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Morphological, Meristic and Genetic Analysis of Stock Structure in Juvenile Atlantic Cod (*Gadus morhua*) from the Newfoundland Shelf

by

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Abstract

We examined joint patterns of variation among eleven morphological variables, one meristic variable (vertebral count), and a genetic variable (DNA sequence variation in the mitochondrial cytochrome b gene) among juvenile (0-group) cod from the northeast Newfoundland shelf and the Grand Banks. Canonical discriminant analysis shows that the group centroids of fish on and off the Grand Banks are significantly different, and that fish from the most southerly region (3O) have a significantly smaller mean vertebral count than fish from the more northerly regions (3K, 3L). However, there is substantial overlap of individuals in canonical variate space and reclassification of fish into their region of origin is successful in less than 50% of cases. Analysis of the distribution of ten DNA sequence genotypes indicates substantial homogeneity of genotypes within localities and little or no genetic subdivision among regions. The pattern of genetic differentiation is consistent with a model of recent origin of most genetic variation following a bottleneck in population numbers. The combination of morphological, meristic, and genetic analysis of juvenile cod in NAFO Division 3K, 3L, and 3O does not support the hypothesis of stock separation among these areas. In particular, our data do not support the use of vertebral counts to define stock separation during early life history.

INTRODUCTION

The capacity of fish populations to adapt and evolve as independent biological entities is limited by the exchange of individuals among populations. A sufficient degree of isolation may result in notable morphological, meristic, and genetic differentiation among populations within species, which may be recognizable as a basis for separation and management of distinct populations. In marine systems, the dispersal ability of animals may be strongly influenced by discontinuities in the physical environment, such as the temperature and salinity differences that define water masses and current boundaries. In extreme cases, such as species which produce planktonic eggs and larvae, individuals may have very limited control over their own horizontal position and dispersal is determined largely by drift patterns in the oceanic environment. The influence of such dispersal patterns on exchange among populations is variable. For species in which the larval retention areas are physically isolated from one another (e.g. herring, Clupea harengus), it has been argued that individuals that drift out of the home area are lost and thus constitute vagrants, whereas individuals that are retained will contribute to the continued survival of the population (Iles and Sinclair 1982; Sinclair 1988). However, for species with more continuous distribution of eggs and larvae (e.g., Atlantic cod, Gadus morhua), reproductive isolation of adjacent populations may be diminished because individuals that drift out of one population can enter another.

Atlantic cod in the western north Atlantic are currently managed as a number of distinct stocks. Templeman (1981) identified 5 populations in the region encompassing the Newfoundland and Labrador shelf, based principally on a meristic analysis of vertebral averages. These populations are found on the Labrador shelf (NAFO regions 2GH), the northeast Newfoundland shelf (2J3KL), the southern Grand Banks (3NO), the Flemish Cap (3M), and St.Pierre Bank (3Ps). Templeman argued that the north/south decline in vertebral averages was indicative of temperature differences among spawning habitats; however, he also believed there was a strong potential for intermixing of the populations because of variations in the transport of eggs and larvae from one region to the next.

Transport of offspring by ocean currents across division boundaries is likely given the notable synchrony in recruitment of groundfish stocks (Koslow et al. 1987; Thompson and Page 1989). Although broad scale fluctuations in environmental factors are generally considered to be the ultimate determinant of recruitment synchrony (Koslow et al. 1987), the degree of environmental synchrony between stock ranges is often poor (Thompson et al. 1988). Thus, it is possible that synchronous recruitment of adjacent populations reflects distributional overlap during the planktonic phase. If correct, this would have important implications for the distinctness of cod populations along the eastern coast of Newfoundland and Labrador.

Templeman's (1981) meristic analysis was based on data collected over multiple years (1947-71) and several age classes. However, he used only a single meristic variable, vertebral average, which can be influenced by interannual variations in temperature (Brander 1979; Frank 1991). Here we ask whether evidence for Templeman's (1981) separation of cod for some regions of the Newfoundland shelf as isolated stocks is supported by other features. Templeman's (1981) proposal predicts that the distribution of eggs, larvae, and juveniles should all coincide with the segregation observed in adults in order to maintain consistent stock differences. To test this hypothesis, we report an analysis of morphological, meristic and genetic analyses of a single year-class of juvenile (0-group) cod from inshore regions of Newfoundland as well as from the Grand Banks. If stock separation is stable, it should be apparent for any given year-class. We limited our study to juveniles because they are likely to have remained in the same areas where they settled following the egg and larval drift period.

MATERIALS AND METHODS

Sampling. Five inshore locations were sampled along the eastern coast of Newfoundland in September and October 1989 (Figure 1). These locations are similar to those sampled by Lear and Wells (1984). Samples were obtained with a modified beach seine 100 m in length, equipped with 7 mm mesh. The seine was extended directly from shore and brought around in a semi-circle. All inshore sampling was conducted after sunset to reduce gear avoidance.

