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Source regions for recruitment of *Calanus finmarchicus* to Georges Bank: evidence from molecular population genetic analysis of mtDNA

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Abstract—Molecular population genetic analysis has provided evidence that the copepod, *Calanus finmarchicus*, of the Labrador Current, Gulf of St Lawrence, Scotian Shelf, Gulf of Maine, and Georges Bank constitute a single, interbreeding population. The DNA sequence of a 350 base pair portion of the mitochondrial large subunit (16S) ribosomal RNA (rRNA) gene was determined for a total of 72 individuals collected in 1992, and 110 individuals collected in 1993 from these regions. There was significant heterogeneity in haplotype frequencies among the samples collected in 1992, but this heterogeneity did not resolve into regional patterns. The only regional differences seen were between pooled samples of the western N. Atlantic and those of the Norwegian Sea. There were no significant differences in haplotype frequencies among the samples collected in 1993, and fewer haplotypes were observed in these samples. Intraspecific molecular variation was typical of other marine species: there were 29 haplotypes among the 182 individuals sequenced. The frequency distribution of the haplotypes was highly skewed: 128 individuals shared one haplotype and 19 individuals were unique. There were 24 variable sites among the 350 bases sequenced; estimated nucleotide diversity was 0.0042. The genetic character of *C. finmarchicus* populations in the western N. Atlantic was stable over time in that three of the haplotypes (including the most abundant) occurred in both 1992 and 1993. However, haplotype frequencies differed significantly between the two years. The lack of regional structure in the 1992 samples and the genetic homogeneity of samples collected in 1993 across the domain from the Labrador Current to the Gulf of St Lawrence to Georges Bank and the Gulf of Maine indicated that there is significant gene flow across this region. The persistent genetic pattern suggests that the Gulf of St Lawrence may be an important source region for recruitment of *C. finmarchicus* to Georges Bank. Determination of zooplankton dispersal patterns within high gene flow species will provide information that may not be determined by conventional oceanographic analyses. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Zooplankton dispersal in the ocean is a complicated mix of passive transport in currents and active swimming by individuals. Biological oceanographers have employed a variety of approaches to study zooplankton dispersal, including tracking of parachute-drogued drifters (Miller, 1976). This study and several previous ones (Bucklin *et al.*, 1989, 1995a; Bucklin, 1991) used population genetic characteristics of plankton to infer patterns of dispersal in the ocean. Population genetic analysis may yield statistical rather than deterministic conclusions about population dynamic phenomena. Thus, the destination of

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a particular plankter may not be predictable, but how frequently a dispersal pathway is used (i.e. estimation of the number of migrating individuals) may be established using population genetic statistics of gene flow among geographic populations.

The use of genetic markers to examine population structure and patterns of gene flow has been an active area of research for many years. Allozymic variation has been used to quantify population structure of a wide variety of organisms (for review, see Avise, 1994). Previous studies of the population genetics of marine copepods using allozymes have demonstrated significant genetic heterogeneity both within and between copepod species (Bucklin and Marcus, 1985; Sevigny and Odense, 1985; Sevigny and McLaren, 1988; Bucklin *et al.*, 1989; Sevigny *et al.*, 1989; Bucklin, 1991). Although these studies have provided some evidence of geographic structure based on allozymic frequencies, patterns of dispersal at meso- and larger scales have been difficult to infer (Bucklin *et al.*, 1989; Bucklin, 1991).

Variation of mitochondrial DNA (mtDNA) also has been employed to examine geographic patterns of intraspecific variation (e.g. Avise *et al.*, 1987). Mitochondrial traits have a distinct advantage as indicators of population structure: the genome is clonally (matrilineally) inherited in most organisms, making it a conserved indicator of breeding patterns in natural populations (see Avise *et al.*, 1979; Wilson *et al.*, 1985). The mode of inheritance of mitochondrial traits has many implications for their distributions in populations. First, when male transmission is negligible, mitochondrial genes will reveal population structure better than nuclear genes (Birky *et al.*, 1989). Also, if females have a lower effective migration rate than males, mitochondrial gene frequencies will be homogenized more slowly than nuclear genes (see Birky *et al.*, 1989).

A variety of technical advances have made feasible the analysis of molecular characteristics for population genetics. Direct sequencing of amplified gene portions without an intermediate cloning step (Innis *et al.*, 1988) is primary among the technical advances. Also of importance are the automation of sequencing protocols (Smith *et al.*, 1986; McBride *et al.*, 1989) and the use of consensus primers for amplification and sequencing, obviating the need for prior sequence data from the species under study (Kocher *et al.*, 1989; Innis *et al.*, 1990). Detailed analysis of DNA sequence variation within species will reveal much about the biology of the species (including reproductive variance, dispersal, and survivorship) and may also reveal the processes by which genes and species evolve.

Restriction fragment length polymorphisms of mtDNA have been assayed for a number of organisms (see review by Avise *et al.*, 1987). Among marine invertebrates, several have been shown to exhibit population genetic structuring on meso- to large (i.e. 100s to 1000s of kilometers) scales, including: the horseshoe crab, *Limulus* (Saunders *et al.*, 1986), the oyster, *Crassostrea* (Reeb and Avise, 1990), the mussel, *Mytilus* (Edwards and Skibinski, 1987), and the copepod, *Calanus pacificus* (Bucklin and Kann, 1991). Restriction fragment polymorphisms also have revealed significant genetic differentiation among geographic populations of marine fish, including herring (Kornfield and Bogdanowicz, 1987), red drum (Gold and Richardson, 1991), haddock (Zwanenburg *et al.*, 1992), and plaice (Stott *et al.*, 1992).

