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Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994

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Abstract

We report on evidence of long term stability in the geographic pattern of genetic differentiation among cod (*Gadus morhua*) collected from 5 spawning banks off Newfoundland and Labrador over a period spanning three decades (1964–1994) and 2 orders of magnitude of population size variation. Six microsatellite DNA loci amplified from archived otoliths (1964 and 1978) and contemporary (1990s) tissue samples revealed fidelity to natal spawning banks over this period. A two level (spawning bank and decade) hierarchical and multilocus AMOVA indicated that 1.55% of the total variation in allele frequencies could be attributed ($P = 0.036$) to spatial structure while no variance component could be attributed to temporal changes. A finer scale analysis among cod from just 3 of these spawning banks reveals, however, evidence consistent with some post-collapse mixing between cod from two banks. In the context of fisheries management and conservation, the survival of the spatial pattern of genetic differentiation during the population collapse suggests that if recovery eventually occurs it will likely be through population re-growth *in situ* rather than by migratory influx.

Introduction

Quantifying long-term variation in the genetic composition of spatially structured migratory marine fish is a recurrent objective in conservation and fisheries genetics largely because of the implications for our understanding of the ecology of populations and their management (Waples 1998). Such an objective is particularly important for an exploited species like Atlantic cod (*Gadus morhua*) which, over the last few decades, has undergone drastic changes in population size throughout its range (Myers et al. 1997). Very abundant and widely distributed migratory marine fish have traditionally been thought to exhibit genetic population differentiation only among distant populations. This view stems largely from consideration of the combined effects of the large potential for long distance dispersal during egg and larval stages and the extremely large effective population sizes characteristic of marine fish. While it is probably true that marine fish exhibit less genetic structure than freshwater and anadromous species (Gyllensten 1985; Ward et al. 1994; DeWoody and Avise 2000), the view that marine fish populations are well connected through larval dispersal (e.g., see Roberts 1997) is challenged by evidence of genetic differentiation among populations at temporal and geographic scales that are relevant to fishery management (Ruzzante et al. 1998, 2000a; Shaw et al. 1999). Such evidence, together with evidence from theoretical arguments (Cowen et al. 2000) and empirical observations of larval retention near local populations (Jones et al. 1999; Swearer et al. 1999) suggest that present day (Hauser and Ward 1998; Ruzzante et

al. 1998) and historical (Barber et al. 2000) oceanographic features should be taken into consideration when making predictions about patterns of genetic structure in marine species.

Several studies have recently demonstrated population differentiation in marine fishes using microsatellite (e.g., Ruzzante et al. 1996, 1998; García de Leon et al. 1997; Shaw et al. 1999) or mitochondrial DNA markers (e.g., Stepien 1999; Nesbø et al. 2000). These studies are generally based on contemporary samples taken over a time lapse of 2 to 3 years with little or no examination of patterns of genetic differentiation over periods spanning several generations during which major changes in population size may have occurred.

The Newfoundland cod population complex comprises several offshore and inshore spawning components that are identified with fishing banks that have been exploited at least since the late fifteenth century (Templeman 1962). Cod populations in this region experienced large decreases in population size during the 3 decades starting in the 1960s and culminating with the collapse of the commercial fishery in the early 1990s (Hutchings and Myers 1994; Hutchings 1996; Myers et al. 1996, 1997). The question we address here by studying preserved historical and contemporary tissues is whether the contemporary genetic population structure, which is known to be statistically significant (Bentzen et al. 1996; Ruzzante et al. 1996, 1998, 1999, 2000b) retains the same spatial components it had before the collapse. We report on an examination of microsatellite DNA variation among cod collected from five regions off Newfoundland and Labrador spanning three decades (1964–1994) and population size variation spanning two orders of magnitude.

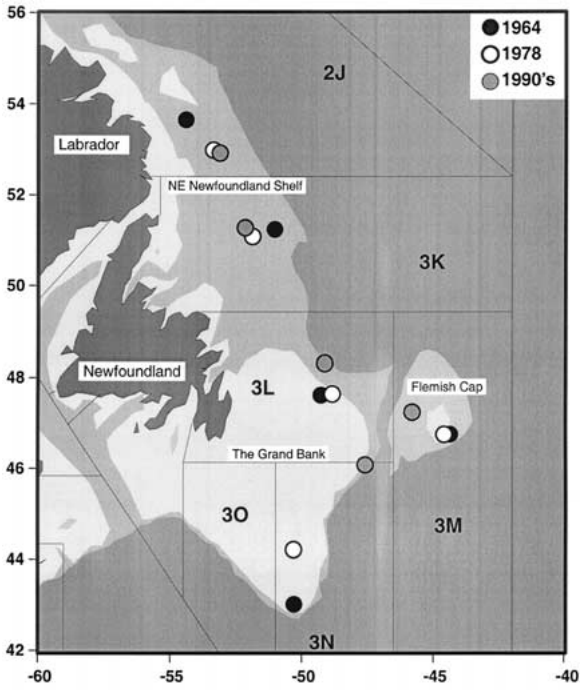

Figure 1. Bathymetric chart of the NE Newfoundland Shelf, Grand Bank and Flemish Cap regions showing the average location of cod (N = 1144) collected in each of five NAFO management divisions in 1964 (N = 254), 1978 (N = 320) and in the 1990's (N = 570). Genetic variability was assessed at six microsatellite DNA loci. See Table 1, for details of sampling locations, sample sizes, etc.

