus (Table 3). In order to demonstrate unequivocally the existence of genetically discrete stocks, it is necessary to demonstrate that the differentiation among putative stocks is greater than the variation observed within putative stocks. We evaluated differentiation among cod from four bays along the northeast coast of Newfoundland in Divisions 3KL relative to variation among cod within each of the bays. We evaluated whether there were distinct "bay stocks" of cod in the four bays. For the microsatellite loci surveyed, the data were consistent with the interpretation of a single inshore population in Conception Bay, Trinity Bay, Bonavista Bay, and Notre Dame Bay ($p>0.05$ for all microsatellite loci; Table 4). Differentiation among locations was observed at PanI ($p<0.05$), but for all loci combined, there was no consistent differentiation among locations. There was no

Table 4. Hierarchical gene-diversity analysis of four putative populations of Atlantic cod along the northeast coast of Newfoundland (Conception Bay, Trinity Bay, Bonavista Bay, and Notre Dame Bay) for seven microsatellite loci and the PanI locus. The relative diversity attributable to sampling years within populations and among populations are indicated, as well as the ratio of among population vs. among years within population diversity.

	Absolute diversity		Relative diversity			
Locus	Total	Within populations	Within populations	Among years within populations	Among populations	Populations/ years
*Gmo*3	0.3378	0.3350	0.9917	0.0083	0.0000	0.0
*Gmo*8	0.9180	0.9167	0.9986	0.0014	0.0000	0.0
*Gmo*19	0.9352	0.9343	0.9991	0.0009	0.0000	0.0
*Gmo*34	0.4686	0.4671	0.9967	0.0009	0.0024	2.7
*Gmo*35	0.7762	0.7761	0.9999	0.0001	0.0000	0.0
*Gmo*36	0.6106	0.6100	0.9991	0.0009	0.0000	0.0
*Gmo*37	0.8653	0.8636	0.9980	0.0020	0.0000	0.0
PanI	0.4391	0.4362	0.9934	0.0000	0.0066	—
All			0.9982	0.0014	0.0004	0.3

Table 5. Hierarchical gene-diversity analysis of four regional groups: Labrador (Gilbert Bay), Northeast inshore Newfoundland (Conception Bay, Trinity Bay, Bonavista Bay, and Notre Dame Bay), southern offshore (Northern Grand Bank, Funk Island Bank), and northern offshore (Belle Isle Bank, Hawke Channel), constituting nine putative populations of Divisions 2J+3KL Atlantic cod for seven microsatellite loci and the PanI locus. The relative diversity attributable to sampling years within populations, among populations within regions, and among regions are indicated, as well as the ratio of among population and region vs. among years within population diversity.

	With Gilbert Bay					Without Gilbert Bay				
Locus	Within poplns	Among years within poplns	Among poplns within regions	Among regions	Poplns+ regions/ years	Within poplns	Among years within poplns	Among poplns within regions	Among regions	Poplns+ regions /years
*Gmo*3	0.9893	0.0078	0.0000	0.0029	0.4	0.9904	0.0096	0.0000	0.0000	0.0
*Gmo*8	0.9806	0.0006	0.0011	0.0177	31.3	0.9984	0.0007	0.0009	0.0000	1.3
*Gmo*19	0.9887	0.0002	0.0005	0.0106	55.5	0.9993	0.0003	0.0004	0.0000	1.3
*Gmo*34	0.9498	0.0002	0.0038	0.0462	421.0	0.9858	0.0006	0.0046	0.0090	22.7
*Gmo*35	0.9961	0.0013	0.0004	0.0022	2.0	0.9984	0.0002	0.0011	0.0003	7.0
*Gmo*36	0.9913	0.0013	0.0021	0.0053	5.7	0.9914	0.0001	0.0032	0.0053	85.0
*Gmo*37	0.9821	0.0088	0.0000	0.0091	1.0	0.9964	0.0016	0.0020	0.0000	1.3
PanI	0.9292	0.0000	0.0087	0.0621	—	0.9875	0.0000	0.0078	0.0047	—
All	0.9798	0.0024	0.0011	0.0167	7.4	0.9957	0.0011	0.0019	0.0013	2.9

convincing evidence for the existence of more than one inshore stock in these bays.

