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HISTORICAL POPULATION SIZE CHANGE OF BOWHEAD WHALES INFERRED FROM DNA SEQUENCE POLYMORPHISM DATA

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Abstract.—Nucleotide sequence data from the mitochondrial control region were used from a phylogenetic context to investigate the long-term history of a population of bowhead whales (*Balaena mysticetus*). In addition, the coalescence time of these sequences was used to estimate the age of the inferred patterns of population size change. The results indicate that mitochondrial genetic polymorphism was not affected by a recent bottleneck that occurred near the turn of the 20th century, thereby preserving the signature of historical population size change in the mitochondrial genome. Further analysis showed that this population underwent an expansion initiated in the Middle to Late Pleistocene. As such, early Holocene changes in Arctic sea ice distribution appear to have had little influence on patterns of genetic variability in this population.

Key words.—Bottleneck, bowhead whale, coalescence time, substitution rate.

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Knowledge of recent and long-term patterns of change in population size is useful for understanding the evolutionary history of a population, and genetic data provide a means of examining historical fluctuations of total population size in response to processes that alter effective population size (Felsenstein 1992; Harpending et al. 1993; Nee et al. 1995). The bowhead whale (*Balaena mysticetus*) provides an interesting case study for examining patterns of long-term change in population size evaluated with genetic polymorphism data. Five currently recognized stocks, or populations, of bowhead whales live in the high latitudes of the Northern Hemisphere and include the Bering-Chukchi-Beaufort Seas (BCB), Okhotsk Sea, Davis Strait, Hudson Bay, and Spitsbergen stocks (Moore and Reeves 1993). With the exception of the Okhotsk Sea population, all others can be classified as high Arctic populations. Although they are geographically disjunct today (Moore and Reeves 1993), the current distributions of these populations have not remained the same, as the pattern of sea ice distribution in the Arctic has changed dramatically over the last 10,500 years (Dyke et al. 1996). In fact, the distribution of bowhead whale fossil remains suggests that the high Arctic populations might have been connected prior to the establishment of the M'Clintock Channel sea-ice plug, around 8500 years ago (Dyke et al. 1996).

All bowhead whale populations have been subjected to commercial whaling over the last few centuries (Woodby and Botkin 1993). The BCB population was the target of unregulated commercial whaling from 1848 to around 1914 (Sonntag and Broadhead 1989; Woodby and Botkin 1993) and as a consequence lost approximately 93% of its total population size (Woodby and Botkin 1993). Nonetheless, a relatively large number of individuals (at least 1000) survived the bottleneck (Woodby and Botkin 1993), suggesting that a large effective size may have been sustained after commercial whaling ceased and the bottleneck ended. The findings of a recent study of microsatellite polymorphism in the BCB population are consistent with this idea (Rooney et al. 1999). However, because the BCB population of bowhead whales is subject to an internationally sanctioned subsistence hunt under the auspices of the International Whaling Commission, there is interest in determining the current population size in comparison to the size prior to the onset of commercial whaling in 1848. Uncovering this relationship is important to gauge the extent of population size recovery subsequent to the cessation of commercial harvesting of BCB bowhead whales in the early 20th century. Therefore, it is important to know whether the pre-1848 population was at equilibrium with respect to population size (cf. Givens et al. 1995). The fact that the BCB population has a large amount of genetic variability at nuclear markers (Rooney et al. 1999) suggests that this polymorphism may be relatively old, in which case patterns of long-term population size change should be apparent from nucleotide sequence data.

As suggested by some authors (Felsenstein 1992; Nee et al. 1995), the phylogenetic structure of genetic polymorphism appears to be effective at detecting fluctuations in population size. One analytical method, lineages-through-time (LTT) analysis, provides an extrapolation of population growth, decline, or stasis by plotting the number of lineages observed in a phylogeny at arbitrarily defined time points (Nee et al. 1995). The subsequent shape of this plot is used to infer the pattern of population size change. If the evolutionary time is short, the effects of mutation and population expansion are negligible. Therefore, if a population is to be analyzed by the LTT method, it should have expanded over a large number of generations, because new allelic lineages must be formed by new mutations.