Methods

Sample collection

Cod samples (Table 1, Figure 1) were collected as part of the Canadian Department of Fisheries (DFO) routine survey cruises within the northern cod region (NAFO Divisions 2J, 3K and 3L), the northern and southern Grand Bank region (Div. 3L and 3N), and the Flemish Cap (3M). Historical samples from 1964 and 1978 were obtained from DFO otolith archives and were chosen to coincide with periods of known extreme values in population size. In 1964 cod populations off Newfoundland and Labrador were at or near a historical maximum. Spawner biomass in 1962, the first year for which estimates are available, is estimated to have been 1.6 million tons. By 1978 these populations had experienced a major decline from which they recovered slightly in the early 1980s (data in Bishop et al. 1993; see Figure 2 in Myers et al. 1997). The samples from 1992–1994 were obtained at a time when the harvestable biomass of cod was at a mere 1% (22 000 tons) of its historical maximum in the 1960s (Bishop et al. 1993; Hutchings and Myers 1994; Myers et al. 1997). Thus, although the collection of samples from 1964, 1978, and 1992–1994 came from the same fishing banks and they originated from populations that differed in size by 1 to 2 orders of magnitude, population sizes in the 1990s were still large enough that genetic effects caused by declines in population size are unlikely. Some, but not all, of the 1992–1994 sample/loci combinations have been used in two recent studies detailing the contemporary genetic structure of cod (Bentzen et al. 1996; Ruzzante et al. 1998).

Table 1. Summary statistics for the cod samples. Median lat. and long.: median latitude and longitude of collection. N: Sample size in number of individuals. Age (yrs) and length (cm): mean and range are given, Prop. mature: proportion of mature fish in the sample. Prop. male: proportion of males in the sample. N by locus: Number of individuals successfully scored by locus. NR: Not run

Sampling year	NAFO division	Median Lat.	Median Long.	N	Age (yrs) Mean	Age (yrs) Range	Length (cm) Mean	Length (cm) Range	Prop. mature	Prop. male	N by locus Gmo2	Gmo4	Gmo132	Gmo145	Gmo1	Gmo 120
1964 (N = 254)	2J	53.3	−54.5	89	5.6	5–6	48.1	34.0–62.0	0.66	0.60	81	60	84	71	83	54
	3K	51.3	−51.5	30	5.7	5–7	58.3	49.0–69.0	0.90	0.50	23	18	25	14	23	22
	3L	48.3	−50.5	68	6.0	6–6	50.0	29.0–84.0	0.19	0.47	58	60	61	58	58	23
	3N	42.8	−50.5	30	–	–	54.7	49.0–60.0	0.13	0.37	29	24	29	26	29	28
	3M	46.8	−44.5	37	6.0	6–6	57.9	49.0–71.0	0.78	0.41	25	24	1	28	34	NR
Amplification success (%)											(85)	(73)	(79)	(78)	(89)	(59)
1978 (N = 320)	2J	53.3	−54.0	50	5.0	5–5	52.0	36.0–63.0	0.64	0.48	46	42	45	46	41	37
	3K	50.8	−52.5	41	5.2	5–6	56.4	41.0–75.0	0.41	0.26	36	38	40	39	39	36
	3L	47.8	−48.5	44	5.0	4–6	51.3	37.0–67.0	0.33	0.47	38	41	42	42	39	40
	3N	44.3	−50.5	30	5.0	5–5	57.8	51.0–65.0	0.13	0.13	26	28	28	25	28	20
	3M	46.8	−44.5	155	4.0	3–5	37.1	23.0–68.0	0.12	0.42	139	98	137	144	146	139
Amplification success (%)											(89)	(77)	(91)	(93)	(92)	(85)
1990s (N = 570)	2J	52.8	−53.8	86	2.5	1–4	35.3	25.0–50.0	0.43	0.49	85	80	82	81	NR	78
	3K	51.2	−53.1	185	2.7	1–5	33.3	21.0–51.0	0.46	0.20	177	178	172	173	29*	164
	3L	48.4	−49.4	218	3.9	1–8	42.9	21.0–75.0	0.49	0.33	214	211	215	211	136**	196
	3N***	46.1	−47.6	30	2.1	1–7	30.1	23.0–69.0	0.03	0.50	28	24	29	25	NR	28
	3M	47.1	−46.2	51	6.1	3–11	60.1	38.0–94.0	0.07	0.55	50	44	50	47	47	44
Amplification success (%)											(97)	(94)	(96)	(94)	(96)	(89)

*Attempted only in a sample of 29 out of the 185 individuals.
**Attempted in a composite sample of 141 out of the 218 individuals. ***The location for this collection is on the border between NAFO Divisions 3N and 3L (see Figure 1). Cod were difficult to find elsewhere within Division 3N in the early 1990s, at the time of the fishery collapse. The sample differed from cod collected at the same time within Division 3L (see Figure 2a and Table 4).

DNA extraction

The procedure for extracting DNA from preserved otoliths was similar to that described by Hutchinson et al. (1999). Cod otoliths from 1964 and 1978 were originally collected by DFO for age determination and were stored dry in paper envelopes (one otolith pair per envelope) in dry and uncontrolled room-temperature conditions. The appearance of the samples varied from clean (presumably rinsed for age reading) to covered with a brown residue of dried tissue (presumably blood). Extraction of DNA and PCR processing of the otoliths was done at high ionic strength and Mg^{++} levels but was otherwise similar to that used for fresh tissue as in Brooker et al. (1994) and Ruzzante et al. (1996).

Details for DNA extraction for contemporary samples are provided elsewhere (Ruzzante et al. 1996, 1998, 1999). Cod blood (∼1 mL) was the primary source of nuclear DNA and was collected from live or recently dead cod. When blood was unavailable we employed soft muscle tissue generally taken from the posterior of the tongue. For reasons unrelated to performance one collection from 1964 (NAFO division 3M, Flemish Cap) was not run for locus Gmo120 (Table 1). Also, for similarly non-technical reasons two collections from the 1990s (NAFO divisions 2J and 3N) were not run for locus Gmo1 (Table 1) and only subsets of the 1990s' collections from divisions 3K and 3L were run for this same locus (Table 1). We estimated amplification success taking these considerations in mind. We tested for the temporal differences in amplification success using two-sample *t*-tests with arcsine transformed proportions.

