Vol. 76: 103–113, 1991 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published September 26

Relationship between spawning mode and phylogeographic structure in mitochondrial DNA of North Atlantic capelin *Mallotus villosus**

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ABSTRACT: Capelin *Mallotus villosus* spawn on beaches in Alaska and British Columbia, but spawn offshore in Icelandic waters and the Barents Sea. Both modes of reproduction co-occur in the northwest Atlantic. The Southeast Shoal population spawns on the Grand Banks 350 km to the SE of Newfoundland at the same time as other stocks, all of which are beach spawners. These observations gave rise to 2 alternative hypotheses concerning the zoogeography and evolution of life cycle in capelin. First, the Southeast Shoal population was originally a beach-spawning population during the late Wisconsinian glaciation and is ancestral to all other northwest Atlantic capelin stocks. In such a case, present-day stocks from this area would represent a monophyletic group derived from a common ancestor no more than 10 000 to 12 000 yr ago. The alternative hypothesis is that the 2 modes of reproduction originally evolved in isolation. Beach spawners are hypothesized to have originated in the north Pacific and recolonized Canadian Arctic waters and the northwest Atlantic following glaciation. Bottom spawners originated in the North Atlantic and continued to reproduce where environmental conditions permitted. In such a case, genetic divergence among bottom-spawners and among beach-spawners from across the North Atlantic would be less than that between beach- and bottom-spawners. We tested these hypotheses by comparing mitochondrial DNA (mtDNA) restriction fragment length polymorphisms among 6 stocks of beach-spawning capelin (St. Lawrence estuary; Gulf of St. Lawrence; Placentia Bay, Conception Bay and Notre Dame Bay, Newfoundland; Nain, Labrador) and 3 stocks of bottom-spawning capelin (Southeast Shoal; Iceland; Barents Sea). We observed 2 major mtDNA genotype groups separated by a mean sequence divergence of 3.42 %, clearly reflecting the genetic separation of the Iceland and Barents Sea stocks from the northwest Atlantic stocks. No geographical heterogeneity in the frequency of mtDNA genotypes was observed among the northwest Atlantic sampling sites. However, differences in nucleon diversities among sites did not support the view that capelin form one large panmictic population in the northwest Atlantic. Although our results do not permit the identification of the Southeast Shoal stock as ancestral to northwest Atlantic capelin, these observations refute the hypothesis that the beach- and bottom-spawning stocks evolved in isolation long before the end of the Wisconsinian glaciation.

INTRODUCTION

Capelin is a small, pelagic schooling osmerid native to the marine waters of the northern hemisphere and considered to be one of the most important forage species in the northwest Atlantic (Frank & Carscadden 1989). The genetic structure of northwest Atlantic capelin remains problematic despite considerable effort over the past 15 yr to discriminate putative stocks. Definition of 5 stocks for management purposes has been based on patterns of the fishery, spawning times, meristics and morphometrics (Misra & Carscadden 1987) but the extent to which environmental variables influence morphometric and meristic characters is unknown. Genetic studies based on protein electrophoresis did not discriminate putative stocks of beach-spawning capelin in the Newfoundland area (Payne 1975, 1976). These same studies, however, did reveal differences between Newfoundland, northern Canadian and Greenland capelin.

* Contribution to the program of GIROQ (Groupe Interuniversitaire de Recherches Océanographiques du Québec)
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0171-8630/91/0076/0103/$ 03.00

The most striking difference that clearly delineates one of the Newfoundland capelin stocks concerns spawning habitat. The Southeast Shoal stock spawns 350 km offshore on one of the shallowest (50 m) areas of the Grand Banks at the same time as other stocks, all of which are beach spawners. For beach spawners, spawning may be subtidal when water temperatures near the beaches become too warm later in the spawning season. Only in the NW Atlantic do both beach and bottom spawning in open-water areas occur. Capelin in Alaska, British Columbia and Greenland are beach spawners while capelin in Icelandic waters and the Barents Sea are bottom spawners. Beach spawning does occur in Norway in cases where capelin spend their entire lives in the same fjord (Carscadden et al. 1989, Stergiou 1989).

The North Atlantic distribution of the 2 reproductive modes and their co-occurrence in Newfoundland waters provides a theoretical framework in which the genetic structure of capelin may be analyzed. Two alternative hypotheses concerning the origin and distribution of the 2 reproductive modes are presented that generate different predictions concerning the definition of capelin stocks.

(1) During the late Wisconsinian glaciation, much of the Grand Banks was exposed. As the glaciers melted and receded about 12000 yr ago, the Grand Banks were flooded. Geologic evidence indicates that the shallow Southeast Shoal was once a beach and Carscadden et al. (1989) proposed that the Southeast Shoal stock was once a beach-spawning stock. The Avalon ice cap extended over Newfoundland onto the Grand Banks and as a result, present day capelin spawning beaches were ice covered. Thus, a logical hypothesis arising from Carscadden et al. (1989) is that all of the stocks now spawning on beaches in the northwest Atlantic originated from the once beach-spawning Southeast Shoal stock during glacial retreat. Thus, present-day NW Atlantic capelin stocks would represent a monophyletic group derived from a common ancestral stock no more than 10000 to 12000 yr ago. This hypothesis predicts that cepelin stocks in the northwest Atlantic form a monophyletic group genetically divergent from other North Atlantic stock-complexes of capelin. The morphological similarity of the Newfoundland stocks (Misra & Carscadden 1987), the inability to discriminate them using biochemical markers and their apparent clear genetic identity relative to Greenland and Arctic Canada capelin (Payne 1976) support this prediction.