Samples were also obtained from the Grand Banks of Newfoundland in August and September 1989 (Figure 1). These samples were obtained from a research vessel survey of juvenile fish over the entire Grand Banks (S. Walsh, Fisheries and Oceans, St. John's, pers. comm.). Sampling sites were determined according to a random stratified design (1° x 1° grid). The survey gear was a two-bridle Yankee 41 (80/104) shrimp trawl with a mesh size of 38 mm throughout and equipped with a 12 mm stretched mesh liner in the codend. Tows were of 30 minutes duration at a speed of 2.5 knots. We also attempted to obtain samples from offshore areas in 3K but were unsuccessful.

All specimens were frozen either at -20°C or at -70°C immediately after capture. Fish were classified into four regions designated 3K, 3L inshore ($3L_i$), 3L offshore ($3L_o$) and 3O, that correspond to the management divisions in the current NAFO scheme. Samples from NAFO Division 3L were sub-divided into inshore and offshore regions to assess possible population differences.

Morphometric characters. Specimens were thawed for 3 hours to ensure uniform condition. Each individual was blotted dry and placed on a flat surface. Data included standard length, distance from snout to the anus, distance from snout to the origin of the third dorsal fin, distance from snout to the origin of the first dorsal fin, distance from snout to the origin of the pelvic fin, length of the head, eye diameter, snout length, jaw length, depth of head and depth of caudal peduncle (Figure 2), measured to three significant digits with electronic callipers accurate to 0.01 mm.

A canonical discriminant analysis was used to assess whether morphological variation was greater within rather than among regions. Morphological features were standardized relative to length with an allometric model fitted by least-squares regression ($\ln Y = \ln a_1 + a_2 \ln L$, where L is standard length (mm), Y is one of the other morphometric features, and a_1 and a_2 are estimated constants). This procedure reduces the potential for analytical error caused by variations in length frequency distributions associated with differences in sampling time or gear efficiency. Analysis of covariance was performed to determine whether there were significant regional differences in allometric parameters. Residuals from the allometric model were used for the analysis. Geisser reclassification probabilities were calculated with the Multiple Discriminant Analysis (MDA) program from the BIOSTAT II package of Pimentel and Smith (1986); prior probabilities of group membership were equalized among regions.

Meristic features. Vertebral counts were determined from x-ray autoradiographs of thawed specimens. Vertebral counts followed the procedure outlined by Templeman (1981) and Lear and Wells (1984) in which the urostylar half-vertebra was included in the count. Two counts were performed independently by each of two technicians and these were then checked by a third. If all three counts did not agree, the specimen was excluded from the analysis. Specimens with fused vertebrae were also excluded. Comparison of the distribution of vertebral counts among regions was performed using the Shapiro and Wilk (1965) test.

Mitochondrial DNA sequence variation. We examined DNA sequence variation in a 307 base pair segment towards the 5' end of the mitochondrial cytochrome b gene. Variation in this segment has been used to assess intraspecific differentiation within other vertebrate species; pairwise sequences divergences for this segment between individuals within species of vertebrates are slightly greater than those estimated from the entire mtDNA molecule (Carr and Marshall 1991a,b; Árnason et al. 1992; Carr and Hughes in press). Samples of brain tissue were removed during measurement of morphological features. Conditions for DNA extraction, amplification by the polymerase chain reaction, and single-stranded DNA sequencing were as described in Carr and Marshall (1991a); some double-stranded DNA templates were sequenced on an Applied Biosystems 373A automated DNA sequencer (Carr and Marshall 1991b). We used as primers the pair of 26-base oligonucleotides described by Kocher et al. (1989). Sequence variants were confirmed by reamplification and resequencing of the same or alternate strand.

Heterogeneity of genotypes within sampling locations was estimated by the heterogeneity index (h) of Nei and Tajima (1981), which is equivalent to the probability that two randomly chosen individuals from the same sample will have different genotypes. The heterogeneity of genotype distributions among regions was tested by the Monte Carlo χ^2 procedure of Roff and Bentzen (1989); a total of 1,000 replications of the matrix were used. Gene flow among sampling localities was estimated as the product $N_e m$ (where

N_e is the effective population number and m is the fraction of migrants per population per generation) by the method of Slatkin and Maddison (1989). This method uses a reconstruction of the phylogeny of genotypes, in combination with a count of the minimum number of migration events (S) necessary to produce the pattern of shared genotypes among populations. For haploid genes such as mtDNA, a value of $N_em = 1.0$, which is equivalent to the exchange of one individual per pair of populations per generation, is regarded as a threshold below which populations would begin to diverge by genetic drift alone (Wright 1931; Slatkin 1985). We estimated N_em using a simulation program estimator supplied by M. Slatkin (Department of Integrative Biology, University of California, Berkeley, pers. comm.); 95% confidence intervals were estimated by bootstraping. We estimated the coancestry coefficient θ, which measures the extent of genotypic differentiation among populations as the probability that two genotypes drawn at random from two different regions are identical by descent, with the HAPLOID.FOR program of Weir (1990); the standard deviation of θ was estimated by jackknifing over populations. For haploid alleles, θ is an equivalent measure to Wright's (1951) F_{ST} for diploid loci.