More recently, the base sequence variation of mtDNA has been used to examine population structure in marine fish and invertebrates. The base sequence of a portion of cytochrome b discriminated populations of cod (Carr and Marshall, (1991a, 1991b) and blue marlin (Finnerty and Block, 1992). Mitochondrial genes have revealed considerable intraspecific sequence variation in the sea urchins, *Strongylocentrotus pallidus* (Palumbi and Kessing, 1991) and *Heliocidaris tuberculata* (McMillan *et al.*, 1992), and the penaeid shrimp, *Penaeus stylirostris* (Palumbi and Brand, 1993); however, little or no geographic structuring was observed for these species. Two general principles emerge from the many studies: first, that both marine fish and invertebrates are quite variable at the protein and molecular level, and second, that this variability is resolved into genetically distinguishable, geographic populations in only some of the species [see Ovenden (1990) for a review of marine stock assessment using mtDNA].

Questions about zooplankton dispersal patterns and source regions for new recruits are important for biological oceanographers who seek to understand the dynamics and fluctuations of secondary production in the ocean. Genetic tags may be useful since they may help elucidate the source populations, i.e. those regions which supply the new recruits to fuel secondary production in the ocean. When source regions are localized and partially isolated, they may contain genetically distinct populations whose individuals have diagnostic genetic characteristics.

This study addresses a specific question: what are the sources of recruitment of *C. finmarchicus* on Georges Bank? The question is particularly interesting (and particularly amenable to genetic analysis) since *C. finmarchicus* does not overwinter on the Bank and must re-populate the area each spring. Where do the new recruits originate each year? Do the source populations and their relative contributions to the Bank population change from year to year? We examine here the question of whether the Gulf of St Lawrence is a source region for *C. finmarchicus* populations on Georges Bank. The importance of the estuary for recruitment of *C. finmarchicus* has been emphasized by Plourde and Runge (1993), who have dubbed it a "*Calanus* pump". Based on circulation patterns (Fig. 1), copepod populations in the Gulf of Maine, the Slope Water, the Scotian Shelf, and the Gulf of St Lawrence may contribute to recruitment of Bank populations (Chapman and Beardsley, 1989). The Nova Scotia Current may provide a conduit for *C. finmarchicus* from the Gulf of St Lawrence, onto the Scotian Shelf (Herman *et al.*, 1991), into the Gulf of Maine, and/or onto Georges Bank.

Circulation patterns in the Gulf of Maine suggest that the dense aggregations of juvenile *C. finmarchicus* that occur at depth in the basins of the Gulf of Maine during the winter may also be important in populating Georges Bank during the spring (Meise-Munns *et al.*, 1990; Batchelder and Miller, 1991). The copepods also may overwinter at depth in the Slope Water (Miller *et al.*, 1991), on the Scotian Shelf (Sameoto and Herman, 1990), and in the Gulf of St Lawrence (Plourde and Runge, 1993). There thus may be multiple source regions for the spring recruitment of the species onto Georges Bank. If these source regions support genetically distinct populations of *C. finmarchicus* (a current topic of investigation by A. Bucklin), it may be possible to identify the source(s) of recruitment for the heterogeneous population over Georges Bank.

Our intention is to use the frequencies of mtDNA genotypes (i.e. haplotypes) to infer patterns of gene flow in *C. finmarchicus*. Our studies focus on the sequence of the 16S rRNA gene, for which there is a large and growing body of information on inter- and intraspecific variation (see e.g. Xiong and Kocher, 1991; Cunningham *et al.*, 1992; Bucklin *et al.*, 1992, 1995a, 1996). We report here on a study of the molecular population genetics of *C. finmarchicus* in the western N. Atlantic. We present information on levels of genetic variation, evidence for sub-division into genetically distinct populations, and estimates of gene flow patterns across the sampling domain.