(2) An alternative hypothesis is that the 2 modes of reproduction evolved in isolation. Stergiou (1989) has suggested that beach spawners originated in the North Pacific and recolonized Canadian Arctic waters and the northwest Atlantic following glaciation. Bottom spawners originated in the North Atlantic and continued to reproduce where environmental conditions permitted. In such a case, present-day northwest Atlantic capelin stocks would represent a polyphyletic group, with the Southeast Shoal stock genetically separated from the beach-spawning stocks before the retreat of the glaciers. As such, genetic divergence among bottom-spawners and among beach-spawners from across the North Atlantic would be less than that between beach- and bottom-spawners. This hypothesis predicts that North Atlantic stocks that exhibit different reproductive modes are more genetically divergent than geographically defined complexes of either bottom or beach spawning stocks. There is not enough evidence in the literature to test this hypothesis. While Payne's (1976) samples came from a large geographical area, he analysed only beach-spawning stocks. Over a much smaller area, Mork & Friis-Sörenson (1983) showed that there was low genetic distance between a beach-spawning fjord stock in northern Norway and the oceanic, bottom-spawning Barents Sea stock.

The analysis of mitochondrial DNA (mtDNA) is recognized as approaching the ideal method for quantifying intraspecific genetic differences. It evolves rapidly, primarily through base substitutions, it is cytoplasmically housed, maternally inherited and haploid in transmission across generations (Avise 1989). Two major aspects of intraspecific mtDNA variability are of interest: (1) the magnitude and pattern of phylogenetic differentiation among the mtDNA haplotypes themselves and (2) the geographic distribution of the mtDNA groupings. The first aspect permits the construction of a gene tree based on the historical relationships among haplotypes deduced from the mutational changes in the molecule. Such gene phylogenies help to reconstruct the historical events that have influenced genetic structure in contemporary populations (Avise 1989). The second aspect permits the comparison of haplotype-frequency differences among locations for stock discrimination.

Previous surveys of mtDNA in freshwater species have shown that mtDNA genotypes often discriminate geographically separated, conspecific populations (e.g. Grewe & Hebert 1988, Bernatchez & Dodson 1990a). This is not always the case in marine and diadromous species. Some show little or no mtDNA differentiation over large areas such as skipjack tuna *Katsuwonus pelamis* (Graves et al. 1984), American eel *Anguilla rostrata* (Avise et al. 1986) and 2 species of marine catfish (Avise et al. 1987). Atlantic cod *Gadus morhua* from the Grand Banks and the North Sea exhibited a lack of genetic divergence based on an analysis of 22 mtDNA restriction fragments (Smith et al. 1989). More recently, however, the proportion of cod genotypes based on variable nucleotide positions within a 298 base region of the mitochondrial cytochrome *b* gene

was shown to differ significantly between Newfoundland and Norwegian samples of cod (Carr & Marshall 1991). More extensive geographic differentiation has been noted for populations of anadromous species such as steelhead trout *Oncorhynchus mykiss* (Wilson et al. 1985), cisco *Coregonus artedii* (Bernatchez & Dodson 1990b) and American shad *Alosa sapidissima* (Bentzen et al. 1989). This variation in mtDNA differentiation resembles the 'population richness' gradient of Sinclair & Iles (1988) who hypothesized that events early in the life history stages, involving retention of eggs or larvae in natal streams or in relation to particular physical oceanographic features, are involved in the definition of the total number of populations within a species' range. Thus, population richness may vary from one population for panmictic species such as the American eel to many populations for anadromous fish homing to natal rivers. Some mtDNA analyses have revealed little or no genetic divergence among supposed stocks of some marine species believed to home to discrete spawning sites (e.g. Atlantic herring *Clupea harengus*; Kornfield & Bogdanowics 1987). However, the level of mtDNA variability influences sampling strategy, and the combination of small sample sizes and high mtDNA genotypic diversity may contribute to masking population structure for purely methodological reasons (Bernatchez et al. 1989). In the case of capelin, their speculated ability to home to natal spawning areas (with spawning beaches usually characterized by freshwater outflow; Carscadden et al. 1988), the demersal nature of their eggs and the possibility of discrete nursery areas for post-larvae and juveniles suggest that the species is potentially population-rich in the North Atlantic.

In this paper, we report the results of mtDNA restriction analysis carried out to test the 2 hypotheses concerning the origin and distribution of beach- and bottom-spawning capelin. The use of mtDNA genotypes as a genetic marker that segregates bottom-spawning stocks from across the North Atlantic into a monophyletic cluster would clearly demonstrate their common origin and refute the hypothesis that northwest Atlantic beach-spawners were recently derived from the ancestral Southeast Shoal stock. Alternatively, the clustering of NW Atlantic stocks into a monophyletic group independently of reproductive mode would demonstrate their common origin and refute the hypothesis that beach- and bottom-spawning stocks evolved in isolation long before the end of the Wisconsinian glaciation.

MATERIALS AND METHODS

Sampling. Approximately 50 specimens each of 6 populations of beach-spawning capelin (St. Lawrence estuary, Ste-Irénée, Québec; Gulf of St. Lawrence, Ste-Flavie, Québec; Fox Harbour, Placentia Bay, Newfoundland; Bryant's Cove, Conception Bay, Newfoundland; Carmanville, Notre Dame Bay, Newfoundland; Nain, Labrador) and 3 populations of bottom-spawning capelin (Southeast Shoal, northwest Atlantic; Iceland; Barents Sea) were collected during spawning in spring and summer 1989 and 1990 (Fig. 1, Table 1). Mature ovaries were shipped on wet ice and mtDNA extracted within 10 d of capture.