Cod in Divisions 2J+3KL had been managed and assessed as a single stock (the northern cod stock), so we evaluated whether there was any evidence of structure among samples from the northern cod stock. Samples were arranged into four regions: Labrador (Gilbert Bay), Northeast inshore Newfoundland (Conception Bay, Trinity Bay, Bonavista Bay, and Notre Dame Bay), southern offshore (Northern Grand Bank, Funk Island Bank), and northern offshore (Belle Isle Bank, Hawke Channel), constituting nine putative populations of northern cod. Significant differentiation within the northern cod stock complex was observed at four microsatellite loci: *Gmo*8, *Gmo*19, *Gmo*34, and *Gmo*36, as well as at PanI ($p<0.05$). On average, regional and population differences were seven times larger than annual variation within populations (Table 5). The Gilbert Bay population was the most distinctive of all populations surveyed in our study (Figure 2), and indeed was distinct from inshore cod along the northeast coast of Newfoundland (Table 6). We examined whether there was still differentiation in northern cod when only the inshore Newfoundland and offshore samples were analysed. Significant differentiation was observed at three microsatellite loci (*Gmo*34, *Gmo*35, *and Gmo*36), as well as PanI ($p<0.05$; Table 5). Although the level of overall differentiation was reduced when the Gilbert Bay

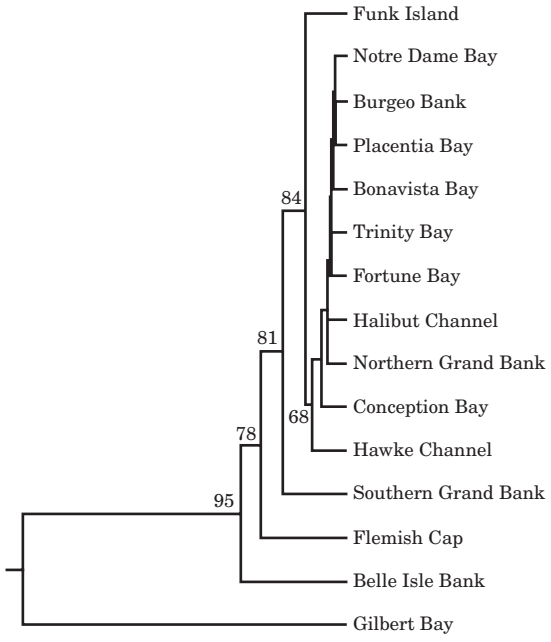

Figure 2. UPGMA dendrogram based on Cavalli-Sforza and Edward's (1967) chord distance for cod from 15 sampling sites in the northwest Atlantic adjacent to Newfoundland and Labrador.

samples were excluded, with regional and population differences three times larger than the annual variation within populations, there was still genetic differentiation in the northern cod stock complex when only the Newfoundland inshore and offshore samples were considered. We compared allele frequencies of the offshore 2J+3KL samples (Hawke Channel, Funk Island Bank, and northern Grand Bank) with the inshore 2J+3KL samples (Gilbert Bay, Notre Dame Bay, Bonavista Bay, Trinity Bay, and Conception Bay). The Belle Isle Bank sample was not considered owing to its small size (<25 fish). Significant differentiation was observed between the Hawke Channel sample and each of the five inshore samples, ranging from three significant differences in comparison with the Trinity Bay and Conception Bay samples to seven significant differences in comparison with the Gilbert Bay sample (Table 6). Similarly, differentiation was also observed between the Funk Island Bank sample and all inshore 2J+3KL populations, ranging from one significant difference in comparison with the Conception Bay population to seven significant differences in comparison with the Gilbert Bay population. Finally, the northern Grand Bank population was also differentiated from most inshore populations, with zero (Trinity Bay) to seven (Gilbert Bay) significant differences in allele frequencies observed.

Among cod in Subdivision 3Ps, no significant, persistent genetic differentiation in cod at the four sampling sites was observed at any locus (all $p>0.05$). Annual variation was larger than any differences among the four sampling sites (Table 7). There was no evidence from this study to reject the hypothesis that cod in the 3Ps management unit constitute a single spawning population. Inshore cod from Placentia Bay were also compared with cod sampled in adjacent bays along the northeast coast of insular Newfoundland (Table 6). There was no significant differentiation between Placentia Bay cod and those from Conception or Trinity Bay, but differences in allele frequencies were observed at two loci when compared with the two more distant locations, Bonavista Bay and Notre Dame Bay.