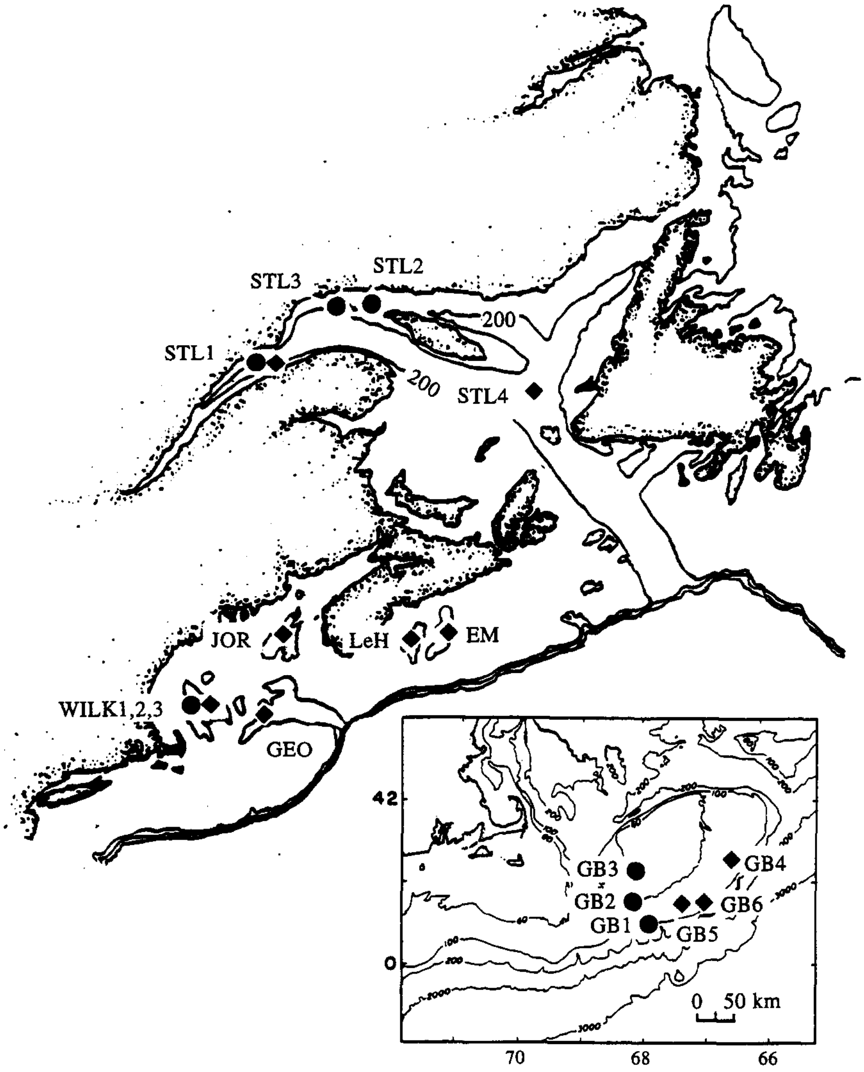

Fig. 1. Locations of collection sites in the western North Atlantic for samples of *Calanus finmarchicus* used in this study. Collection locations for 1992 (circles) and 1993 (diamonds) samples are shown; details are given in Table 1. Sample name abbreviations are: Georges Bank (GB), Gulf of St Lawrence (STL), Emerald Basin (EM), LeHave Basin (LeH), Georges Basin (GEO), Wilkinson Basin (WILK), and Jordan Basin (JOR). The collection locations for the Labrador Current samples are not shown.

MATERIALS AND METHODS

Sample collection and preservation

Samples for this study were collected in 1992 and 1993 during separate cruises to the Labrador Current, Gulf of St Lawrence, Scotian Shelf, Gulf of Maine, and Georges Bank (Fig. 1, Table 1). Immediately after collection, the seawater was drained from the samples

Table 1. Collection dates, locations, and depths for the seven zooplankton samples sorted for adult females of the copepod, Calanus finmarchicus. *The samples were preserved in 95% ethanol immediately following collection. Numbers of individuals sequenced from each sample are indicated by* N

Station name	Number	Date	Latitude	Longitude	Depth (m)	*N*
1992						
St Lawrence River Estuary						
STL1	92–02	6/2/92	48°40′	68°35′	300	10
Northern Gulf of St Lawrence						
STL2	4M	8/16/92	50°01.62′	64°23.90′	165	7
STL3	F921065	8/17/92	50°00.01′	64°46.92′	175	8
Gulf of Maine / Wilkinson Basin						
WILK1	2462	8/26/92	42°29′	69°43′	50	10
WILK2	2459	8/25/92	42°30′	69°48′	25	9
Georges Bank						
GB1	MOC 974	5/21/92	40°39.03′	67°51.82′	surface	10
GB2	MOC 995	5/26/92	40°41.84′	67°52.74′	surface	9
GB3	MOC 998	5/27/92	40°59.34′	68°00.95′	surface	10
1993						
St Lawrence River Estuary						
STL1		5/9/93	48°36.29′	68°37.60′	250	10
Gulf of St Lawrence—Lawrentian Channel						
STL4	FX-A	5/19/93	48°47.80′	61°20.08′	150	15
Deep Basins of the Scotian Shelf and Gulf of Maine						
Emerald Basin (EM)		1/6/93	43°27.0′	63°09.47′	250	10
LaHave Basin (LeH)		1/10/93	43°45.0′	63°54.0′	240	7
Georges Basin (GEO)		1/7/93	42°07.5′	68°01.6′	222	10
Wilkinson Basin (WILK3)		1/8/93	42°39.0′	69°33.0′	280	12
Jordan Basin (JOR)		1/9/93	42°36.0′	67°57.0′	220	3
Georges Bank						
GB4	St. 11	5/19/93	41°09.0′	66°40.50′	50	9
GB5	St. 16	5/20/93	40°42.00′	67°35.00′	50	11
GB6	St. 46	5/21/93	40°40.50′	67°18.50′	50	6
Labrador Current						
Labrador no. 1	137 247	7/10/93	48°43.0′	51° 03.50′	100	10
Labrador no. 2	137 248	7/11/93	48°11.7′	52° 38.00′	100	7

using nitex of the same mesh size as the collecting net, and the plankton were placed in 95% ethanol. The alcohol was changed once within 24 h for all the samples. Individual adult females of *C. finmarchicus* were sorted from each collection under a dissecting microscope at 60 × magnification. Confusion with *C. glacialis* was avoided by selecting only those adult females whose thoracic length was less than 3 mm.

Molecular techniques

The DNA amplifications were done without purification of the DNA. Individual copepods were sorted from the samples in the laboratory, and allowed to rehydrate in

0.5 ml distilled water for 6–12 h. The water was then pipetted from the tube, and replaced with 88 μl of PCR amplification buffer (78 μl distilled water and 10 μl 10 × Perkin Elmer PCR buffer). The copepods were homogenized using a pipette tip. The remaining ingredients for the polymerase chain reaction (PCR) were added (10 μl 2 mM deoxynucleotide triphosphates, 1 μl of 100 μM solutions of each primer, and 0.5 unit Taq polymerase), and the reaction mix was covered with 2 drops of mineral oil. The amplification reaction was run in a Perkin Elmer 480 PCR machine with the following program: 94°C (1 min); 37°C (2 min); 72°C (3 min) for 40 cycles.