Isolation and restriction enzyme analysis of mtDNA. Mitochondrial DNA was purified according to González-VillaSeñor et al. (1986) as modified by Bernatchez et al. (1988). Aliquots of mtDNA were digested

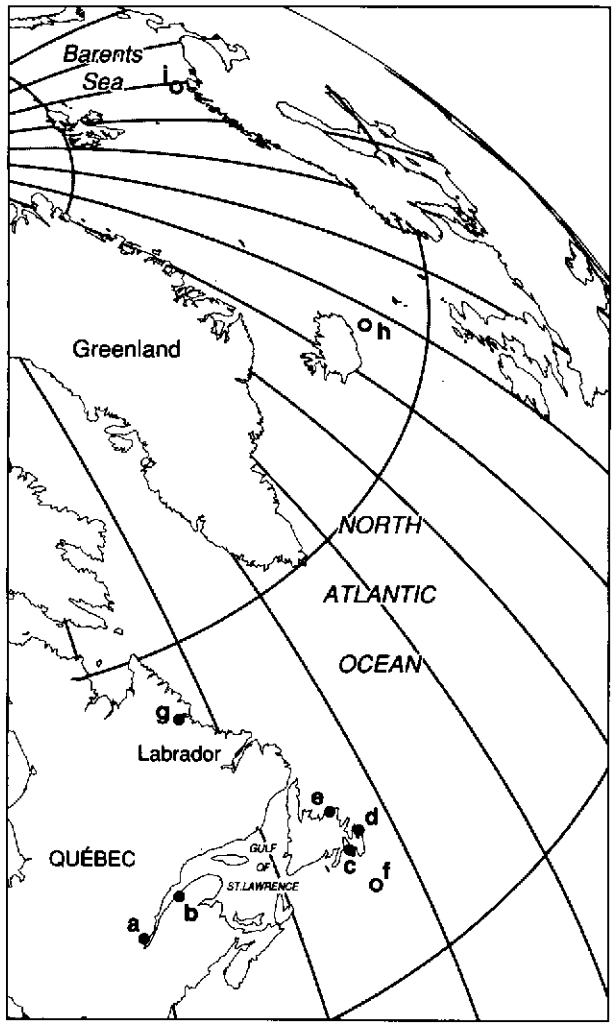

Fig. 1. *Mallotus villosus*. Location of sampling sites for populations of beach-spawning (●) and bottom-spawning (○) capelin in the North Atlantic Ocean. a, St. Irénée, Québec; b, St. Flavie, Québec; c, Fox Harbour, Placentia Bay, Newfoundland; d, Bryant's Cove, Conception Bay, Newfoundland; e, Carmanville, Notre Dame Bay, Newfoundland; f, Southeast Shoal; g, Nain, Labrador; h, Iceland; i, Barents Sea

Table 1. *Mallotus villosus*. Sample locations, spawning type, sample sizes, number of mtDNA genotypes and estimated nucleon diversity for capelin populations sampled in the North Atlantic. Be: beach spawners; Bo: bottom spawners. Letters a to i refer to sampling sites illustrated in Fig. 1

Population	Type	Sample size	No. of genotypes	Nucleon diversity
a St. Irénée, Qué.	Be	50	26	0.840
b St. Flavie, Qué.	Be	40	16	0.765
c Placentia Bay, Nfld	Be	48	26	0.844
d Conception Bay, Nfld	Be	48	22	0.823
e Notre Dame Bay, Nfld	Be	41	13	0.666
f Southeast Shoal	Bo	59	28	0.808
g Nain, Labrador	Be	43	24	0.891
h Iceland	Bo	48	35	0.967
i Barents Sea	Bo	47	36	0.983

separately with 6 hexameric (*Apa*I, *Bg*I, *BstE*II, *Dra*I, *EcoR*V, *Pst*I) and 4 multihexameric restriction endonucleases (*Ava*II, *Ban*I, *Ban*II, *Hinc*II). Mitochondrial DNA fragments were electrophoretically separated on 0.8 or 1.2% agarose gels run for 4 h at 75 V.

Visualization of restriction fragments. When mtDNA was obtained in sufficient quality and quantity, ethidium bromide staining was sufficient to reveal the digested fragments. In cases of low or poor-quality yield of mtDNA, DNA was transferred to nylon membranes under alkaline conditions. Restriction fragments of mtDNA were denatured under alkaline conditions for 20 min in a denaturation transfer solution (0.4 M NaOH, 0.6 M NaCl) and transferred from the agarose gel to a positively charged nylon membrane using the same solution (Sambrook et al. 1989). The nylon membrane was then neutralized for 15 min at room temperature and dried for 30 min. Nylon membranes were hybridized with a highly purified radiolabelled (^{32}P) total capelin mtDNA probe prepared with the multiprime (Amersham) DNA labelling reaction (Feinberg & Volgestein 1983, 1984). Membranes were autoradiographed using an intensifying screen (Cronix Lightning-plus) for 5 to 16 h at −70°C.

Data analysis. Size estimates of fragments were made by running simultaneously into the agarose gel digests of phage lambda DNA with *Hind*III and *EcoR*I-*Hind*III double digest. Fragments less than 300 base pairs (b.p.) in length were not scored. Distinct single endonuclease patterns were identified by a specific letter. Each fish was assigned a multi-letter code which described its composite mtDNA genotype.

Nucleotide sequence divergence (p) between genotypes was estimated according to Upholt's (1977) fragment method. Sequence divergence and standard deviation were estimated independently for hexameric and multihexameric enzymes and the estimates were then pooled following weighting for the number of base pairs sampled by each type of enzyme. The resulting distance matrix was clustered by UPGMA (Sneath & Sokal 1973) using the average linkage algorithm of the SAS statistical package. The diversity of mtDNA lineages within each population was estimated with Nei & Tajima's (1981) nucleon diversity index which takes into account both the number and frequency of mtDNA genotypes. Geographical heterogeneity in the frequency of mtDNA genotypes among sampling sites was analyzed using the likelihood ratio chi-square test (G^2; SAS statistical package).

We also estimated the likelihood of detecting mitochondrial DNA diversity at the population level using the combinatorial approach of Hebert et al. (1988) as applied by Bernatchez et al. (1989) to mtDNA data. The relationship between the number of fish sampled and the number of mtDNA genotypes detected was estimated for each population by an incremental random choice of fish (increments of 4 fish to a maximum of 40 fish). The procedure was repeated 10 times for each sampling intensity to generate an estimate of random sampling variance. The relationship between the number of genotypes detected as a function of genome sampling was estimated for each population by incremental random choice of restriction enzymes. Results were obtained by an incremental choice of one enzyme repeated 10 times for each incremental step. As different restriction enzymes produced different numbers of fragments, we attributed the fragment numbers to their respective enzymes and summed the fragments for any enzyme combination.

