Low Mitochondrial Diversity and Small Effective Population Sizes of the Copepods *Calanus finmarchicus* and *Nannocalanus minor:* Possible Impact of Climatic Variation During Recent Glaciation

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Molecular population genetic diversity of two planktonic copepods of the North Atlantic, *Calanus finmarchicus* and *Nannocalanus minor* (Crustacea, Copepoda, Calanoida), was characterized using the sequence variation in a 350 bp region of the mitochondrial 16S rRNA gene. The subarctic species, *C. finmarchicus,* shows lower population genetic diversity (haplotype diversity, $h = 0.368$, SD = 0.043; nucleotide diversity, $\pi = 0.00370$, SD = 0.0026) than the temperate/subtropical species, *N. minor* ($h = 0.824$, SD = 0.024; $\pi = 0.00502$, SD = 0.0032). Effective population sizes (N_e, estimated from numbers of haplotypes) and effective female population sizes ($N_{f(e)}$, estimated from nucleotide diversities) for the two species are 10^7 to 10^{10} smaller than census female population sizes (N_f) estimated from observed densities and areal distributions. For both *C. finmarchicus* and *N. minor,* $N_f \sim 10^{15}$, $N_e \sim 10^8$, and $N_{f(e)} \sim 10^5$. We hypothesize that the cause of both low levels of molecular diversity and small effective population sizes of the two species is the impact of glaciation during the past 20,000 years. *C. finmarchicus* may have experienced 75% range reduction and latitudinal displacement during the last glacial maximum at 18,000 years BP, giving rise to a genetic bottleneck; this may explain low diversity and an L-shaped distribution of pairwise haplotype differences. In contrast, *N. minor* may have experienced range reduction of only 30% and less change in latitudinal extent, with less impact of levels of molecular diversity and the shape of the pairwise difference distribution. Although marine zooplankton species are highly abundant, conservation biologists should note that their numbers may vary significantly on climatic to evolutionary time scales, generating low levels of molecular genetic diversity.

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 89:383–392

Molecular diversity of the mitochondrial 16S rRNA gene was studied in two species of planktonic copepods (Calanoida, Copepoda, Crustacea) that are widely distributed across the North Atlantic: *Calanus finmarchicus* (Bucklin and Kocher 1996; Bucklin et al. 1996b) and *Nannocalanus minor* (Bucklin 1998; Bucklin et al. 1996a). The two species differ in latitudinal range, geographic distribution, and ecological habitat preferences. *N. minor* is a warm-water cosmopolite (Ashjian and Wishner 1993; Sewall 1929) continuously distributed between 10°N and 43°N in the western North Atlantic (Owre and Foyo 1967) and is abundant in both temperate (transition zone) and subtropical waters (Grice and Hulsemann 1965; van der Spoel and Heyman 1983). In contrast, *C. finmarchicus* is a subarctic species occurring throughout much of the ocean basin between 38°N and 78°N, as well as on the eastern and western North Atlantic continental shelves and (Fleminger and Hulsemann 1977). *C. finmarchicus* shows three distinct centers of abundance in the Norwegian Sea, Labrador–Irminger Seas, and northwest Atlantic, with sparser populations outside these regions (Planque 1996).

The two species also differ in many aspects of their life histories, including numbers of generations per year, rates of egg production, and diapause stages. *C. finmarchicus* juveniles enter diapause and overwinter at depth. The species has one generation per year in some portions of its range, such as the northern latitudes and offshore waters, while multiple generations—probably up to three per year—may occur in coastal regions such as Georges Bank without an intervening diapause period (Miller et al. 1991). In contrast, *N. minor* reproduces continuously throughout much of its range, producing multiple generations without apparent pause (Asjian and Wishner 1993).

A significant body of work has addressed how genes and species change

over evolutionary time. Collectively hypotheses regarding the consequences of random evolutionary changes are known as neutral theory (Nei 1987); they may be used to predict amounts, types, and patterns of genetic diversity. In particular, neutral theory may predict (1) the number of alleles, (2) the frequency distribution of alleles in a population, and (3) the distribution of pairwise differences between alleles. These predictions are based on several poorly known quantities, including mutation rates of the genetic character used and the species' effective population size (i.e., the size of an ideal population in which all parents have an equal expectation of being the parent of any given progeny). Comparisons between observed and predicted values for each characteristic may be used to understand the ecological and evolutionary dynamics of the species and to assess the importance of evolutionary forces (i.e., mutation, migration, genetic drift, and natural selection) in determining population genetic makeup.

Given differences in patterns of life history, distribution, and abundance, we might expect that the two species would differ in patterns of population genetic diversity and structure. To test this, the DNA sequences of homologous 350 bp regions of mitochondrial 16S rRNA were analyzed to allow direct comparison of *C. finmarchicus* and *N. minor.* Patterns of population genetic diversity were compared between the two species of copepods in light of the evolutionary forces that may determine their present population genetic composition. These data and the predictions of neutral theory were then used to examine how these species have responded to environmental variation on recent and geologic time scales. The comparison of systematically related (i.e., same family) but ecologically different species may allow us to consider what can be learned of a species' evolutionary history from its present population genetic composition. Here we examine patterns of molecular genetic diversity in *C. finmarchicus* and *N. minor* for the signatures of evolutionary events and processes in the histories of the two species. We consider paleo-oceanographic evidence of the effects of climatic variation resulting from the glacial maximum approximately 18,000 years BP on the planktonic assemblages of the North Atlantic.

Materials and Methods

For *C. finmarchicus,* DNA sequences were analyzed for 216 individuals collected during 1992 (110 individuals) and 1993 (106 individuals) from the northwest Atlantic, including the Gulf of St. Lawrence, Gulf of Maine, and Georges Bank; the central North Atlantic; the Labrador Sea; and the Norwegian Sea. For *N. minor,* DNA sequences were analyzed for 158 individuals collected in 1993 from the Florida Straits, the Gulf Stream, and the Sargasso Sea. The samples used for this study were thus collected from diverse regions of each species' distributional range in the North Atlantic. Collection information for *C. finmarchicus* is described in Bucklin and Kocher (1996) and Bucklin et al. (1996b); and for *N. minor* in Bucklin et al. (1996a).

For this study, an homologous 350 bp region of the mitochondrial 16S rRNA gene was examined and statistically analyzed for the two species. The question of whether the sites of substitutions along this sequence were similar between the two species was addressed by analysis of covariance using both a nonparametric rank sum test and a parametric analysis of covariance (Sokal and Rohlf 1981). Molecular diversity was estimated as (1) the number of haplotypes [i.e., haploid genotypes of mitochondrial DNA (mtDNA)], (2) haplotype diversity, (3) nucleotide diversity, and (4) frequency distributions of pairwise nucleotide differences between haplotypes. Statistical methods are described in the original studies of *C. finmarchicus* (Bucklin and Kocher 1996; Bucklin et al. 1996b) and *N. minor* (Bucklin 1998; Bucklin et al. 1996a).

