

Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic

Paul Bentzen, Christopher T. Taggart, Daniel E. Ruzzante, and Doug Cook

Abstract: Allelic variation in six highly polymorphic microsatellite loci (mean heterozygosity, 86%) provided evidence that cod (*Gadus morhua*) in the northwest Atlantic belong to multiple genetically distinguishable populations, and further that genetic differences may also exist between northwestern and southeastern cod aggregations within the northern cod stock complex off Newfoundland. Cod were sampled from winter aggregations ranging from Hamilton Bank to the northern Grand Bank in the northern cod complex, and from the Flemish Cap, the Scotian Shelf, and the Barents Sea. Tests of allele frequency homogeneity (χ^2), F_{ST} , and $(\delta\mu)^2$, allele sharing, and Rogers' distance measures revealed significant differences among northern cod, Flemish Cap, Scotian Shelf, and Barents Sea samples. Within the northern cod complex, two pooled samples, NORTH (Hamilton, Funk, and Belle Isle banks) and SOUTH (northern Grand Bank area), were distinguishable using χ^2 , $(\delta\mu)^2$, and allele sharing measures. Both $(\delta\mu)^2$ and Rogers' distances clustered western Atlantic samples in two groups distinct from the divergent Barents Sea sample; one comprised NORTH, SOUTH, and Scotian Shelf, and the other, Flemish Cap.

Résumé : La variation allélique de six loci de microsatellites fortement polymorphes (hétérozygotie moyenne de 86%) a mis en évidence le fait que les morues (*Gadus morhua*) du nord-ouest de l'Atlantique appartiennent à de multiples populations génétiquement distinctes, et qu'il peut en outre exister des différences génétiques entre les concentrations de morues du nord-ouest et du sud-est dans le complexe de stocks de morue du nord situé près de Terre-Neuve. Des morues ont été échantillonnées dans le complexe de stocks de morue du nord, du banc Hamilton au nord du Grand Banc; sur le Bonnet flamand; sur la plate-forme néo-écossaise; enfin, en mer de Barents. Les tests de l'homogénéité de la fréquence allélique (χ^2), de F_{ST} et des distances $(\delta\mu)^2$, du partage des allèles et de Rogers ont révélé des différences importantes entre les échantillons du complexe de morue du nord, du Bonnet flamand, de la plate-forme néo-écossaise et de la mer de Barents. Dans le complexe de morue du nord, deux échantillons combinés, NORTH (bancs Hamilton, Funk et de Belle-Isle) et SUD (région du nord du Grand Banc) se distinguaient grâce au calcul de χ^2 , de $(\delta\mu)^2$ et du partage des allèles. Le calcul des distances $(\delta\mu)^2$ et de Rogers regroupaient les échantillons de l'ouest de l'Atlantique en deux groupes distincts de l'échantillon particulier de la mer de Barents; l'un rassemblait les échantillons NORTH, SUD et néo-écossais, l'autre le Bonnet flamand.

[Traduit par la Rédaction]

Introduction

Despite considerable study in the past, basic features of the population structure of Atlantic cod (*Gadus morhua*) remain controversial. Although spawning areas for cod in many parts of its North Atlantic range are known to be relatively discrete, stable over time, and often relatively distant from other spawning areas (Brander 1994; Serchuck et al. 1994; Frank et al. 1994; Taggart et al. 1994; Buch et al. 1994; Schopka 1994), whether or not such spawning aggregations constitute reproductively and genetically distinct populations remains uncertain.

Proper resolution of these uncertainties is important for a detailed understanding of the biology of the species and ultimately for successful management (Angel et al. 1994). These issues are exemplified by the seriously depleted northern cod stock complex (Northwest Atlantic Fisheries Organization (NAFO) divisions 2J, 3K, and 3L).

The distribution and migration patterns of northern cod are complex. Most mature northern cod overwinter in warm (>2°C) slope water along the margin of the continental shelf, but some remain inshore all winter, relying on serum antifreeze proteins to survive in cold (<0°C) coastal water (Goddard et al. 1994). Spawning occurs over a 3- to 4-month period from winter through early summer, and generally begins earlier in the north than in the south (Myers et al. 1993). Spawning takes place in offshore bank areas and along the continental shelf break, but some also occurs in the interior of the Grand Bank and in coastal regions (reviewed in Taggart et al. 1994). Cod that have overwintered and spawned offshore migrate inshore in the spring to coastal feeding grounds, then return offshore in late autumn – early winter (Templeman 1966). Juvenile cod may remain inshore for several years before adopting the migratory behaviour of mature cod around age 4 (Lear and Green 1984; Rose 1993).

Existing data on population structure within the northern cod are equivocal. On the one hand, geographic surveys

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P. Bentzen.¹ Marine Molecular Biotechnology Laboratory, University of Washington, 3707 Brooklyn Avenue Northeast, Seattle, WA 98105-6715, U.S.A.

C.T. Taggart. Department of Oceanography, Dalhousie University, Halifax, NS B3H 4J1, Canada.

D.E. Ruzzante. Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.

D. Cook. Marine Gene Probe Laboratory, Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.

¹ Author to whom all correspondence should be addressed.
e-mail: pbentzen@fish.washington.edu

(Hutchings et al. 1993), vertebral data (Templeman 1981; Lear and Wells 1984), and tag recovery data (Templeman 1974, 1979; Lear 1984; reviewed in Lear and Green 1984 and Taggart et al. 1995) all suggest that northern cod are divided into several distinct offshore spawning units; on the other hand, none of these observations constitute evidence of reproductive isolation among spawning units. Cod-tagging studies have provided evidence of population fidelity to particular overwintering-spawning areas on offshore banks and in some inshore bays, as well as evidence of vagrant movements among these areas (Lear 1984; Taggart et al. 1995; Wroblewski et al. 1996). However, tag recovery data yield no direct evidence of the extent of gene flow among spawning units.

In theory, allele frequency data from neutral genetic markers may provide the most stringent test of population differentiation. Thus far, however, evidence of population structure gained from genetic studies of northern and other western Atlantic cod has been mixed, and subjected to various interpretations. Data from a variety of allozyme (Mork et al. 1985) and mitochondrial DNA (mtDNA) studies (Smith et al. 1989; Carr and Marshall 1991*a*, 1991*b*; Pepin and Carr 1993; Carr 1995; Carr et al. 1995) have not permitted rejection of the null hypothesis that no genetic differentiation exists among various samples drawn from northwest Atlantic populations. In contrast, highly significant differences among populations have been detected with a small number of protein loci (Jamieson 1975; Cross and Payne 1978), more recently with a suite of nuclear DNA restriction fragment length polymorphism (RFLP) loci (Pogson et al. 1995), and most recently with nuclear microsatellite loci (Ruzzante et al. 1996*a*).

Several authors have invoked selection (non-neutrality of genetic loci) as an explanation for the contrast in the results obtained with different genetic markers, but differ in their view of the direction taken by selective forces. Some (e.g., Mork and Sundnes 1985; Mork et al. 1985) have suggested that selection is acting to increase heterogeneity at some loci (thereby exaggerating the distinctiveness of populations), whereas Pogson et al. (1995) have argued that balancing selection may be acting to homogenize variation expressed by some protein loci across different locales (thereby underrepresenting the isolation of populations).

Notwithstanding the possible role of selection, loci that exhibit significant geographic variation in cod have tended to exhibit moderate to high levels of polymorphism, whereas those that do not have tended to be less polymorphic, with low heterozygosities and (or) low frequency of variant alleles or haplotypes (Jamieson 1975; Cross and Payne 1978; Mork et al. 1985; Smith et al. 1989; Carr and Marshall 1991*a*; Pepin and Carr 1993; Carr 1995; Carr et al. 1995; Pogson et al. 1995). This would suggest that studies of cod population structure at less than ocean basin scales might be more informative if highly polymorphic, and preferably neutral, markers are used.

We have initiated such studies utilizing highly polymorphic microsatellite markers. The attributes of microsatellites as genetic markers and their application to fisheries have been reviewed elsewhere (Park and Moran 1994; Wright and Bentzen 1994; O'Reilly and Wright 1995). Briefly, microsatellites are repetitive arrays of di-, tri-, or tetra-nucleotide sequences in the nuclear genome. They exhibit high levels of allelic polymorphism in repeat number, are presumed to be selectively neutral (but see Warren 1996), and are readily assayed from

small amounts of fresh or preserved tissue using the polymerase chain reaction (PCR). Recent studies employing microsatellites have detected high levels of genetic variation in cod (Brooker et al. 1994; Ruzzante et al. 1996*a*), as well as genetic differences among some moderately distant locations in the western Atlantic (S.K. McConnell, Department of Biology, Dalhousie University, Halifax, N.S., personal communication) and even between northern cod aggregations overwintering inshore and offshore spawning aggregations (Ruzzante et al. 1996*a*).

Here we report on polymorphism at six microsatellite loci in northern cod sampled from offshore overwintering-spawning locations spanning their range from the northern Grand Bank northwest to Hamilton Bank off Labrador, as well as from two other northwest Atlantic locations, Flemish Cap and the Scotian Shelf, and from one northeast Atlantic location in the Barents Sea. We report results that lead us to reject the null hypothesis of no genetic differentiation among cod from various northwest Atlantic regions, and hence to conclude that northwest Atlantic cod belong to multiple, genetically distinguishable populations. Further, we describe evidence that northern cod may comprise more than one population. We also use the results of our study to address questions concerning the relative utility of microsatellites as genetic markers for cod, and the various approaches that can be taken in analyzing microsatellite data.