RESULTS

The 10 enzymes used generated a total of 247 restriction fragments with a mean of 78 per individual. A high degree of polymorphism was observed among the 424 capelin analyzed. All enzymes were polymorphic generating a total of 179 mtDNA genotypes. The estimated mtDNA nucleon diversity among all NW Atlantic fish analyzed was 0.805 and that of Icelandic and

Barents Sea fish was 0.975. Mitochondrial DNA diversity varied widely among northwest Atlantic populations sampled, from a low of 0.666 in Notre Dame Bay to a high of 0.891 in Labrador (Table 1). The mean size of the mtDNA genome as estimated by averaging the sums of all digestion patterns except those of *Ban*II (omitted because of the number of small, undetected fragments) was 17575 b.p. (SD = 626). This length estimate for the mtDNA molecule falls within the values obtained for a variety of other fish species (Bernatchez & Dodson 1990b).

Clustering of the distance matrix by UPGMA

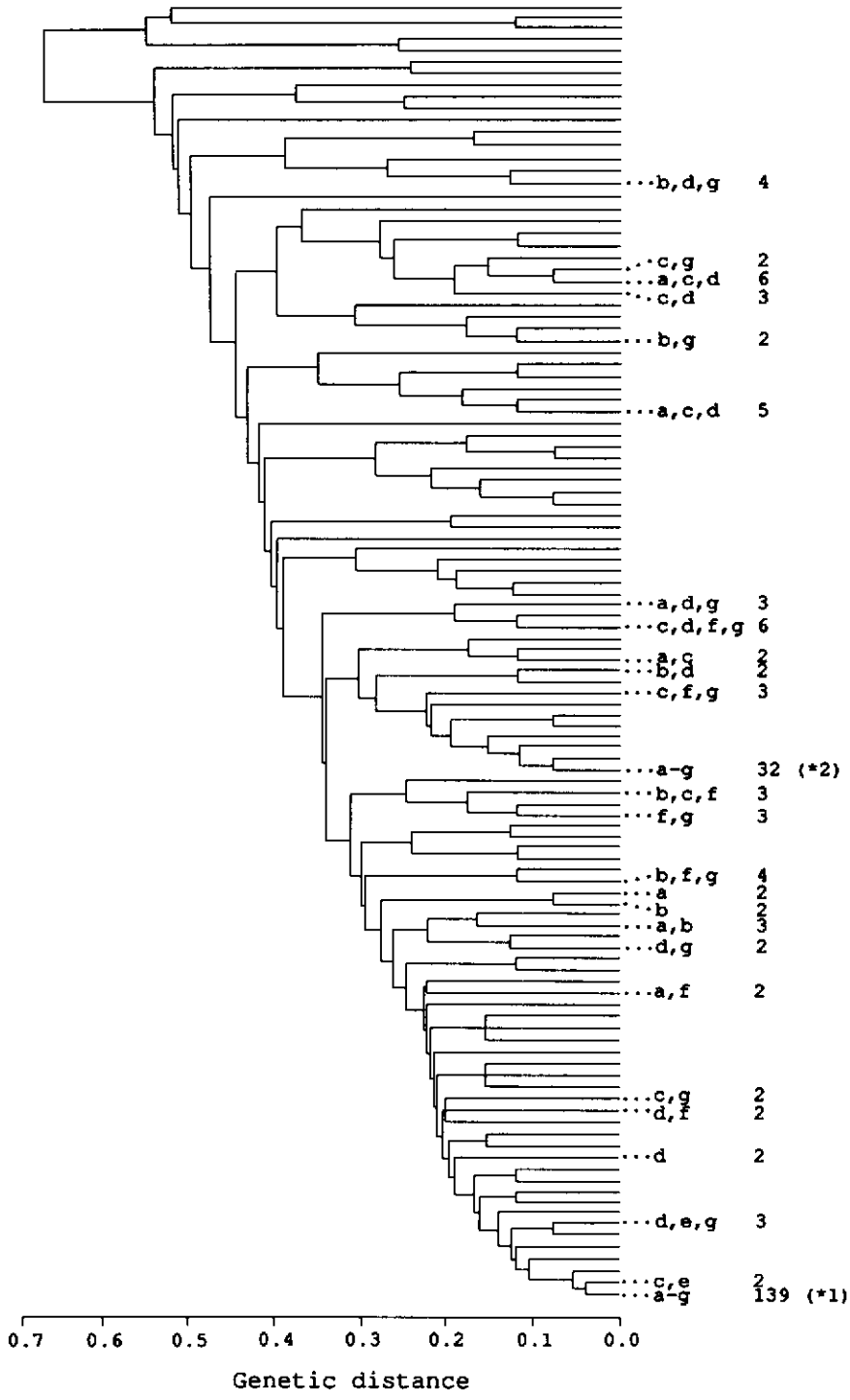

Fig. 2. *Mallotus villosus*. UPGMA dendrogram clustering the distance matrices of sequence divergence estimates calculated for 7 northwest Atlantic capelin stocks and the geographical distribution of genotypes characteristic of more than one fish. a to g: sampling sites indicated in Fig. 1; numbers: absolute abundance of each genotype; *1, *2: 2 most common genotypes observed in northwest Atlantic capelin

revealed 2 major mtDNA phylogenetic groups separated by a mean sequence divergence of 3.42 %. Each phylogenetic group was uniquely associated with either the northeast or northwest Atlantic. No mtDNA genotypes were common to both geographical areas. Thus, these 2 groups clearly reflect the genetic separation of the Iceland and Barents sea stocks from the northwest Atlantic stocks. Little phylogenetic substructuring was observed within either major phylogenetic group (Figs. 2 & 3). Pairwise sequence divergence estimates between all northwest Atlantic genotypes was low (0.42 %, SD = 0.16 %) as was that between the northeast Atlantic genotypes (0.51 %, SD = 0.20 %). Among the 329 fish from northwest Atlantic stocks, 42.2 % (139 specimens) belonged to one mtDNA genotype (Fig. 4) and 9.7 % (32 specimens) belonged to a second genotype. The remaining 158 fish were characterised by the remaining 106 northwest Atlantic mtDNA genotypes. Only 24 of these genotypes were characteristic of more than one fish. Northwest Atlantic genotypes were no more than 5 mutation step variants of the most common genotype.