Regional and population differentiation was examined for cod in the Northwest Atlantic from seven regional groups: Labrador (Gilbert Bay), Northeast inshore Newfoundland (Conception Bay, Trinity Bay, Bonavista Bay, and Notre Dame Bay), southern offshore (Northern Grand Bank, Funk Island Bank), and northern offshore (Belle Isle Bank, Hawke Channel, Division 2G), southern Newfoundland (Fortune Bay, Placentia Bay, Burgeo Bank, Halibut Channel, and Subdivision 3Pn), southern Grand Bank (Divisions 3N and 3O), and the Flemish Cap (Flemish Cap), a total of 18 putative populations. Significant regional and population differentiation was observed at most loci, with the combined differences on average about 11 times greater than the annual variation within locations (Table 8). As the geographic area of sample coverage expanded over that of previous analyses, greater differentiation among regions and populations was observed compared with annual variation within locations.

Allele frequencies of cod from offshore banks were compared with those from the Flemish Cap, with the expectation that genetic differentiation would be observed, because on the basis of tagging studies and other biological characters, the Flemish Cap stock is considered to be distinct. There was significant genetic differentiation in allele frequencies between the Flemish Cap population and each of four offshore bank locations (Hawke Channel, Funk Island Bank, northern Grand Bank, southern Grand Bank) for at least four of the loci surveyed in each comparison. This is strongly indicative of restricted gene flow between the Flemish Cap population and the offshore banks, and provides confirmation of the Flemish Cap population as a separate breeding stock (Table 6). The question then arises whether the offshore 2J+3KL populations constitute a single breeding population. Pairwise population comparisons of the allele frequencies of the three offshore populations indicated that there were 1–3 significant differences, suggesting that there may be at least three breeding populations or stocks offshore in 2J+3KL (Table 6).

Significant abundances of cod in Placentia Bay were observed subsequent to the decline in cod abundance offshore in 2J+3KL, prompting some speculation about

Table 6. Probability of homogeneity of allele frequencies estimated from pairwise probability tests derived from GENEPOP version 3.1 with the Markov–Chain approach using χ^2 probability values (Raymond and Rousset, 1995). Values considered statistically significant are emboldened.