Materials and methods

Sample collections

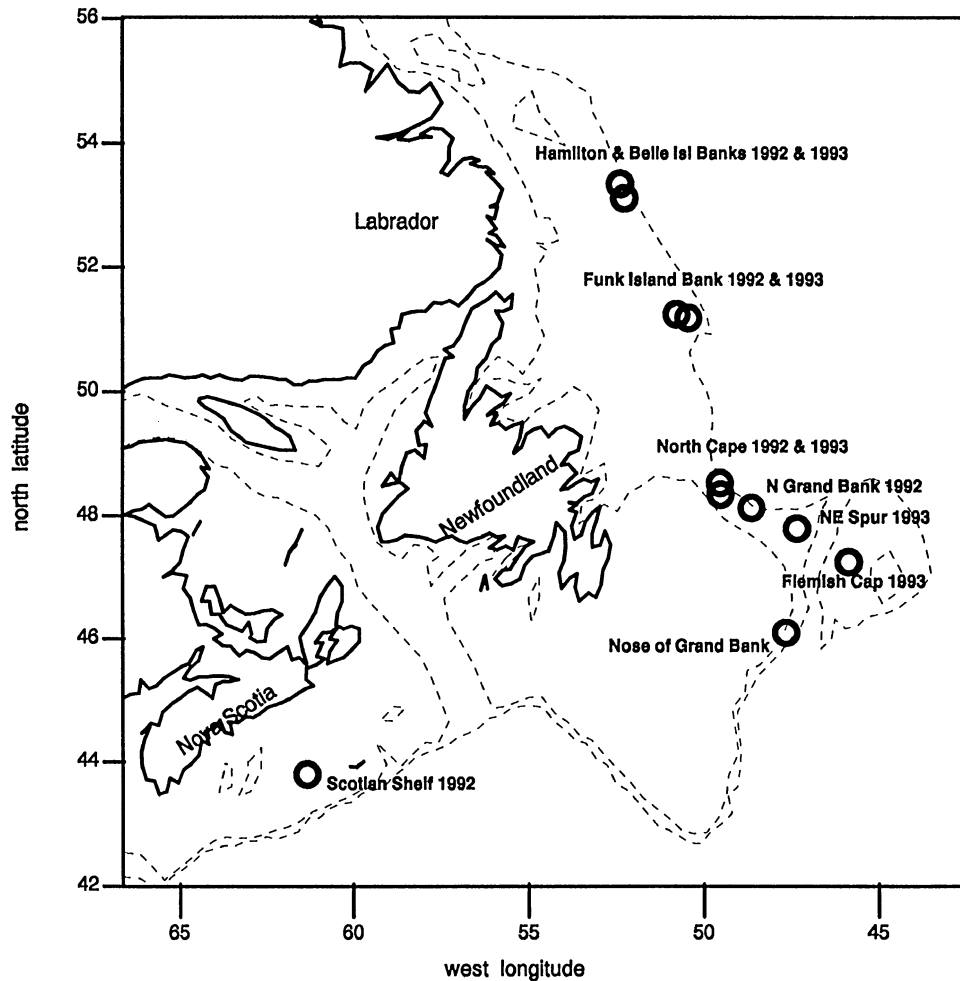
Northern cod were collected during January and February of 1992 and 1993 from nine different sample populations stretching from Hamilton Bank in the north to the "Nose" of the Grand Bank in the south (Fig. 1, Table 1). These samples were collected from prespawning aggregations using an otter trawl fishing at depths between 300 and 500 m. Tissue samples of cod from the Flemish Cap (Fig. 1, Table 1) were secured in the summer of 1993 from the commercial trawl fishery bycatch (nominal depths of 30 m) and were collected by onboard fisheries observers retained by the Canada Department of Fisheries and Oceans. Samples from the Scotian Shelf (Fig. 1, Table 1) comprised recently hatched cod larvae collected within a gyrelike water mass on Western Bank in November of 1992 (Taggart et al. 1996; Ruzzante et al. 1996*b*). Tissue samples of Barents Sea cod were taken from frozen cod heads that were off-loaded from a Russian freezer trawler and were provided by National Sea Products Ltd., Arnolds Cove, Newfoundland, in July 1993 (lot No. 686). The fish had been captured some time during the first half of 1993 at an unknown location in the Barents Sea (International Council for the Exploration of the Seas (ICES) area I).

The average fork lengths of cod were highly variable among samples (Table 1) and (excluding the larval cod sample) ranged from 30 cm (Nose of the Grand Bank in 1993) to 60 cm (Flemish Cap in 1993). The estimated ages of these cod (derived from length at age relationships) were equally variable (Table 1) and ranged from 2 to 6 years. The sex ratio of the analyzed samples was approximately 1:1 for samples where the sex of the individual was known, although the majority of samples were from immature or first time spawning fish (Table 1).

Sample tissues and genetic analyses

Blood and muscle from juvenile and mature cod and eye tissues from larval cod were used as the DNA source for genetic analysis. Blood samples were collected from live, or recently dead, cod using a sterile 2-mL hypodermic needle (21 gauge) inserted ventrally into the blood vessels that run in the haemal arches of the vertebrae between the anal fin and the caudal peduncle, and ~1 mL of blood was withdrawn and

Fig. 1. Bathymetric chart (200- and 1000-m isobaths) of the northwest Atlantic shelf regions showing the dates and bank affiliations for the cod samples collected in 1992 and 1993 and used for microsatellite analyses.



preserved immediately in 5 mL of 95% ethanol and stored. Muscle tissue samples for genetic analysis were taken from the anterior of the tongue of dead cod and preserved in 95% ethanol. Larvae were preserved in liquid nitrogen immediately following collection at sea (Taggart et al. 1996). DNA extraction from alcohol-preserved tissue was as described in Bentzen et al. (1990) and Ruzzante et al. (1996a). DNA was released from eyeballs of larval cod as described in Ruzzante et al. (1996b).

PCR was carried out on 1–2 μ L of purified or crude cod DNA as previously described by Brooker et al. (1994) using six sets of cod microsatellite primers: Gmo2, Gmo132, and Gmo145 (Brooker et al. 1994), Gmo4 (Wright 1993), Gmo120 (Ruzzante et al. 1996a), and Gmo141 (Ruzzante et al. 1996b). Gmo2, Gmo4, Gmo120, Gmo132, and Gmo141 are perfect GT repeats, and Gmo145 is a compound $G_x(GA)_x$ repeat as defined by Tautz (1989). The PCR products were resolved on 6.5% denaturing polyacrylamide gels and the alleles sized relative to a sequence ladder generated from M13mp18.

Data analyses

Tests for departures from Hardy–Weinberg equilibrium (HWE) and deficiencies or excess of heterozygotes, and tests for heterogeneity in allele frequencies among samples were conducted using the χ^2 pseudoprobability programs CHIHW and CHIRXC (Zaykin and Pudovkin 1993). Both programs use randomization procedures to estimate the significance of the χ^2 statistic (Roff and Bentzen 1989). In all cases, 1000 randomizations were used to estimate probability (P) values.

Genetic distances among population samples were estimated using Rogers' (Rogers 1972), allele sharing (Bowcock et al. 1994), average squared (Goldstein et al. 1995a), and $(\delta\mu)^2$ (Goldstein et al. 1995b) measures. Estimates of subpopulation structure were obtained using F_{ST} following Weir and Cockerham (1984) and the related R_{st} measure recently proposed for microsatellite data by Slatkin (1995). Significance values for estimates of genetic distance and subpopulation structure were determined as described in Ruzzante et al. (1996b) using Monte Carlo simulations and the bootstrap method (minimum 1000 resampling trials per individual comparison; Manly 1991), and critical significance levels for simultaneous statistical tests were assessed using sequential Bonferroni adjustments as outlined in Rice (1989).

The genetic relationships among cod populations were evaluated using the various genetic distance measures listed above as inputs for neighbour-joining and Fitch–Margoliash trees constructed using programs in the PHYLIP software package (version 3.5C; Felsenstein 1994). As these two methods of tree construction yielded the same topologies only the Fitch–Margoliash trees are presented here.

Results

Variability of microsatellite loci within and among populations

All six microsatellite loci surveyed were highly polymorphic

Table1. Cod sample collection statistics.