In Icelandic waters and the Barents Sea, only 5 of a total of 71 genotypes were shared by fish sampled from both stocks. Only 13 % (12 of 95 fish) were characterised by the most common genotype (Fig. 4). The remaining 83 fish were characterised by the remaining 70 northeast Atlantic mtDNA genotypes. Only 8 of these genotypes were characteristic of more than 1 fish (range 2 to 8 fish). Northeast Atlantic genotypes were no more than 5 mutation step variants of the most common genotype.

Geographical heterogeneity in the distribution of mtDNA genotypes among the northwest Atlantic stocks was evaluated by analyzing the distribution of the 2 most common genotypes (designated Genotypes 1 and 2, respectively) and all remaining genotypes pooled as a third category (Table 2). These genetic groupings were not specifically associated with given stocks and were distributed among all spawning sites. No significant difference was detected in their geographic distribution (G^2 = 15.36, df = 12, $0.10 < p > 0.25$). Lack of population structuring was further illustrated by the distribution of rare genotypes. Excluding the 2 most common genotypes, of the 24 mtDNA genotypes representative of more than 1 fish (range 2 to 7 fish), only 3 were uniquely associated with one population. All others were distributed among several sampling sites (range 2 to 5 sampling sites; Fig. 2).

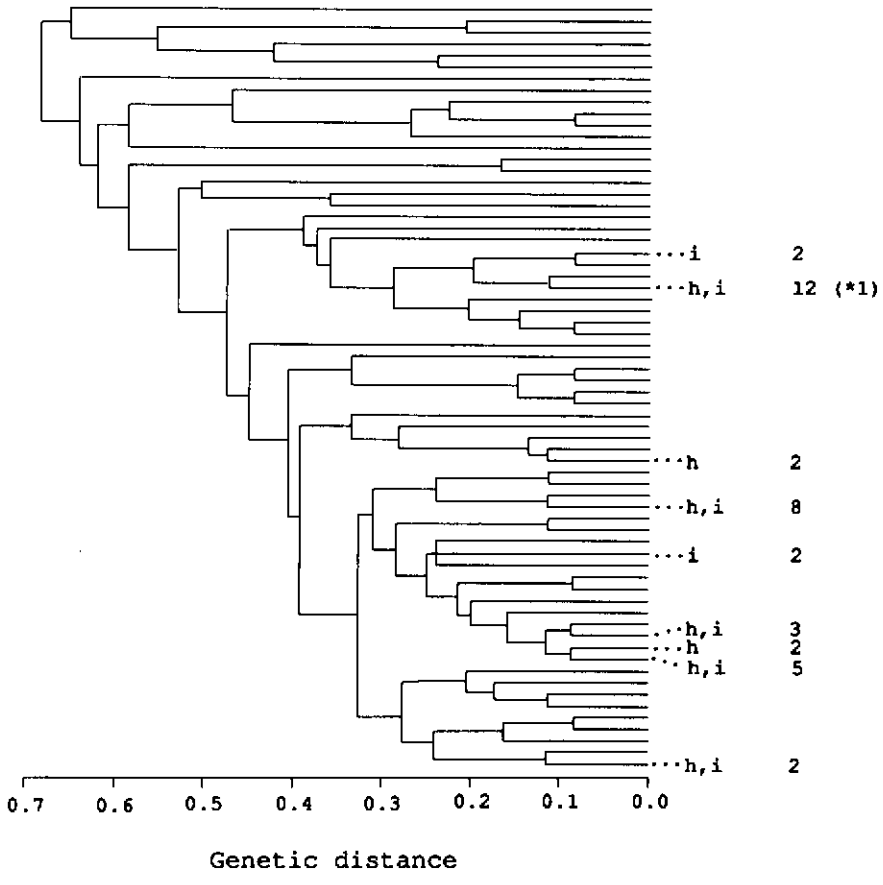

Fig. 3. *Mallotus villosus*. UPGMA dendrogram clustering the distance matrices of sequence divergence estimates calculated for Icelandic and Barents sea capelin stocks and the geographical distribution of genotypes characteristic of more than one fish. h, i: sampling sites indicated on Fig. 1; numbers: absolute abundance of each genotype; *1: most common genotype observed in northeast Atlantic capelin