The haplotypes for each species were defined by identifying all substitutions in a multiple alignment of the same region of 16S rRNA. Haplotype frequencies were computed and haplotype diversity (h) was calculated by

$$h = (n/(n-1))\left(1 - \sum f_i^2\right) \quad (1)$$

where n is the number of haplotypes sequenced and f_i is the frequency of the ith variant (Nei 1987). Variance of h was estimated according to the method of Nei (1987). Pairwise differences (p_{ij}) were calculated as the proportion of different nucleotides between the ith and jth haplotypes. Frequency distributions of p_{ij} were plotted for each species. Nucleotide diversity (π) was calculated according to Nei (1987) by

$$\pi = \sum_{i<j} p_{ij}/n_c \quad (2)$$

where n_c is the total number of sequence comparisons [$n(n-1)/2$]. Variance was calculated according to Tajima (1983). The pairwise difference distributions were compared by a parametric F test of the sample variances and a nonparametric Kolmogorov–Smirnov test of cumulative frequencies (Sokal and Rohlf 1981).

Census female population sizes were estimated based on published observations of densities and depth distributions for *C. finmarchicus* (Casas et al. 1995; Meise and O'Reilly 1996; Miller et al. 1991; Tande 1991) and *N. minor* (Ashjian and Wishner 1993; Copley et al. 1989), and the areal extent of subarctic and temperate (transition zone) plus subtropical biogeographic distributions (from McIntyre et al. 1976). The estimates were calculated by

$$N_f = C \times A, \quad (3)$$

where N_f is the female census population size, C is the observed mean density of females under 1 m^2 area of surface integrated over the depth distribution of the species, and A is the areal extent (in m^2) of the species distribution estimated from maps of McIntyre et al. (1976). Unfortunately the observations were not sufficiently numerous nor appropriately spaced to allow analysis of variance or confidence limits of these estimates. Estimates of changes in distributional range, including range compression, experienced by the species during glaciation were made by comparing the areas of current distributional ranges of subarctic and temperate/subtropical planktonic coccolithophorid assemblages with those of 18,000 years BP (McIntyre et al. 1976).

The expected effective population size (N_e, including both females and males) was estimated following the method of Ewens (1972):

$$\pi_i = l_i 2_n \theta^i / (l_{2,2n}\theta + l_{2,2n}\theta^2 + \ldots + l_{2n,} 2_n \theta^{2n}) \quad (4)$$

where π_i is the probability that the number of haplotypes observed in the sample is i (where $i = 1, 2, \ldots, 2n$); the coefficients, l_i, are Stirling's numbers of the first kind; $\theta = 4N_e u$; and where the mutation rate (u) is assumed to be 10^{-8} substitutions per generation (see Avise et al. 1988). The value of N_e was estimated by generating curves using a range of N_e values, and selecting the curve that predicted the observed number of haplotypes (k).

Effective female population sizes ($N_{f(e)}$) were estimated according to the approach of Avise et al. (1988). Observed pairwise haplotype difference distributions were

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                                                                    20
                *
N.minor   AATGAATTTT TTAAATAGCC
                 *
C.fin     AGTGAA-TAG TTAAACAGCC

                                                                    70
                  *          *     *                    *       *
N.minor   GCTTTAGTGC TAAGGTAGCA TAATAATTAG TTTTTTAATT GAAAAATAGA
                           *          *     *
C.fin     GCGTTAGTGT TAAGGTAGCA TAGTAATTAG TTTCTTAATT GGGAAATAGG

                                                                   120
                           *     *            *  *   * *
N.minor   ATGAATGGTT TTACTAAAAT TGATATTTTA AAATAATTAG ACCAAAATTT
          *        *                          * *
C.fin     ATGAATGGTT TCACTAAAAT ATAGTTTTTA TCCTCATTTG --CGAAATTT

                                                                   170
                                 * *                   *
N.minor   TAATTTTAGT GAAAATACTA AGATAATATT TTTAGACGAG AAGACCCTAT
                   *     *       **                  *
C.fin     TAATCTAAGT GAAAATACTT AGCAGTTGTA CTAGGACGAG AAGACCCTAT

                                                                   220
                        * ** **        *        *    **
N.minor   GAAGCTAAAA ATCACTATAA AAATTATAAA TTTAT---CA GATTTATTTT
             *                    *
C.fin     GAAGCTGGCA AACTATTAAT ACATATTCCT ATTATTTATT AGTTTATTTT

                                                                   270
                  *     *                          *         *
N.minor   TTGGGGAAAA AATTAATAAA TATATTAATA ATCATTTATA AAAACTTATC
                   *                    *         *
C.fin     TTGGGGTAAA ATTTAATAAT ACTATTAACA CAATTGTACT AAATTACATC

                                                                   320
             * *       *  *
N.minor   CTTCTAGGAA TGTTGAAAAA GCTCCTCTAG GGATAACA-G CATAATATTT
                           *                      *
C.fin     CTT-TAGGAA TTATGAAGAA GCTCCTCTAG GGATAACATG CATTATGCTT

                                                                   350
                   *
N.minor   ATTAGAGTTC TTATCAGAAT AAATGTTTGT
                              **
C.fin     AAAAGAGTTC TTATCAGAAT AAGCGTTTGT
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Figure 1. DNA sequence for 350 bp region of the mitochondrial 16S rRNA for *C. finmarchicus* (data from Bucklin and Kocher 1996) and *N. minor* (data from Bucklin et al. 1996a). The most abundant haplotype is shown for each species; sequences for the two species differ by 25% of the bases. There is no evidence of covariance in the substitution sites between the two species. Intraspecifically variable sites are indicated by an asterisk (*) above the base; hyphens (-) indicate alignment gaps between the sequences.

used to estimate the distribution of time to coalescence (G; time to common ancestry in the female lineages within a species). The relative probability of coalescence [$f(G)$] was determined for the range of observed G values using the equation from Avise et al. (1988, after Tajima 1983) and Nei (1987):

$$f(G) = (1/N_{f(e)})(1 - 1/N_{f(e)})^{G-1} \quad (5)$$

and $N_{f(e)}$ was estimated by curve-fitting the observed distribution of G to expected distributions based on a range of $N_{f(e)}$ values.

Results

The 16S rRNA sequence differed by 25% between *C. finmarchicus* and *N. minor.* This level of difference is typical of that between noncongeneric species of the superfamily Calanidae (Bucklin et al. 1998). Variable sites (i.e., sites that varied *within* each species) were distributed throughout the 350 bp sequence (Figure 1); the two species did not show the same pattern of variable sites based on analysis of covariance (covariance coefficient, $r = -0.006$, by analysis of covariance; $P < .46$ by rank sum test; Sokal and Rohlf 1981).

Table 1. Patterns of molecular genetic diversity of *C. finmarchicus* and *N. minor* based on a 350 bp region of mitochondrial 16S rRNA

Species	N	k	h (SD)	π (SD)
Calanus	216	31	0.368 (0.043)	0.00370 (0.0026)
Nannocalanus	158	51	0.824 (0.024)	0.00502 (0.0032)

Numbers of haplotypes (k) were determined from alignments of sequences from the individuals assayed (N). Haplotype diversity (h), nucleotide diversity (π), and their standard deviations (SD) were calculated according to Nei (1987).