In this study, nucleotide sequences of the mitochondrial control region are used to examine bowhead whale historical population size fluctuations in a phylogenetic context. Be-

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cause mitochondrial DNA (mtDNA) exists in the haploid condition in the cell, it is generally more sensitive to population size fluctuations than nuclear DNA (Avise et al. 1984; Wilson et al. 1985). Moreover, because the control region is the fastest evolving portion of the mitochondrial genome (Kocher and Wilson 1991), it serves as a very informative marker for population studies. Therefore, LTT analyses of mtDNA phylogenies should be useful for inferring patterns of historical population size change that persist over the long-term evolution of bowhead whale populations. The age of these patterns can then be estimated by using methods to date time to coalescence.

Materials and Methods

Data Collection

Bowhead whale tissue (skin, liver, kidney, or muscle) from 98 individuals was obtained from the Department of Wildlife Management, North Slope Borough, Alaska. Identification numbers are listed in Rooney (1998). Tissues were preserved in 70–100% ethanol, a −80°C freezer, or a solution of 20% dimethyl sulfoxide and saturated NaCl stored at 4°C. DNA was extracted from samples according to the protocol of Hillis et al. (1996). The following primers were designed to amplify the entire mtDNA control region: CR1 (5′-CCTCCCTAAGACTCAAGG-3′) and CR2 (5′-GAGGGCATTCTCACTGG-3′). Polymerase chain reaction (PCR) conditions included one initial denaturation at 96°C for 2 min followed by 35 cycles of 30 sec at 94°C, 30 sec at 50°C, 45 sec at 72°C, and a final extension of 15 min at 72°C. Reactions were carried out in 50-μl volumes and reagent concentrations were: 50–100 ng of DNA, 250 μM dNTPs, 1.5 mM $MgCl_2$, 1 μM of each of the above listed primers, 0.2 units of *Taq* polymerase (Promega, Inc., Madison, WI); and 1× reaction buffer (Promega, Inc.). PCR products were electrophoresed through 1.5% agarose gels and stained with ethidium bromide for visualization. PCR bands were purified with the Promega Wizard PCR Preps DNA Purification System. DNA from some of the samples initially would not amplify. However, the addition of bovine serum albumin at a final concentration of 25 ng/ml resulted in successful amplification in most of those cases. Subsequently, 98 purified products were sequenced on an ABI 377 automated sequencer (Applied Biosystems, Inc., Foster City, CA). Hypervariable region I and flanking regions were sequenced on both strands for all individuals. The forward strand was sequenced with primer CR1 and the reverse strand with the Dlp5 primer (Baker et al. 1993) modified to match the published bowhead whale control region sequence (Arnason et al. 1993).

Data Analysis

Genetic variability was estimated by using two parameters: the average pairwise sequence divergence (π), also known as nucleotide diversity (Nei and Li 1979; Nei 1987), and haplotype diversity (H). Estimates of these parameters and their standard errors were obtained with the computer program DnaSP 3.51 (Rozas and Rozas 1999), using the formulas from Nei (1987). If a bottleneck occurred in the recent history of a population, the ability to detect historical population size changes could be confounded or limited if this recent bottleneck was severe enough to have impacted genetic polymorphism. Therefore, we plotted the frequency spectrum of control region haplotype classes (Luikart et al. 1998) to test for potential bottleneck effects on genetic variability. We used this test because the loss of allelic variation is a more sensitive indicator of bottleneck effects than overall estimates of heterozygosity (Nei et al. 1975; Luikart et al. 1998).