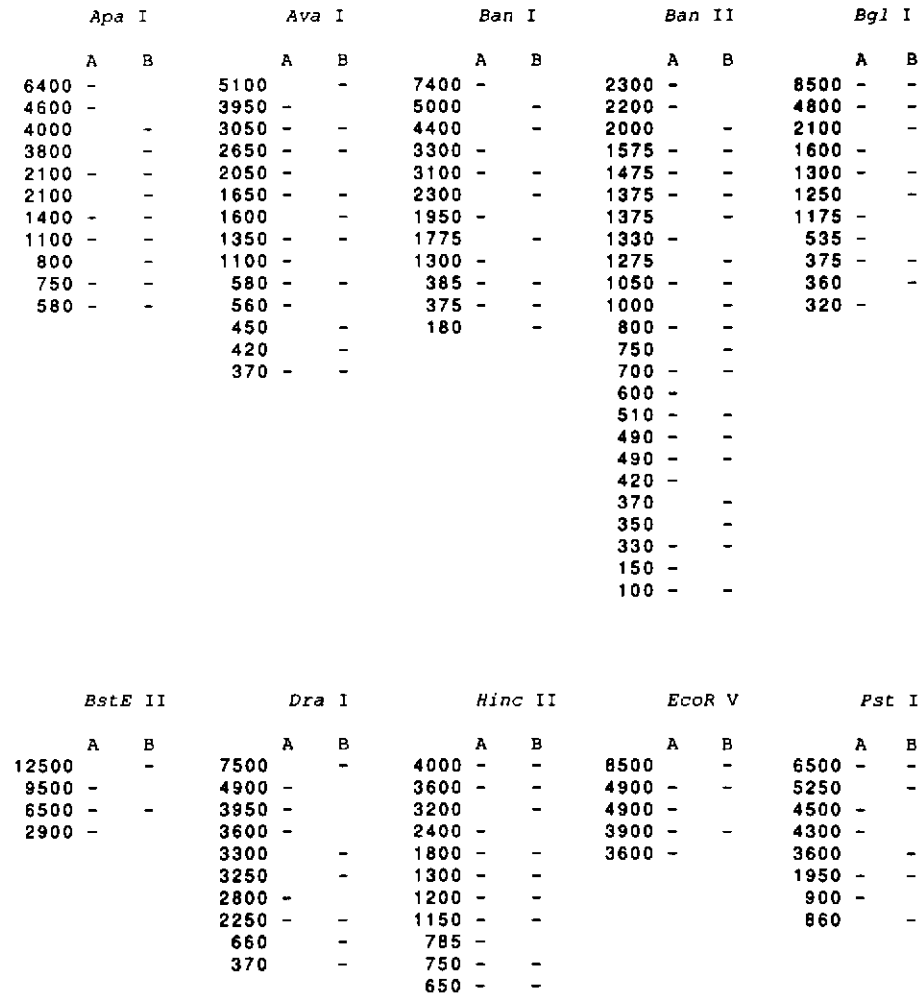

Fig. 4. *Mallotus villosus*. Fragment size estimates (in base pairs) of the restriction fragment patterns (produced following digestion with each of 10 enzymes) characterising the most common mtDNA genotype observed among northwest Atlantic capelin (A) and those characterising the most common mtDNA genotype observed among Icelandic and Barents sea capelin (B)

Table 2. *Mallotus villosus*. Frequency distribution of the 2 most common mtDNA composite genotypes (designated Genotypes 1 and 2, respectively) and the remaining genotypes (others) observed among capelin sampled from 7 spawning sites in the northwest Atlantic. Letters a to g refer to sampling sites identified in Fig. 1

Population	Genotype 1	Genotype 2	Others
a St. Irénée, Qué.	20	3	27
b St. Flavie, Qué.	19	5	16
c Placentia Bay, Nfld	19	3	26
d Conception Bay, Nfld	20	4	24
e Notre Dame Bay, Nfld	22	7	12
f Southeast Shoal	25	8	26
g Labrador	14	2	27
Totals	139	32	158

The number of mtDNA genotypes detected as a function of the sample size of specimens and restriction fragments increased sharply in all sampling sites with the possible exception of Notre Dame Bay, Newfoundland and Ste. Flavie, Québec (Figs. 5 & 6). Sample sizes of up to 40 specimens and 78 restriction fragments were generally inadequate to detect all mtDNA variants within stocks. In the case of the Southeast Shoal, sample sizes of up to 59 specimens were inadequate to detect all mtDNA variants within the stock (Fig. 7).

DISCUSSION

The results of the mtDNA restriction analysis clearly demonstrate that 1 bottom-spawning and 6 beach-

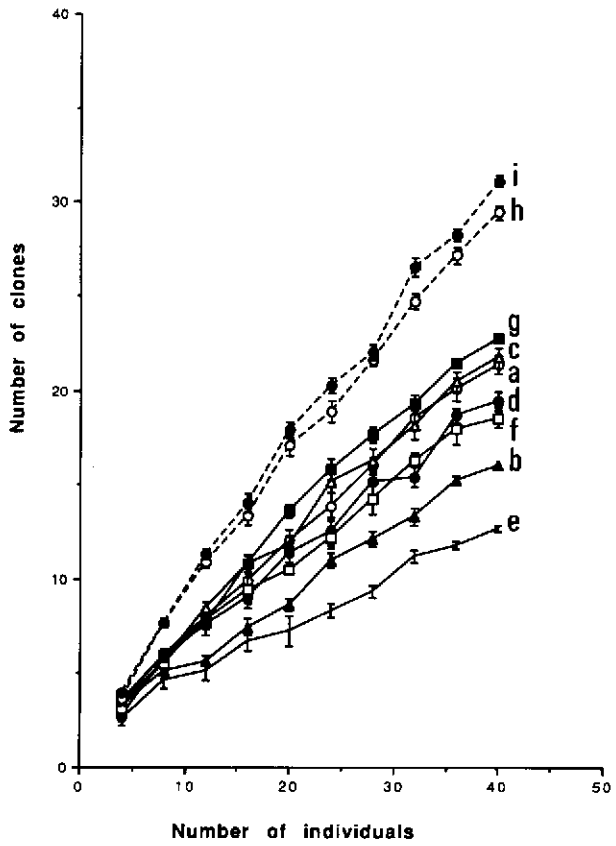

Fig. 5. *Mallotus villosus*. Effect of fish sample size on the number of mtDNA clonal lines detected within populations. Incremental steps were stopped at 40 fish. a to i: sampling sites indicated on Fig. 1. Vertical bars: standard deviations based on 10 repetitions at each incremental step