Sequence variation among 216 individuals of *C. finmarchicus* resolved 31 haplotypes for 16S rRNA, while there were 51 haplotypes among 158 individuals of *N. minor* (Table 1). The shape of the haplotype frequency distributions of the two species differed, but both distributions were highly skewed, with a few haplotypes at high frequency and numerous haplotypes at low frequency (Figure 2). Haplotype diversity was 0.368 (SD = 0.043) for *C. finmarchicus* and 0.824 (SD = 0.024) for *N. minor* (Table 1).

A further characteristic of the molecular variation in the two copepods was the low level of divergence among most of the haplotypes. For *C. finmarchicus,* nearly 80% of the individuals shared the same haplotype and many haplotypes differed by only one base (Bucklin and Kocher 1996). Although the frequency distribution of pairwise haplotype distances for *N. minor* appeared more normal (Figure 3), 35% of the individuals shared the same haplotype; most individuals differed by two or three bases among the

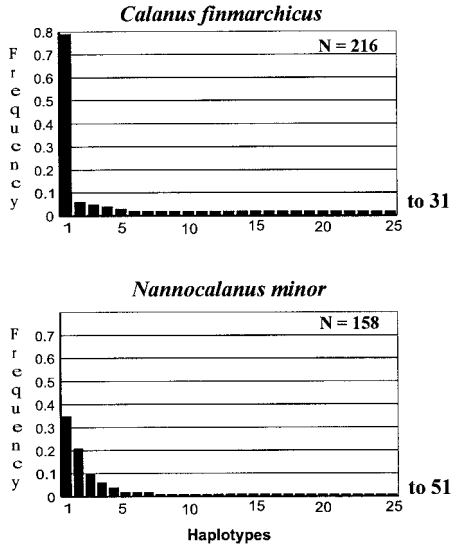

Figure 2. Frequency distributions of the first 25 haplotypes of *C. finmarchicus* and *N. minor* based on the 350 bp region of 16S rRNA shown in Figure 1. All additional haplotypes, to a total of 31 haplotypes for *C. finmarchicus* and 51 haplotypes for *N. minor,* are unique.

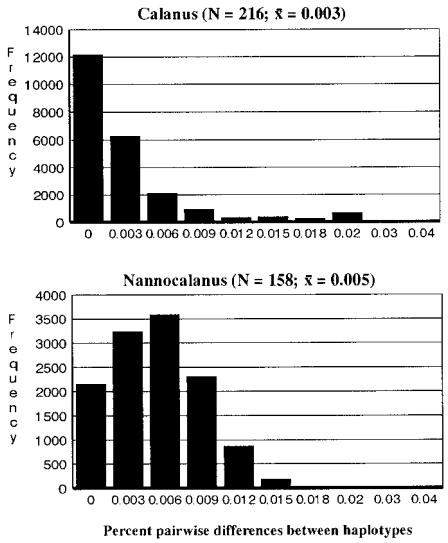

Figure 3. Frequency distributions for pairwise haplotype distances for 16S rRNA for *C. finmarchicus* and *N. minor.* The means (π), variances, and shapes of the distributions of pairwise distances (p_{ij}) differ between the two species. The shapes of the distributions, in particular, may reflect the evolutionary histories of the species.

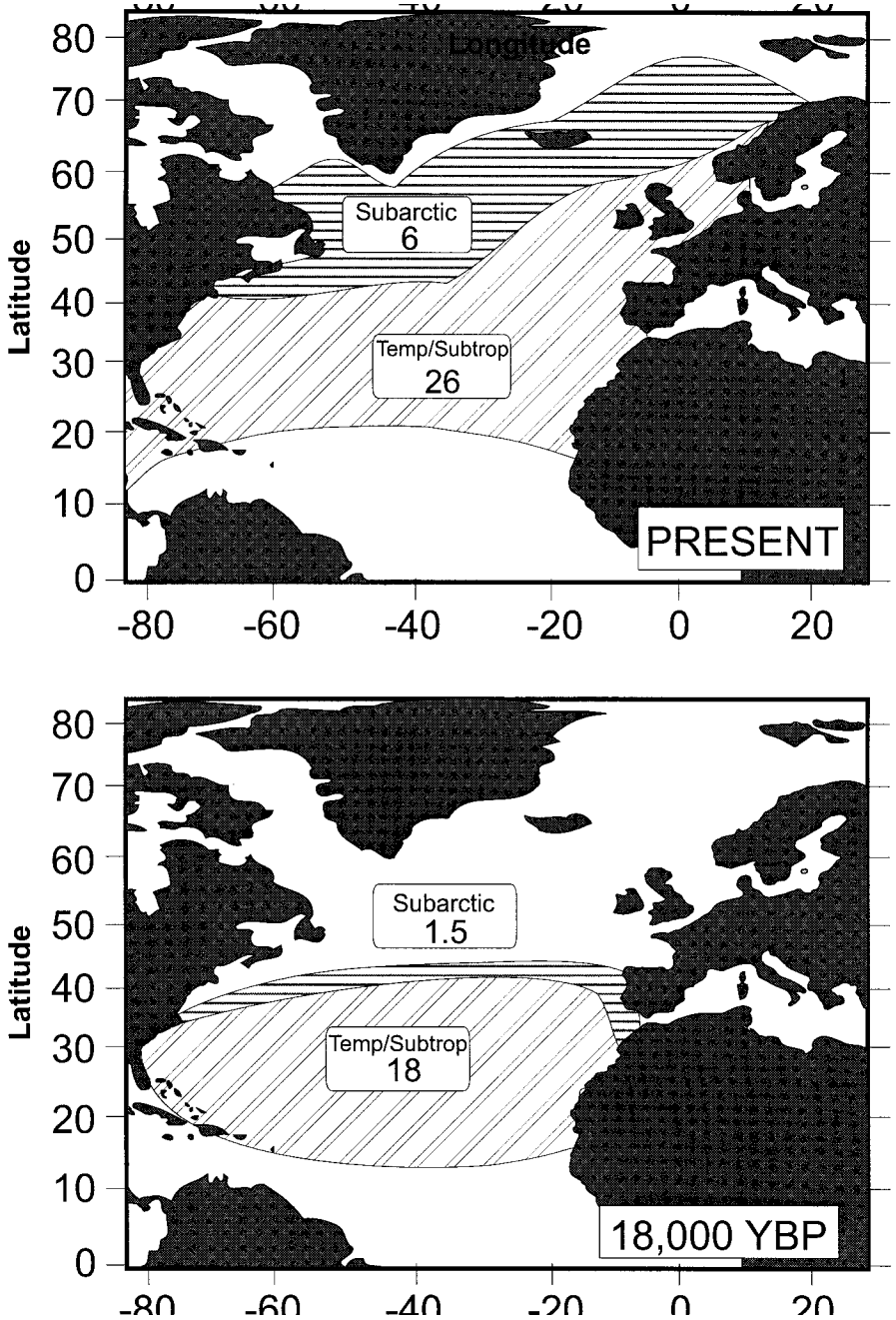

Figure 4. Distributions of subarctic and temperate/subtropical (Temp./Subtrop.) assemblages of coccolithophores at present and 18,000 years BP. Estimations of areal dimensions of the ranges are given as 10^6 km^2 for each distribution. (Figure modified from McIntyre et al. 1976.)