Data analysis

We tested each sample for deviations from Hardy-Weinberg expectations (HWE) and for genotypic disequilibrium between any two loci using χ^2 pseudo-probability contingency tests following Weir (1996). Tests of HWE were done using goodness of fit and log-likelihood ratio tests and were conducted by randomization of alleles within populations (1000 bootstrap samples, Manly 1991). Tests of genotypic disequilibrium were done by permutation of alleles across individuals within populations. These analyses were conducted using Splus© (MathSoft, Inc. 1996) functions written by DR. Genotypic disequilibrium can occur in individual samples due to sampling error associated with small sample size and these effects are minimised when an entire data set is pooled into a single sample. If genotypic disequilibrium occurs with the entire data set (N = 1144), then it is less likely to result from sampling error and more likely to result from physical linkage. Thus, to discriminate sampling

error from physical linkage, tests of genotypic disequilibrium were also conducted for the entire data set. We conducted a two level (spawning location and decade) hierarchical AMOVA using Arlequin version 2.000 (Schneider et al. 2000). Estimates of subpopulation structure were obtained using F_{ST} following Weir and Cockerham (1984). Significance for F_{ST} was estimated by resampling alleles across individuals and populations and for each locus separately. Multilocus estimates of F_{ST} were calculated by first summing the variance components across loci (Weir and Cockerham 1984) and then dividing. Confidence intervals for F_{ST} were estimated by resampling (1000 bootstrap samples) for each locus independently alleles across individuals *within* populations. The 95% CI was defined as the interval between the 25th and the 975th bootstrapped F_{ST} estimate. We used F_{ST} simply to describe the genetic composition of our samples and the extent of allele frequency differences among populations but did not use these estimates to extrapolate to the number of migrants among populations (c.f. Waples 1998; Whitlock and McCauley 1999). We also estimated pairwise genetic distances among populations using D_A (Nei et al. 1983), a non-SMM (stepwise mutation model) estimate of genetic distance with low variance relative to other non-SMM measures (Takezaki and Nei 1996; see also Ruzzante 1998). To visualize these pairwise relationships in a synthetic yet comprehensive way we applied multidimensional scaling (MDS) analysis to the D_A matrix. This multivariate method simplifies data with minimum loss of information and is likely to describe data more accurately than trees when there is considerable genetic exchange between close geographic neighbours (Cavalli-Sforza et al. 1994), as is likely to be the case in our study. Using MDS we illustrate relationships among populations in more than two orthogonal dimensions, dimensions that represent the effect of historical and recurrent mixing and migration on observed gene frequencies (Menozzi et al. 1978; Cavalli-Sforza et al. 1993, 1994). This information is fundamentally different from that obtained from an F_{ST} analysis and other equilibrium measures. When appropriate, significance levels ($\alpha < 0.05$) were adjusted for multiple comparisons using the sequential Bonferroni approach (Rice 1989). Occasionally, however, to facilitate interpretation we also report precise *P*-values (e.g., HWE and genotypic disequilibrium tests in Table 2). Unless stated otherwise (e.g., for the AMOVAs) statistical tests and analyses of genetic distances and population structure were conducted using Splus© (Mathsoft Inc. 1996) standard code or functions written *ad hoc*.

Results

Amplification success

Table 1 reports the number of individuals successfully amplified per locus and sample as well as their percentages. Over all loci the median amplification success increased from 78.5% (1964) to 90% (1978) and to 95% for the contemporary samples (means: 77%, 87.8%, 94.3%, for 1964, 1978 and 1990s, respectively) and the differences were significant ($P < 0.011$ and $\alpha = 0.017$ after correction for 3 simultaneous tests) when comparing the contemporary samples with either historical collection. There was also a tendency ($P = 0.022$) for an increase in amplification success between the 1964 and 1978 samples. When individual loci were considered, most loci showed at least a tendency for higher amplification success in the contemporary than in either historical collection, this difference was significant ($P < 0.011$) for Gmo2 (see Table 1).

Single locus statistics

The number of alleles per locus for the entire data set (N = 1144) ranged from 17 for Gmo1 to 62 for Gmo4 (Table 2). No individual sample contained all alleles present in the entire data set (Table 2). Averaged over 6 loci, observed and expected heterozygosities ranged from 0.737 to 0.794. When Gmo1 is excluded (1990s samples from NAFO Divisions 2J and 3N) observed and expected heterozygosities were higher and ranged between 0.864 and 0.900 (Table 2). When Gmo120 and Gmo132 are excluded (1964 sample from NAFO Division 3M, Flemish Cap), observed and expected heterozygosities, instead, decreased to 0.648 and 0.698, respectively (Table 2).