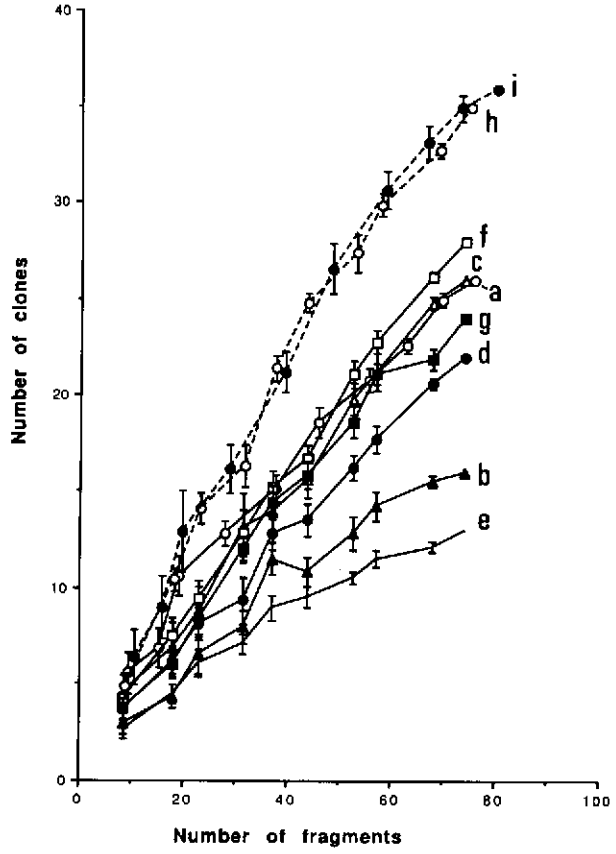

Fig. 6. *Mallotus villosus*. Effect of number of restriction fragments assayed on the number of mtDNA clonal lines detected within populations. a to i: sampling sites indicated on Fig. 1. Vertical bars: standard deviations based on 10 repetitions at each incremental step

spawning capelin populations from the northwest Atlantic form a monophyletic group genetically divergent from the bottom-spawning stocks of Iceland and the Barents sea. Although our results do not permit the identification of the Southeast shoal stock as ancestral to northwest Atlantic capelin (Carscadden et al. 1989), the observation that the genetic divergence between the mtDNA genotypes of Icelandic and Barents sea bottom-spawning stocks and the Southeast Shoal is 7 times greater than that among the mtDNA genotypes of the northwest Atlantic clearly refutes the hypothesized relationship between spawning mode and geographic origin proposed by Stergiou (1989). Spawning mode may be largely facultative and may be determined by appropriate environmental features rather than an obligatory life history adaptation. This view is consistent with the observation that when beach spawning is completed on the south and west coasts of Newfoundland due to the rise in water temperatures at the beaches, spawning may continue at increasing depths down to at least 35 to 55 m until the end of August (Templeman 1968). In addition, Carscadden et

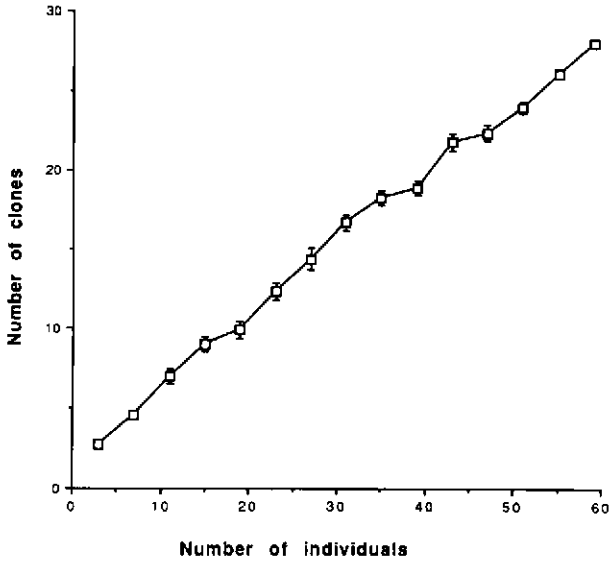

Fig. 7. *Mallotus villosus*. Effect of fish sample size on the number of mtDNA clonal lines detected among 59 capelin sampled on Southeast Shoal. Vertical bars: standard deviations based on 10 repetitions at each incremental step

al. (1989) noted that principally water temperature, and substrate type and size, characterised suitable spawning habitat for capelin on Newfoundland beaches as well as on the Southeast Shoal.

Homogeneity of mtDNA genotype distribution among northwest Atlantic capelin may be due to present or past gene flow, 2 phenomena that are not easily distinguishable. On one hand, it is evident that ample oppurtunity for contemporary gene flow among stocks occurs in Newfoundland and Labrador waters. While the latitudinal range of capelin spawning in these waters extends several hundred kilometers, individual spawning beaches are often separated by only a few kilometers. Juvenile capelin feed in offshore areas and undergo extensive migrations (in the order of 100 to 200 km) to spawning grounds. Juveniles of the bottom-spawning Southeast Shoal stock are thought to mix with juveniles of beach spawners on the feeding grounds of the northern Grand Banks (Carscadden 1984). Mature pre-spawning capelin tagged near Newfoundland spawning beaches a few weeks before the reproductive season have been recovered near other spawning areas several kilometers away and in adjacent bays (B. Nakashima pers. comm.). This indicates that during the spawning season, migrations are extensive and ripe fish probably occur close to a number of spawning beaches, thus affording an opportunity for gene flow among putative stocks. The extensive distribution of rare mtDNA genotypes observed in the northwest Atlantic may be interpreted as supporting this view of ongoing gene flow among spawning sites.

Alternatively, it is possible that genotypes common to many populations are ancestral mtDNAs which remained widespread following the genetic isolation of populations. Avise et al. (1984) demonstrated that intraspecific mtDNA variability observed today is greatly influenced by stochastic lineage extinction which in turn is a function of generation time, density regulation, long-term effective population size, time since population founding and population subdivision. Present day population sizes of capelin in the northwest Atlantic are huge. For example, the total 1989 biomass estimate for capelin populations from southern Labrador to the southern Grand Banks was 5600000 tons (Miller & Carscadden 1989, Miller 1990). Thus, large effective population sizes may have acted to buffer stochastic lineage extinction among populations such that the 10000 to 12000 yr since population founding is not sufficient to discriminate present day populations based on frequency distributions of ancestral mtDNA genotypes. The widespread distribution of rare mtDNA genotypes may also be the result of widely dispersed ancestral mtDNA genotypes rather than contemporary gene flow. The larvae of capelin spawning in the estuary of the St. Lawrence estuary (here represented by the St. Irénée sample) are rapidly transported downstream following hatching and juveniles are subsequently concentrated in a large counterclockwise gyre in the northwestern Gulf of St. Lawrence (Jacquaz et al. 1977). Despite the spatial isolation of the St. Lawrence estuary stock from the Grand Banks' stocks, it was not genetically distinct from other northwest Atlantic stocks. Furthermore, 5 of the 6 rare genotypes sampled at St. Irénée and characteristic of more than 1 sampling site were also found in Labrador, Conception Bay, Placentia Bay and on the Southeast Shoal. It is difficult to reconcile such apparently extensive gene flow among contemporary stocks with a life-history pattern characterised by multiple, discrete spawning and larval retention areas. A more parsimonious hypothesis may be that ancestral mtDNAs remained widespread following the founding of present-day capelin populations in the northwest Atlantic.