Comparison		*Gmo*3	*Gmo*8	*Gmo*19	*Gmo*34	*Gmo*35	*Gmo*36	*Gmo*37	PanI
Flemish Cap	Funk Island	**0.0028**	**0.0075**	0.0717	**<0.0001**	0.2380	**0.0003**	0.0316	**<0.0001**
	Hawke Channel	**<0.0001**	**0.0014**	**0.0015**	**<0.0001**	0.3309	**<0.0001**	**0.0001**	**<0.0001**
	N. Grand Bank	**<0.0001**	0.0316	0.0142	**<0.0001**	0.1274	**<0.0001**	0.2308	**<0.0001**
	S. Grand Bank	**0.0011**	0.4690	**<0.0001**	**<0.0001**	0.1534	**<0.0001**	0.0246	**<0.0001**
Offshore vs. inshore Newfoundland									
Funk Island Bank	Gilbert Bay	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0271	**<0.0001**	**<0.0001**
	Notre Dame	0.2046	**0.0015**	0.4281	**0.0046**	0.5535	**0.0004**	**<0.0001**	**<0.0001**
	Trinity	0.6565	0.4943	0.6933	0.1405	0.8484	0.0284	**<0.0001**	**0.0068**
	Bonavista	**0.0002**	0.0203	0.7194	**<0.0001**	0.1966	0.1900	**<0.0001**	**<0.0001**
	Conception	0.1349	0.6128	0.3507	0.3270	0.0902	0.2216	**0.0001**	0.3408
Hawke Channel	Gilbert Bay	0.0352	**<0.0001**	**<0.0001**	**<0.0001**	**0.0014**	**<0.0001**	**<0.0001**	**<0.0001**
	Notre Dame	0.9852	0.0173	**0.0044**	**0.0005**	0.3059	**<0.0001**	**0.0021**	<0.0001
	Trinity	0.7056	0.1113	0.0251	0.0888	0.3550	**0.0001**	**0.0001**	**0.0002**
	Bonavista	0.2835	0.8738	**0.0037**	**<0.0001**	0.0917	**<0.0001**	**<0.0001**	**<0.0001**
	Conception	0.3542	0.5966	**0.0001**	0.3605	0.2112	**<0.0001**	**<0.0001**	0.1037
N. Grand Bank	Gilbert Bay	0.0992	**<0.0001**	**<0.0001**	**<0.0001**	**0.0008**	**<0.0001**	**<0.0001**	**<0.0001**
	Notre Dame	0.4161	0.4046	0.6139	0.5941	0.0231	0.0219	0.0857	**0.0015**
	Trinity	0.5986	0.6692	0.1439	0.9896	0.0191	0.1109	0.2648	0.3783
	Bonavista	0.3640	0.9480	0.0729	0.0721	**0.0062**	0.0207	0.1840	**<0.0001**
	Conception	0.0897	0.9543	0.0515	0.9789	0.1107	**0.0008**	0.2174	0.6158
Single offshore popln 2J+3KL?									
Hawke Channel	Funk Island	0.4998	0.1546	0.4151	0.9187	0.1684	**0.0004**	**<0.0001**	0.5676
	N. Grand Bank	0.8214	0.6057	0.5419	0.2454	0.3748	0.0840	**0.0004**	0.0118
Funk Island	N. Grand Bank	0.0878	0.4595	0.5527	0.2906	0.0586	**0.0044**	**<0.0001**	0.0923
Inshore population structure									
Gilbert Bay	Notre Dame	0.0451	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0391	**<0.0001**	**<0.0001**
	Bonavista	0.5171	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0236	**<0.0001**	**<0.0001**
	Trinity	**0.0066**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0254	**<0.0001**	**<0.0001**
	Conception	0.0400	**<0.0001**	**<0.0001**	**<0.0001**	**0.0049**	0.0432	**<0.0001**	**<0.0001**
Placentia Bay and offshore samples									
Placentia	Flemish Cap	**<0.0001**	**0.0031**	0.0407	**<0.0001**	0.3240	**<0.0001**	0.1684	**<0.0001**
	Hawke Channel	0.8544	0.1806	0.0170	**<0.0001**	0.7494	**<0.0001**	**<0.0001**	**<0.0001**
	Funk Island	0.1670	**0.0058**	0.6126	**0.0020**	0.7085	0.0816	**<0.0001**	**0.0021**
	N. Grand Bank	0.7206	0.7746	0.3625	0.6696	0.1676	**<0.0001**	0.0996	0.0408
	Division 3O	0.9190	0.0880	**<0.0001**	0.2666	0.0306	**0.0030**	0.2454	0.4314
Placentia Bay and inshore samples									
Placentia	Notre Dame	0.4855	0.1066	0.4562	0.9895	**0.0053**	**<0.0001**	0.1076	0.0098
	Bonavista	0.4900	0.4292	0.05566	0.8402	**<0.0001**	0.4105	0.8418	**0.0040**
	Trinity	0.4562	0.1576	0.5419	0.4452	0.4162	0.0340	0.3927	0.6621
	Conception	0.2822	0.3462	0.3263	0.1895	0.1322	0.0676	0.0127	0.0308

the movement of cod from offshore into Placentia Bay. Allele frequencies were therefore compared between the Placentia Bay sample and offshore spawning samples in 2J+3KL and the Flemish Cap. There was little similarity between the Placentia Bay and offshore northern cod samples, with significant genetic differentiation at 1–4 loci observed between the Placentia Bay sample and the offshore Newfoundland samples examined (Table 6). The genetic evidence is not consistent with the hypothesis of extensive movement of cod from offshore in 2J+3KL into Placentia Bay.

Distinctiveness of the Gilbert Bay population was apparent through analysis of CSE distance (Figure 2). The distinctiveness of the rather small Belle Isle Bank sample (n=10) likely reflects both geographic differentiation and sampling error. The southern Grand Bank and Flemish Cap samples were distinct from samples from other locations. There was little differentiation between inshore cod from the four bays off northeastern Newfoundland and cod from Subdivision 3Ps (Figure 2).