Collection location and date	<i>N</i>	Mean location ^a		Length (cm)		Estimated age (years)		Number mature
		Latitude	Longitude	Range	Mean	Range	Mean	
Hamilton and Belle Isle banks, 1992	8	53.31	-52.38	25-48	33.4	1.7-4.4	2.7	3
Funk Island Bank, 1992	30	51.25	-50.51	28-51	41.0	2.0-4.8	3.5	12
North Cape, 1992	84	48.49	-49.46	40-75	53.9	3.4-8.2	5.2	21
Northern Grand Bank, 1992	56	48.11	-48.50	25-63	40.0	1.7-6.4	3.4	20
Belle Isle Bank, 1993	30	53.11	-52.17	27-43	34.9	1.9-3.7	2.8	16
Funk Island Bank, 1993	60	51.22	-50.76	21-48	33.6	1.4-4.4	2.7	14
North Cape, 1993	30	48.4	-49.5	21-47	34.3	1.4-2.4	2.8	7
Northeast Spur, 1993	30	47.79	-47.26	23-58	32.0	1.5-5.7	2.5	4
Nose of Grand Bank, 1993	30	46.09	-47.59	23-69	30.1	1.5-7.3	2.3	1
Barents Sea, 1993	100	—	—	43-82	57.0	3.0-9.0	5.2	—
Flemish Cap, 1993	51	47.25	-45.75	38-94	60.1	3.1-11.4	6.1	3
Scotian Shelf, 1992	193	43.75	-61.35	Larvae	—	<30 days	—	0

^aDecimal degrees.

in all cod populations. The number of alleles observed per locus ranged from 24 for Gmo132 to 58 for Gmo141 (40.8 ± 14.9 (mean \pm SD); Table 2). Observed heterozygosities (H_o) were correspondingly high, ranging from 0.369 for Gmo132 in the Barents Sea sample to 1.0 for Gmo4, Gmo120, Gmo141, and Gmo145 in several populations (mean = 0.86; Table 3). In all cases variation among the microsatellite alleles occurred in size increments consisting of multiples of two base pairs (bp).

We pooled some samples of northern cod to facilitate further statistical analyses, including broader geographic comparisons. Pairwise comparisons using χ^2 pseudoprobability tests were made first across sampling years at the same location, and then among geographically related sampling areas. No significant differences in allele frequencies were found among any of the six microsatellites among the 1992 and 1993 samples from Hamilton, Belle Isle, and Funk Island banks; hence, these samples were pooled to form a single sample (hereafter, NORTH). Similar analyses revealed no significant differences among the 1992 and 1993 samples from the North Cape, Grand Bank, and Nose of the Bank, so these samples were combined to form a single pooled sample (SOUTH). A sample from the northeast Spur area located between the northern Grand Bank and the Flemish Cap did differ from SOUTH in allele frequencies for Gmo141 ($P = 0.005$; Table 4) and consequently was excluded from the pooled SOUTH sample.

Pseudoprobability tests revealed no significant departures from HWE for the pooled NORTH, pooled SOUTH, or Scotian Shelf samples (Table 3). Pseudoprobability tests also indicated that measures of heterozygote excess or deficiency (D) did not deviate significantly from zero for five of the microsatellites in all populations. The exception was Gmo141, for which D was negative (indicating a deficiency of heterozygotes) in five of six population samples; four of these departures were significant (Table 3).

Geographic variation in alleles: size and frequency

The size-frequency distributions of microsatellite alleles varied among NORTH, SOUTH, and the four unpooled samples (Table 3, Fig. 2). Variation in mean allele size was significant (Kruskal-Wallis test, $P \leq 0.006$) for all loci except Gmo2 among the six populations (Tables 2, 3).

Pairwise pseudoprobability tests revealed significant differences in allele frequencies at one or more loci for each pair of

Table2. Single locus statistics for cod microsatellites.

Locus	<i>N</i>	<i>n</i>	H_e	<i>P</i>
Gmo2	664	25	0.803	0.123
Gmo4	646	57	0.957	<0.001*
Gmo120	622	44	0.953	0.006*
Gmo132	672	24	0.769	<0.001*
Gmo141	640	58	0.97	<0.001*
Gmo145	647	37	0.938	<0.001*
Mean	648.5	40.8	0.898	—

Note: *N*, number of individuals; *n*, number of alleles; H_e , expected heterozygosity; *P*, probability that mean allele size is identical among NORTH, SOUTH, northeast Spur, Flemish Cap, Scotian Shelf, and Barents Sea samples, determined using Kruskal-Wallis tests (5 df). Tests considered significant following adjustment for six simultaneous tests ($\alpha = 0.008$) are indicated with an asterisk.

populations, with the exception of the NORTH versus northeast Spur comparison (Table 4). Pairwise comparisons involving the Barents Sea sample were most likely to be significant: 16 of 30 comparisons involving this population revealed significant differences in allele frequencies. With one exception, Gmo120, all six microsatellites exhibited significant heterogeneity in allele frequencies in at least some pairwise comparisons.

Broad-scale geographic variation was strongest for Gmo132 (Fig. 2). Although the allele distributions for NORTH, SOUTH, and northeast Spur were very similar ($P \geq 0.133$, Table 4), they differed sharply from those seen in other populations. In the three northern cod samples (north, south, and northeast Spur) the allele distribution for Gmo132 consisted of a single mode dominated by two alleles of 111 and 117 bp at 24-28 and 38-44% frequency, respectively. In contrast, the frequencies of the 111- and 117-bp alleles were 76 and 5% in the Barents Sea sample, and 55 and 19% in the Flemish Cap sample. The Scotian Shelf sample exhibited multiple modes of alleles at this locus, separated from each other by intervals of approximately 20 bp. One of these modes, dominated by 111- and 117-bp alleles, corresponded to the only mode seen in the other populations. The shape of this mode was similar to that seen in the northern cod samples. The two modal allele groups unique to the Scotian Shelf sample comprised alleles close in size to 137 and 157 bp, respectively, with a collective frequency of 22.1%.

Table 3. Summary statistics for genetic variation in Atlantic cod population samples.

	Northern cod			Scotian Shelf	Flemish Cap	Barents Sea
	Northeast Spur	NORTH	SOUTH			
			Gmo2			
<i>N</i>	28	119	195	193	50	79
<i>n</i>	7	18	20	20	12	17
Allele size	110.83	111.90	112.54	111.91	112.14	113.39
H_o	0.679	0.798	0.749	0.725	0.92	0.759
H_e	0.764	0.794	0.805	0.773	0.827	0.842
<i>D</i>	-0.112	0.005	-0.07	-0.062	0.113	-0.097
<i>P</i> (HW)	—	0.386	0.757	0.681	—	—
<i>P</i> (D)	0.345	0.797	0.031	0.074	0.11	0.037
			Gmo4			
<i>N</i>	28	116	187	191	44	80
<i>n</i>	2	37	40	40	24	31
Allele size	205.46	208.32	212.63	209.88	203.42	211.18
H_o	0.929	0.966	0.925	0.958	0.864	0.963
H_e	0.926	0.955	0.953	0.957	0.938	0.939
<i>D</i>	0.003	0.011	-0.03	0.001	-0.079	0.026
<i>P</i> (HW)	—	0.985	0.98	0.977	—	—
<i>P</i> (D)	0.763	0.686	0.021	1	0.022	0.495
			Gmo120			
<i>N</i>	30	108	176	192	44	72
<i>n</i>	20	28	36	32	26	32
Allele size	189.55	188.47	189.85	187.67	191.72	187.51
H_o	0.967	0.972	0.92	0.938	0.841	0.972
H_e	0.934	0.946	0.95	0.953	0.938	0.95
<i>D</i>	0.035	0.028	-0.031	-0.016	-0.104	0.023
<i>P</i> (HW)	—	0.985	0.98	0.977	—	—
<i>P</i> (D)	0.729	0.686	0.021	1	0.022	0.495
			Gmo132			
<i>N</i>	30	118	197	193	50	84
<i>n</i>	7	10	12	18	8	10
Allele size	115.325	114.887	114.912	120.434	113.660	111.858
H_o	0.767	0.729	0.766	0.824	0.62	0.369
H_e	0.746	0.723	0.751	0.797	0.646	0.414
<i>D</i>	0.028	0.008	0.021	0.033	-0.041	-0.109
<i>P</i> (HW)	—	0.356	0.116	0.341	—	—
<i>P</i> (D)	0.818	0.915	0.646	0.322	0.669	0.127
			Gmo141			
<i>N</i>	28	114	194	193	32	79
<i>n</i>	26	46	47	53	28	39
Allele size	168.05	174.26	172.44	172.91	173.15	181.16
H_o	0.75	0.93	0.897	0.933	0.844	0.975
H_e	0.947	0.966	0.967	0.967	0.952	0.96
<i>D</i>	-0.208	-0.037	-0.073	-0.036	-0.114	0.015
<i>P</i> (HW)	—	0.972	0.972	0.997	—	—
<i>P</i> (D)	<0.001*	0.021	<0.001*	0.009*	0.003*	0.617
			Gmo145			
<i>N</i>	28	118	191	189	47	74
<i>n</i>	19	25	31	31	21	22
Allele size	180.80	176.79	175.31	176.23	176.17	174.03
H_o	0.964	0.932	0.916	0.937	0.957	0.851
H_e	0.928	0.932	0.94	0.934	0.921	0.921
<i>D</i>	0.039	0.001	-0.025	0.002	0.04	-0.075
<i>P</i> (HW)	—	0.995	0.978	0.992	—	—
<i>P</i> (D)	0.591	1	0.152	0.88	0.464	0.011
Mean H_e	0.874	0.886	0.894	0.897	0.87	0.838
SE	0.038	0.042	0.038	0.036	0.049	0.086

Table3 (concluded).

	Northern cod			Scotian Shelf	Flemish Cap	Barents Sea
	Northeast Spur	NORTH	SOUTH			
Mean H_o	0.842	0.888	0.862	0.886	0.841	0.815
SE	0.051	0.041	0.033	0.038	0.048	0.096
Mean D	-0.036	0.003	-0.035	-0.013	-0.031	-0.036
SE	0.041	0.009	0.014	0.014	0.014	0.026

Note: N , number of individuals; n , number of alleles; H_o , observed number of heterozygotes; H_e , expected number of heterozygotes; D , measure of heterozygote deficiency or excess $((H_o - H_e)/H_e)$; $P(HW)$ and $P(D)$, estimated probability levels based on pseudoprobability tests. Allele sizes are given as the mean. Tests considered significant following sequential Bonferroni adjustment for six tests per locus (initial $\alpha = 0.008$) are indicated with an asterisk (*).