The program END-EPI (ver. 1.0; Rambaut et al. 1997) was used to conduct a LTT analysis to infer historical population size change patterns from mtDNA polymorphism data. Prior to conducting LTT analysis, a phylogenetic tree was constructed. The neighbor-joining method (Saitou and Nei 1987) with Tamura and Nei (1993) gamma distances, as implemented in the computer program MEGA2 (Kumar et al. 2001), was used to construct this tree. Statistical reliability was assessed using 1500 bootstrap pseudoreplicates with a randomized taxon input order. The shape parameter, α, for the gamma distribution was estimated using the method of Sullivan et al. (1995). The Tamura-Nei distance was chosen because it was developed specifically for the control region (Tamura and Nei 1993). The phylogenetic tree was divided into a set of time points so that the number of lineages could be counted at each point. The number of lineages was then plotted against time under a semilogarithmic transformation (Nee et al. 1995). Depending on whether the shape of this transformation was convex or concave, an epidemic or endemic transformation was applied, respectively (Nee et al. 1995; Rambaut et al. 1997). Finally, a Wilcoxon signed-ranks test was applied to the upper 35% of the curve under a null hypothesis of accelerating, decelerating, or linear slope, which corresponds to an increasing, decreasing, or stable growth rate in the most recent 35% of nodes (Ong et al. 1996). This proportion was used because changes in linearity should only occur in the recent past, relative to the time scale applied (Ong et al. 1996). Ong et al. (1996) showed that 0.5% of the total population size must be sampled for Type II error to be less than or equal to 5% in LTT analyses. Because current estimates of the BCB population size are near 8200 individuals (International Whaling Commission 1997), two groups of 40 individuals, approximating 0.5% of the total population size, were analyzed. Group I was composed of samples collected during the years 1990, 1995, and 1996, and group II was composed of samples collected during the years 1993 and 1997.

Coalescence Time and Mutation Rate

To infer the coalescence time of mtDNA control region haplotypes, an estimate of the mutation rate was obtained. In mammals, the transition/transversion ratio in the mtDNA control region is very high, and transitional changes often quickly reach the saturation level when two species are compared (Vigilant et al. 1991). Therefore, we used the following formula to estimate the number of nucleotide substitutions per site from comparisons of the focal species and an outgroup species:

$$d = (Tv + TvR)/m = p(1 + R), \quad (1)$$

where Tv is the number of transversions between species, R

is the transition/transversion ratio within the focal species (in this case, bowhead whale), m is the sequence length, and $p = Tv/m$ (Nei 1992). To estimate the d-value, we chose the northern right whale (*Eubalaena glacialis*) as the outgroup species because the genus *Eubalaena* is the closest extant relative to *Balaena* (Arnason et al. 1993; McLeod et al. 1993).

Once an estimate of d was obtained, the rate of nucleotide substitution per site per lineage per year (λ) was estimated by $\lambda = d/2T$, where T is the time since divergence between the two species used. Therefore, if t_g is the generation time measured in years, the mutation rate per nucleotide site per generation is given by $\mu = \lambda t_g$, whereas the mutation rate per haplotype is given by $\nu = m\mu$. We also used the gamma distance model of Tamura and Nei (1993) to compute the mutation rate. This was done by estimating the average pairwise Tamura-Nei gamma distance (d) between all bowhead whale sequences and an outgroup sequence. Subsequently, the formula $\lambda = d/2T$ was applied to obtain an estimate of the mutation rate.

Once this mutation rate estimate ($\hat{\nu}$) was obtained, we estimated the coalescence time in generations by solving for the equation $\hat{t} = \hat{\tau}/2\hat{\nu}$(Rogers and Harpending 1992; Harpending et al. 1993). The parameter τ (Rogers and Harpending 1992; Harpending et al. 1993) was estimated by using the computer program DnaSP 3.14 (Rozas and Rozas 1999). By multiplying $\hat{t}$ by the generation time, a coalescence time estimate in years was obtained.

Results

Control Region Polymorphism

A total of 453 nucleotides were sequenced from the 5′ end of the control region for all 98 samples (GenBank accession numbers AF355204–AF355271). These 98 sequences consisted of 68 unique haplotypes with a total of 59 segregating sites. Because the control region sequences were highly conserved with respect to length and overall variability, constructing an alignment was straightforward. For the entire dataset (all 98 new sequences plus the published sequence of Arnason et al. 1993), the average nucleotide frequencies were 0.30 for adenine, 0.22 for cytosine, 0.17 for guanine, and 0.31 for thymine, and base composition bias averaged 0.14. The average number of transversions estimated from pairwise comparisons of unique bowhead whale alleles was 3.2 when the northern right whale was used as the outgroup, and the average transition/transversion ratio (R) was 16.1 for bowhead whale haplotypes only.