350 bp sequenced. Nucleotide diversity was within the range typically observed: π = 0.00370 (SD = 0.0026; range 0–0.046) for *C. finmarchicus* and 0.00502 (SD = 0.0032; range 0–0.017) for *N. minor* (Table 1; Figure 3). The distributions of the pairwise haplotype differences for *C. finmarchicus* and *N. minor* were statistically compared: they differ in variance (by *F* test, $P < .0001$) and in shape (by Komogorov–Smirnov test, $P < .0001$; Sokal and Rohlf 1981). The *C. finmarchicus* distribution was more skewed (skewness parameter, $g_1 = 2.64$) than that of *N. minor* ($g_1 = 0.36$; Sokal and Rohlf 1981).

Observed densities of female *C. finmarchicus* (integrated over the depth of the water column under 1 m^2 of sea surface) depended upon the region sampled: 2,000/m^2 (average over 10 months and 75 collections on Georges Bank; Casas et al. 1995); 3,000/m^2 (average over nearly 10 years and 6,000 collections over the northwest Atlantic continental shelf and slope; Meise and O'Reilly 1996; Meise CJ, personal communication); 2,000/m^2 (average used to model Norwegian and Barents Sea populations by Tande 1991); 500/m^2 (average of 15 collections in northwest Atlantic Slope Water; Miller et al. 1991). The overall present female census population size (N_f) for *C. finmarchicus* was estimated to be 6×10^{15}, using an average density of 1,000/m^2 and an estimated areal distribution of 6×10^6 km^2 (Figure 4). The observed densities of female *N. minor* ranged from 500/m^2 (average of 14 tows in the Gulf Stream system; Ashjian and Wishner 1993) to 100/m^2 (average of 11 collections in Gulf Stream warm-core rings; Copley et al. 1989). Using an overall average female density of 100/m^2 and an estimated areal distribution of 2.6×10^7 km^2 (Figure 4), the estimated female census population size (N_f) of *N. minor* was 2.6×10^{15} (Table 2).

Estimated effective population sizes (N_e), including both males and females, of *C. finmarchicus* and *N. minor* were much smaller than the calculations based on observed distribution and abundance. $N_e = 2 \times 10^8$ for *C. finmarchicus* and 4×10^8 for *N. minor* (Figure 5), based on Ewens (1972). In contrast, using the approach of Avise et al. (1988), the estimated effective female population sizes ($N_{f(e)}$) were even smaller: $N_{f(e)} = 1.4 \times 10^5$ for *C. finmarchicus* and 2.5×10^5 for *N. minor* (Figure 6).

Evidence of changes in planktonic species' distributions as a result of climatic

Table 2. Estimated female census and effective population sizes at present and 18,000 years BP for *C. finmarchicus* and *N. minor*

Species	Female density	Area present	Area past	N_f present	N_e	$N_{f(e)}$
C. finmarchicus	$10^3/m^2$	6.0×10^6 km^2	1.5×10^6 km^2	6×10^{15}	2×10^8	1.4×10^5
N. minor	$10^2/m^2$	2.6×10^7 km^2	1.8×10^7 km^2	2.6×10^{15}	4×10^8	2.5×10^5

Estimated census female population size (N_f) was calculated using female density [i.e., numbers under 1 m^2 of ocean surface to the depth range of the species (based on citations given in the text)] and areal extent of subarctic and temperate/subtropical planktonic assemblages today (area present) and 18,000 years BP (area past) from McIntyre et al. (1976). Effective population size (N_e) was estimated from numbers of haplotypes based on Ewens (1972). Effective female population size ($N_{f(e)}$) was estimated from nucleotide diversity based on coalescence theory after Avise et al. (1988).

variation in the North Atlantic was evaluated using published maps of coccolithophorid assemblage distributions (McIntyre et al. 1976). For the subarctic assemblage, there was a 75% decrease in areal extent during glaciation, while the temperate/subtropical (including both the transition and subtropical zones) assemblage was estimated to have experienced a 30% decrease in areal extent (Table 2; Figure 4). The subarctic assemblage was displaced almost completely from its present range, while the latitudinal range of the central assemblage was truncated on the northern edge.

Discussion

Patterns and Causes of Molecular Diversity in *C. finmarchicus* and *N. minor*

Both *C. finmarchicus* and *N. minor* showed intraspecific variation in the DNA sequences of the same 350 bp region of mitochondrial 16S rRNA in terms of both haplotype diversity (h) and nucleotide diversity (π; Table 2). The skewed haplotype frequency distributions observed for both *C. finmarchicus* and *N. minor* are typical of many other marine species, both vertebrate and invertebrate, and have been observed for fish (Camper et al. 1993; Zwanenburg et al. 1992) and a sea urchin (Palumbi and Kessing 1991), among others. Explanations for the occurrence of a large number of low-frequency haplotypes may lie in the enormous population sizes of marine organisms, including *C. finmarchicus* (one of the largest census populations in the animal kingdom), which may cause retention of numerous haplotypes and result in undersampling of the populations.

Levels of nucleotide diversity were low to moderate for both *C. finmarchicus* (π = 0.00370, SD = 0.0026) and *N. minor* (π = 0.00502, SD = 0.0032; Table 1); these values are within the range, 0.0005 to 0.020, typically observed for a wide variety of organisms (Stephan and Langley 1992). The original studies on *C. finmarchicus* reported values of π = 0.004 for the same 350 bp region (Bucklin and Kocher 1996) and π = 0.006 (Bucklin et al. 1996b). The difference in mean values resulted from the sampling of different numbers of individuals collected from different geographic regions during different years. Bucklin and Kocher (1996) considered *C. finmarchicus* from the northwest Atlantic collected during 1992 and 1993, while Bucklin et al. (1996b) considered collections from the NW Atlantic and the Norwegian Sea in 1992 only. This study represents the most complete assessment of the species' molecular diversity. The original study of *N. minor* by Bucklin et al. (1996a) yielded π values of 0.0045 and considered the same individuals as this study, but the analysis was based on a 450 bp region of 16S rRNA. In this study the region analyzed was reduced to be homologous with *C. finmarchicus*.

Although the mean value of π varied in previous studies, the shape of the pairwise difference distribution did not change with the individuals sequenced or the length of the sequences: for *C. finmarchicus,* the large majority of individuals shared a single haplotype and generated an L- shaped pairwise difference distribution; for *N. minor,* the distribution was a curve with equal mean and mode. An important result of this comparison is that, although the mean value of π for a species depends on the degree to which the entire species' population is sampled, the shape of the distribution may not be so dependent. For this analysis we used the mean value representing all individuals sequenced to date, and will rely primarily on the shape of the distribution of pairwise differences for this discussion of differences between the two species.