Isolation by distance

The importance of geographic separation in accounting for genetic differentiation among populations was

Table 7. Hierarchical gene-diversity analysis of four putative populations of Atlantic cod along the southern coast of Newfoundland in Subdivision 3Ps (Fortune Bay, Placentia Bay, Burgeo Bank, and Halibut Channel) for seven microsatellite loci and the PanI locus. The relative diversity attributable to sampling years within populations and among populations are indicated, as well as the ratio of among population vs. among years within population diversity.

	Absolute diversity		Relative diversity			
Locus	Total	Within populations	Within populations	Among years within populations	Among populations	Populations/ years
*Gmo*3	0.3316	0.3305	0.9966	0.0034	0.0000	0.0
*Gmo*8	0.9169	0.9163	0.9993	0.0007	0.0000	0.0
*Gmo*19	0.9359	0.9349	0.9989	0.0011	0.0000	0.0
*Gmo*34	0.4605	0.4596	0.9980	0.0007	0.0013	1.9
*Gmo*35	0.7673	0.7663	0.9987	0.0011	0.0002	0.2
*Gmo*36	0.6027	0.6010	0.9971	0.0029	0.0000	0.0
*Gmo*37	0.8641	0.8612	0.9967	0.0033	0.0000	0.0
PanI	0.4716	0.4581	0.9714	0.0286	0.0000	0.0
All			0.9959	0.0041	0.0000	0.0

Table 8. Hierarchical gene-diversity analysis of seven regional groups: Labrador (Gilbert Bay), Northeast inshore Newfoundland (Conception Bay, Trinity Bay, Bonavista Bay, and Notre Dame Bay), southern offshore (Northern Grand Bank, Funk Island Bank), and northern offshore (Belle Isle Bank, Hawke Channel, Division 2G), southern Newfoundland (Fortune Bay, Placentia Bay, Burgeo Bank, Halibut Channel, Subdivision 3Pn), southern Grand Bank (Divisions 3N and 3O), and the Flemish Cap (Flemish Cap), constituting 18 putative populations of cod in the northwest Atlantic for seven microsatellite loci and the PanI locus. The relative diversity attributable to sampling years within populations, among populations within regions, and among regions are indicated, as well as the ratio of among population and region vs. among years within population diversity.

	Absolute diversity		Relative diversity				
Locus	Total	Within populations	Within populations	Among years within populations	Among populations within regions	Among regions	Populations +regions/ years
*Gmo*3	0.3401	0.3359	0.9877	0.0039	0.0000	0.0084	2.2
*Gmo*8	0.9163	0.9036	0.9861	0.0001	0.0040	0.0098	138.0
*Gmo*19	0.9352	0.9258	0.9899	0.0001	0.0036	0.0064	100.0
*Gmo*34	0.4646	0.4482	0.9647	0.0000	0.0049	0.0304	—
*Gmo*35	0.7713	0.7674	0.9949	0.0000	0.0049	0.0002	—
*Gmo*36	0.5964	0.5914	0.9916	0.0004	0.0039	0.0041	20.0
*Gmo*37	0.8628	0.8411	0.9749	0.0048	0.0167	0.0036	4.2
PanI	0.4593	0.4195	0.9133	0.0072	0.0000	0.0795	11.0
All			0.9799	0.0017	0.0045	0.0139	10.8

evaluated by examination of the correlation between pairwise linearized F_{ST} values based on the seven microsatellite loci surveyed and the pairwise distance (km) between samples. The distance between samples was determined as the most direct migration route, which included distances around all the headlands to make the values more biologically meaningful. For all samples, the positive correlation between F_{ST} and geographic distance was significant ($r=0.09$, Mantel's test, $p<0.05$). However, the Gilbert Bay population was very distinct, and F_{ST} values incorporating this population clustered separately from all other comparisons (Figure 3). When the Gilbert Bay sample was removed from the analysis, the positive correlation increased ($r=0.30$, $p<0.01$). Cod from more distant locations tend to be more distinct genetically than cod from locations nearby.