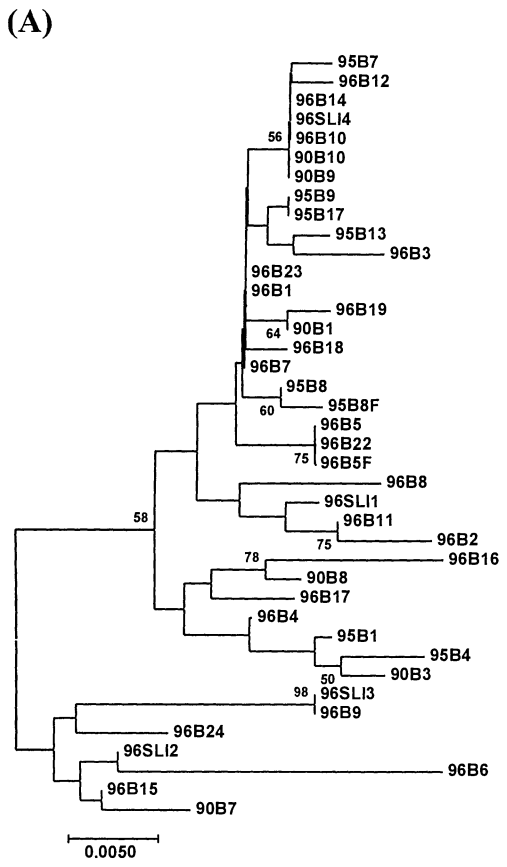
Long-Term History of Population Size Change

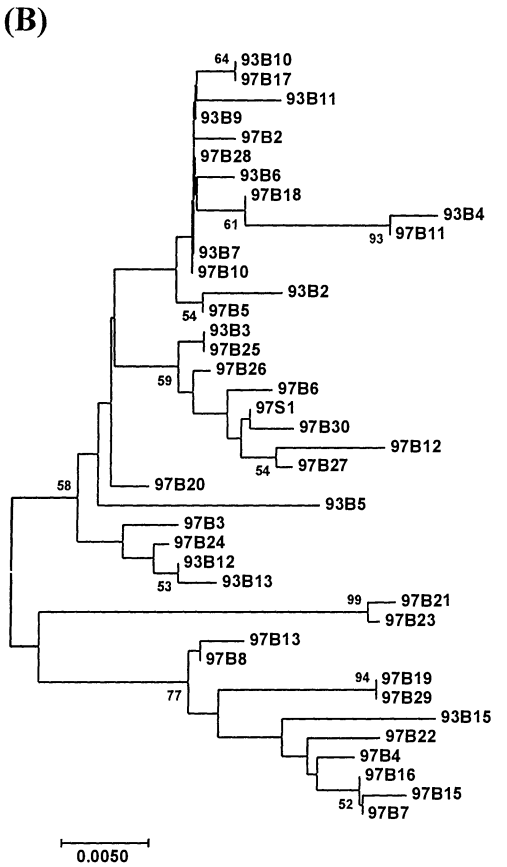
The phylogenetic trees reconstructed for groups I and II are shown in Figure 1. When the semilogarithmic transformation was used in the LTT analysis, both groups revealed a convex curvature, suggesting a population size expansion for the BCB stock (Fig. 2A). An epidemic transformation was used to investigate the population growth rate (Nee et al. 1995; Rambaut et al. 1997), resulting in a concave curvature for both groups (Fig. 2B). These results indicate a decelerating growth rate overall. Although these patterns apply to the entire history of the expansion, from its initiation to the present, it would be interesting to know whether the rate was accelerating, decelerating, or remaining constant over the most recent evolutionary past. To explore this question further, tests of linearity were applied over the most recent 35% of nodes in the phylogeny (Ong et al. 1996; Rambaut et al. 1997). In the case of group I, the hypothesis of an increasingly steep curvature could not be rejected when a uniform rate across nucleotide sites was assumed. However, this hypothesis was rejected ($P < 0.01$; Table 1) when mutation rate heterogeneity was accounted for in the phylogenetic analysis. In contrast, the hypothesis of a decreasingly steep curvature could not be rejected in either case. Thus, exponential growth at a constant rate resulted when a uniform rate model was used, whereas exponential growth at a decelerating rate resulted when a model of mutation rate heterogeneity was used. For group II, the hypothesis of a decreasingly steep curvature could not be rejected when either mutation rate heterogeneity or a uniform rates model was assumed in the phylogenetic analysis. However, the hypothesis of an increasingly steep curvature was rejected ($P < 0.05$; Table 1) under both assumptions. Thus, a model of exponential growth at a decelerating rate was inferred for group II.