Patterns of population genetic diversity are caused by ecological and evolutionary processes acting on a species over many time and space scales. The well-spring of genetic diversity is mutation; mutation rates are treated as a constant for most population genetic studies, which typically assume values ranging from 10^{-6} to 10^{-8} substitutions per generation (Avise 1994; Brown et al. 1979). Gene flow (migration) can rearrange the distribution of genetic diversity across a species, but cannot—in the absence of recombination—create new genetic types. Random genetic changes

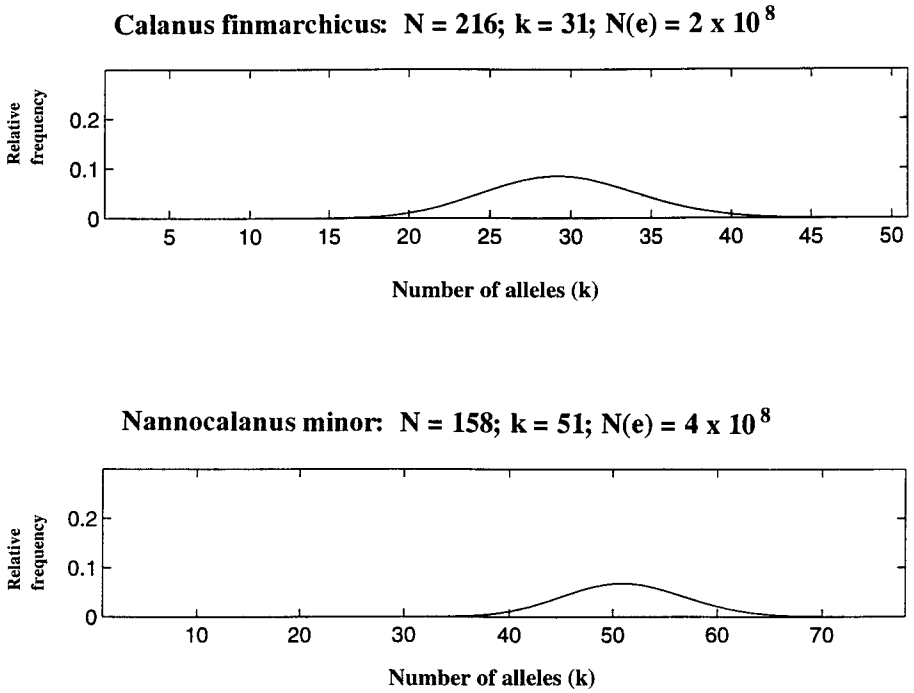

Figure 5. Estimation of effective population sizes (N_e) for *C. finmarchicus* and *N. minor* based on the approach of Ewens (1972) using numbers of alleles or haplotypes (k), numbers of individuals assayed (N), and an assumed mutation rate (u) of 10^{-8} substitutions per generation.

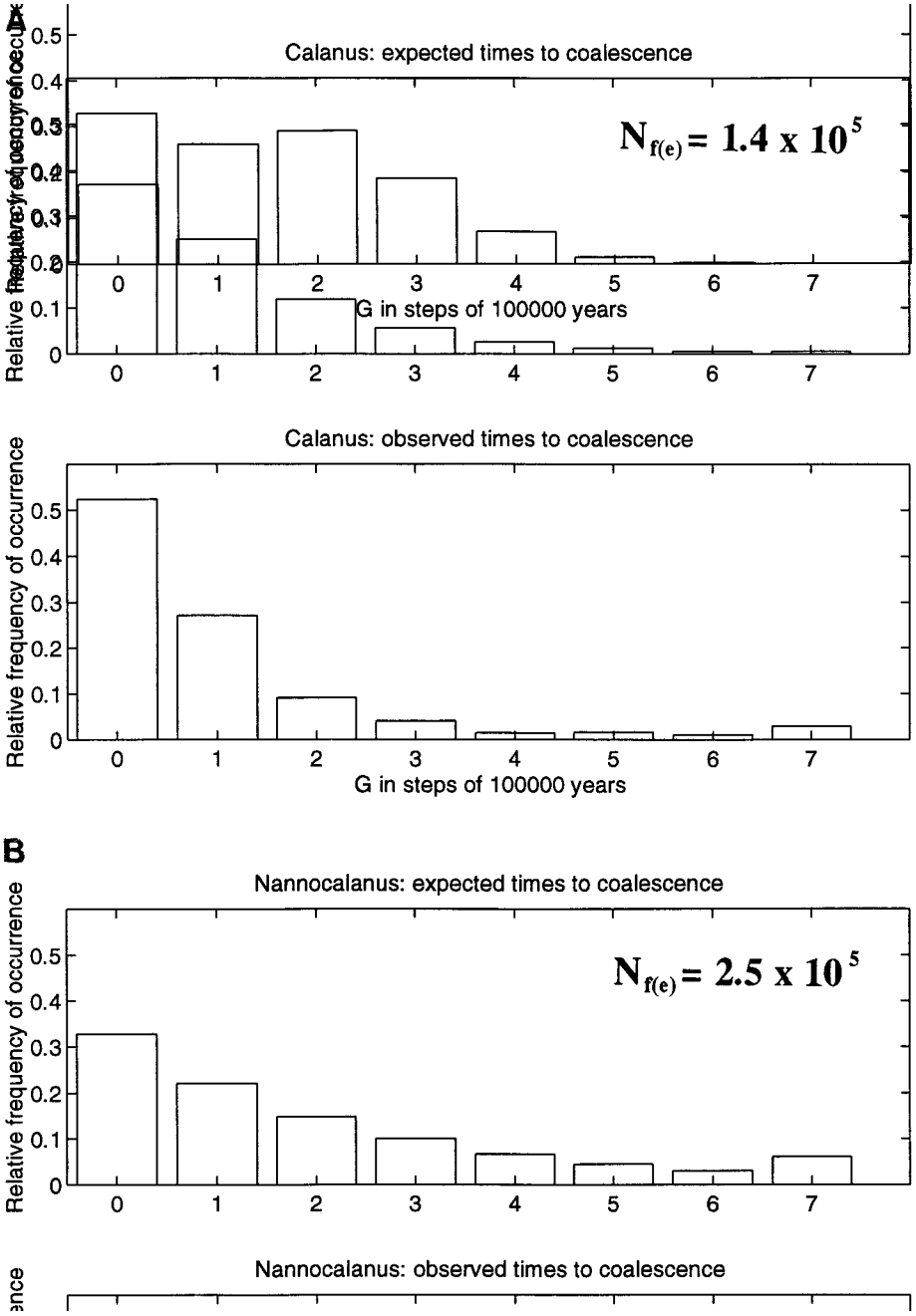

Figure 6. Expected (top) and observed (bottom) relative frequency distributions of time to coalescence (*G*, time to common ancestry of the gene variants present in the species) for *C. finmarchicus* **(A)** and *N. minor* **(B)**. The observed distributions were calculated from the distribution of pairwise haplotype distances (shown in Figure 3) using neutral theory expectations and an assumed mutation rate (u) of 10^{-8} substitutions per generation. The expected distributions were calculated according to the approach of Avise et al. (1988). The estimated effective female population size [$N_{f(e)}$] was determined by curve-fitting the observed distribution to expected distributions based on a range of values of $N_{f(e)}$. *G* is given in units of 10^5 years.