Table4. Probability of homogeneity of allele frequencies estimated from pairwise pseudoprobability tests.

Comparison		Gmo2	Gmo4	Gmo120	Gmo132	Gmo141	Gmo145
NORTH	SOUTH	0.552	<0.001*	0.443	0.643	0.195	0.214
NORTH	Northeast Spur	0.923	0.311	0.479	0.133	0.057	0.469
NORTH	Scotian	0.57	0.186	0.501	<0.001*	0.923	0.238
NORTH	Flemish	0.455	0.21	0.71	<0.001*	0.111	0.327
NORTH	Barents	0.002*	0.183	0.058	<0.001*	0.073	<0.001*
SOUTH	Northeast Spur	0.908	0.049	0.787	0.22	0.005*	0.321
SOUTH	Scotian	0.352	0.017	0.107	<0.001*	0.121	0.311
SOUTH	Flemish	0.229	<0.001*	0.269	<0.001*	0.032	0.789
SOUTH	Barents	<0.001*	<0.001*	0.024	<0.001*	<0.001*	0.001*
Northeast Spur	Scotian	0.822	0.217	0.208	0.066	0.017	0.323
Northeast Spur	Flemish	0.533	0.184	0.775	0.023	<0.001*	0.651
Northeast Spur	Barents	0.011	0.642	0.404	<0.001*	0.001*	0.001*
Scotian	Flemish	0.123	0.058	0.035	<0.001*	0.142	0.483
Scotian	Barents	<0.001*	0.001*	0.262	<0.001*	0.018	0.006
Flemish	Barents	0.707	0.012	0.405	<0.001*	0.002*	0.005

Note: Probability estimates considered significant following sequential Bonferroni adjustments for 15 simultaneous tests per locus (initial $\alpha = 0.003$) are indicated with an asterisk (*).

The heterogeneity of Gmo132 allele distributions across the range of populations examined was apparent in the results of the pairwise pseudoprobability tests (Table 4). Among a total of 15 pairwise comparisons between populations, 10 tests were significant for Gmo132, whereas the number of significant results obtained with the other five loci ranged from 0 to 5 (mean = 3).

Temporal variability

The majority (67%) of the cod in the northeast Spur sample were estimated to be from the 1990 year-class (age 2+ in 1993), and most of the remaining fish were from the 1989 and 1991 year-classes (17 and 10%, respectively; Fig. 3). In contrast, cod in the pooled SOUTH sample were broadly distributed among year-classes 1985–1990 (mean age = 5+ in 1993), with small contributions from 1991 and pre-1985 year-classes as well. Finally, we estimated that the pooled NORTH sample was composed primarily of the three year-classes 1988, 1989, and 1990 (17, 32, and 35, respectively), along with a small number of 1986, 1987, and 1991 fish.

We used χ^2 pseudoprobability tests to evaluate whether variation in allele frequencies among year-classes might confound the interpretation of genetic variation among geographic samples. Although the estimated year-class compositions of the northeast Spur, SOUTH, and NORTH samples differed from each other (Fig. 3), heterogeneity of allele frequencies among age-classes was not significant for any of the six microsatellite loci in a pooled sample of SOUTH and northeast

Spur fish (adjusted $\alpha = 0.008$, $P \geq 0.027$). Likewise, allele frequencies did not vary significantly for any of the loci among the 1989 and 1990 year-classes of cod in the NORTH sample ($P \geq 0.143$).

To further evaluate whether temporal variability of allele frequencies was a factor in our results, we split the NORTH and SOUTH pools into their 1992 and 1993 components, then conducted pairwise χ^2 pseudoprobability tests of allelic heterogeneity for Gmo4, the locus that differed between NORTH and SOUTH. Pairwise comparisons revealed significant differences in allele frequencies for NORTH92 versus SOUTH92, NORTH92 versus SOUTH93, and NORTH93 versus SOUTH93, but not for NORTH92 versus NORTH93, NORTH93 versus SOUTH92, and SOUTH92 versus SOUTH93 (Table 5).

Estimates of genetic distance

When applied to our cod data, four measures of genetic distance (allele sharing, $(\delta\mu)^2$, Rogers', and average squared) yielded results that varied markedly in at least two ways (Table 6). First, the number of pairwise distances that were significantly greater than zero differed among the measures. Among 10 pairwise comparisons, all 10 genetic distances were significantly greater than zero for the $(\delta\mu)^2$ and allele sharing measures. For the Rogers' and average squared distances, the corresponding numbers of significant tests were nine and zero, respectively (Table 6). The second difference was apparent when we summarized the pairwise distances using Fitch–

Fig. 2. Allele frequencies of microsatellite loci in cod populations. Shown are the absolute allele frequencies for SOUTH, and for other populations, the difference between the allele distribution in each population and the observed distribution in SOUTH (i.e., other-SOUTH). Absolute/relative frequencies and allele size in base pairs (bp) are given by the ordinate and abscissa, respectively, for each locus.

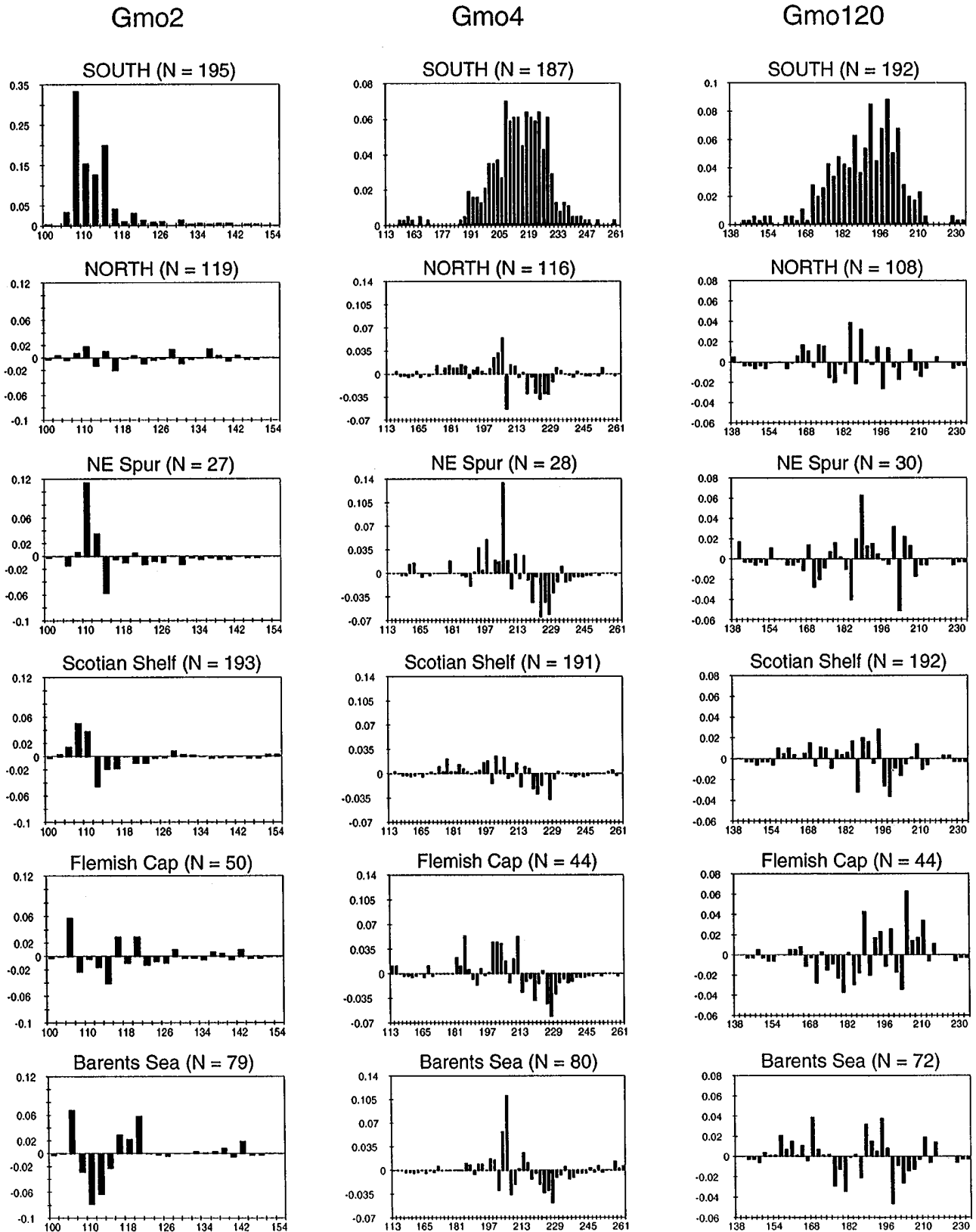


Fig. 2 (concluded).