Hardy-Weinberg expectations

For HWE tests we report the precise *P*-values for those cases likely to be significant after a row-wide Bonferroni correction for 6 loci ($\alpha = 0.008$) with at least one of the goodness-of-fit or likelihood-ratio tests (Table 2). With this correction for 6 simultaneous tests there was evidence for a departure from HWE with one or both tests at 1 to 3 loci in 7 collections (13 loci/collection cases, Table 2) and no

Table 2. Single locus statistics: Number [range in length, i.e., number of basepairs] of alleles per locus. H_{obs} and H_{exp}: Observed and expected heterozigosities (averaged over loci) estimated for all samples pooled (overall) and for each location and year separately. D: Deficiency (<0) or excess (>0) of Heterozygotes; HWE: loci out of Hardy-Weinberg expectations; disequilibrium: pairs of loci in disequilibrium frequencies. For Hardy Weinberg tests, $\alpha = 0.008$ after Bonferroni correction for 6 (i.e., 6 loci) simultaneous tests (initial K = 6). For pairwise tests of Disequilibrium $\alpha = 0.003$ after Bonferroni correction for 15 simultaneous tests (initial K = 15). NA: not available

		Number [range] of alleles per locus						Heterozygosity (#)				Genotypic disequilibrium
		Gmo2	Gmo4	Gmo132	Gmo145	Gmo1	Gmo120	Observed	Expected	D(#)	HWE (*P*-value)†	
Overall		25 [92–148]	62 [109–301]	16 [99–129]	41 [137–241]	17 [70–126]	48 [128–238]	0.756 (##)	0.761 (##)	−0.007 (##)	**Gmo1 (0.003, 0.001)** Gmo132 (0.013, 0.002)	
1964	2J	14 [106–138]	24 [189–237]	8 [107–123]	20 [159–199]	7 [94–108]	31 [142–222]	0.742	0.735	−0.005	–	–
	3K	10 [106–136]	20 [161–235]	6 [105–121]	16 [155–193]	4 [96–106]	20 [158–210]	0.776	0.756	0.035	Gmo145 (0.017, 0.008) Gmo120 (0.072, 0.004)	–
	3L	10 [108–130]	31 [109–301]	6 [109–121]	27 [147–211]	7 [86–110]	17 [148–206]	0.770	0.734	0.045	–	–
	3N	9 [106–122]	23 [151–241]	9 [109–129]	23 [149–221]	3 [90–104]	22 [158–206]	0.750	0.737	<0.001	**Gmo4 (<0.001, 0.002)** **Gmo145 (0.001, 0.005)** Gmo120 (0.095, 0.002)	
	3M	8 [106–124]	22 [115–243]	2 [111–115]*	21 [155–241]	5 [96–110]	NA	0.648 (§§)	0.698 (§§)	−0.045 (§§)	**Gmo4 (<0.001, 0.001)** Gmo145 (0.030, 0.009)	
1978	2J	11 [106–138]	25 [187–239]	6 [111–121]	23 [155–215]	6 [94–110]	26 [160–220]	0.759	0.750	0.006	Gmo120 (0.039, 0.002)	
	3K	14 [92–138]	26 [173–237]	7 [109–121]	23 [153–201]	4 [94–106]	23 [158–218]	0.794	0.747	0.065	–	–
	3L	9 [106–138]	24 [183–249]	9 [105–123]	21 [151–211]	8 [84–106]	23 [162–214]	0.769	0.746	0.025	–	–
	3N	13 [100–142]	19 [187–237]	7 [105–121]	16 [159–207]	3 [94–104]	21 [154–238]	0.763	0.755	0.019	**Gmo120 (<0.001, 0.006)**	
	3M	18 [106–144]	35 [153–267]	13 [99–129]	15 [145–205]	11 [70–126]	41 [128–222]	0.752	0.759	−0013	Gmo2 (0.024, <0.001) **Gmo120 (<0.001, 0.001)**	Gmo2–Gmol120
1990s	2J	19 [104–142]	34 [155–275]	10 [105–127]	24 [155–207]	NA	28 [154–210]	0.882 (§)	0.864 (§)	0.016 (§)	–	–
	3K	19 [102–140]	42 [113–259]	11 [105–125]	32 [145–221]	4 [94–104]	32 [138–218]	0.769	0.752	0.029	–	–
	3L	21 [100–148]	38 [141–259]	12 [103–125]	30 [137–215]	10 [92–110]	35 [142–238]	0.737	0.763	−0.031	–	–
	3N	10 [106–126]	19 [169–245]	7 [111–123]	21 [157–211]	NA	22 [154–230]	0.900 (§)	0.867 (§)	0.037 (§)	**Gmo4 (<0.001, 0.005)** Gmo145 (0.005, 0.022)	
	3M	12 [106–142]	24 [113–223]	8 [107–121]	20 [153–199]	6 [96–108]	26 [148–214]	0.739	0.748	0.001	–	Gmo4–Gmo120

#: Values for observed and expected heterozygosities and heterozygote deficiency are averaged over 6 (§: 5 or §§: 4) single locus estimates of H_{obs}, H_{exp}, and D, respectively. Estimates averaged over 5 loci exclude locus Gmo1, those averaged over 4 loci exclude Gmo120 and Gmo132.

†Loci out of HWE with two tests are in bold characters.

##: Single locus observed and expected heterozygosity estimates, and estimates of heterozygote deficiency (<0) or Excess (>0) for the whole collection of samples pooled (N = 1144) are, respectively, as follows: Gmo2: 0.755, 0.798, −0.053. Gmo4: 0.963, 0.957, 0.007. Gmo132: 0.716, 0.722, −0.009. Gmo145: 0.946, 0.939, 0.007. Gmo1: 0.197, 0.198, −0.006. Gmo120: 0.956, 0.952, 0.004.

*Only one individual successfully scored.

evidence for departures from HWE in the 8 remaining collections. When applying a more conservative table-wide Bonferroni correction for 90 (6 loci and 15 collections) simultaneous tests (i.e., $\alpha = 0.00055$), 6 loci/collections are significant with one of the tests (5 with goodness of fit, 1 with log-likelihood ratio test) and none was significant with both tests (Table 2). Given that with 90 simultaneous comparisons, nearly 5 of them are expected by chance alone to be significant at an overall $\alpha = 0.05$, we conclude that there is no substantial evidence for departures from HWE in any of our historical or contemporary samples.