RESULTS

Morphometric characters. All body features were significantly correlated with standard length (Table 1). Analysis of covariance indicated no evidence of significant differences in allometric parameters among regions. After accounting for differences in body length using allometric models pooled over regions, a canonical discriminant analysis using all residual elements indicated significant differences in the centroid positions among the four geographic regions (Wilk's $\lambda = 0.80$, $F_{27, 1014} = 2.96$, $p < 0.001$) (Figure 3). The residuals of the positions of the first dorsal and pelvic fins as well as the head, snout, and eye dimensions contributed most strongly to the first two canonical variables (Table 2). Regions 3K and $3L_i$ were not significantly different from one another, nor were regions $3L_o$ and 3O. However, the differences between these two pairs ($3K/3L_i$ vs $3L_o/3O$) were significant, suggesting that juvenile cod captured on the Grand Banks are different morphometrically from individuals captured in the same NAFO division but in inshore habitats. Despite the significant differences among centroid positions, overlap in the distribution of individuals from different regions in the space of the first two canonical variables is substantial (Figure 4). Reclassification of animals into the region of origin based on morphological features is highly ineffective in separating individuals from random unknown samples. Table 3 shows that, on average, less than 50% of reclassifications are correct. Animals from the northern Grand Banks were correctly reclassified in 69% of cases, but this is due partly to the small sample size.

Meristic features. Trends in vertebral averages correspond to those obtained by both Templeman (1981) and Lear and Wells (1984): fish sampled in the most southerly region (3O) have significantly fewer vertebrae than the animals sampled in the three more northerly regions (Figure 5). However, there are no significant differences in the distribution of the number of vertebrae among any of the other regions. These results are in contrast with the morphometric analysis, which separate juvenile cod on and off the Grand Banks. Canonical discriminant analysis indicates that morphological characteristics are unrelated to vertebral counts (analysis not shown).

Mitochondrial DNA sequence variation. Within the middle 307 bp of the amplified segment, 11 variable positions were identified among 103 cod (Figure 6). Of these positions, one (9%) is a purine to pyrimidine transversion mutation (G↔T), four (36%) are purine transition mutations (A↔G), and six (55%) are pyrimidine transition mutations (C↔T). Two of the purine transitions occur at first positions in their respective codons, and result in amino acid substitutions; neither of these is in a region considered crucial to the function of cytochrome b (Howell and Gilbert 1988). All of the remaining substitutions occur at the third codon position, except for a single first-position change for an alternative leucine codon.

The variable positions define 10 distinct sequence genotypes among cod, which differ by between one and five nucleotide substitutions (Figure 6). Genotypes A, C, E, G, and J have been reported previously (Carr and Marshall, 1991a). Genotypes C, J, and M form a clade defined by a shared nucleotide substitution at position 246. Genotype J differs from C by two additional substitutions, and genotype M differs from genotype C by three additional substitutions (Figure 7), making it the most divergent genotype yet noted in Gadus morhua. The distribution of these genotypes among sampling locations is given in Table 4. Genotype A is the most common genotype in all geographic regions (overall mean 84%, range 71-88%). The most common alternative genotype (G) occurs at an overall frequency of 5.8% and is found in all three NAFO Divisions examined. Genotype E occurs at an overall frequency of 3.9%, and is confined to division 3L, where it occurs in both inshore and offshore sampling locations. The remaining genotypes occur in only one or two individual fish.

Nei and Tajima's (1981) heterogeneity indices (h) for regions 3K, $3L_i$, $3L_o$, and 3O are 0.22, 0.37, 0.40, and 0.35, respectively; the aggregate index is 0.33. The Monte Carlo test indicates no significant heterogeneity of genotype distributions among the four major regions ($\chi^2 = 23.8$, df = 27, $P > 0.63$). Non-significant results are also obtained if the data are pooled by the three NAFO Divisions ($\chi^2 = 18.3$, df = 18, $P > 0.44$) or if they are separate along management units (3KL vs 3O, $\chi^2 = 13.0$, df = 9, $P > 0.21$) . The coancestry coefficient θ among the four regions is -0.0122 (s.d ± 0.0199): this result indicates that the proportion of genotypic differentiation due to subdivision into regions is negligible.

From the phylogenetic relationships shown in Figure 7 and the sample sizes and distribution of 10 genotypes among the four regions given in Table 4, we calculate $S = 10$, from which we estimate $N_em = 0.7$ (95% confidence interval 0.2 - 1.1). Pairwise estimates of N_em among the four regions are similar.

DISCUSSION

The combination of morphological, meristic, and genetic data does not identify any consistent pattern of variation among juvenile cod (0-group) in NAFO regions 3K, 3L, and 3O. Despite the limited sample sizes, meristic variations are entirely consistent with Templeman's (1981) and Lear and Wells' (1984) observations of adult and juvenile fish: cod from NAFO Division 3O have significantly fewer vertebrae than cod in divisions further north. This pattern is different from that observed in morphometric characters in which juvenile cod captured on the Grand Banks are different from individuals captured in inshore areas of the same Division. Finally, the

genetic analysis shows no evidence of any differentiation among Divisions. As such, the analysis does not support Templeman's (1981) proposal that the distribution of pre-recruit stages of cod on the Newfoundland shelf represents that of reproductively isolated stocks.