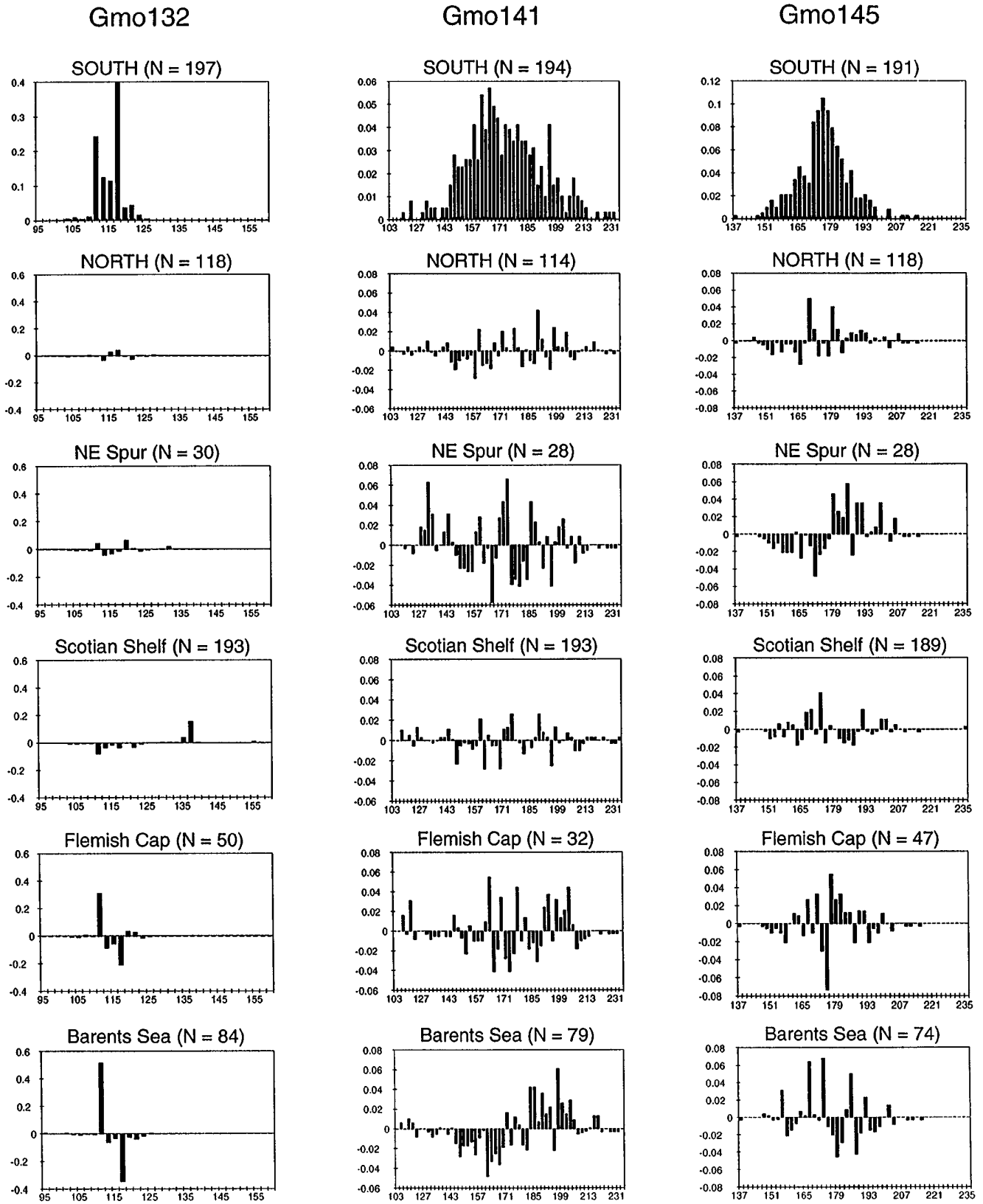
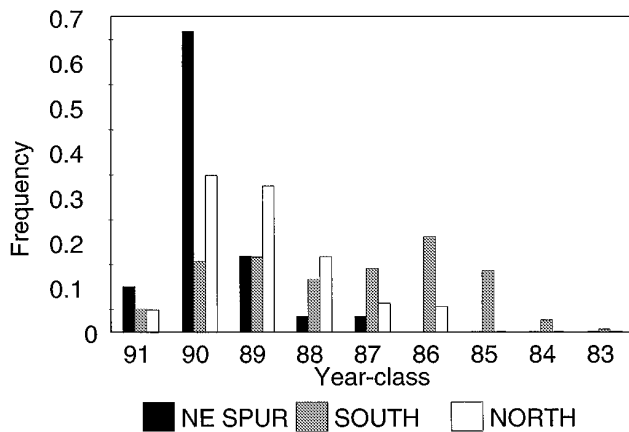


Fig. 3. Year-class contributions (distributions) for the northern cod samples as determined according to length at age estimates.



Margoliash trees. Both the $(\delta\mu)^2$ and the Rogers' tree aligned northwest Atlantic populations into two groups distinct from the relatively divergent Barents Sea sample (Figs. 4A and 4C, respectively). One group consisted of the NORTH, SOUTH, and Scotian Shelf samples, and the other consisted of the Flemish Cap sample. In contrast, the allele sharing tree presented little evidence of hierarchical population structure, with only a weak indication of a NORTH, SOUTH, Scotian Shelf cluster and no indication of divergence between the Barents Sea sample and the four western Atlantic samples (Fig. 4B). Finally, the average squared distance tree portrayed the four western and one eastern Atlantic population as an unresolved polytomy, with a single central node and terminal branches of similar length (Fig. 4D).

Estimates of population substructure

F_{ST} estimates calculated for six different populations ranged from 0.0 for Gmo120 to 0.092 for Gmo132 (Table 7). Except for Gmo2, all nonzero F_{ST} values were significant, as was the combined F_{ST} ($= 0.015$, $P < 0.001$). We found that excluding the small northeast Spur sample from the analysis had little overall effect (combined $F_{ST} = 0.016$, $P < 0.001$). F_{ST} values were larger between a pooled western Atlantic sample (NORTH, SOUTH, and Scotian Shelf, but excluding the Flemish Cap sample) and the Barents Sea ($F_{ST} = 0.037$, $P < 0.001$). On a finer geographic scale, F_{ST} values for a comparison between all northern cod samples and the Flemish Cap sample were only marginally smaller than in the larger scale six-population analysis, but only significant because of a large contribution from Gmo132 ($F_{ST} = 0.090$, $P < 0.001$). The overall F_{ST} between all northern cod and the Scotian Shelf sample was significant ($F_{ST} = 0.003$, $P < 0.001$) but also entirely dependent on Gmo132 ($F_{ST} = 0.021$, $P < 0.001$). Finally, the overall F_{ST} was significant for a comparison among samples in the NORTH group ($F_{ST} = 0.007$, $P = 0.002$), but not for a comparison among samples in the SOUTH group ($F_{ST} = 0.002$, $P = 0.080$) or between NORTH and SOUTH ($F_{ST} = 0.000$).

The R_{ST} analyses (Table 7) presented a more complex picture than that obtained with F_{ST} . In various analyses, the single-locus R_{ST} estimates ranged from large and positive to large and negative values for all loci except Gmo145. The most extreme example of this effect involved Gmo132, for which

Table 5. Probability of homogeneity of allele frequencies for Gmo4 estimated from pairwise pseudoprobability tests.

Comparison		<i>P</i>
NORTH92	NORTH93	0.116
NORTH92	SOUTH92	<0.001*
NORTH92	SOUTH93	0.004*
NORTH93	SOUTH92	0.188
NORTH93	SOUTH93	0.036*
SOUTH92	SOUTH93	0.707

Note: Probability (*P*) estimates considered significant are indicated with an asterisk (*).

the estimated R_{ST} was 0.208 ($P < 0.001$) in a three-way comparison between NORTH, SOUTH, and Scotian Shelf but assumed a value of -0.131 when all northern cod were pooled into a single sample and compared with the Scotian Shelf sample.

In comparison to the results obtained with F_{ST} , fewer single- and multi-locus R_{ST} values were significantly greater than zero (Table 7). The overall R_{ST} was not significant in comparisons of six (mean $R_{ST} = 0.034$, $P = 0.060$) or five populations (excluding northeast Spur, mean $R_{ST} = 0.021$, $P = 0.126$) despite large positive (and highly significant) contributions from Gmo132 and Gmo145. The overall R_{ST} was also not significant in a comparison between pooled NORTH, SOUTH, and Scotian Shelf samples and the Barents Sea (mean $R_{ST} = 0.037$, $P = 0.119$). Overall R_{ST} values were negative and hence not significantly different from zero in comparisons between pooled northern cod and Flemish Cap, and between pooled northern cod and Scotian Shelf. By contrast, the overall R_{ST} was positive ($= 0.018$) and significant ($P = 0.003$) in a three-way comparison between NORTH, SOUTH, and Scotian Shelf, owing to contributions from Gmo132 ($R_{ST} = 0.208$, $P < 0.001$) and Gmo145 ($R_{ST} = 0.027$, $P = 0.015$). Single- and multi-locus R_{ST} values were not significant in other comparisons within NORTH or SOUTH, or between NORTH and SOUTH (Table 7).

Discussion

Our results lead us to reject the null hypothesis of no genetic differentiation among northern cod in particular, and northwest Atlantic cod in general, a result contrary to that obtained in previous studies employing less variable mtDNA or allozyme markers (Mork et al. 1985; Smith et al. 1989; Carr and Marshall 1991a, 1991b; Árnason et al. 1992; Carr 1995; Carr et al. 1995; but see Cross et al. 1978; Pogson et al. 1995). We employed a variety of measures to assess genetic differentiation and population structure in cod using the microsatellite data, and most, but not all, of the measures revealed significant genetic differences among population samples at large (ocean basin) and meso (regional bank) scales. Our results, therefore, have various implications for the population biology of cod, the management of cod stocks, the utility of microsatellites as genetic markers in population studies, and the statistical methods used to interpret the data. We consider these issues below.