Genotypic disequilibrium

For genotypic disequilibrium tests we report the precise *P*-value for the cases that showed evidence of disequilibrium after a row-wide Bonferroni correction for 15 simultaneous tests (i.e., $\alpha = 0.003$). Two samples, both from NAFO division 3M (Flemish Cap), one from 1978 and the other from the 1990s, showed evidence of genotypic disequilibrium in one pair of loci, Gmo2 and Gmo120 for the 1978 collection, and Gmo4 and Gmo120 for the 1990s collection (Table 2).

Given that nearly one test in 15 is expected to be significant by chance at an overall $\alpha = 0.05$, and that there was no evidence of genotypic disequilibrium for any pairwise combination of loci when all N = 1144 individuals were pooled into a single sample (Table 2), we also conclude that there is no evidence of genotypic disequilibrium in any of our historical or contemporary collections. Together, the results of HWE tests and of tests of genotypic disequilibrium indicate that errors or bias in population allele frequency estimates due to small sample sizes are unlikely to be of biological significance.

We examined the data for evidence of temporal change in allele composition. We found no decreases in expected heterozygosity ($P > 0.351$ in single locus Kruskal Wallis rank tests and $\chi^2_{[12]} = 9.9974$, $P > 0.500$, when combining probabilities across the six loci using Fisher's method) or in the number of rare (i.e., present with $P < 0.05$) alleles (Kruskal Wallis rank test $\chi^2_{[2]} = 2.21$, $P = 0.33$). These results indicate that despite the commercial extirpation of cod populations in the 1990s, populations are still large enough that bottleneck effects are unlikely (Nei et al. 1975).

Spatial and temporal population structure

A two level (spawning bank and decade) hierarchical and multilocus AMOVA indicated that 1.55% of the total variation in allele frequencies could be significantly ($P = 0.036$) attributed to spatial structure while the variance component attributed to temporal changes within regions was negative. Standard analyses of population differentiation provided similar results: F_{ST} (95% CI) estimates among the five spawning banks were, on average, larger than the estimates among temporal collections (Table 3). The average of the 1964, 1978, and 1990s multilocus F_{ST} estimates ranges between 0.0039 and 0.0053 depending on the loci included in the overall estimate (Table 3), while the average of the five temporal estimates ranges between 0.0022 and 0.0023 (Table 3). These results collectively suggest that the spatial genetic structure among cod populations from these 5 NAFO Divisions is greater than the temporal genetic changes experienced by these same populations over the 3 decades starting in 1964.

Genetic distances

We next examined the genetic relationships among all samples using Nei et al.'s D_A genetic distances. Collections from 1964 and 1978 were indistinguishable within each of regions 2J, 3K and 3L, northern Grand Bank (all considered as northern cod) when assessed with D_A ($P > 0.300$, 1000 permutations of individual locus genotypes across all temporal and spatial samples). The three pairs of temporal samples were therefore pooled within each region to increase the sample sizes and allow D_A estimates over all 6 loci. Collections from region 3N (southern Grand Bank) and 3M (Flemish Cap) from 1964 and 1978 differed when assessed with D_A ($P = 0.026$) and were therefore not pooled (Table 4). There are 7 D_A estimates of temporal changes within the 5 NAFO Divisions (bold estimates in Table 4). The three estimates for the NE Newfoundland shelf (Divisions 2J, 3K, and 3L) are smaller than the 4 temporal estimates involving the southern Grand Bank (Division 3N) and Flemish Cap (Division 3M, $\mu_1 = 0.047$, $\mu_2 = 0.172$, $P < 0.001$, one-tailed, non-paired *t*-test).

A more synthetic description of the genetic relationships among samples is provided by a multidimensional scaling (MDS) of the D_A matrix (Figure 2). The samples from regions 3N and 3M (southern Grand Bank and Flemish Cap, respectively) lie at opposite extremes along dimension 1 of the MDS plot independent of collection date (Figure 2a). The Southern Grand Bank and Flemish Cap are separated by a 1000 m deep submarine trench (Figure 1). The