Discussion

A requirement for development of genetic differentiation among putative cod stocks is fidelity to specific spawning locations, with a resulting restriction in gene flow

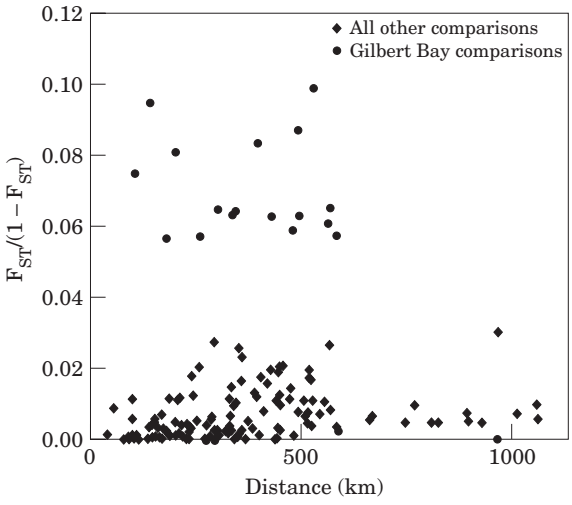

Figure 3. Relationship between pairwise F_{ST} and pairwise distance for cod from 18 locations in the northwest Atlantic adjacent to Newfoundland and Labrador.

among stocks. Most samples examined in the current study, particularly inshore ones, were adult fish collected during the spring spawning season, when stocks should have returned to their spawning sites from overwintering locations. Single spawning populations appear to have been sampled, with the exception of the Burgeo Bank and southern Grand Bank (Division 3O) samples. One component of the Burgeo Bank sample was collected between 6 and 7 April 1998, and this may have contained an admixture of populations, prior to their return to separate spawning sites. Tagging of fish captured on Burgeo Bank, conducted at the same time as the DNA sample collection, indicated that some fish subsequently moved northwest into the Gulf of St Lawrence, whereas others moved northeast to the south coast of Newfoundland (Brattey *et al*., 2000). Ten locations were sampled in at least two years, and the limited evaluation of annual variation in allele frequencies within sampling locations relative to differentiation among all sampling locations indicated substantially greater genetic differentiation among sampling locations than among sampling years within locations. Temporal stability of microsatellite variation in cod has also been observed by Ruzzante *et al*. (1997). Demonstration that population differentiation is persistent over time increases the likelihood that the appropriate population structure has been elucidated (Waples, 1998).

At PanI, there were significant departures from the expected Hardy–Weinberg distribution of genotypic frequencies. In each case, there was an excess of heterozygotes, indicating that balancing selection favouring heterozygotes may operate at PanI in some populations. In Icelandic cod, observed frequencies of heterozygotes were in most cases higher than those expected under Hardy–Weinberg equilibrium, but the discrepancies were not significant (Jónsdóttir *et al*., 1999). If the locus is under selection, it may be that selection intensity is higher in populations adjacent to Newfoundland than in those adjacent to Iceland. Fevolden and Pogson (1997) reported that the frequencies of the 475 bp fragment at PanI were substantially higher in northeast Arctic (offshore) cod than they were in coastal (inshore) cod. Similar results were observed in our study, with the frequency of the 475 bp fragment 0.90 in the most offshore population (Flemish Cap), whereas in the most inshore population (Gilbert Bay), the frequency of the same fragment was zero. Offshore cod populations were characterized by having higher frequencies of the 475 bp fragment than inshore populations. There may be some selective advantage associated with the 475 bp fragment in offshore environments. Cod off south Iceland have also been reported to be distinct at this locus, with the differentiation stable annually (Jónsdóttir *et al*., 2001).

The question is still whether the "bay" stocks of cod in Divisions 3KL are genetically distinct. Inshore, our results and those from a previous study that used a different group of microsatellite loci (Ruzzante *et al*., 2000b) revealed the Gilbert Bay population to be distinct. The earlier survey indicated no differentiation among cod from Trinity Bay, St Anthony Basin, and the Notre Dame Channel (Ruzzante *et al*., 1998), although sample sizes were small relative to the number of alleles present, decreasing the power of the tests to detect differentiation. Our study, based on larger sample sizes and more extensive geographic coverage, also provides no evidence of more than one stock of cod in adjacent bays along the northeast coast of Newfoundland. Recent tagging studies indicate extensive movement of cod between adjacent bays around insular Newfoundland, including those on the south coast (Brattey *et al*., 2000; Lilly *et al*., 2001). Levels of migration (resulting in gene flow) among cod spawning around insular Newfoundland are apparently sufficiently high to preclude genetic differentiation, except between the most geographically separated bays. However, there was clear genetic differentiation between inshore cod populations along the northeast coast of Newfoundland and the inshore population from Gilbert Bay, Labrador. The relationship between the Placentia Bay population and offshore-spawning populations of northern cod seems to be clearer. Significant genetic differentiation between the Placentia Bay population and the offshore populations was observed at 3–6 loci in comparisons with five offshore populations surveyed, suggesting that the high catch rates and abundance of cod in Placentia Bay in recent years (Brattey *et al*., 2000) are not the result of offshore-spawning cod from the Newfoundland shelf migrating inshore and subsequently breeding and remaining in Placentia Bay. Increased

abundance of cod in Placentia Bay is likely the result of local recruitment.