Population structure: ocean basin scales

We observed the strongest differentiation between the Barents Sea sample and the northwest Atlantic samples, a feature seen in χ^2 tests of allele frequency homogeneity, $(\delta\mu)^2$, and Rogers'

Table 6. Genetic distances (above diagonal for each section) and probability estimates (below diagonal for each section).

	NORTH	SOUTH	Flemish	Scotian	Barents
			$(\delta\mu)^2$		
NORTH		5.37	7.77	6.24	13.35
SOUTH	0.008*		17.85	7.78	18.62
Flemish	0.042*	<0.001*		16.85	27.54
Scotian	0.003*	<0.001*	<0.001*		29.03
Barents	<0.001*	<0.001*	<0.001*	<0.001*	
			Allele sharing		
NORTH		0.303	0.386	0.286	0.326
SOUTH	0.010*		0.460	0.307	0.322
Flemish	0.015*	<0.001*		0.448	0.383
Scotian	0.038*	<0.001*	<0.001*		0.344
Barents	0.004*	<0.001*	0.013	<0.001*	
			Rogers'		
NORTH		0.057	0.128	0.072	0.159
SOUTH	0.37		0.122	0.070	0.157
Flemish	<0.001*	<0.001*		0.138	0.126
Scotian	0.001*	<0.001*	<0.001*		0.159
Barents	<0.001*	<0.001*	<0.001*	<0.001*	
			Averagesquared		
NORTH		312.045	336.892	341.100	321.870
SOUTH	0.948		346.102	341.778	326.275
Flemish	0.445	0.277		373.290	357.642
Scotian	0.332	0.298	0.037		364.872
Barents	0.787	0.715	0.139	0.034	

Note: Probability values judged significant following sequential Bonferroni adjustment (initial $\alpha = 0.005$) are indicated with an asterisk (*).

genetic distances. This result is consistent with genetic differentiation of cod at Atlantic basin scales that has been previously documented using allozymes (Mork et al. 1985), mtDNA (Carr and Marshall 1991a, 1991b; Carr 1995), and nuclear RFLP markers (Pogson et al. 1995). Cod from the northwest Atlantic during the 1950s and 1960s, but not since 1966, have been known to stray to Greenland, Iceland, the North Sea, and the Barents Sea, but with extremely low and decreasing (from west to east) frequency (Taggart et al. 1995).

Mitochondrial DNA studies have revealed differing levels of genetic diversity across the eastern and western portions of the range of cod. Nucleon diversities (analogous to heterozygosity for nuclear loci) off Newfoundland and Nova Scotia are lower (18–38%) than they are in the northeast Atlantic (82–85%), a pattern that suggests that cod in the northwest Atlantic have been subject to one or more population bottlenecks (Carr and Marshall 1991a, 1991b; Carr 1995; Carr et al. 1995). Such a loss of genetic variation in northwest Atlantic cod is not evident in the microsatellite loci: mean expected heterozygosity among all six loci in our northwest Atlantic samples was 88%, compared with 84% for the Barents Sea sample. Allozyme and cDNA loci similarly show no evidence of a genetic bottleneck in the northwest Atlantic (Mork et al. 1985; Pogson et al. 1995), implying that any bottleneck(s) that may have occurred only influenced genetic diversity in mtDNA.

Population structure: northwest Atlantic

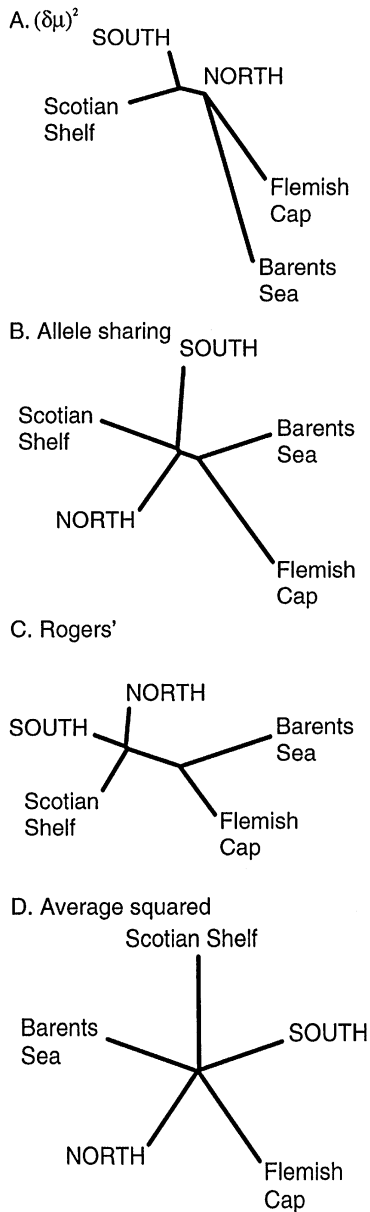
Comparisons of single-locus allele frequencies, single- and multi-locus F_{ST} values, and $(\delta\mu)^2$, allele sharing, and Rogers' genetic distances revealed significant genetic differences among northwest Atlantic population samples. Flemish Cap,

Scotian Shelf, and northern cod samples all differed significantly from each other. Each of these regions are separated from each other by wide and deep submarine trenches. Within the northern cod complex, χ^2 , $(\delta\mu)^2$, and allele sharing analyses suggested the presence of at least two genetically distinguishable groups: one comprising our pooled NORTH samples (Hamilton, Belle Isle and Funk Island banks; NAFO divisions 2J, 3K) and the other consisting of our SOUTH samples from the northern Grand Bank area (North Cape, Grand Bank, and Nose of the Bank; NAFO division 3L). One other northern cod sample, northeast Spur (division 3L), also differed at one locus (Gmo141) from the adjacent SOUTH pool of northern cod samples but not from the NORTH pool; this result could be an artifact of the small sample size for this group (Table 3) and (or) allele scoring problems affecting this sample (see below).

The genetic variation we observed in northwest Atlantic cod could conceivably result from three different causes: (i) selective effects on non-neutral genetic markers, (ii) temporal or "chaotic" (*cf.* Hedgecock 1994) variation in allele frequencies among samples not associated with stable spatial differentiation, or (iii) divergence through the effects of genetic drift and mutation acting on reproductively isolated populations. We consider each in turn below.

We have no direct evidence of the neutrality or non-neutrality of the microsatellite markers employed in this survey, but since they all consist of dinucleotide repeats, they cannot be part of the protein-coding regions of genes. It is possible that they might be indirectly involved in selection through close linkage to a selectively important gene, but this is unlikely, at least statistically. Moreover, the extreme degree

Fig. 4. Fitch–Margoliash phenograms depicting relationships among cod populations based on four measures of genetic distance.



of polymorphism exhibited by these microsatellites argues against fitness effects associated with particular alleles.

We evaluated the relative significance of temporal or chaotic variation versus stable geographic variation to our results in two ways. First, we conducted χ^2 tests of allele frequency heterogeneity among the inferred year-classes of cod within the NORTH and SOUTH (in this case including northeast Spur) pools of samples and we observed no significant variation among the year-classes for any of the six loci. Second, we split the SOUTH and NORTH pools into their 1992 and 1993 components, then conducted pairwise χ^2 tests of allele frequency heterogeneity for Gmo4, the single locus that differed between the NORTH and SOUTH pools. Differences in allele frequency were significant for NORTH92 versus SOUTH92, NORTH92 versus SOUTH93, and NORTH93 versus SOUTH93 comparisons, but not significant for

NORTH93 versus SOUTH92, NORTH92 versus NORTH93, and SOUTH92 versus SOUTH93 comparisons (Table 5). In general, these results are more consistent with the hypothesis that the difference seen with Gmo4 between the NORTH and SOUTH pools reflects stable spatial rather than temporal or chaotic variation among samples; however, the lack of significance in the NORTH93 versus SOUTH92 comparison leaves some uncertainty over this issue. The χ^2 results and direct inspection of the allele frequency data both suggest the following two points: (i) the SOUTH92 and SOUTH93 pooled samples were closely similar and (ii) the NORTH93 sample was somewhat more similar to the two SOUTH samples than was the NORTH92 sample. Clearly, the tentative conclusion that the difference between NORTH and SOUTH is a stable phenomenon needs independent testing using surveys of additional samples drawn from the areas and populations we have examined here.

Our data are not sufficient to permit a direct evaluation of the relative importance of spatial versus temporal variation for comparisons outside of northern cod. However, a number of independent surveys have confirmed the presence of the large (>131 bp) Gmo132 alleles in Scotian Shelf cod and their absence in northern cod (Brooker et al. 1994; Taggart 1995; Ruzante et al. 1996a, 1996b), indicating that this difference between northern and Scotian Shelf cod is a stable phenomenon.

The genetic differentiation we observed among northwest Atlantic cod populations using microsatellite markers stands in contrast with the results of surveys of mtDNA sequence variation in northwest Atlantic cod (Carr and Marshall 1991a, 1991b; Pepin and Carr 1993; Carr 1995; Carr et al. 1995). Conversely, our microsatellite analyses show some similarities to studies of Cross and Payne (1978) and Pogson et al. (1995) that surveyed protein and nuclear RFLP loci, respectively. Both of these studies detected highly significant genetic differences among northwestern Atlantic populations of cod.