Our results contrast with studies on adult fish, which have shown north/south clines suggestive of stock differences in a suite of parameters such as age and size at maturity (Fleming 1960), growth (May et al. 1965), fecundity (May 1967), and parasites loads (Kahn et al. 1980, but see Brattey et al. 1990). However, all of these features can be strongly influenced by variations in environmental and biotic conditions and are in themselves insufficient to define reproductive segregation in the study area. Average vertebral count has also been used as a meristic indicator of stock differences in cod: eggs spawned in colder water produce fish with higher mean vertebral counts as adults than those spawned in warmer water (Tåning 1944, 1952; Dannevig 1950; Lindsay 1954; Brander 1979). Templeman (1981) has argued therefore that variation in vertebral counts is a good indication of physical separation during early life history. For example, eggs spawned in 2J3KL earlier in the year will occur in colder water than those spawned in 3NO later in the year, and will be expected to produce higher vertebral averages in the adults.

The only significant difference in vertebral averages observed in the present study does indicate that the juvenile cod caught on the southern Grand Banks (3O) developed at warmer temperatures than animals caught further north. Brander's empirical model predicts that these cod underwent embryonic development at an average temperature 4°C warmer than fish from the other three regions. However, this difference is in itself not conclusive evidence that the 3O fish came from a distinct spawning population. There is considerable overlap in the spawning times of cod northeast of Newfoundland and on the Grand Banks, which occur in March/May and April/June (Templeman 1981). In a typical year, surface temperature rises sharply during the period from April to June on the northeast Newfoundland shelf and the Grand Banks (Petrie et al 1991): in 1989, surface temperature on the northern edge of the Grand Banks rose by 6°C between April and June (recorded at Station 27: 47° 32.7'N, 52° 35.2'W). Therefore, local temporal variations in the rate of water warming during the spawning period could result in substantially different developmental profiles and produce variations in vertebral averages among cod from the same spawning aggregations that are equal to or greater than the variations observed among regions. Vagaries of current drift due to climatic fluctuations could result in differential dispersal of eggs and larvae produced during different portions of the spawning season within a region. Templeman (1979) reports finding mature and immature cod with a wide range of vertebral counts, typical of the north to south cline observed among regions, within a single region of the northern Grand Banks. Templeman (1981) himself clearly recognized that there was potential for inter-mixing of populations along the Newfoundland and Labrador shelf regions as well as for variations in developmental conditions within a given spawning region.

Morphometric differences might reflect different adjustments by the animals to their feeding environment, prey types and availability, or other features associated with the pre- or post-settlement of juvenile cod. If so, it appears that the juvenile cod see the environment as relatively homogeneous. Although some group centroids differ significantly, and some features or combinations of features suggest regional differences in morphology, the extensive overlap among groups makes it difficult to assign any particular animal to a given group with any confidence. Juvenile cod may not develop distinct morphometric characteristics because features that influence development (e.g. food abundance, temperature) may not be sufficiently heterogeneous in the pelagic environment to result in notable differences among young fish. Alternatively, life history variations observed in adult fish may be due to prolonged exposure to environmental conditions not yet experienced by 0-group cod.

It is possible that small sample sizes from the Grand Banks could limit our ability to detect significant differences in morphometric characters. However, the degree of overlap in canonical variables is in contrast with the results of other studies of stock discrimination that show distinct separation of morphometric features among populations, independently of age or year-class (Meng and Stocker 1984; Taylor and McPhail 1985; Surrf et al. 1986; Fabrizio 1987; Schaefer 1991; Roby et al. 1991). It is also possible that interannual variations in cohort characteristics would be missed by focusing on a single year-class. However, stable stocks should be observable over any year-classes. Failure to observe consistent differences would indicate that reproductive isolation has not been established.

Numerical simulations by Helbig et al. (1992) show that average current structure on the Newfoundland shelf could act as an isolation mechanism between stocks on and off the Grand Banks. Tagging studies indicate that, although there is broad scale dispersion during the inshore feeding migration of adult cod, there is strong fidelity for reproductive sites, which may produce some separation of spawning components (Lear 1984, 1986). Nonetheless, transport and diffusion are probably sufficient to prevent any substantial degree of differentiation among juvenile cod populations on the Newfoundland shelf. If Templeman's (1981) proposal for stock definition is correct, we should be able to find distinct spawning aggregations of adults as well as distinct nursery populations of juveniles. Absence of the latter does not preclude existence of the former.

Recent studies of mtDNA in Atlantic cod have revealed variable amounts of genetic diversity within and among populations in the vicinity of Newfoundland and elsewhere in the North Atlantic. Smith et al. (1989) found no variation among a limited number of restriction fragment length polymorphism (RFLP) markers in a single sample from the Grand Banks ($\underline{h}=0.0$). In a study of the same piece of DNA described here, Carr and Marshall (1991a,b) documented a pattern among adult cod in the western Atlantic that is qualitatively similar to that observed here in juvenile cod. Although multiple genotypes are observed, genotype A predominates in all populations, no alternative genotype occurs at an overall frequency of more than a few percent, and most genotypes are observed only once (mean $\underline{h}<0.40$). In contrast, genetic variation in populations elsewhere in the North Atlantic appears to be substantial, with no single genotype predominating and several co-existing in the same population at moderate frequencies. For a single population from Norway, Carr and Marshall (1991a) measured $\underline{h}=0.88$; for eight populations from Iceland, we calculate $\underline{h}$ = 0.92 from the RFLP data in Figure 4 of Árnason et al. (1992). Substantial mtDNA variation among Arcto-Norwegian cod has also been reported by Dahle (1991).