(genetic drift) may result from sampling error with stochastic variation in reproductive success (Hedgecock 1994). Natural selection can act to increase or decrease genetic diversity, depending upon the environmental constraints. How these evolutionary forces of mutation, migration, drift, and natural selection interact to determine the population genetic makeup of a species depends on many factors, including the species' response to environmental variation, and the past and present sizes of the population. To explain the observed differences in molecular population genetic character, we must examine both the ecological and the evolutionary dynamics of the species. Relevant ecological processes include life history, population dynamics, and environmental interactions. The geological and paleontological history of the species are the basis of the evolutionary biology, which is usually inferred from the fossil records of analogous species and from climatic indicator species.

Importantly, inferences about species' evolution based on molecular diversity share the assumption of mutation-drift equilibrium conditions (Nei 1987). Lack of equilibrium in *C. finmarchicus* and *N. minor* is evident from the observed haplotype frequency distributions and suggested by the lower-than-expected number of haplotypes and pairwise difference distributions. Many terrestrial and aquatic species and populations studied to date show evidence of lack of equilibrium conditions. Although lack of equilibrium will contribute to the consistent differences between observed patterns of molecular diversity and theoretical expectations, this comparison is nevertheless useful for understanding species' evolution, particularly for comparisons among species based on similar analyses.

Estimation of Census and Effective Female Population Sizes

Estimations of the current census female population sizes (N_f) of *C. finmarchicus* and *N. minor* were made based on observed densities, depth distributions, and geographic ranges of the species (Ashjian and Wishner 1993; Casas et al. 1995; Copley et al. 1989; Meise and O'Reilly 1996; Miller et al. 1991; Tande 1991). The difficulty of accurately estimating species' concentrations across ocean basins necessitates caution in the interpretation of our female census population sizes. Species' densities vary widely across the geographic range, and observations are insufficient

or lacking for both *C. finmarchicus* and *N. minor* for some portions of the range. Our calculations are conservative and may underestimate actual census populations by orders of magnitude. However, our values are among the only such estimates we know of and are useful for comparative purposes. We have not attempted to provide an estimate of variance or error on these estimates.

The areal distribution for each species was calculated from published distributions of subarctic and temperate/subtropical assemblages of planktonic coccolithophorids (McIntyre et al. 1976). Descriptions of current biogeographic provinces of the North Atlantic are based on persistent physical structure and current patterns in the ocean, which determine to a large extent the distributions of all zooplankton groups (van der Spoel and Heyman 1983). Although we cannot be sure that historical copepod distributions were identical to coccolithophorid distributions, this comparison represents our best estimate of distributions of the biogeographic provinces 10,000 to 20,000 years BP.

The estimates of current census population sizes indicate that there are approximately two times as many females of *C. finmarchicus* ($N_f = 6 \times 10^{15}$) compared to *N. minor* ($N_f = 2.6 \times 10^{15}$) at any given time (Table 2). In order to compare census female population size (N_f) with effective population size (N_e) and effective female population size ($N_{f(e)}$)—two important characteristics for the ecology and evolution of species—we used population genetic predictions based on neutral theory. Since these predictions are based on an assumption of mutation-drift equilibrium, which is unlikely for these two species, our discussion is limited to consideration of differences of many orders of magnitude. There are several methods for the estimation of effective population size based on a variety of molecular population genetic parameters; we used two methods based on numbers of haplotypes and nucleotide diversity (π).

Based on the observed numbers of haplotypes, the effective population size (N_e), including both males and females, was 2×10^8 for *C. finmarchicus* and 4×10^8 for *N. minor* (Ewens 1972; Table 2, Figure 5), about seven orders of magnitude below our estimates of census population size. Thus there were far fewer haplotypes observed in these populations than expected for species of their abundance. Estimates of effective female population size based on nucleotide diversity, using coalescence theory and distributions of pairwise distances between haplotypes (see Avise et al. 1988, for a description of methods), also departed markedly from estimated census population sizes. The effective female population size ($N_{f(e)}$) for *C. finmarchicus* was 1.4×10^5 and 2.5×10^5 for *N. minor* (Figure 6). Thus mitochondrial 16S rRNA haplotypes are much less numerous and less divergent than predicted by neutral theory for species with large population sizes.

Pairwise Difference Distributions

The two species, *C. finmarchicus* and *N. minor,* differ in the distribution of pairwise differences between haplotypes (Figure 3). The distribution for *C. finmarchicus* is L-shaped, with most individuals (80%) having the same haplotype. In contrast, the majority of *N. minor* haplotypes differ by two or three substitutions among 350 bp. The mean pairwise differences are 0.00370 (SD = 0.0026) for *C. finmarchicus* and 0.00502 (SD = 0.0032) for *N. minor* (Table 1). The pairwise distance distributions of the two species differ in variance (by an F test; $P < .0001$) and shape (by the nonparametric Komogorov–Smirnov test; $P < .0001$; Sokal and Rohlf 1981; Figure 3). Interestingly, the most divergent haplotypes for *N. minor* differed by only 6 bases among the 350 bp sequenced, while for *C. finmarchicus,* the most divergent haplotype differed by 12 bases. The reason for this difference can only be speculated about: perhaps the highly divergent haplotypes are relicts of older populations (see discussion below).

Rogers and Harpending (1992) find evidence of species' evolutionary histories, including recent genetic bottleneck events and rapid population expansion, in the shape of the pairwise difference distributions. Based on theory and computer simulations, Rogers and Harpending (1992) hypothesize that L-shaped distributions may result from a recent genetic bottleneck; rapid population growth generates a "wave" in the distribution that propagates to the right over time. Equilibrium conditions also result in an L-shaped curve. If we evaluate our observed distributions for the two species in this light, we may infer that *C. finmarchicus,* which exhibits an L-shaped distribution of pairwise differences, may have experienced a recent genetic bottleneck. Preliminary evaluation of the pairwise difference distribution for *C. finmarchicus* using the Arlequin (version 1.1) software for population genetic data analysis (see Excoffier: http://anthropologie.unige.ch/arlequin), which calculates several parameters from the Rogers and Harpending (1992) and Rogers (1995) analyses, confirmed that the pairwise haplotype difference distribution does not reflect equilibrium conditions, and provides support for our hypothesis that *C. finmarchicus* has experienced a genetic bottleneck in the recent past. In contrast, the unimodal distribution for *N. minor* (Figure 3) suggests that, although the species' population is not in equilibrium—perhaps as a result of rapid population expansion in the more distant past—the displacement of the mode toward larger pairwise haplotype differences suggests an older event.

Evolutionary Explanations for Observed Patterns

Why do both *C. finmarchicus* and *N. minor* have fewer 16S rRNA haplotypes than theoretically predicted? Why do haplotype frequency distributions and pairwise difference distributions differ between these two species? Comparison between species using identical analyses may reveal much about their evolutionary history and ecological functioning. There are a number of phenomena that may influence population genetic diversity; several of the most significant are considered here.