The amplification primers used were 16SAR and 16SBR (Palumbi *et al.*, 1991) based on the Drosophila yakuba sequence (Clary and Wolstenholme, 1985). The sequences are:

16SAR 5′-CGCCTGTTTAACAAAAACAT-3′
16SBR 5′-CCGGTTTGAACTCAGATCACGT-3′

Amplification products to be sequenced were checked for size and purity by loading 10 μl on a 2% agarose gel. The remaining 90 μl of product were then loaded into wells of a 1% Nusieve gel with ethidium bromide and electrophoresed at 44 volts. Product bands were cut from the gel; the DNA was hot phenol extracted from the agarose and precipitated with isopropanol. The DNA was resuspended in 20 μl of distilled water and quantified spectrofluorometrically; approximately 250 ng of DNA were used for the cycle sequencing step.

The sequencing reaction was done in a Perkin Elmer 480 Thermal Cycler using a Cycle-Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). Fluorescently labelled dideoxynucleotides were incorporated during an asymmetrical amplification using one of the amplification primers. One cycle-sequencing reaction, using the 16SBR primer, was done for all templates. In addition, the complementary reaction using the 16SAR primer was done for seven individuals in order to verify substitutions at particular sites. One variable site was removed from consideration on this basis; all other substitutions were not dependent on sequencing direction.

Nucleotide sequencing was carried out in an Applied Biosystems 373 DNA sequencer. The sequencer uses a 6% acrylamide gel; gels are electrophoresed for 10 h. The sequences are shown as fluorescent emission spectra for each base, resulting in a 4-color chromatogram. The sequence was further compiled using SeqEd (version 2.0) and checked thoroughly for accurate machine reading.

The GCG Sequence Analysis Software Package was used for alignments and preliminary analyses. The programs are based on those by Smithies *et al.* (1981); they have been published by Devereaux *et al.* (1984) and are now commercially available (Genetics Computer Group, Madison, WI). The multiple sequence alignment program, PileUp, was used to align the sequences for each individual. PileUp is a simplification of the progressive alignment method of Feng and Doolittle (1987). Although the program has limitations, it is a safeguard against human subjectivity in the alignment process. Alignment was done with sequences from one individual of *C. glacialis* and one of *C. pacificus* for comparison. Alignments were done to maximize the similarity of the sequences by allowing alignment gaps between sequences in the multiple alignment.

Nucleotide diversity (π) was calculated as an estimate of genetic variation according to Nei (1987) by the formula:

$$\pi = \sum_{i<j} \pi_{ij}/n_c \tag{1}$$

where π_{ij} is the proportion of different nucleotides between the *i*th and *j*th haplotypes and n_c is the total number of sequence comparisons $[n(n-1)/2]$.

A cluster diagram was generated by PileUp to identify distinct haplotypes. The unique haplotypes were pooled into a single class, since the unique haplotypes are not geographically informative. The geographic distributions of the haplotypes (including the one pool of all unique haplotypes) were statistically evaluated by a chi-square test using a Monte Carlo simulation (Roff and Bentzen, 1989). Comparisons among all samples and regions were done to identify significant geographic partitioning. Each comparison used 1000 replicates and generated a p value ± the standard deviation of results from the 1000 simulations.

RESULTS

Molecular variation of Calanus finmarchicus

Among the individuals of *C. finmarchicus*, 29 different haplotypes were defined by a 350 bp section of the mitochondrial 16S rRNA (Fig. 2). Nineteen of the haplotypes occurred in only one individual; 128 individuals shared a single haplotype; nine additional haplotypes were shared by 2–6 individuals. There was thus a highly skewed distribution of haplotype frequencies, with a single ubiquitous haplotype and numerous unique haplotypes (Fig. 3). The two most different haplotypes differed by 5 bases (1.4%). For comparison, a 450 bp region of this same gene (including this 350 bp section) differed by 12–18% among the species of *Calanus* (Bucklin *et al.*, 1992, 1996). The numerous, genetically similar haplotypes result in a moderate nucleotide diversity (π) of 0.0042. There were 24 variable sites among the 182 sequences of 350 base pairs, including 13 unique substitutions (i.e. those occurring in only one individual; Fig. 2).

Evidence of population genetic spatial structure

The samples collected in 1992 differed significantly in the relative haplotype abundances exhibited by the assayed individuals (based on chi-square tests using Monte Carlo simulations; Roff and Bentzen, 1989) (Table 2, Fig. 4). However, the differences were due to one divergent sample from the northern shore of the Gulf of St Lawrence (STL3). Haplotype frequencies among the other western N. Atlantic samples did not differ significantly ($p = 0.298$) (Table 2). A comparison of the pooled western N. Atlantic samples vs samples of *C. finmarchicus* collected from the Norwegian Sea (data from Bucklin *et al.*, 1996) indicated differentiation of populations in the different oceanographic domains, as shown by significant differences in haplotype frequencies ($p = 0.044$; Table 2).