The degree of genetic subdivision among populations (measured as θ) is an important indication of the extent to which animals subdivided among different geographic areas exist as separate breeding populations (Wright 1951; Weir 1990). Gene flow among populations (estimated by $\underline{N_em}$) tends to reduce such subdivision and can result in a substantially homogeneous distribution of genotypes (Slatkin 1985) as measured by chi-square (Roff and Bentzen 1989). Following the lead of Árnason et al. (1992) based on the method of Slatkin and Maddison (1989), we estimated $\underline{N_em}$ among the four Newfoundland regions as 0.7 (95% confidence interval 0.2 - 1.1). We also re-estimated gene flow among the eight Icelandic populations from Figure 6 of Árnason et al. (1992) as $\underline{N_em} = 1.1$. For exchange between Newfoundland and Norway, based on a revised phylogeny of the genotypes in Carr and Marshall (1991a), we obtained $\underline{N_em} = 6.8$. These estimates of $\underline{N_em}$ raise several questions. First, gene flow among Icelandic populations seems substantially less than that previously suggested ($\underline{N_em} = 40$) by Árnason et al. (1992); this is due in part to the small sample sizes for which a well-resolved phylogeny based on completely comparable RFLP data was available. The extent of stock differentiation of Icelandic cod may need to be reconsidered. Second, the estimate of transatlantic gene flow is substantially greater than that among either Iceland or Newfoundland populations (i.e., gene flow appears to be less between inshore and offshore localities in Newfoundland than between either of these areas and Norway). However, the χ^2 analysis shows that genotype distributions are highly heterogeneous between transatlantic populations ($p << 0.001$) whereas they are substantially homogeneous among the four Newfoundland regions ($p > 0.63$), which is consistent with substantial mixing among components of the Newfoundland cod. Monte-Carlo χ^2 analysis of the Icelandic localities also indicates non-significant heterogeneity ($\chi^2 = 210$, df = 176, $p > 0.07$). Finally, the very small estimate of $\underline{N_em}$ among Newfoundland populations suggests that they should be diverging evolutionarily. However, the very small estimate of θ also indicates little or no differentiation among populations.

One explanation of the disparity among the various indicators of differentiation is that Newfoundland cod have undergone a period of exponential growth following a relatively recent "bottleneck" in population numbers. Most genotypic variants will then be newly derived from a common surviving lineage, and the result is a "star phylogeny" (Slatkin and Hudson 1991) of the type seen in Figure 7 and in Carr and Marshall (1991b). Genetic relationships among individuals will not be well resolved, and the estimator of migration ($\underline{S}$) cannot be very large no matter how extensive actual migration. Under these conditions, Slatkin and Maddison's (1989) method may not be appropriate: $\underline{S}$ probably underestimates actual migration (M. Slatkin, pers. comm.). Further, the algorithm does not take into account the frequency of genotypes, especially rare genotypes confined to single populations. Finally, studies of adult fish indicate that several of the genotypes that in this analysis are confined to particular regions also occur in other regions (Carr and Marshall 1991a and unpub. data). $\underline{N_em}$ will rise if some of the rarer genotypes are in fact more widely distributed. The bottleneck theory is also consistent with the low genetic heterogeneity ($\underline{h}$) of mtDNA, and the absence of detectable allozyme differentiation (Cross and Payne 1978), among cod populations in the western North Atlantic, as previously discussed (Carr and Marshall 1991a).

An alternative explanation for the low genetic heterogeneity of cod populations from Newfoundland is that the segment of the cytochrome b gene examined is too evolutionarily conservative to distinguish populations within species. Comparative data on the same segment from other vertebrate species indicate this is not the case. Pairwise sequence divergences for this segment are slightly greater than those estimated for the mtDNA molecule as a whole (Wayne et al. 1990; Carr and Hughes in press). Among four species of tuna (Thunnus sp.), Nei and Tajima's (1981) $\underline{h}$ index ranged from 0.28 to 0.84 (calculated from Bartlett and Davidson 1991). Among capelin (Mallotus villosus) from a single sampling location in Newfoundland, 20 different genotypes were found among 48 individuals ($h = 0.74$) (T.P. Birt and W.S. Davidson, Department of Biochemistry, Memorial University, pers. comm). Variation within marlin (Makaira nigricans) is similarly high (Finnerty and Block 1992). The low genetic variability of cod on the northeast Newfoundland shelf is thus unlikely to be a consequence simply of a low rate of molecular evolution.