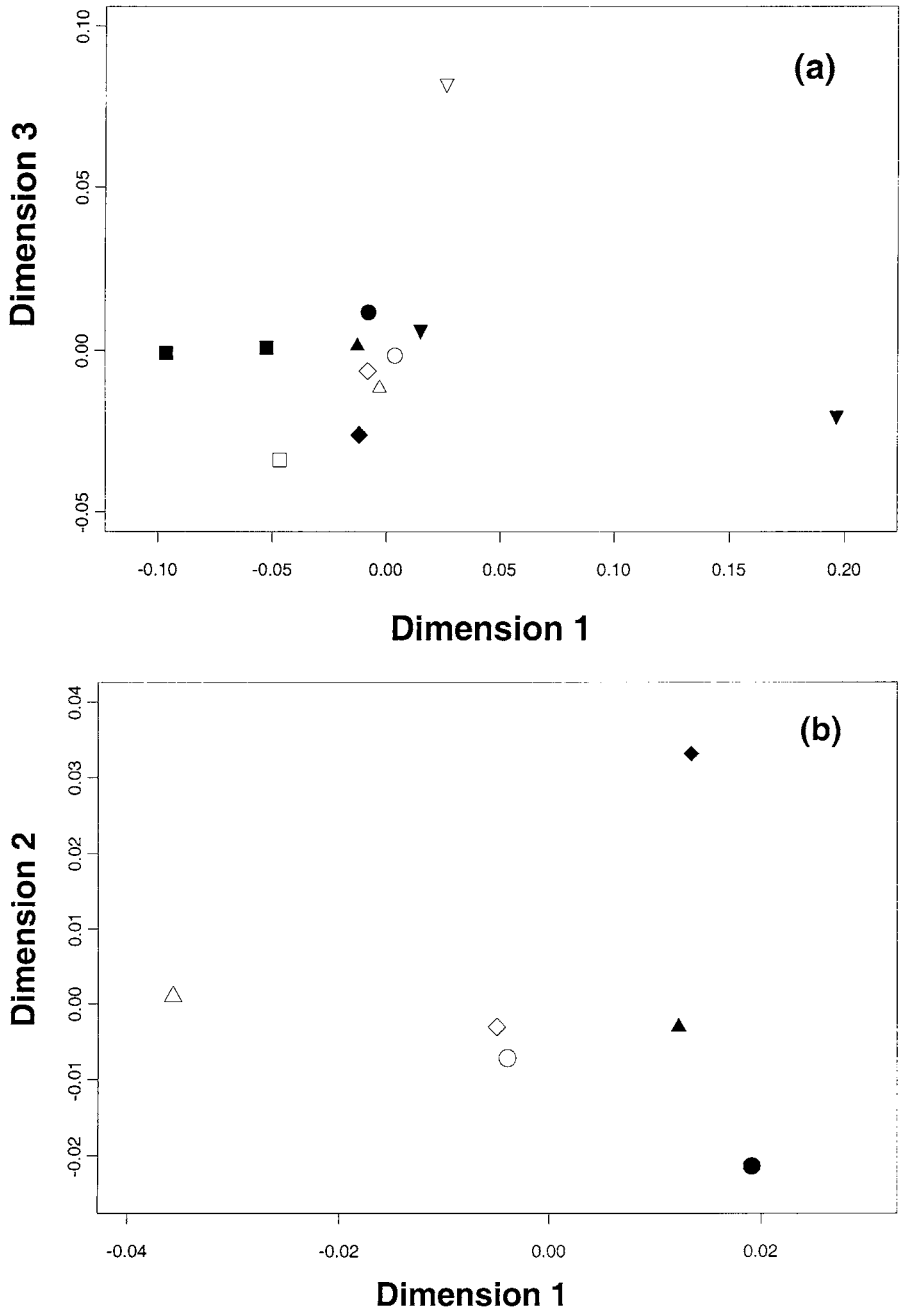

Figure 2. (a) Multidimensional scaling (MDS) plot (dimension 1 vs. 3) of the matrix of estimates of genetic distance (D_A) among local populations from spawning regions 2J (▲ and △), 3K (● and ○), 3L (♦ and ◊), 3N (■ and □), and 3M (▼ and ▽) collected in 1964 and 1978 (solid symbols), and 1992–1994 (open symbols). Cod collections from 1964 and 1978 were genetically indistinguishable ($P > 0.300$) within regions 2J, 3K and 3L. These collections were therefore pooled within each region. Dimension 1 explains 60% of the total variance, while dimension 3 explains 10% of variance. Samples represented with solid symbols were taken before the main collapse, samples represented with open symbols were taken in the early 1990s. Dimension 2 (not shown) explains 18% of the variance, the most divergent sample along this axis is that from the southern Grand Bank (region 3N) from 1964. Dimensions 4 and 5 (not shown) explain, respectively, 9%, and 3% of the total variance. (b) MDS plot (dimension 1 vs. 2) of only the samples from spawning regions 2J (▲ and △), 3K (● and ○), 3L (♦ and ◊). Note the closer grouping along dimension 2 of the 1990s samples from 3K and 3L, suggesting that some mixing has taken place between cod from these regions. Dimensions 1 and 2 explain 37% and 30% of the variance, respectively. Dimensions 3, 4, and 5 (not shown) explain 14%, 11%, and 7% of the variance, respectively.

Table 3. Single locus and multilocus F_{ST} estimates (*P*-values within brackets) and 95% Confidence intervals (CI) for the multilocus estimates

Group	loci†	Gmo2	Gmo4	Gmo132	Gmo145	Gmo1	Gmo120	Overall	95% CI
1964 (N = 254)	Gmo120	0.0047 (0.134)	0.0037 (0.058)	0.0056 (0.061)	0.0005 (0.385)	0.0132 (0.028)	NA#	0.0039 (0.011)	0.0036–0.0043
	Gmo120, Gmo132							0.0035 (0.029)	0.0033–0.0039
	Gmo120, Gmo1							0.0034 (0.020)	0.0031–0.0038
1978 (N = 320)	All 6 loci included	0.0077 (0.017)	0.0018 (0.131)	0.0248 (<0.001)	0.0042 (0.017)	0.0005 (0.372)	0.0033 (0.040)	0.0072 (<0.001)	0.0070–0.0074
	Gmo1							0.0075 (<0.001)	0.0073–0.0077
	Gmo132							0.0039 (0.001)	0.0037–0.0041
1990s (N = 570)	Gmo1	−0.0014 (NA)	0.0028 (<0.001)	0.0229 (<0.001)	0.0007 (0.204)	NA	−0.0000 (NA)	0.0044 (<0.001)	0.0043–0.0045
2J (N = 225)	Gmo1	0.0089 (0.022)	0.0036 (0.029)	0.0052 (0.105)	0.0044 (0.032)	NA##	−0.0022 (NA)	0.0037 (0.005)	0.0035–0.0038
	Gmo1, Gmo132							0.0034 (0.005)	0.0031–0.0034
3K (N = 256)	All 6 loci included	−0.0010 (NA)	−0.0019 (NA)	−0.0054 (NA)	−0.0029 (NA)	0.0149 (0.105)	0.0030 (0.106)	−0.0007	–
3L (N = 330)	All 6 loci included	0.0022 (0.186)	0.0005 (0.328)	0.0031 (0.184)	0.0049 (0.005)	0.0016 (0.263)	0.0033 (0.073)	0.0028 (0.013)	0.0028–0.0030
3N (N = 90)	Gmo1	0.0124 (0.076)	0.0005 (0.421)	−0.0012 (NA)	−0.0009 (NA)	NA###	0.0006 (0.403)	0.0022 (0.194)	0.0015–0.0027
3M (N = 243)	Gmo120	−0.0034 (NA)	0.0030 (0.097)	0.0064 (0.035)	0.0091 (0.002)	0.0010 (0.320)	NA	0.0034 (0.014)	0.0034–0.0040