The samples collected in 1993 similarly showed no significant differences in haplotype frequencies among the four regions: Labrador Current, Gulf of St Lawrence, deep basins of the Scotian Shelf and Gulf of Maine, and Georges Bank ($p = 0.242$; Table 2). One haplotype (the most abundant haplotype of 1992 samples also) predominated in all samples, ranging from 71–92% of the individuals (Fig. 5).

```
#1   CTCAGTGAATATTTAAACAGCCGCGTTAGTGTTAAGGTAGCATAGTAATTAGTTTCTTAATTGGGAAATAGGATGAATGGTTTCACTAAAATATAGTTTT 100
#2   ....................................................................................................
#3   ....................................................................................................
#4   ....................................................................................................
#5   .....................................................C..............................................
#6   ....................................................................................................
#7   ....................................................................................................
#8   ...........G........................................................................................
#9   ....................................................................................................
#10  ....................................................................................................
POOL ...........G.....................................A.C.C..................G........C.............A....
                1                                     1 1 1                  2        1
                                                                                      G
                                                                                      1

#1   TATCCTCATTTGCGAAATTTTAATCTAAGTGAAAATACTTAGCAGTTGTACTAGGACGAGAAGACCCTATGAAGCTGGCAAACTATTAATACATATTCCT 200
#2   .................................T..................................................................
#3   ........C...........................................................................................
#4   ....................................................................................................
#5   ....................................................................................................
#6   ...........C........................................................................................
#7   ...........................................................................................T........
#8   .................................T..................................................................
#9   ....................................................................................................
#10  ........C..................................................................................T........
POOL ........C....................C...T......CC..................C............C.................T........
             2                    1   4      11                  1            1                 2
                                              T
                                              1
```

```
#1   CTCAGTGAATATTTAAACAGCCGCGTTAGTGTTAAGGTAGCATAGTAATTAGTTTCTTAATTGGGAAATAGGATGAATGGTTTCACTAAAATATAGTTTT 100
#2   ....................................................................................................
#3   ....................................................................................................
#4   ....................................................................................................
#5   .....................................................C..............................................
#6   ....................................................................................................
#7   ....................................................................................................
#8   ...........G........................................................................................
#9   ....................................................................................................
#10  ....................................................................................................
POOL ...........G.....................................A.C.C..................G........C.............A....
                1                                     1 1 1                  2        1
                                                                                      G
                                                                                      1

#1   TATCCTCATTTGCGAAATTTTAATCTAAGTGAAAATACTTAGCAGTTGTACTAGGACGAGAAGACCCTATGAAGCTGGCAAACTATTAATACATATTCCT 200
#2   .................................T..................................................................
#3   ........C...........................................................................................
#4   ....................................................................................................
#5   ....................................................................................................
#6   ...........C........................................................................................
#7   ...........................................................................................T........
#8   .................................T..................................................................
#9   ....................................................................................................
#10  ........C..................................................................................T........
POOL ........C....................C...T......CC..................C............C.................T........
             2                    1   4      11                  1            1                 2
                                              T
                                              1
```

Fig. 2. Base sequence variation of the 350 base pair portion of the mitochondrial 16S rRNA gene used for this study. The reference sequence is the most abundant haplotype, which occurs in over one-half of the individuals. The sequence designated "pool" indicates the sites of all substitutions for the 19 unique haplotypes, which are not shown individually. The number of times each substitution was observed is shown below the line. Abundances for all haplotypes are given in Table 3. Bases that are identical to the reference sequence are shown as "."; unknown/unreadable bases are shown as "*N*".

Temporal variation in Calanus finmarchicus *populations*

Three haplotypes (including the most abundant one) occurred in samples for both 1992 and 1993. However, haplotype frequencies were significantly different between the two years ($p=0.000$; Table 2). The most frequent haplotype constituted 49% of 72 individuals in 1992 and 85% of 110 individuals in 1993; the frequency of genetically unique individuals also differed between 1992 (19%) and 1993 (2%) (Table 3).

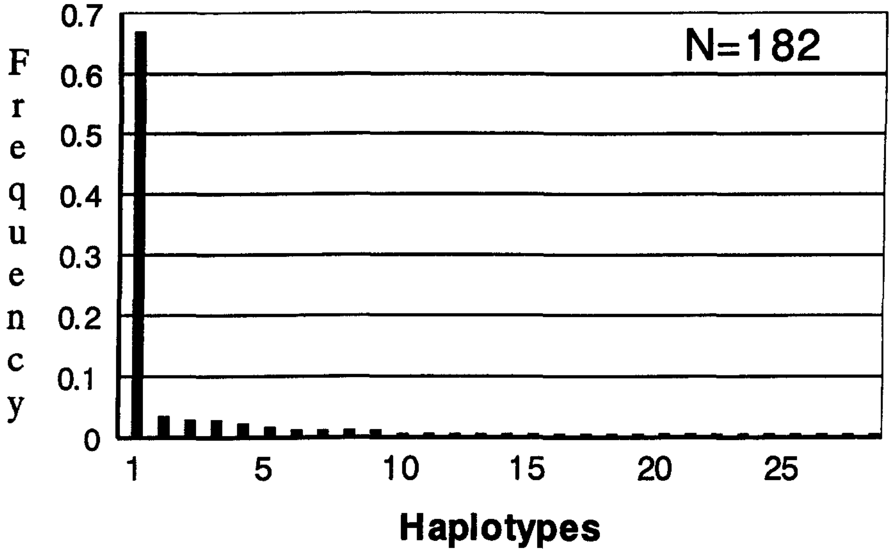

Fig. 3. Haplotype frequency distribution for 182 individuals of *Calanus finmarchicus* collected in the western North Atlantic in 1992 and 1993. A skewed haplotype frequency distribution, with one abundant and nearly ubiquitous haplotype and numerous unique haplotypes, is typical of many of the marine invertebrates studied to date.