Cross and Payne (1978) studied variation at two loci, transferrin (*Tf*) and phosphoglucose isomerase (*PGI-1*), across a range of locations that included several that corresponded closely to the locations we sampled in the northwest Atlantic, but some 20 years earlier. In Cross and Payne's study, as in ours, significant genetic differences were detected between Flemish Cap, Scotian Shelf, and northern cod populations. Cross and Payne's results suggested a split between populations south of the Laurentian channel (including the Scotian Shelf) and those to the north. In our study, such a distinction is only weakly suggested by the $(\delta\mu)^2$ distances, and not by the other distance measures. However, as noted above, the Laurentian channel does mark a discontinuity in geographic variation at the Gmo132 locus, with alleles larger than 131 bp frequently observed south of the Laurentian channel, but never to the north (Fig. 3).

With respect to the three conceivable explanations outlined earlier for the genetic variation that we detected in our analyses of northwest Atlantic cod, and on the basis of the ensuing arguments, we conclude that (iii) divergence through the effects of genetic drift and mutation acting on reproductively isolated populations is the simplest and most consistent explanation.

Our results also show some similarity to those of Cross and Payne (1978) regarding relationships among populations

Table 7. F_{ST} and R_{ST} values for cod microsatellite loci.

	1	2	3	4	5	6
	F_{ST}					
Gmo2	0.003 (0.030)	0.011 (0.005*)	0.000 (na)	0.000 (na)	0.001 (0.130)	-0.002 (na)
Gmo4	0.003 (<0.001*)	0.005 (0.001*)	0.002 (0.132)	0.002 (0.003*)	0.000 (na)	0.004 (0.004*)
Gmo120	0.000 (na)	0.000 (na)	0.001 (0.318)	0.000 (na)	0.000 (na)	0.000 (na)
Gmo132	0.092 (<0.001*)	0.200 (<0.001*)	0.090 (<0.001*)	0.015 (<0.001*)	0.021 (<0.001*)	-0.001 (na)
Gmo141	0.003 (0.004*)	0.004 (0.001*)	0.002 (0.139)	0.000 (na)	0.000 (na)	0.000 (na)
Gmo145	0.003 (0.004*)	0.007 (<0.001*)	0.002 (0.177)	0.006 (0.179)	0.000 (na)	0.000 (na)
Overall	0.015 (<0.001*)	0.037 (<0.001*)	0.014 (<0.001*)	0.003 (<0.001*)	0.003 (<0.001*)	0.000 (na)
	R_{ST}					
Gmo2	0.102 (0.137)	-0.129 (na)	-0.057 (na)	0.014 (0.408)	-0.061 (na)	-0.006 (na)
Gmo4	0.026 (0.368)	0.085 (0.142)	-0.117 (na)	0.008 (0.296)	-0.003 (na)	0.006 (0.385)
Gmo120	0.0112 (0.391)	-0.090 (na)	0.001 (0.496)	0.016 (0.137)	0.004 (0.407)	0.025 (0.093)
Gmo132	0.415 (<0.001*)	0.387 (<0.001*)	-0.034 (na)	0.208 (<0.001*)	-0.131 (na)	0.013 (0.207)
Gmo141	-0.017 (na)	0.039 (0.233)	-0.075 (na)	-0.002 (na)	-0.013 (na)	-0.012 (na)
Gmo145	0.098 (0.002*)	0.049 (0.216)	0.117 (0.024)	0.027 (0.015)	-0.012 (na)	0.037 (0.009)
Overall	0.034 (0.060)	0.037 (0.119)	-0.051 (na)	F0.018 (0.003*)	-0.016 (na)	0.005 (0.319)

Note: P values are in parentheses. 1, among six populations (NORTH, SOUTH, Flemish Cap, Scotian, Barents, northeast spur); 2, between all northern cod and Scotian Shelf (pooled) and Barents Sea; 3, between all northern cod (pooled) and Flemish Cap 94; 4, between NORTH, SOUTH, and Scotian Shelf; 5, between all northern cod (pooled) and Scotian Shelf; 6, between NORTH and SOUTH. Probability values judged significant following sequential Bonferroni adjustment (initial $\alpha = 0.005$) are indicated with an asterisk (*).

within the northern cod complex. Their results, like ours, suggest a subdivision between cod from the northeastern Newfoundland Shelf (corresponding to our NORTH pool) and cod from the more southerly Grand Bank areas. Their study, however, included samples from the southern Grand Bank. Moreover, cod in Cross and Payne's study that came from our SOUTH area (North Cape and northern Grand Bank) showed mixed affinities between the more northerly and southerly groups.

The genetic differentiation between northern cod, Flemish Cap, and Scotian Shelf populations suggests that the deep ocean trenches that separate these populations act as barriers to gene flow. A similar conclusion was reached for haddock (*Melanogrammus aeglefinus*) in the western Atlantic by Zwanenburg et al. (1992) on the basis of mtDNA data.

The isolating effect of ocean channels or other hydrographic features appears most extreme for the Flemish Cap. In both our study and that of Cross and Payne, Flemish Cap cod were more divergent from geographically proximate Grand Bank samples than the latter were from more spatially distant Scotian Shelf and Labrador samples. Cross and Payne (1978) speculated that the distinctiveness of Flemish Cap cod might result from their separate descent from an isolated glacial refuge around Flemish Cap. Regardless of the historical and hydrographic factors involved, the genetic distinctiveness of the Flemish Cap cod demonstrate that genetic differentiation of cod populations can occur on geographic scales of 200 km or less. The relative isolation of cod on the Flemish Cap is also supported by tagging studies that show that all but 4% of cod tags reported recaptured ($n = 132$) from a release ($n = 704$) on the Flemish Cap were reported from the Flemish Cap. In addition, of the tens of thousands of cod tagged and released over the last 40 years in the Newfoundland and Grand Banks region (excluding Flemish Cap), none (0%) of 4836 recaptures in the 1950s, 4 (0.03%) of 13 600 recaptures in the 1960s, and 4 (0.02%) of 17 620 recaptures in the 1970s and 1980s were reported as captured from the Flemish Cap (Taggart et al. 1995), despite a history of intensive fishing activity on the Cap.

Although our results are consistent with the existence of reproductively isolated populations of cod, they also indicate that the extent of genetic differentiation between populations is of low magnitude. Both direct inspection of allele frequency distributions (see Fig. 2) and F_{ST} and R_{ST} measures indicate that the proportion of total genetic variation occurring among populations is small: approximately 1.5 and 3% for range-wide estimates of F_{ST} and R_{ST} , respectively (Table 7, column 1). The low level of genetic differentiation in cod undoubtedly stems, at least in part, from gene flow (straying), but may also reflect the relatively recent origin of cod populations (Pogson et al. 1995) and (or) the effects of convergent mutations acting on the microsatellite loci (see below).

Utility of microsatellites as genetic markers

We observed an average of 40.8 alleles per locus in the six microsatellites that we surveyed in cod. Heterozygosities averaged 86% across all loci and population samples and reached 100% in some cases. This level of variation is doubly remarkable. First, as noted elsewhere, cod microsatellites appear to be unusually variable relative to comparable dinucleotide microsatellites in many other species (Brooker et al. 1994; see also O'Reilly and Wright 1995). Second, the variation exhibited by the cod microsatellites substantially exceeds that seen with most other genetic markers in cod, although it is comparable to that observed with a minisatellite locus assayed in cod using the PCR (Galvin et al. 1995). By contrast, average heterozygosity and the average number of alleles per locus for 10 polymorphic allozyme loci were 13% and 1.9, respectively, for a basin-scale survey of cod (Mork et al. 1985). In a similar study involving nuclear RFLP loci detected with cDNA probes, average heterozygosities and numbers of alleles per locus (36% and 4.3) were intermediate between the values for allozyme and microsatellite loci (Pogson et al. 1995).

Our microsatellite survey as well as that of Ruzzante et al. (1996a) of cod microsatellite loci detected genetic differentiation in northwest Atlantic cod populations not evident with less

variable genetic markers. These results suggest that hypervariable microsatellite markers offer some advantages of sensitivity in contexts where genetic variation exhibited by other genetic markers is low, or where genetic differentiation, at least in terms of neutral markers, is marginal. In other circumstances, however, hypervariability may be suboptimal for at least two reasons. (i) Large numbers of alleles create statistical problems in the form of large sampling errors. We countered this problem by pooling some samples, at the cost of forgoing both serial replication and analysis on some spatial scales for the northern cod complex (e.g., among the multiple marine banks encompassed within the NORTH pool). (ii) Hypervariability implies high mutation rates at these loci. The step-mutational process believed to prevail in microsatellites may result in many convergent or parallel mutations; hence, identity of allelic state does not necessarily denote identity by descent (Di Rienzo et al. 1994). In this regard, it is worth noting cases where the microsatellites did not reveal genetic differentiation. Although northwest Atlantic populations were individually distinguishable from the Barents Sea sample at two to five loci, only one microsatellite locus, Gmo132, distinguished all northwest Atlantic populations from the Barents sample. Since eastern and western Atlantic cod are known to be well differentiated at protein, nuclear RFLP, and mtDNA loci, the lack of consistent east–west differences in microsatellite loci could reflect the action of convergent mutations erasing the effects of genetic drift, and in a practical sense, it suggests that hypervariable microsatellites may not be the markers of choice for broad-scale surveys.