A further question is whether there are genetically distinct "inshore" and "offshore" stocks of cod in Divisions 2J+3KL. The microsatellite loci and PanI surveyed in the current study indicate that offshore populations were genetically distinct from most inshore populations. Genetic differentiation at microsatellite loci between inshore and offshore cod in Divisions 2J+3KL was previously observed by Ruzzante *et al.* (1996) at one of five microsatellite loci surveyed. The microsatellite loci and the pantophysin locus were able to detect genetic differentiation on a finer geographic scale than was apparent in a survey of mitochondrial DNA variation (Pepin and Carr, 1993; Carr *et al.*, 1995). However, our results are more consistent with an isolation-by-distance population structure for northern cod than a strict inshore–offshore division. There were no differences between samples from Trinity Bay and the northern Grand Bank, but differentiation was generally greater between more northern offshore populations and the inshore populations along the northeast coast of Newfoundland. Furthermore, the microsatellite data in the current study and those of Bentzen *et al.* (1996) and Ruzzante *et al.* (1998), as well as the tagging data of Lear (1984) and Taggart (1997) all suggest that there is more than one offshore spawning stock. Although the current data indicate that there could be at least three spawning stocks of cod offshore in Divisions 2J+3KL, the samples were collected during autumn and their exact spawning locations are not known. More intensive sampling of spawning cod there is required before any definitive conclusions can be drawn, but sizes of offshore spawning populations are currently so low that it remains very difficult to obtain adequate samples.

Whether the inshore spawning stock can contribute to rebuilding the offshore spawning stock is a topical question. Given the population substructure we have detected between most inshore and offshore areas, and among offshore areas themselves, the likelihood that the inshore-spawning stock will contribute to offshore recovery is low. Even a small number of migrants per generation would eliminate the population structure we have detected. Unless the patterns of migration between the inshore- and offshore-spawning cod changed greatly in recent years, our results are more consistent with the notion that any rebuilding of offshore spawning groups can only result from a resurgence of the remnant offshore stock itself. Similarly, it is unlikely that the Flemish Cap population will contribute significantly to recovery of the other offshore populations, given its degree of genetic differentiation. For the microsatellite loci, such differentiation indicates a significant restriction of gene flow between cod of the two areas. At the PanI locus, selection may have contributed to the observed differences in allelic frequencies, providing an example of the adaptive differentiation that can occur between stocks once gene flow between them is reduced. Tagging data also indicate little exchange between the Flemish Cap and offshore populations (Taggart *et al.*, 1995).

In conclusion, the results of the current study indicate considerable genetic heterogeneity among cod sampled from various locations around Newfoundland and Labrador. They are not consistent with a panmictic model for cod population structure in this region, and therefore generally agree with the conclusions of previous studies that have used microsatellites to elucidate population structure of cod in the Northwest Atlantic (Bentzen *et al.*, 1996; Ruzzante *et al.*, 1998; Taggart *et al.*, 1998). However, our findings are more consistent with an isolation-by-distance population structure for cod in Newfoundland waters, rather than a strict inshore-offshore division (Ruzzante *et al.*, 1996, 1997), and they do not support the suggestion that separate "bay" stocks exist in adjacent bays off the northeast coast of Newfoundland (Ruzzante *et al.*, 2000b). Although the uniqueness of cod in Gilbert Bay is confirmed herein, we found no evidence that cod in adjacent bays are genetically distinct; only the most geographically separated bays show evidence of significant genetic differences. Finally, the results of the current study do not support the hypothesis of multiple stock structure among cod in NAFO Subdivision 3Ps off the south coast of Newfoundland (Ruzzante *et al.*, 2000a), although there is some evidence that mixed stocks were sampled in the most western portion of this management unit.

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