Table 2. Chi-square tests for geographic homogeneity of mtDNA haplotype frequencies for 182 *individuals representing* 20 *separate collections.* p-*values are given* ± *one standard deviation, as determined by Monte Carlo simulations (Roff and Bentzen, 1989). Asterisks at right indicate significance level:* *** *(*p<0.001*);* ** *(*p<0.01*);* * *(*p<0.05*). Data for the comparison with samples of* C. finmarchicus *from the Norwegian Sea (Norway) are from Bucklin* et al. (1996)

Comparison	*p*-value
1992	
Among northwest Atlantic samples	0.000±0.000***
GB1,2,3, WILK1,2, STL1,2	0.298±0.015
STL1,2,3	0.000±0.000***
Northwest Atlantic vs Norway samples	0.044±0.005*
1993	
Among all samples	0.242±0.014
1992 vs 1993	
Among all samples	0.000±0.000***
1992 vs 1993 pooled	0.000±0.000***

Table 3. *Numbers of individuals of each haplotype by sample. The haplotype designated "P" is the pool of all unique haplotypes. Haplotype #12 was found only in Norwegian Sea samples; these data are not included in any of the totals and are shown here for comparison (data from Bucklin* et al., 1996*). Explanations for the sample name abbreviations can be found in Table* 1

	Samples—1992									Samples—1993						
No.	GB1	GB2	GB3	WIL1	WIL2	STL1	STL2	STL3	(NOR)	GB4	GB5	GB6	DB	STL1	STL4	LAB
1	6	5	4	5	6	6	3	—	(24)	8	10	5	35	9	14	12
2	3	—	1	—	1	—	1	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	—
4	—	—	1	—	1	1	—	—	—	—	—	—	2	—	—	1
5	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—
8	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	1	—	—	—	—	1	—	1	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	2	1	1	1
P	1	2	2	2	1	1	2	3	(6)	—	—	1	1	—	—	3
12	—	—	—	—	—	—	—	—	(2)	—	—	—	—	—	—	—

DISCUSSION

Molecular population genetic analysis of marine plankton is useful in understanding dispersal processes of otherwise untrackable organisms. Genetic characteristics can be used to delimit and distinguish populations (i.e. groups of randomly mating individuals) within a species by assessing the differences in the frequencies of alleles (i.e. forms of a gene) in samples collected within the geographic distribution of the population. Populations are genetically homogenized by dispersal of individuals within their boundaries; populations become genetically differentiated when there is little or no dispersal between them—or when forces that counterbalance dispersal, such as differential natural selection and/or drift, are strong.

These results indicate that *Calanus finmarchicus* of the Labrador Current, Gulf of St Lawrence, Gulf of Maine, and Georges Bank constitute a single, interbreeding population. Patterns of genetic variation in populations of *C. finmarchicus* in the western N. Atlantic are consistent with the hypothesis that copepods recruit to Georges Bank from all of these "upstream" areas in the western N. Atlantic. The estuary of the St Lawrence River, in fact, may be a *Calanus* "pump" (Plourde and Runge, 1993), i.e. a source region supplying copepods to the next spring increase on Georges Bank.

Despite dispersal across the domain that is sufficiently frequent to prevent regional differentiation of populations, *C. finmarchicus* populations were not genetically homogeneous. There was significant sample-to-sample variation in haplotype frequencies (Table 2), but this patchiness did not resolve into regional patterns of population genetic structure (Figs 4 and 5). This result—the lack of significant population structure within the western N. Atlantic region—was consistent with other biological and physical evidence of circulation in the region. Recent studies of the zooplankton assemblages of the Gulf of St Lawrence by Runge and Simard (1990) and of the Scotian Shelf by Herman *et al.* (1991) confirmed their similarity in terms of species composition. It is likely that a large portion of the zooplankton assemblage of the Scotian Shelf is supplied primarily by export from the St Lawrence Estuary via the Laurentian Channel (Herman *et al.*, 1991; Plourde and Runge, 1993). However, analysis by Herman *et al.* (1991) indicated that some of the plankton biomass must be generated locally: local reproduction by zooplankton retained in the deep basins (which may also ultimately originate from the Gulf of St Lawrence) also may contribute to Scotian Shelf populations.

Based on historical and recent observations (Bisagni *et al.*, 1996) reported that water may require on the order of 9 months to be transported between the Gulf of St Lawrence and Georges Bank. Whether the copepods are transported at the same rate—and whether they require an overwintering stop along the way—is unknown. The large volume of transport from the Gulf of St Lawrence, across the Scotian Shelf, and either directly onto Georges Bank or into the Gulf of Maine, argues that zooplankton dispersal across this region may be extensive.

Further support for the validity of our conclusion is provided by evidence of genetic differentiation between populations of *C. finmarchicus* in the western N. Atlantic and the Norwegian Sea. Pooled samples collected from the Norwegian Sea differed from pooled samples from the western N. Atlantic ($p = 0.044$; Table 2). (See Bucklin *et al.*, 1995, 1996) for a discussion of the population genetics of *C. finmarchicus* in the Norwegian Sea.) There was one genetically divergent sample within each domain (one from the northern Gulf of St Lawrence [STL3], one from the Norwegian Sea [NOR2]). Based on these preliminary data, we can partition the *C. finmarchicus* population into two domains—one on either side of the North Atlantic.