The absence of consistent patterns among meristic, morphological, and genetic analyses of stock structure in juvenile cod is similar to the results obtained in other marine species (Leslie and Grant 1990; Roby et al. 1991). In contrast, analyses of anadromous fish tend to show consistent patterns of stock structure revealed in both physical and genetic markers (Reddin et al. 1990; Nolan et al. 1991; Melvin et al. 1992). The contrast suggests that the degree of stock separation may be determined by features in early life history (Sinclair 1988). For example, whereas anadromous fish populations tend to spawn in distinct and isolated areas, spawning in marine fish species generally occurs over much broader geographic areas. In marine species, therefore, extensive egg and larval drift across management unit boundaries may limit the potential for genetic differentiation of such stocks, despite differences in subsequent environmental histories that may affect phenotypic features. Attempts to define populations based on differences in meristic or morphological features must be verified by genetic evidence in order to confirm that the former differences reflect some degree of reproductive isolation rather than simply environmental distinctiveness.

Our analysis also suggests that the reality of reproductive isolation among cod on the Newfoundland shelf south of 52°N may need to be reconsidered. Although the present study gives no indication that 0-group cod on the east coast of Newfoundland are separated into different stock components, it has yet to be determined whether fish that occur in separate spawning aggregations represent distinct populations. There is considerable dispersal of adult fish among regions 2J, 3K, and 3L from year to year, and no such exchange occurs between 2J3KL and 3NO spawning stocks (Lear 1984). Physical isolation during the early life is unlikely because of the highly variable currents into which eggs and larvae are released (Helbig et al. 1992). Whether individuals spawned on the northeast Newfoundland shelf return to this area to spawn, or whether they would contribute substantially to the Grand Banks population if they were to settle in that area, is unknown. Such long-distance movements are possible: Harden Jones (1968) suggests that offspring from the Icelandic cod stock that drift to Greenland may return to their parental spawning grounds to reproduce. Such a hypothesis cannot yet be rejected in the case of cod in NAFO regions 3K, 3L, and 3O. Furthermore, it is possible that the ecological dynamics of separate management units reflect distinct regional differences in post-settlement habitat quality, and hence production, despite the lack of genetic differentiation among these units. Adult cod on the northeast Newfoundland shelf exhibit variations in life history features that affect the production of exploitable biomass in the different regions (Lear 1986). Thus, geographic complexes of phenotypically homogeneous adult cod can be recognized, and can form the basis for the management of fishing patterns and effort. However, it is essential to recognize that these complexes are not genetically differentiated and thus do not represent distinct populations. The strong correlation of recruitment among Newfoundland cod stocks may represent similar responses to similar environmental conditions (Koslow et al. 1987). Alternatively, this pattern may also represent spatial variation in reproductive success or failure of a single large population. The information obtained in this study suggests that the interrelationships of cod along the Newfoundland shelf, both in terms of genetic and ecological relatedness of adults and pre-recruits, should be addressed in future. It is essential to understand the potential interactions of adults and offsprings throughout their range.

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Table 1. Regressions equations and r^2 of morphometric features in relation to length. Data from all regions were pooled based on the results of an analysis of covariance. All variables were log-transformed ($Y=\ln(y)$; $X=\ln(x)$). Three hundred and fifty-seven points were used in the analysis. All relationships were significant ($p<0.01$).

Relationship	Regression	r^2
Position of 3rd Dorsal vs Length	$Y = -0.21 + 1.03\,X$	0.99
Position of 1st Dorsal vs Length	$Y = -0.52 + 1.01\,X$	0.95
Position of Anus vs Length	$Y = -0.37 + 1.02\,X$	0.96
Position of Pelvic Fin vs Length	$Y = -0.54 + 0.95\,X$	0.89
Length of Head vs Length	$Y = -0.59 + 1.01\,X$	0.96
Eye Diameter vs Length	$Y = -0.86 + 0.88\,X$	0.82
Length of Snout vs Length	$Y = -1.27 + 1.08\,X$	0.86
Length of Jaw vs Length	$Y = -0.94 + 0.98\,X$	0.89
Depth of Caudal Peduncle vs Length	$Y = -1.18 + 0.95\,X$	0.84

Table 2. Results of canonical discriminant analysis performed using all morphological variables. The latter are the residuals from the general allometric relationships reported in Table 1.

Canonical Variable	Likelihood Ratio	Approx. F	D.F. (Num)	D.F. (Den)	Pr > F
1	0.80	2.96	27	1014	0.001
2	0.92	1.87	16	696	0.02
3	0.98	1.26	7	349	0.27

Total Canonical Structure

Morphological Feature	Canonical Variable		
	1	2	3
Position of 3rd Dorsal Fin	-0.067	0.043	0.53
Position of 1st Dorsal Fin	-0.40	-0.056	0.22
Position of Anus	0.17	0.34	0.086
Position of Pelvic Fin	0.20	-0.54	0.31
Length of Head	0.47	0.30	0.050
Eye Diameter	0.55	-0.50	-0.19
Length of Snout	0.52	0.098	0.54
Length of Jaw	0.28	0.13	0.38
Depth of Caudal Peduncle	0.24	0.043	0.43

Table 3. Results of reclassification of individual fish into regional groups based on discriminant analysis (Table 2). Numbers represent percent reclassification into each region relative to the region of origin. Overall hits 48%, misses 52%.