Mutation Rate, Coalescence Time, and Historical Female Effective Size

Using the control region sequence for the northern right whale (Arnason et al. 1993) and equation (1), we computed the average number of nucleotide substitutions per site (d) between all bowhead whale and the northern right whale control region sequences and obtained $d = 0.12$. The fossil record suggests that *Eubalaena* and *Balaena* diverged in the Pliocene (McLeod et al. 1993), which spans 1.64 to 5.2 million years ago. Although no definitive date is available, the split most likely occurred less than four million years ago (MYA) (McLeod et al. 1993; F. C. Whitmore Jr., pers. comm.). Therefore, the average of the beginning and ending dates of the Pliocene (3.4 million years ago) was chosen as a rough estimate. This gives an estimate of the rate of nucleotide substitution per site per lineage per year (λ) to be $0.12/(2 \times 3{,}400{,}000) = 1.8 \times 10^{-8}$ (range = 1.2×10^{-8}–3.0×10^{-8} based on a 2–5 million years divergence range). Interestingly, λ was estimated to be 2.2×10^{-8} (range = 1.5×10^{-8}–3.7×10^{-8} based on a 2–5 million years divergence range) using gamma-corrected Tamura-Nei distances with an estimated gamma shape parameter, $\alpha = 0.116$. Here, we used the average (2×10^{-8}) of the two estimates (gamma and Tv/R-based). This estimate is higher than the estimate (average = 7.5×10^{-9}; range = 5×10^{-9}–1.0×10^{-8}) obtained by Hoelzel and Dover (1991) and the estimate (average = 8.5×10^{-9}; range = 7×10^{-9}–1.0×10^{-8}) obtained by Baker et al. (1993).

The time to female maturation in bowhead whales has been estimated as 17 to 20 years (Schell and Saupe 1993). This indicates that the generation time in bowhead whales is longer than 20 years, with a magnitude that depends on longevity and time to reproductive senescence. Although there is little evidence of obvious female reproductive senescence in bowhead whales (Tarpley and Hillman 1998; J. C. George, pers.