The lack of genetic structure at sub-regional scales is not typical of all marine organisms—not even all planktonic copepods. Chapman and Bortone (1994) found significant genetic structuring of red snapper along the Gulf of Mexico using 16S rRNA gene sequences that was not apparent based on sequences of regions of the protein-coding mitochondrial genes, cytochrome b and 6-adenosine triphosphatase. The estuarine copepod, *Acartia tonsa*, exhibited significant structure among estuaries of the east coast of the U.S. based on mitochondrial 16S rRNA sequence data (Caudill and Bucklin, 1994). The best explanation for the lack of structure within the western N. Atlantic domain from the Gulf of St Lawrence to the Gulf of Maine and Georges Bank is significant gene flow across this range. Additional mitochondrial genes are currently being sequenced for these same individuals to determine whether genes with more variation will indicate the same pattern.

The pattern exhibited by *C. finmarchicus* has been demonstrated for other species with different mitochondrial genes, providing strong evidence of the validity of the patterns observed in terms of ocean processes. In particular, a study using DNA sequence variation of the mitochondrial cytochrome oxidase I gene of the euphausiid, *Meganyctiphanes norvegica*, has demonstrated similar patterns of large-scale population structure, including differences between western N. Atlantic and Norwegian Sea populations, and homogeneity within the western N. Atlantic (Bucklin, unpublished data). The approach of using multiple species and genes to demonstrate a population genetic phenomenon has been called "coherence" by Avise (personal communication, 1994; presented at the Third International Marine Biotechnology Conference, Tromso, Norway, 1994). Comparisons across genes and species yields strong conclusions about gene flow patterns in different environments. It seems likely that widely distributed, highly abundant zooplankton species may partition their world on large scales: i.e. they are structured at oceanographic spatial scales on the order of 1000s of km.

Molecular variation of Calanus finmarchicus

The pattern of intraspecific molecular genetic variation for *Calanus finmarchicus* has some similarity with that exhibited by other marine species. There was considerable base sequence variation in the 350 base pair portion of the mitochondrial 16S rRNA. This variation occurred in the form of numerous haplotypes, many of them unique, which differed by only a few bases (Fig. 3). Explanations for the presence of numerous unique haplotypes may lie in the enormous population sizes of *Calanus* (one of the highest among animals) and other marine organisms, which may allow retention of numerous haplotypes. The skewed haplotype frequency distribution is not unusual among marine species, either vertebrate and invertebrate. A similar haplotype frequency distribution was observed for red snapper (Camper *et al.*, 1993) and haddock (Zwanenburg *et al.*, 1992). A similar pattern, albeit with small sample sizes, was observed for the urchin, *Strongylocentrotus pallidus* (Palumbi and Kessing, 1991).

A further characteristic of the molecular variation was the low level of divergence among most of the haplotypes. The most divergent haplotypes differed by only 5 of 350 bases or 1.4% of the sequenced bases. Nei's measure of nucleotide diversity (π) was 0.008 for *Drosophila* and 0.004 for *Peromyscus* (Nei, 1987; p.267), based on restriction fragment length polymorphisms; we calculated π to be 0.0042 for *C. finmarchicus*. Lack of divergence among haplotypes may be explained by small effective population size (caused by historical variation in population size, natural selection, and/or population genetic structure). Avise *et al.* (1988) explain low molecular distances among mtDNA lineages in fish by variation in reproductive success, which acts to reduce effective population size. Similar forces may be operating in marine plankton, although the life history data required to test these hypotheses are largely lacking.

Temporal variation in genetic characteristics

A persistent question about marine organisms concerns the temporal stability of their population genetic characteristics. For many marine organisms, including the holoplankton, there may be marked variation in population census size (i.e. the number of individuals alive at any given time) on seasonal to annual time scales. There may also be extreme variation in the success of reproduction and survivorship of the offspring. This population dynamic phenomenon creates the opportunity for rapid change in the genetic makeup of the population, through either genetic drift (if there are very few surviving offspring) or natural selection. This question may be resolved by repeated sampling of a species in a given oceanographic domain.

For this study, we assayed similar sample sizes over the same domain for two successive years. Our conclusion is that there was genetic stability in the genetic makeup of *C. finmarchicus* populations in the western N. Atlantic since three of the haplotypes (including the predominant haplotype) occurred in samples collected in both 1992 and 1993. However, haplotype frequencies differed significantly between the pooled samples from 1992 and 1993 ($p = 0.000$; Table 2). Genetic assessment of populations for at least 5–10 years will be required to establish interannual patterns of variation. This may be most easily done using samples from museum collections; adaptation of molecular techniques for formalin-preserved samples is part of an ongoing study (by A. Bucklin).