#: F_{ST} for Gmo120 among 4 populations = 0.0025 (0.187).
##: F_{ST} for Gmo1 between 2 temporal groups = 0.0172 (0.057).
###: F_{ST} for Gmo1 between 2 temporal groups = 0.0198 (0.115).
†: Loci excluded in overall estimate.

Table 4. Pairwise D_A genetic distances between cod collections from 5 NAFO divisions (2J, 3K, 3L, 3N, and 3M see Figure 1) obtained in 1964, 1978, and in the early 1990s. Collections from NAFO Divisions 2J, 3K, and 3L from 1964 were indistinguishable from those from their respective regions from 1978 and were thus pooled within regions to increase sample sizes and allow D_A estimates over all 6 loci (see Table 1 for sample sizes per locus and region). D_A estimates between temporal samples within regions are in bold

	3K 64–78	3L 64–78	3N 64	3M 64	3N 78	3M 78	2J 90s	3K 90s	3L 90s	3N 90s	3M 90s
2J 64–78	0.040	0.046	0.107	0.208***	0.100	0.061***	**0.054**	0.039	0.034	0.088	0.092***
3K 64–78	0	0.056	0.117	0.211***	0.124	0.069**	0.060	**0.041**	0.043	0.118	0.100*
3L 64–78	0	0	0.109	0.208***	0.110	0.079***	0.059	0.049**	**0.047****	0.098	0.122***
3N 64	0	0	0	0.272***	**0.180***	0.114*	0.099	0.099	0.101	**0.125**	0.151**
3M 64	0	0	0	0	0.301***	**0.183*****	0.202***	0.188***	0.202***	0.255***	**0.199*****
3N 78	0	0	0	0	0	0.134**	0.127*	0.115*	0.105	0.139	0.170**
3M 78	0	0	0	0	0	0	0.071***	0.053***	0.057***	0.107	0.092**
2J 90s	0	0	0	0	0	0	0	0.044	0.042	0.116	0.115**
3K 90s	0	0	0	0	0	0	0	0	0.032	0.091	0.087**
3L 90s	0	0	0	0	0	0	0	0	0	0.095	0.101***
3N 90s	0	0	0	0	0	0	0	0	0	0	0.146*
3M 90s	0	0	0	0	0	0	0	0	0	0	0

*P <0.05; **P < 0.01; ***P < 0.001

genetic differences between cod from these two locations were relatively large and geographically stable between from 1964 through to 1994. Collections from the northern cod region and the northern Grand Bank (NAFO Divisions 2J, 3K, and 3L) are spread between these two extremes on dimension 1 (Figure 2). Dimension 1 explains a large proportion (60%) of the total genetic variance among all the samples. Thus, these results indicating temporal stability on the broad geographic scale that includes cod aggregations from all 5 NAFO Divisions are consistent with the AMOVA, which assigned a significant proportion of the explained variance to spatial effects. As is typical with MDS and principal coordinate analyses, the higher the dimension number (i.e., the lower the importance of the dimension) the lower the percentage of the total variance that is explained by that dimension. Dimension 2, which explains 18% of the total variance separates the 1964 southern Grand Bank (3N) sample from the rest, most of which are so tightly grouped along this axis that they can not be easily distinguished (data not shown). Dimension 3, which only explains 10% of the total variance distinguishes the post-collapse Flemish Cap from all other samples. The relationship between dimensions 1 and 3 is depicted in Figure 2a where the samples from the 1990s preserve their order (Figure 2a), a pattern consistent with the F_{ST} analysis that indicates that the overall broad scale structure among cod from the 5 NAFO divisions has remained approximately the same over three decades and collapse in population size. We also conducted a finer scale analysis by examining the relationship among the pre- and post-1990s collapse samples of northern cod (NAFO divisions 2J, 3K and 3L) exclusively (i.e., we excluded Flemish Cap and southern Grand Bank cod). An MDS plot of dimensions 1 and 2 (37% and 30% of variance, respectively) suggests that, though there was stability in the overall spatial genetic pattern, there was also a decrease in the 1990s in the genetic differentiation between cod from divisions 3K and 3L (see also Table 4) as would be expected under the hypothesis of increased mixing between cod from these two regions in the years leading to, and including the collapse of the fishery (Rose et al. 2000).

Discussion

Published studies of long term temporal changes in the genetic composition of fish populations have thus far been limited to freshwater and anadromous species, and in all cases DNA was extracted from old scales (Miller and Kapuscinski 1997; Nielsen et al. 1997, 1999; Tessier and Bernatchez 1999). We have used DNA extracted from archived otoliths and from contemporary samples to show that a heavily exploited and highly migratory marine fish, Newfoundland cod, has historically (over 3 decades) retained a geographic structure that is most easily explained by fidelity to natal spawning grounds. The overall AMOVA of allele frequencies shows a significant geographical effect but no significant temporal effect. When measured with F_{ST}, the spatial genetic structure averaged over the three decades ranges between 0.0039 and 0.0053 depending on which loci are included, while the

estimate of temporal structure averaged across the 5 NAFO Divisions is approximately 0.0022–0.0023. Multidimensional scaling analysis shows the stability of the broad scale geographical pattern over time. Analysis at the finer geographical scale that excludes Flemish Cap (3M) and southern Grand Bank (3N) cod suggests, however, the possibility of an increased mixing between cod from divisions 3K and 3L (but not cod from 2J) and is consistent with the hypothesis of a disruption of the seasonal (feeding) migratory behaviour following the disappearance of older cod hypothesised to sustain migratory routes (Rose 1993), or of a southern shift in distribution of 3K cod concomitant with the fishery collapse in the late 1980s and early 1990s (Rose et al. 2000).