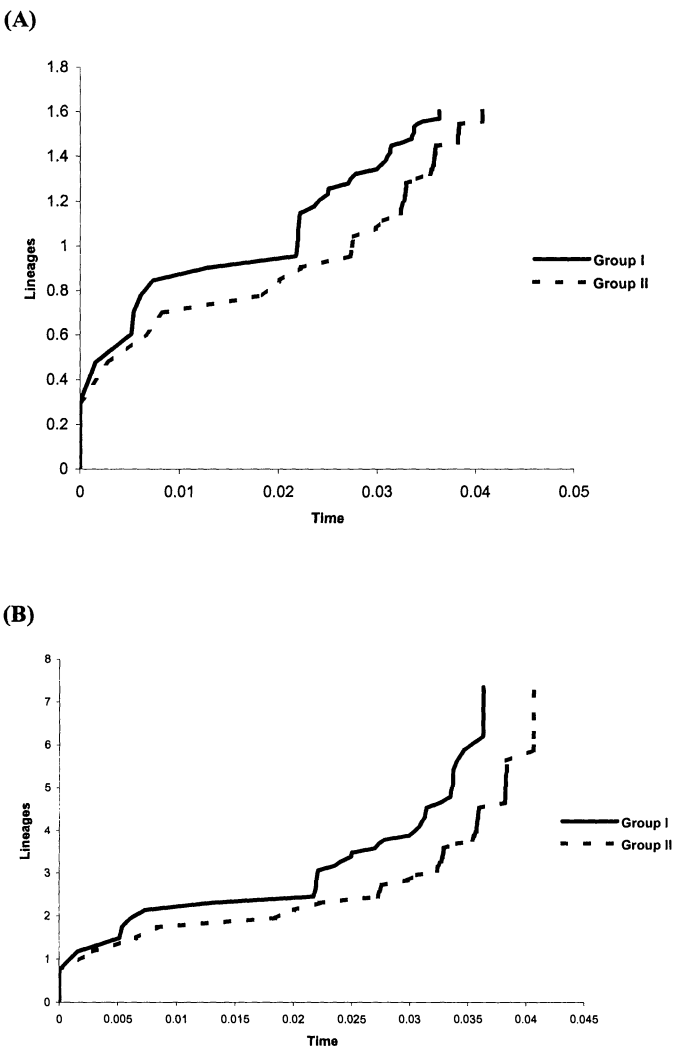


FIG. 2. (A) Semilogarithmic transformations and (B) epidemic transformations of the phylogenetic trees (Fig. 1) reconstructed for groups I and II.

comm.), it does not mean that reproductive senescence does not occur. Nevertheless, given the delayed age at first reproduction (Schell and Saupe 1993), a three- to four-year calving interval (Rugh et al. 1992; Koski et al. 1993), and strong evidence of animals living over 100 years (George et al. 1999), female reproduction past 60 years of age is not unlikely (George et al. 1999). Therefore, the most likely generation time probably lies somewhere between 30 and 40 years, an average of the age at female sexual maturity and suspected age of female reproductive senescence. Although it is a crude approximation, such an average can be chosen to estimate the mutation rate, because the generation time will not affect our coalescence time calculations. This is because the generation time is multiplied by the inferred number

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FIG. 1. Phylogenetic trees for individuals from (A) group I and (B) group II. Trees were reconstructed using the Tamura-Nei gamma distance with $\alpha = 0.116$.

TABLE 1. Results of lineages-through-time (LTT) analysis. In cases where a uniform rate was not assumed, a gamma shape parameter of 0.116 was estimated from the data by the Sullivan et al. (1995) method and applied to the Tamura-Nei distances.

	Hypothesis of linearity	
Uniform rate	More steep	Less steep
(A) Group I		
yes	rejected*	not rejected
no	not rejected	not rejected
(B) Group II		
yes	rejected**	not rejected
no	rejected**	not rejected

* $P < 0.01$; ** $P < 0.05$.

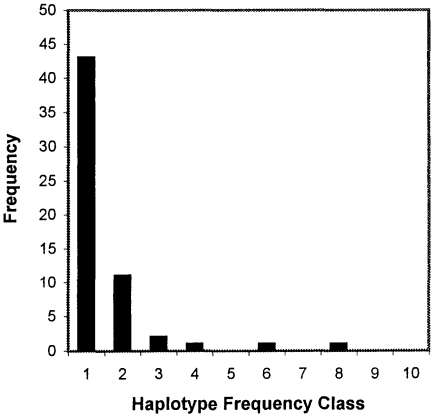

FIG. 3. Control region haplotype frequency distribution. The L-shaped distribution indicates that the BCB population did not experience a bottleneck (Luikart et al. 1998). Values along the x-axis are the maximum values for each haplotype class (e.g., the first class represents haplotypes with frequencies between zero and one).

of coalescent generations to convert the coalescent into years; thus, the estimate of generation time cancels out. This can be shown as follows. Our estimate of the substitution rate per year ($\lambda = 2 \times 10^{-8}$) gives an estimate of the mutation rate per generation (μ) as 6×10^{-7}, using a generation time estimate of 30 years and an estimate of the mutation rate per sequence per generation (ν) as $m\mu = 2.7 \times 10^{-4}$. Using an estimate of $\hat{\tau} = 4.802$, the coalescence time in generations is 8892.6, which yields an estimated coalescence time of approximately 267,000 years when multiplied by the assumed generation time of 30 years. If a generation time of 40 or 50 years is assumed, μ and ν will change, but the coalescence time remains the same.