The least variable microsatellite, Gmo132, exhibited the greatest genetic differentiation in comparisons outside the northern cod complex. In the basin-scale comparisons of five populations (excluding northeast Spur), F_{ST} and R_{ST} values for Gmo132 were 45 and 37 times higher than the averages for the other five loci, respectively (Table 7). For the most part, basin-scale differences in the allele distributions of Gmo132 were due to marked differences in the frequencies of alleles common to multiple populations (Fig. 3), a pattern consistent with divergence primarily by genetic drift. One population, however, Scotian Shelf, exhibited a unique group of large alleles, indicating a role of (divergent) mutations in establishing genetic differentiation in this case. The unusual Gmo132 alleles present in the Scotian Shelf cod may have arisen through a mutational mechanism involving changes in the flanking sequence (D. Cook, unpublished observation) rather than within the repeat sequence, making this aspect of Gmo132 differentiation atypical.

Two complications that may attend the use of some microsatellite loci in population surveys are the occurrence of null alleles (alleles that fail to amplify) and the presence of artifactual “stutter” bands on gels that can make some loci difficult to score (both reviewed in O’Reilly and Wright 1995). Both phenomena can lead to scoring errors marked by an apparent deficiency of heterozygotes. We tested the inheritance of the microsatellite markers used in this study with captive families involving six parents, and the reproducibility of gel scoring with blind comparisons between two gel readers. The former test revealed no evidence of null alleles at any locus; the latter supported the reproducibility of gel scores (D. Cook, unpublished data). Nonetheless, one of the microsatellite loci we assayed, Gmo141, exhibited a significant deficiency of

heterozygotes in four populations (see Table 3), suggesting the occurrence of null alleles and (or) scoring problems. Either problem could bias the results of allele frequency comparisons. In this regard, it may be noteworthy that the genetic difference that led to the exclusion of the northeast Spur sample from the SOUTH pool involved Gmo141; northeast Spur also showed the strongest heterozygote deficiency at this locus (Table 3).

For the reasons outlined above, the more moderately variable nuclear RFLP loci studied by Pogson et al. (1995) may present statistical and analytical advantages for population discrimination over the microsatellites employed in this study. On the other hand, the practical and economic advantages of PCR-based microsatellite assays over the Southern blot based RFLP technology are compelling (Wright and Bentzen 1994; O’Reilly and Wright 1995). Indeed, only PCR-based methods could allow the assay of the disparate tissue sources (including the larval cod eyeballs) used in this study.

The speed and modest cost of microsatellite analyses allowed us to achieve relatively large sample sizes in this study. This factor, coupled with differing levels of genetic variability, may account for the difference between our results and those obtained with mtDNA. The sample sizes in this study, which averaged 169 for NORTH, SOUTH, and Scotian Shelf, are at least 5 times greater than those reported to date for similar locations in the mtDNA studies (Carr 1995; Carr et al. 1995). Further, this study surveyed six (presumably) unlinked loci, whereas mtDNA is equivalent to a single locus. Finally, although the mtDNA studies have detected numerous haplotypes, the frequency distribution of these haplotypes around Newfoundland and Nova Scotia is characterized by a single haplotype that prevails everywhere at frequencies close to 85%, with individual variant haplotypes frequently observed only once, and invariably at $\leq 3\%$ frequency (Carr and Marshall 1991a, 1991b; Carr 1995; Carr et al. 1995). Skewed haplotype distributions of this type may not offer the statistical power needed to detect the low (but significant) levels of population differentiation revealed with microsatellite and RFLP markers.

Statistical methods for analyzing microsatellite data

We employed a variety of analyses to reach the conclusions above. In general, familiar measures such as χ^2 contingency tests, F_{ST} , and Rogers’ genetic distance not only proved to be sensitive indicators of genetic differentiation when evaluated using Monte Carlo methods, they also yielded similar results. The genetic distance measure, $(\delta\mu)^2$, recently proposed by Goldstein et al. (1995b) for microsatellites, also revealed significant genetic differentiation in a pattern largely congruent with the previous methods. Three other measures proposed specifically for microsatellite data gave variable results; these included two other genetic distance measures, allele sharing (Bowcock et al. 1994) and average squared (Goldstein et al. 1995a), as well as R_{ST} , an analog of F_{ST} (Slatkin 1995).

R_{ST} , like F_{ST} , estimates the proportion of total genetic variation that occurs between populations. Unlike F_{ST} , however, R_{ST} incorporates information about the size of microsatellite alleles. The inclusion of allele size is expected to make R_{ST} more informative (Slatkin 1995). However, variances of estimates of R_{ST} in pairwise comparisons can be relatively large when sample sizes differ, even when both samples are large. By contrast, variances on F_{ST} estimates appear to be much less

affected by unequal sample sizes (D.E. Ruzzante, unpublished data). In this study, R_{ST} estimates were relatively variable in comparison to F_{ST} estimates (Table 7). The variability inherent in the R_{ST} estimate was most striking in the different results obtained for two analyses, one in which NORTH, SOUTH, and Scotian Shelf were compared, and another in which the northern cod complex was pooled as a single group and compared with Scotian Shelf. R_{ST} estimates for Gmo132 in these two analyses were 0.208 and -0.131 , respectively (Table 7). In contrast, F_{ST} estimates varied little from each other (0.015 and 0.021, respectively) and were both statistically significant ($P < 0.001$, Table 7). A comparison of the R_{ST} and F_{ST} estimates from the present study with those from Ruzzante et al. (1996a) suggests that the two estimates may perform differently under different conditions such as number of populations considered, nature of the differences among populations, and degree of population subdivision. It appears, however, that for analyses of R_{ST} it may be important to equalize sample sizes (D.E. Ruzzante, unpublished data).

Two criteria, the number of pairwise distances significantly greater than zero and concordance with previous genetic data and zoogeographic factors, suggest that the $(\delta\mu)^2$ and Rogers' genetic distances were approximately equally effective in resolving relationships among cod populations, and that the average squared distance was not useful. The numbers of pairwise distances significantly greater than zero among 10 comparisons were 10, 10, 9, and 0 for the $(\delta\mu)^2$, allele sharing, Rogers', and average squared distances, respectively (Table 6). Further, the $(\delta\mu)^2$ and Rogers' trees were both in accord with previous protein, mtDNA, and nuclear RFLP analyses as well as geographic considerations in depicting the Barents Sea sample as most divergent, and the three populations from the northwest Atlantic continental shelf (NORTH, SOUTH, and Scotian Shelf), as closely related (Figs. 4A, 4C). This pattern was not evident in the allele sharing tree (Fig. 4B). It should be added that the allele sharing distance has the limitation that its expectation is greater than zero even for within-population comparisons. Finally, the average squared distance tree revealed no structure at all among cod populations (Fig. 4D).

The Rogers' tree was very similar to a maximum likelihood tree based on allele frequencies (data not shown). The latter approach to tree construction corresponds to a model in which populations diverge by genetic drift alone, and it is insensitive to the genealogical relationships of the alleles involved (Felsenstein 1994). In contrast, the $(\delta\mu)^2$ measure is based on differences in mean allele size between populations and hence is explicitly based on the step mutational model (Goldstein et al. 1995b). Hence, for these populations and loci, drift- and mutation-based models appear to work equally well in portraying reasonable relationships among populations.

Implications for northern cod

This study was motivated in large part by interest in the underlying population structure of the northern cod stock complex, which has undergone intense fishing pressure over the last four decades and has reached an all-time low in abundance (Taggart et al. 1995). It was recently suggested by deYoung and Rose (1993) that northern cod population structure is formed by extensive egg and larval drift, followed by opportunistic (and nonphilopatric) recruitment of juveniles to whatever adult

assemblages they find themselves near. Consequently, deYoung and Rose predicted that population structure in northern cod is neither genetically based nor temporally stable, particularly following what they showed to be an apparent southward shift in abundance occurring after 1988. Our data, which include cod from a variety of year-classes, but a majority from after 1988, are inconsistent with both predictions. We have shown evidence for at least two genetically discernable groups within the northern cod complex, our NORTH and SOUTH pools that correspond to the 2J3K and 3L management divisions. A related microsatellite study demonstrated genetic differences between two overwintering cod aggregations within this complex in NAFO division 3L, one inshore and the other offshore (Ruzzante et al. 1996a). The differences occurred despite the fact that cod of both migratory types intermingle inshore during the summer as a result of the feeding migration by offshore cod. As noted above, these joint findings deserve independent testing with additional samples, and ideally with additional markers. Such work, now in progress at Dalhousie University, will serve to refine our knowledge of population structure within the northern cod complex. At present, however, the results reported here, those of Ruzzante et al. (1996a), and the previous work of Cross and Payne (1978) collectively support, with some weight, the conclusion that northern cod are not comprised of a single, panmictic assemblage, but rather are composed of genetically distinguishable subunits, each of which appear to be geographically affiliated with spawning area. This suggests that it may be wise to seriously reconsider the current (and historical) management scheme for northern cod, which presumes one stock distributed throughout the inshore and offshore regions stretching from Hamilton Bank to the Grand Bank through NAFO divisions 2J, 3K, and 3L (Taggart et al. 1994).

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