	Reclassification				
Origin	3K	3L inshore	3L offshore	3O	n
3K	45%	24%	16%	15%	80
3L inshore	20%	48%	14%	18%	221
3L offshore	0%	13%	69%	19%	16
3O	22%	17%	10%	51%	42

Table 4. Frequency of mtDNA sequence variants for the seven major sampling locations.

Locality	NAFO	mtDNA genotype										
	Div.	A	G	E	P	X	U	N	M	C	J	Totals
St.Anthony	3K	15	2	0	0	0	0	0	0	0	0	17
Springdale	3K	7	1	0	0	0	0	0	0	0	0	8
Centerville	$3L_i$	10	0	2	1	1	0	0	0	0	0	14
Bellevue Beach	$3L_i$	13	0	0	0	0	1	0	1	0	0	15
Brigus South	$3L_i$	15	1	1	0	0	1	1	0	0	0	19
NE Grand Banks	$3L_o$	11	1	1	0	0	0	1	0	0	0	14
SE Grand Banks	3O	13	1	0	0	0	0	0	0	1	1	16
Total		84	6	4	1	1	2	2	1	1	1	103

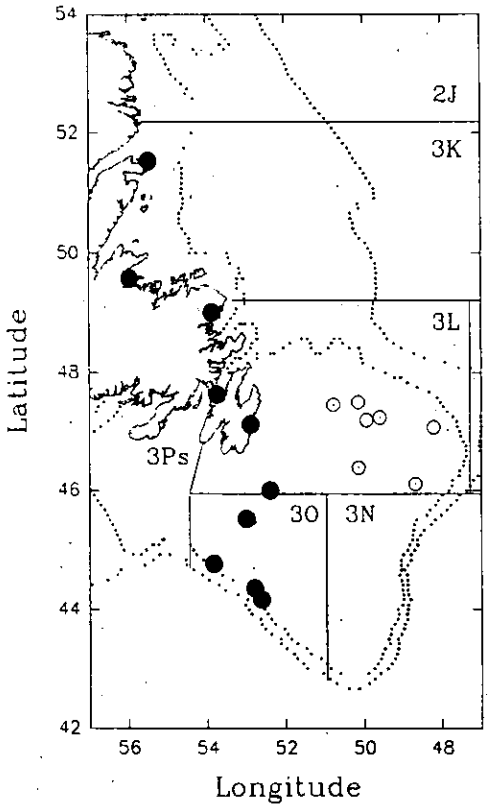

Figure 1. Sampling locations from which juvenile 0-group cod were obtained. Contours shown are of the 200 m and 1000 m isobaths. Stations were located throughout the Grand Banks but no fish were caught in the southeastern region (3N). NAFO divisions are shown for reference. The open symbols in division 3L represent the sampling locations that were classified as 3L offshore.

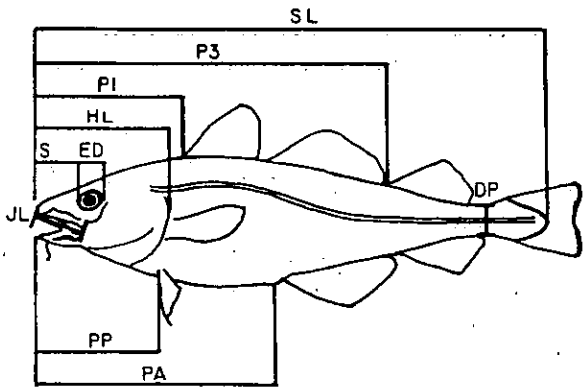

Figure 2. Diagram of a cod showing measurements taken for the morphometric analysis; SL, standard length; P3, distance from the snout to the origin of the third dorsal fin; P1 distance from the snout to the origin of the first dorsal fin; PA, distance from the snout to the anus; PP, distance from the snout to the origin of the pelvic fin; HL, length of the head; ED, eye diameter; S, snout length; JL, jaw length; DP, depth of body at the caudal peduncle.

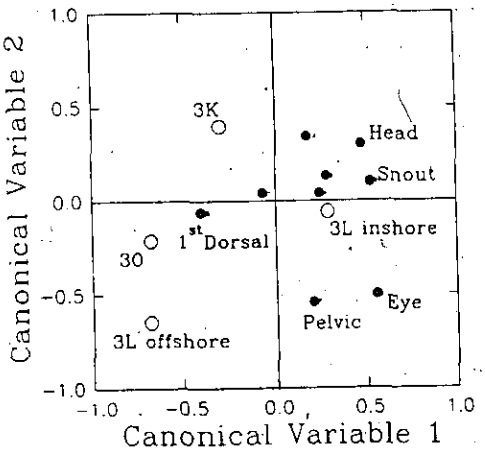

Figure 3. Centroid position of the 4 major sampling regions along canonical variables 1 and 2 based on an analysis of residual morphometric characteristics. The position of morphometric features that contributed significantly to the regional separation are shown (closed circles with labels).