The historical female effective size can be estimated from $\theta = 2N_e\nu$ provided that one has an estimate of initial θ ($\hat{\theta}_0$) and final θ ($\hat{\theta}_T$; Rogers and Harpending 1992; Harpending et al. 1993). The estimate $\hat{\theta}_1$ is determined from patterns of extant polymorphism, whereas $\hat{\theta}_0$ is estimated by setting $\hat{\theta}_1$ as infinite and then working through a series of formulas (for a complete description see Rogers 1995). We obtained $\hat{\theta}_0 = 2.583$ and $\hat{\theta}_1 = 11.418$, using DnaSp 3.14 (Rozas and Rozas 1999). This corresponds to effective sizes of approximately 3590 and 15,860, respectively.

DISCUSSION

Givens et al. (1995) suggested a pre-1848 population size of about 15,000 individuals in the BCB population, nearly twice the current population size estimate of 8200 (Raftery and Zeh 1998). Key concerns are whether the population was at equilibrium with respect to the level of genetic variability before it was reduced and how this may have affected interpretations of population trends derived from genetic polymorphism data. Our ability to infer patterns of historical population size change from control region polymorphism data would be confounded if the BCB bottleneck that occurred near the turn of the 20th century had affected mtDNA genetic variability. In this case, recent bottleneck effects on genetic variability would have obscured historical patterns of population size change.

The results from this study suggest that patterns of genetic variability in the mtDNA control region were not substantially influenced by the presumed bottleneck occurring at the turn of the 20th century. First, the observed haplotype frequency distribution is L-shaped (Fig. 3), providing strong evidence for the absence of bottleneck effects on genetic variability (Nei et al. 1975; Luikart et al. 1998). Second, estimates of $\hat{\pi}$ and $\hat{H}$ (0.0163 ± 0.0009 and 0.986 ± 0.005, respectively) in the BCB population are as high or higher than cetacean populations that did not experience a bottleneck (e.g., Antarctic minke whales; Bakke et al. 1996; Table 2) or that experienced a bottleneck but retained large amounts

TABLE 2. Control region genetic variability in various cetacean populations. These data were compiled from Bakke et al. (1996), Baker et al. (1993), Rosel et al. (1994), and Lyrholm et al. (1996).

Species	Bottleneck effects	$\hat{\pi}$	$\hat{H}$	Sample size	Unique haplotypes	Sequence length
Humpback whale, NP[1]	no	0.014	0.75	31	7	283
Humpback whale, NA	no	0.020	0.87	34	15	283
Humpback whale, SH	no	0.023	0.95	25	16	283
Minke whale, NA	yes	0.006	0.86	87	25	345
Minke whale, Ant	no	0.016	1.0	23	23	345
Sperm whale, NA	yes	0.002	0.74	10	4	954
Sperm whale, NP	yes	0.002	0.74	16	6	954
Sperm whale, SH	yes	0.002	0.68	11	5	954
Long-beaked dolphin	no	0.012	0.94	11	10	404
Short-beaked dolphin	no	0.018	0.97	18	17	404

[1] NP, North Pacific; NA, North Atlantic; SH, Southern Hemisphere; Ant, Antarctic.

of genetic variability (e.g., humpback whales; Baker et al. 1993; Table 2). In contrast, the BCB population shows much higher estimates of genetic variability than bottlenecked populations with low levels of genetic variability (e.g., sperm whales; Lyrholm et al. 1996). These results and comparisons suggest that the BCB population retained a large amount of genetic variability despite the bottleneck, which further suggests that the effective population size was either unaffected or only negligibly so. This is supported by the fact that the estimated female effective size (15,860) is almost twice as large as the current total population size estimate (8200). We would not have obtained this result if the population had lost a substantial amount of genetic variability as a consequence of the bottleneck. As a result, the signature of historical population size change was preserved in the control region sequence data.