Evidence for genetic differentiation among contemporary Newfoundland cod aggregations

We have recently provided evidence of genetic differentiation between contemporary inshore and offshore cod in Newfoundland (Ruzzante et al. 1996) and Labrador (Ruzzante et al. 2000b). Where patterns have been examined over time (short term), these differences have been shown to be temporally stable over a period from 2 to 3 years (Ruzzante et al. 1997). Genetic differences have also been documented among contemporary offshore aggregations in Labrador and Newfoundland waters (Bentzen et al. 1996; Ruzzante et al. 2000a). Elsewhere (Ruzzante et al. 1998, p. 1676) we have hypothesised that for northern cod and neighbouring regions an appropriate stock structure is likely to comprise: (1) a non-migratory Flemish Cap component (NAFO Division 3M); (2) a cross-shelf migrant component with spawning (winter) fidelity to the various banks on the NE Newfoundland Shelf (Divisions 2J and 3K); (3) a cross-shelf migrant component with spawning (winter) fidelity to the northern Grand Bank region (Division 3L); and (4) an along-shelf migrant component that has inshore or nearshore winter and spawning fidelity along coastal Newfoundland (i.e., inshore cod). This latter group may exhibit structure at the scale of neighbouring bays, though such structure may be ephemeral. Our findings and predictions have recently been confirmed by a study on the population structure of Labrador and Newfoundland cod conducted by a different laboratory that used over 5000 cod collected in the late 1990s from 19 putative inshore and offshore population components (Beacham et al. 2000). They screened for variation in seven new microsatellite loci (i.e., not used in our studies), one major histocompatibility (MHC) locus, and one locus presumed to be under selection and found that all inshore cod collections they examined were genetically distinct from all offshore collections of northern cod. These authors also concluded that there may be at least three distinct offshore spawning populations of northern cod (Beacham et al. 2000).

The increasing collection of recent studies largely employing microsatellite DNA markers and published by different authors provides strong support to reject the null hypothesis of no genetic differentiation among contemporary populations of Atlantic cod in the northwest Atlantic. Further support for the hypothesis that Newfoundland cod is a complex of distinct spawning components is provided by evidence based on tagging studies of spawning fidelity at offshore bank scales (Templeman 1979; Lear 1984; Taggart et al. 1995; Taggart 1997), by evidence of bank-scale spatial and temporal differences in spawning (Hutchings et al. 1993; Myers et al. 1993), and by evidence of variation in vertebral counts (Templeman 1981; Lear and Wells 1984).

Here, we have attempted to examine the evidence back through time. We have used DNA extracted from archived otolith collections together with contemporary collections to provide evidence that the broad scale genetic structure among the 5 NAFO Divisions that include the northern cod region (2J, 3K, and 3L), the southern Grand Bank (3N), and the Flemish Cap (3M) has remained temporally stable over a period of three decades starting in 1964 and encompassing very large changes in population size.

Implications for recovery of Newfoundland cod

With one exception, the estimated F_{ST} values are less 0.5% (Table 4). Although relatively low, these values are in line with those reported for cod at comparable geographic distances in other regions of the NW Atlantic [e.g., Scotian Shelf (Ruzzante et al. 1998), Bay of Fundy and adjacent areas (Ruzzante et al. 2000a)] as well as of the NE Atlantic (Hutchinson et al. 2001). Two issues should be taken into consideration before interpreting these relatively low values as indicating high gene flow. First, for any given level of gene flow, the magnitude of F_{ST} is inversely related to the number of alleles and thus, to heterozygosity (Jin and Chakraborty 1995; Hedrick 1999) and the average number of alleles in the six microsatellite loci employed here is relatively high (34.8). Second, in

some cod genetics studies gene flow and geographic distance have been shown to be correlated at relatively small (e.g., Pogson et al. 1995; see also the cline in genetic differentiation within the Scotian Shelf in Ruzzante et al. 1998), and large (Mork et al. 1985; Pogson et al. 2001) geographic distances. Such correlations suggest that dispersal distances and effective population sizes are likely much smaller that predicted for the species. It is likely that the low F_{ST} values can therefore be ascribed, at least in part, to the recent age of populations rather than to extensive gene flow (Pogson et al. 2001).

Cod populations in the northwest Atlantic are presumably still large enough that biological extinction is not an immediate threat. We do know, however, that contrary to early 1990s predictions (Lear and Parsons 1993; deYoung and Rose 1993) there is no evidence so far for a recovery of northern cod (Rose et al. 2000; Hutchings 2000). The survival of the broad scale spatial pattern of the genetic differentiation during the population collapse may provide some insight into why population recovery is not occurring, or is not occurring faster. Depensatory mechanisms or Allee effects (see Myers et al. 1995), whereby below a certain threshold in population size, per capita reproductive success declines due to behavioral or physiological mechanisms (e.g., difficulties in finding a mate) are likely operating not at the population complex level, but at the level of its individual components (Frank and Brickman 2000). The survival of the broad scale geographic pattern of genetic structure may also provide some insight into how population recovery could possibly occur in the future. For example, if recovery eventually takes place, it will likely do so mostly through population re-growth *in-situ*, rather than by immigration.

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