Origin of species. Coalescence theory indicates that all variants of a gene originated from a single copy of that gene at some point in the evolutionary history of the lineage, assuming no convergence (see Avise 1994). The more numerous and more highly diverged the variants, the further in the past is the progenitor gene. In concept, differences between *C. finmarchicus* and *N. minor* in the numbers of haplotypes and distributions of pairwise haplotype differences may be explained by the different ages of the lineages (Figure 7). However, comparisons between each species and its most closely related species (in both cases, a sibling species) indicated that speciation in both *Calanus* and *Nannocalanus* was accompanied by similar levels of divergence in the mitochondrial 16S rRNA gene: *C. finmarchicus* differed from its nearest sibling species, *C. marshallae* (Frost 1974), by 12–13% of a 450 bp region of 16S rRNA (Bucklin et al. 1995), while *N. minor* differed from two undescribed sibling species by 10–12% of the identical gene region (Bucklin et al. 1996a). Using the analysis of 16S rRNA divergence in hermit crabs versus evidence of speciation in the fossil record by Cunningham et

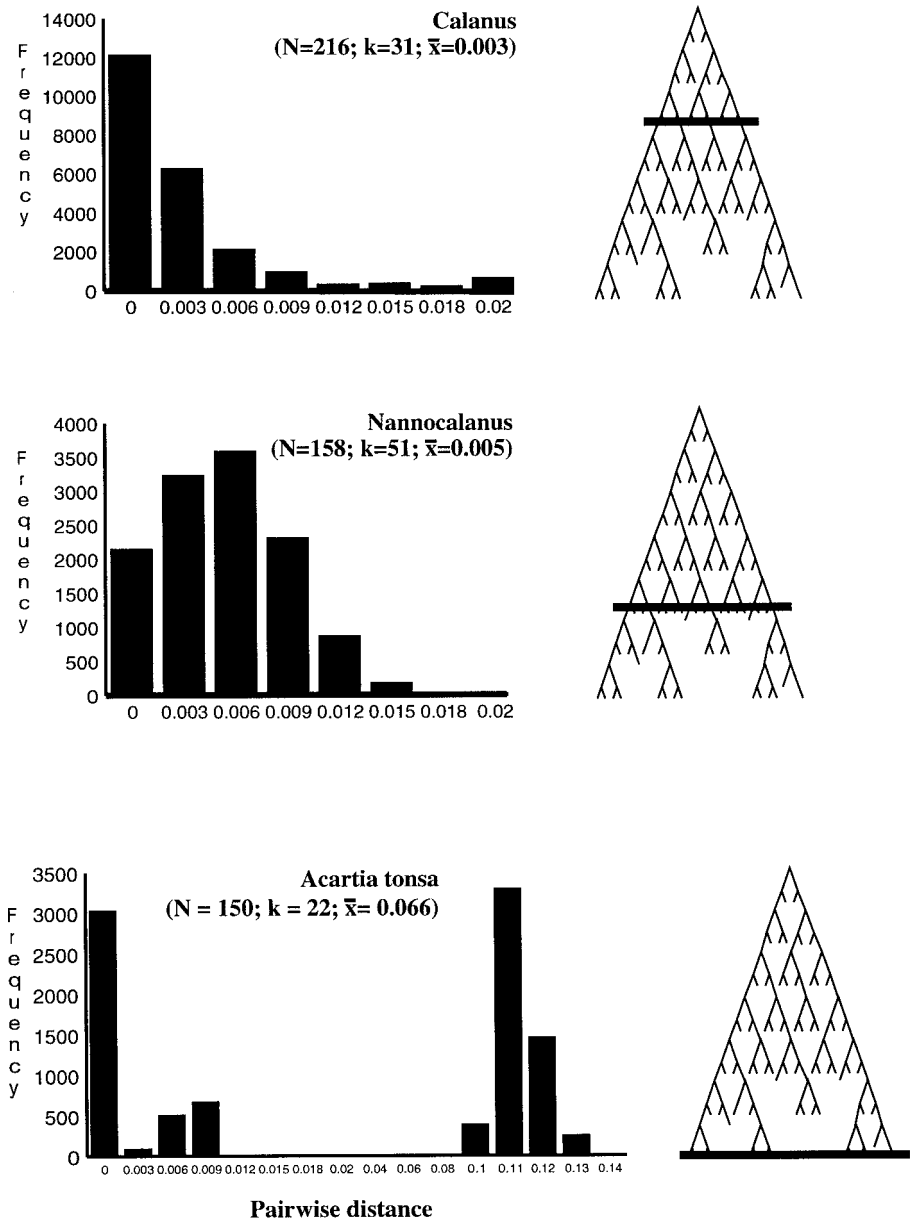

Figure 7. The evolution of molecular diversity interpreted in light of the distribution of pairwise differences between haplotypes (shown at left) and diagrammatic gene trees (at right) showing the creation and divergence of haplotypes by accumulation of base substitutions. The frequency distribution of haplotype differences of *C. finmarchicus* suggests a shorter evolutionary process, and shorter time to coalescence. The current population genetic composition is shown in evolutionary perspective by the position of the bar across the gene tree diagram. The presence of a number of highly divergent haplotypes suggests that there may be "relict" haplotypes retained in the species population. The frequency distribution of haplotype differences for *N. minor* has a different shape, with fewer individuals sharing the same haplotype, more haplotypes in low frequency, and a concentration of haplotypes differing by 2 or 3 bp. The longer time to coalescence is indicated by the lower position of the bar across the gene tree. The frequency distribution for *A. tonsa* [included here for comparison and based on data from Caudill (1995)] shows evidence of multiple haplotypes in each of several distinct lineages, with loss of intermediate lineages. (Note: Figure for *A. tonsa* is based on a 220 bp sequence of 16S rRNA; scale differs for pairwise haplotype differences.) Letters indicate number of individuals sequenced (*N*), number of haplotypes (*k*), mean value of pairwise differences between haplotypes (*x*).

al. (1992)—and assuming some degree of rate constancy in the evolution of the mitochondrial 16S rRNA within the copepod superfamily, Calanidae—both *C. finmarchicus* and *N. minor* are likely to have diverged from their nearest relative more than 10 million years ago (Bucklin et al. 1992). It is thus unlikely that patterns and levels of molecular population genetic diversity result from the recent origin of the species.

Natural selection. Haplotypes are created by mutations (i.e., nucleotide base substitutions); haplotype divergence is the result of accumulation of mutations over time. Natural selection may remove some mutations, and thus some molecular diversity, from the gene pool. A possible explanation for the smaller-than-predicted numbers and divergences of mtDNA haplotypes in planktonic copepod populations, or any other taxon, may be the constraints of natural selection. Natural selection may act directly on individuals with deleterious forms of a gene or linked groups of genes. In the case of mtDNA, natural selection may act upon the entire molecule (Brown et al. 1979). Natural selection acting on traits encoded in the nuclear genome may also constrain mtDNA variation (Kilpatrick and Rand 1995). It is possible that the low molecular diversity of mtDNA may result not from small effective population sizes, but from natural selection preventing the accumulation of numerous genetic types in the species, no matter how large the breeding population.