What historical population patterns can be inferred from the control region data? Our results suggest that the BCB population experienced a size expansion based on the LTT analysis (Fig. 2). Similar conclusions were reached in a separate study of microsatellite polymorphism patterns in the BCB population (Rooney et al. 1999). Further support for a population expansion is provided from the difference in magnitude between the initial (3590) and current (15,860) female effective size estimates, in which the latter is four times larger than the former. The interpretation of whether the historical population growth rate was accelerating, decelerating, or remaining constant over the most recent evolutionary past was influenced by the choice of substitution model (mutation rate heterogeneity or uniform rate; Table 1). This is not surprising given that the choice of substitution model can affect phylogeny reconstruction (Nei 1996; Huelsenbeck and Rannala 1997), upon which LTT analysis depends. Nevertheless, because it is known that mutation rate heterogeneity is characteristic of mtDNA control region sequence evolution (Tamura and Nei 1993), it is likely that the results using this model are more accurate than a uniform rates model. Thus, according to the results from both groups, it can be inferred that population growth had been approaching equilibrium in the BCB stock at a decelerating rate prior to its reduction in size.

Because population size fluctuations as well as certain population-level processes (e.g., selection; Slatkin and Hudson 1991; Marjoram and Donnelly 1994) can influence LTT analysis, it would be helpful to infer the coalescence time of the sequences used to date the age of the inferred historical population trends. The time to coalescence of BCB mtDNA haplotypes was estimated to lie within the Middle to Late Pleistocene. It was speculated in Rooney et al. (1999) that the pattern of BCB microsatellite polymorphism might have been influenced by changes in sea-ice distribution dating to approximately 8500 years ago, which effectively isolated the populations of the high Arctic from each other (Dyke et al. 1996). If this is true for mtDNA haplotypes, the coalescence time and the time since the sea-ice distribution change should approximately coincide. Therefore, estimating the coalescence time of the mtDNA haplotype sequences should provide an opportunity to test whether this prediction holds. Results of this study suggest that changes in sea-ice distribution that occurred 8500 years ago did not influence the signature of historical population size change in the mtDNA sequence data, as our estimated coalescence time (approximately 267,000 years) for BCB mtDNA haplotypes falls within the Middle to Late Pleistocene.

We showed that the mutation rate had no effect on estimating the coalescence time in our study. However, the estimation of substitution rate λ does have an effect. In this case, the divergence time estimate between the bowhead and northern right whale has the most significant impact on estimating λ and therefore coalescence time. Due to the uncertainty of the divergence time between the bowhead and northern right whales, we selected the average of a range of divergence dates (2 to 5 million years). If we use the fastest (3.7×10^{-8}) and slowest (1.2×10^{-8}) of the λ estimates, which are derived from the earliest (2 MYA) and latest (5 MYA) divergence dates, respectively, we obtain coalescence times of 144,000 years using the fastest rate and 445,000 years using the slowest rate.

If we use previous estimates of the cetacean control region substitution rate, we obtain a coalescence time estimate of 711,000 years (range = 534,000–1,067,000 years), when using the λ estimates generated by Hoelzel and Dover (1991) and 628,000 (range = 534,000–762,000 years) when using the λ estimates generated by Baker et al. (1993). These coalescence time estimates are substantially higher than our estimate (267,000 years; range = 144,000–445,000 years) because our estimate of λ is faster by an approximately 2.5 to 4.0-fold difference from these previous estimates. These slower λ estimates may have resulted from estimation procedures based on comparisons of relatively distantly related taxa, whereas we used sister taxa for our study comparisons. Comparisons of distantly related taxa may have confounded these earlier estimates due to problems of multiple substitutions, among-site rate heterogeneity, and transition/transversion bias. Consequently, substitution rates appear to have been underestimated in these earlier studies because they did not consider some or all of these factors. In fact, our average estimate of λ (2×10^{-8}) is close to the average estimate (1.5×10^{-8}; range = $0.8–2.2 \times 10^{-8}$; Pesole et al. 1999) obtained from comparisons of fin and blue whales, which are also sister taxa. This lends further support to our results, because there is no reason to believe that bowhead and northern right whales will undergo substantially different rates of control region nucleotide substitution than fin and blue whales.

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