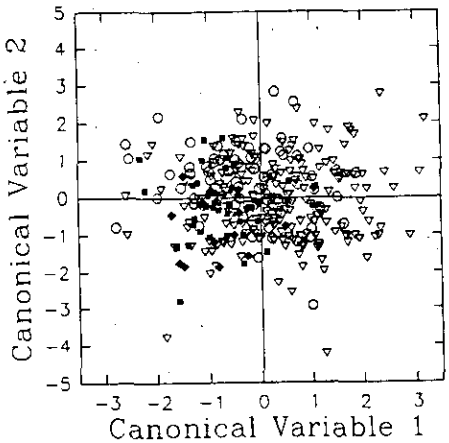

Figure 4. Loading of individual fish on canonical variables 1 and 2. Each specimen is identified by the region in which it was caught (3K o; 3L inshore ▼; 3L offshore ◆; 3O ●).

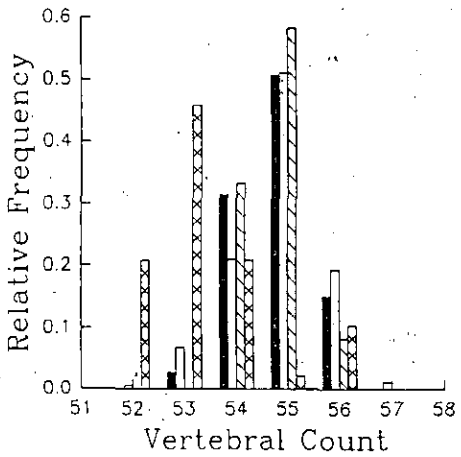

Figure 5. Relative frequency distribution of vertebral counts for samples from region 3K (closed bars), 3L inshore (open bars), 3L offshore (single hatched bars), 3O (cross hatched bars).

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      F  G  S  L  L  G  L  C  L  I* T  Q  L  L  T  G  L  F  L  A  M  H  Y  T  S  D  26
A  at ttt ggc tct ctt cta ggc ctt tgc tta att act caa ctt cta aca gga cta ttt cta gcc ata cac tat acc tca gac  80
C  .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
E  .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
G  .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
J  .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
M  .. ... ... ... ... ... ... ... .t ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
N  .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... t.. ... ... ... ... ... ... ... ...
P  .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
U  .. ... ... ... ... ... ... ... g.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X  .. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... g ... ... ... ... ... ... ...

      I  E  T  A  F  S  S  V  V  H  I  C  R  D  V  N  Y  G  W  L  I  R  N  M  H  A  N  53
A  atc gag aca gcc ttc tca tcc gta gtc cac atc tgt cgt gat gta aac tac ggc tga cta att cgg aat ata cat gct aat 161
C  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
E  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
G  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
J  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
M  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
N  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
U  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

      G  A  S  F  F  F  I  C  L  Y  M  H  I  A  R  G  L  Y  Y  G  S  Y  L  F  V  E  T  80
A  ggt gcc tct ttc ttt ttc att tgt ctt tat atg cac att gcc cga ggt ctc tat tat ggt tcc tat ctt ttt gta gag aca 242
C  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
E  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
G  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
J  ... ... ... ... ... ... ... ... ... ... ... ... ... ... g ... ... ... ... ... ... ... ... ... ... ...
M  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ... ... ... ... ... ...
N  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P  ... ... ... ... ... ... ... ... ... ... ... ... ... c ... ... ... ... ... ... ... ... ... ... ... ...
U  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
X  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

      W  N  I  G  V  V* L  F  L  L  V  M  M  T  S  F  V  G  Y  V  L                                    101
A  tga aac atc ggg gtt gtc ctt ttc ctt tta gta ata ata acc tct ttc gta ggt tat gtc ctc cc                 307
C  ... ..t ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
E  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
G  ... ... ... ..t ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
J  ... ..t ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
M  ... ..t ... ... ... a.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
N  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
P  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
U  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
X  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .. ..
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Figure 6. Variation in DNA sequence of Gadus morhua within a 307 base pair region of the mitochondrial cytochrome b gene. In each of the 10 genotypes, each nucleotide is identical to that in genotype A except where indicated. The top line gives the inferred amino acid sequence according to the International Union of Biochemists single-letter code; variable amino acid residues are indicated by asterisks. Numbers adjacent to the first and second lines indicate position numbers in the protein and nucleotide sequences.

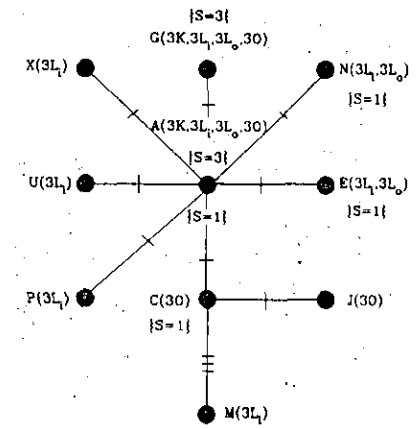

Figure 7. Phylogenetic relationships of ten cod genotypes and inferred migration events among four regions. A maximum parsimony network is shown; numbers of nucleotide substitutions occurring along each network branch are indicated by crosshatches. Distributions of genotypes among regions are summarized from Table 4. The occurrence of migration events (S) is deduced by the method of Slatkin and Maddison (1989): genotypes found in n populations each imply a minimum of n-1 events, and two additional events are implied by the distribution of genotype M relative to genotype C, and by that of genotypes C, J, and M relative to genotype A (total S = 10).