The observation of different nucleon diversities among geographic sites is also contradictory to the view that capelin form 1 large panmictic population in the northwest Atlantic (Table 1, Fig. 5). Samples from both Notre Dame Bay and St. Flavie, Quebec, showed much lower diversity than observed at all other sites. Assuming temporal stability in nucleon diversity estimates, such differences in the diversity of mtDNA lineages suggests a certain degree of population structure. In conclusion, these observations suggest that spatial homogeneity in the distribution of mtDNA genotypes among capelin is not sufficient evidence to identify panmixis, as was concluded in the case of American eels (Avise et al. 1986). Although the spatial homogeneity of the mtDNA genotypes of the American eel conforms to the conventional view of their panmictic, catadromous life cycle, capelin are characterised by a very different life history pattern that does not conform to the view of panmixis suggested by the lack of mtDNA differentiation among widely separated conspecific samples. It is possible that little or no geographic differentiation in mtDNA genotypes may be typical of highly abundant marine species, or subgroups of a species, that have colonized marine habitats made accessible following the last glaciation events.

A striking characteristic of the genetic structure revealed in this study is the discrepancy between the time of divergence of the 2 major mtDNA phylogenetic groups and the time of divergence among mtDNA haplotypes observed within each phylogenetic group. It is assumed that genetic distance is proportional to time since lineage separation. In some birds and mammals, the rate of evolution of mtDNA has been estimated at about 2 % nucleotide substitutions per million yr (Brown et al. 1979, Shields & Wilson 1987). This rate is currently widely applied to fish (e.g. Avise et al. 1987, González-VillaSeñor & Powers 1990). Applying this

rate to capelin, the 2 major mtDNA phylogenetic groups diverged ca 1.75 million yr ago, whereas the mtDNA haplotypes observed within each group diverged only ca 0.25 million yr ago. This suggests that the 2 phylogenetic groups experienced a demographic event about 1.5 million yr after they diverged that depressed the numbers of female ancestors that contributed to the present-day gene pool through female lines (Avise 1989). This is consistent with the general view that for phylogenetic groups with large population sizes and high rates of gene exchange, the evolutionary effective population size of females is much smaller than the current population size of females (Avise et al. 1988).

Despite the emerging consensus that marine fishes experience greater genetic interconnectedness among conspecific populations than that observed among populations of freshwater species, historical biogeographical factors may nevertheless have played a role in the genetic structuring of both marine and freshwater species (Avise et al. 1987). This view is illustrated by the geographic division of the toadfish *Opsanus tau* (Avise et al. 1987) and the horseshoe crab *Limulus polyphemus* (Saunders et al. 1986), distributed along the eastern North American coast, into northern and southern genotypic groupings approximately coincident with conventionally-recognized zoogeographic provinces. Similarly, the common mummichog *Fundulus heteroclitus*, a coastal, euryhaline species, is divided along the eastern North American coast into a northern and southern form most probably related to some past zoogeographic event (González-VillaSeñor & Powers 1990). In the present study, historical events may be responsible for the major genetic divergence separating northwest Atlantic capelin from Icelandic and Barents Sea capelin, a divergence similar in magnitude to that separating the American eel from the European eel *Anguilla anguilla* (genetic divergence = 3.7%; Avise et al. 1986). Avise et al. (1990) speculated that separation of the 2 North Atlantic eel forms may be explained by cooling during Pleistocene glaciation events that forced a southern retreat and disjunction of spawning areas of a single ancestral population. A similar phenomenon may have influenced North Atlantic capelin. Alternatively, Stergiou (1989) speculated that capelin from the Pacific Ocean repopulated the Canadian Arctic and northwest Atlantic during the warming up of the Canadian Arctic Archipelagos during the holocene hypsithermal some 4500 to 6000 yr ago. Although the relationship between spawning mode and geographic origin proposed by Stergiou (1989) is refuted by the present results, northwest Atlantic capelin, regardless of spawning mode, may nevertheless have been derived from Pacific populations. The present-day genetic structure of capelin may thus be related to the use of the Arctic Ocean as an intermittent dispersal route between the Pacific and Atlantic Oceans following the opening of the Bering Strait about 3.0 million yr ago (Grant 1987). We are presently extending our sampling to Arctic Canada and Alaska to test these hypotheses.

Acknowledgements. We gratefully acknowledge Chantal Ouellet and Marie-Claire Baby for laboratory assistance and Brian Dempson, Milton Peach and technicians of the Pelagic Section (Department of Fisheries and Oceans, St. John's, Newfoundland), Jean-Denis Lambert (Department of Fisheries and Oceans, Mont-Joli, Québec), Noël Gauthier (St-Irénée, Québec), Hjalmar Vilhalmsson and Sveinn Sveinbjornsson (Marine Research Institute, Reykjavik, Iceland), and Harald Gjøsæter (Institute of Marine Research, Bergen, Norway) for providing fish samples. This research was funded by a Natural Sciences and Engineering Research Council (Canada) grant, a Canadian Department of Fisheries and Oceans Subvention Program grant and a Canadian Department of Supply and Services contract to J. J. Dodson.

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This article was submitted to the editor

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Manuscript first received: December 10, 1990
Revised version accepted: July 8, 1991