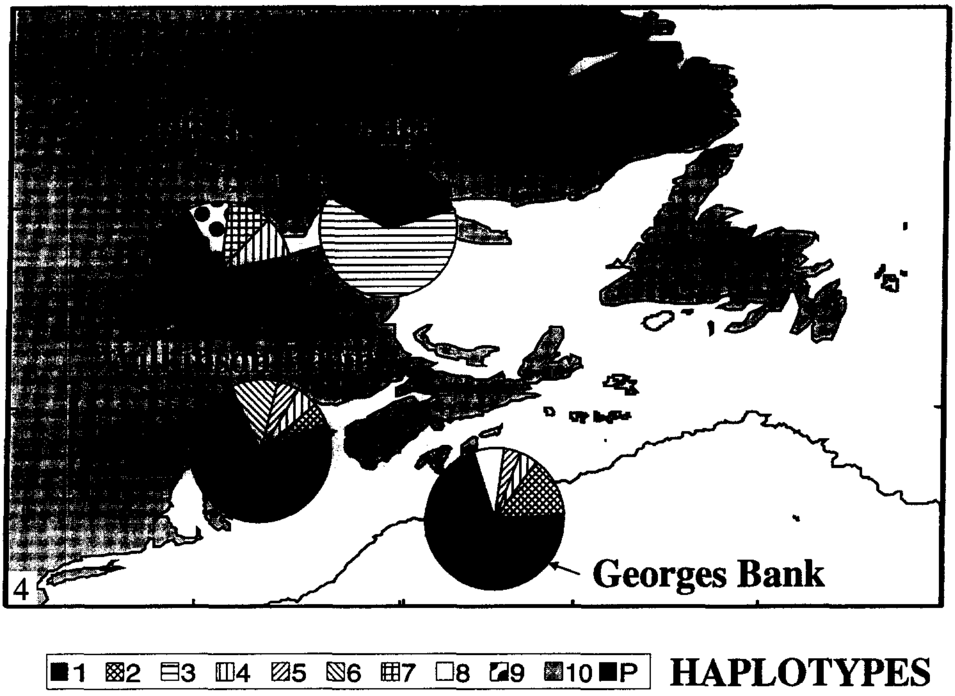

Fig. 4. Haplotype abundances by region for 1992. Samples within each region were pooled to yield the regional pattern of haplotype distribution shown. The regions are the estuary of the Gulf of St Lawrence (STL1), the northern Gulf of St Lawrence (STL2 and STL3), Georges Bank (GB), and Wilkinson Basin in the Gulf of Maine (WILK). Collection details are given in Table 1.

CONCLUSIONS

There was no evidence of significant population genetic structure of *C. finmarchicus* across the domain from the Labrador Current and the Estuary of the St Lawrence River to Georges Bank and the Gulf of Maine, despite the presence of one very distinctive sample (STL3 in 1992). The genetic data indicated that there was considerable dispersal (gene flow) by the copepod across this region, which supported physical and satellite-based evidence of rapid transport of water and biota from the Gulf of St Lawrence onto Georges Bank. It seems likely that the estuary of the St Lawrence River is an important source region for recruitment of *C. finmarchicus* onto Georges Bank. An expanded sampling regime is planned for future studies to evaluate the importance of this source and to resolve further patterns of dispersal of *C. finmarchicus* in the N. W. Atlantic. Additional samples from the Labrador Current, the Scotian Shelf, and the western N. Atlantic Slope Water will be used to address questions of the importance of various source regions for recruitment of the copepod to Georges Bank.

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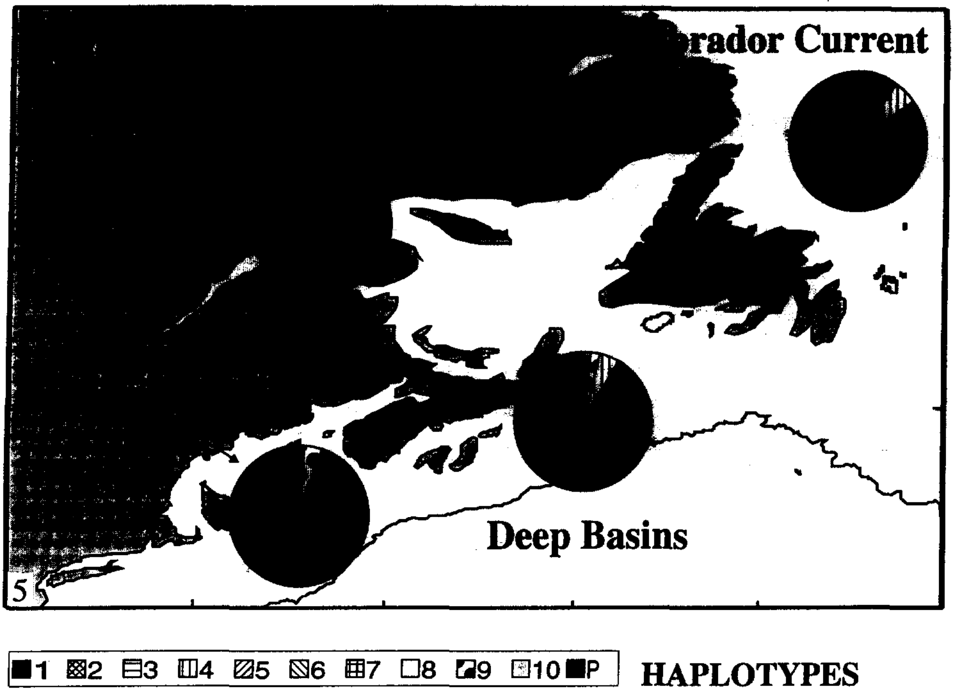

Fig. 5. Haplotype abundances by region for 1993. Samples within each area were pooled to reveal regional patterns of haplotype distribution. The regions are the Labrador Current, the Gulf of St Lawrence (STL1 and STL4), Georges Bank (GB), and the deep basins the Scotian Shelf and the Gulf of Maine (DB). Collection details are given in Table 1.

B. W. Frost (University of Washington) confirmed the identification of the copepods. T. C. LaJeunesse and J. Conroy (University of New Hampshire) provided technical assistance. This work was supported by an NSF Research Fellowship in Biotechnology and Ocean Sciences (OCE-9018528), the Office of Naval Research Oceanic Biology Program (N00014-93-1-0178), and the Office of Global Programs of NOAA (NA36GP0298-01).

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