The forces of natural selection may be distinguished from other evolutionary forces, as a first cut, by the directed nature of the changes (Endler 1986). In contrast, genetic drift, gene flow, and mutation are random forces that occur without respect to the environmental conditions of the organisms. At the molecular level, variation in a DNA sequence constrained by natural selection may be expected to exhibit similar patterns regardless of species or geography. Thus we may look for correlation with environmental conditions and covariance in molecular characters for evidence of natural selection.

Evidence of selective constraints on evolutionary changes of the 16S rRNA molecule is difficult to obtain. A simple test is to evaluate patterns of base substitutions along the aligned sequence to determine whether different species show covariance in the sites of variation. This analysis for the sequences for *C. finmarchicus* and *N. minor* indicated that the patterns of substitutions were different, with no significant covariance in variable sites (Figure 1). This simple test does not prove that the 16S rRNA molecule is not selectively constrained, but does suggest that these two species show different patterns of variation in base substitutions of this 16S rRNA region.

Small or historically variable effective population size. Lack of deep divergence among haplotypes may be explained by small effective population size resulting from historical variation in population size (Avise et al. 1988) and/or variance in reproductive success (Hedgecock 1994). Avise et al. (1988) hypothesized that the small molecular distances among mtDNA

lineages in some fishes resulted from variation in reproductive success.

Even for "old" species resulting from taxonomic divergences far back in time—as we hypothesize *C. finmarchicus and N. minor* to be—observed population genetic diversity may be low if the species have experienced a genetic bottleneck (i.e., a precipitous decline in population size and/or a strong selective event) in its recent evolutionary past. Repeated episodes of genetic bottlenecks will significantly decrease the temporally averaged effective population size of the species (see Avise et al. 1988).

Range compression. The paleontological history of the North Atlantic basin suggests that there may have been a significant shift in geographic range and environmental conditions associated with the glacial maximum approximately 20,000 years BP. Depositional records of planktonic coccolithophorids have recorded these biogeographic transformations (McIntyre et al. 1976). Range compression of subarctic species, with distributions similar to that of *C. finmarchicus,* was notable in that it not only decreased the areal distribution 75%, but also displaced the entire species' population from its current geographic range (Table 2, Figure 4). Range compression for the temperate/subtropical assemblage, similar to *N. minor,* was only about 30%, and was even less profound because the latitudinal range was not changed as much as for the subarctic assemblage (Table 2, Figure 4). This degree of population decrease alone would not be expected to cause significant alteration in the population genetic composition of the species, especially given the enormous population sizes typical of both species. However, population decrease in association with changes in the selective regime (due to changed environmental conditions and geographic ranges) may have caused a type of bottleneck and resulted in loss of genetic diversity.

Support for this hypothesis may be found in a recent study by Planque (1996) showing significant correlation between population densities of *C. finmarchicus* and climatic variation in the form of the North Atlantic Oscillation (NAO). Based on collections by the Continuous Plankton Recorder survey, *C. finmarchicus* densities showed marked interannual variation, with fivefold changes in abundance, in concert with the NAO index (Planque 1996). Year-to-year variation in population abundances of this magnitude may also be sufficient to generate some of the conditions that lower effective population size, including variance in reproductive success and historical variation in the breeding population size.

Comparison with Acartia tonsa. It is useful to compare the observed patterns of population genetic diversity with that of other marine species with different ecological and evolutionary histories. A study by Caudill (1995) examined DNA sequence variation of the obligately estuarine, planktonic calanoid copepod, *A. tonsa,* for a 220 bp region of the mitochondrial 16S rRNA within the 350 bp region sequenced for *C. finmarchicus* and *N. minor.* In marked contrast to the latter two species, *A. tonsa* showed deep divergences between 16S rRNA haplotypes, with pairwise haplotype differences of 15–18% (Caudill 1995) versus 1–3% between 16S rRNA haplotypes of *N. minor* (Bucklin et al. 1996a) and *C. finmarchicus* (Bucklin et al. 1996b). The estimated effective female population size ($N_{f(e)}$) for *A. tonsa* [based on coalescence theory and using the approach of Avise et al. (1988)] would be many orders of magnitude larger than for either *C. finmarchicus* or *N. minor,* because of these deep divergences and long times to coalescence. This is in direct contrast to observed census populations, which are many orders of magnitude larger for the two oceanic species than for the obligately estuarine one.

An explanation for these differences may be that *A. tonsa* differs from *C. finmarchicus* and *N. minor* in both habitat (coastal estuaries versus the open ocean) and life history (long-lived diapause eggs may accumulate in estuarine sediments and form an "egg bank"; Marcus et al. 1994). Because of its estuarine distribution, *A. tonsa* may have experienced more significant range compression during glaciation over the past 20,000 years than the other two species: McAlice (1981) hypothesized that the latitudinal range of *A. tonsa* decreased markedly due to ice cover of the continental shelves of what is now the northern United States. Despite these changes in geographic distribution, the species' egg bank may have buffered historical variation in effective population size (for discussion see Caudill 1995; Hairston and DeStasio 1988). Sequestration of genetic diversity in diapause eggs may have allowed retention of multiple old mitochondrial lineages in different, geographically isolated estuaries at the southern end of the range. Populations of each of these lineages would then expand as the glaciers retreated, generating the patterns observed today (Figure 7; Caudill 1995).

Conclusions

The primary result of this study is that both *C. finmarchicus* and *N. minor* exhibit levels of molecular diversity that would be expected for much, much less abundant species. Estimated effective populations sizes are 10^7 to 10^{10} lower than estimated census populations for both species. Based on mitochondrial 16S rRNA sequence variation, both *C. finmarchicus* and *N. minor* have fewer haplotypes, smaller divergences between haplotypes, and lower nucleotide diversity than would be predicted based on observed census population sizes. If these parameters are used to predict effective population sizes, the resulting estimates range from 1.4×10^5 to 2×10^8 for *C. finmarchicus* and from 2.5×10^5 to 4×10^8 for *N. minor.* These low estimates may result from a variety of ecological and evolutionary phenomena, including genetic drift, historical variation in effective population size, variance in reproductive success, and/or constraining natural selection. Because differences between theoretical predictions and observations may also result from error and inaccuracy in the theoretical assumptions (including assumptions of mutation-drift equilibrium) and inferences, it is most informative to compare related species using homologous molecular characters and similar statistical analyses.

A secondary result of this study is that levels of molecular diversity (especially the distribution of pairwise differences between haplotypes) are significantly lower for *C. finmarchicus* than for *N. minor.* We hypothesize that the primary reason for the difference in the molecular diversity between the two species is their evolutionary histories, and especially the impact of glaciation during the past 20,000 years. The subarctic species, *C. finmarchicus,* may have experienced a 75% range compression during glacial periods (McIntyre et al. 1976) and may have been entirely displaced from its geographic range, while the temperate/subtropical *N. minor* may have lost only 30% of its range through truncation of the northern limits of the species. The dual impact of range compression and geographic displacement for *C. finmarchicus* may have produced a genetic bottleneck, resulting in a loss of genetic diversity that is reflected in the present population genetic composition of the species. The possibility of sig-

nificant reduction of molecular diversity during genetic bottleneck, even for very abundant species, serves as a warning for conservation biologists that large population does not necessarily confer a high level of genetic diversity.

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