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Evaluating a Putative Bottleneck in a Population of Bowhead Whales from Patterns of Microsatellite Diversity and Genetic Disequilibria

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Abstract. A size-selected *Balaena mysticetus* genomic library was screened for clones containing simple sequence repeat, or microsatellite, loci. A total of 11 novel loci was identified. These loci were combined with a set of 9 published loci, for a total of 20 markers, and were scored across a sample of 108 bowhead whales from the Bering–Chukchi–Beaufort Seas population of bowhead whales. Genetic variability was measured in terms of polymorphism information content values and unbiased heterozygosity. From the latter, estimates of long-term effective population size were obtained. In addition, gametic phase disequilibrium among loci was investigated. Moderate to high levels of polymorphism were found overall, and the long-term effective size estimates were large relative to total population size. Tests of heterozygosity excess (Cornuet and Luikart 1996) and allele frequency distribution (Luikart et al. 1998) indicated that the possibility of a recent genetic bottleneck in the Bering–Chukchi–Beaufort Seas population of bowhead whales is highly unlikely. However, the fact that five loci displayed a statistically significant heterozygote deficiency remains to be explained.

Key words: Bottleneck — Genetic diversity — Linkage disequilibrium — Null allele — Bowhead whale

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Introduction

Cetaceans of the suborder Mysticeti, commonly known as mysticetes or baleen whales, first appear in the fossil record during the Late Oligocene (Van Valen 1968; Barnes 1987; McLeod et al. 1993). Among the extant families of mysticetes, the Balaenidae is the oldest as evidenced by fossils that date to the Early Miocene (McLeod et al. 1993). This family is comprised of three recognized extant species that are thought to have evolved in the Pliocene (McLeod et al. 1993): the northern right whale, *Eubalaena glacialis;* the southern right whale, *Eubalaena australis;* and the bowhead whale, *Balaena mysticetus.* The bowhead whale is unique among mysticetes in terms of morphology and life history. For example, the head comprises about a third of the total body length, thus accommodating baleen that can measure up to 4.6 m long (Haldiman and Tarpley 1993; Lowry 1993). The blubber and skin layers also represent remarkable adaptations, where in certain parts of the body the former is up to 28 cm thick and the latter is up to 24 mm thick (Haldiman and Tarpley 1993). This morphology has allowed the bowhead whale to exploit the Arctic environment, allowing it to inhabit waters that often drop below 4°C (Niebauer and Schell 1993).

The distribution of the bowhead whale is limited to the high latitudes of the Northern Hemisphere (Moore and Reeves 1993). It is divided into five "stocks," or management populations: the Spitsbergen stock, the Davis Strait stock, the Hudson Bay stock, the Okhotsk Sea stock, and the Bering–Chukchi–Beaufort Seas (BCB) stock. The BCB stock is by far the largest, num-

bering approximately 8200 whales (International Whaling Commission 1997). The Davis Strait and Hudson Bay stocks combined number at least 450 animals (Zeh et al. 1993), the Sea of Okhotsk Stock numbers approximately 150–200 (Berzin et al. 1986), and the Spitsbergen stock, which was last estimated to be in the tens (Christensen et al. 1990), may have become extinct. The Okhotsk Sea and BCB stocks were isolated from the Davis Strait, Hudson Bay, and Spitbergen stocks subsequent to the establishment of the M'Clintock sea-ice plug, approximately 8500 years ago (Dyke et al. 1996). Genetic studies of the relationships among stocks have yet to be completed.

The BCB population has been subject to both aboriginal subsistence harvests and commercial whaling during the course of its history. The contemporary Eskimo peoples of Alaska have hunted the bowhead whale throughout their known history (Stoker and Krupnik 1993). Today, they hunt whales under internationally sanctioned harvests and voluntarily agree to follow the quotas set by the International Whaling Commission. In contrast, the commercial Yankee whaling era resulted in an intense harvest of the BCB stock throughout the mid to late 1800s and early 1900s in order to supply the oil and baleen markets in the United States (Sonntag and Broadhead 1989). After nearly 70 years of unregulated commercial whaling, the BCB population dropped from an estimated size of approximately 15,000 individuals (Givens et al. 1995) to an estimated minimum size of 1000 individuals (corresponding to a total population loss of 93%) when commercial whaling ceased, near 1914 (Woodby and Botkin 1993). Despite the fact that an estimated 93% of the BCB total population size may have been lost, a total population size reduction to 1000 whales may not have been extensive enough to have depleted genetic diversity. However, estimating the extent of the bottleneck is extremely difficult, as this requires data obtained from mid- to late nineteenth- and early twentieth-century whaling records, which are of questionable accuracy. Therefore, the population size reduction may have been more extensive than originally thought. In fact, a recent report suggests that substantial amounts of genetic diversity may have been lost in the BCB population due to the commercial whaling era bottleneck (LeDuc et al. 1998). Today, the BCB stock is increasing at a rate of approximately 3.2% annually and represents the world's largest remaining population of bowhead whales (International Whaling Commission 1997). However, there is continued concern for the conservation status of the BCB bowhead whale population, due primarily to the turn-of-the-century bottleneck. Because the other stocks have yet to show significant recovery (Zeh et al. 1993), the bowhead whale has remained in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora since it was first added in 1975 and in the United States Endangered Species Act since it was listed in 1973.

Studies of cetacean populations have shown that simple sequence repeats (SSRs), or microsatellites, can be informative elucidating kin, social, or population structure (e.g., Amos et al. 1993; Richard et al. 1996; Valsecchi et al. 1997). However, empirical studies of effective population size in cetaceans are lacking. The effective population size controls the level of diversity maintained in a population and is influenced by demographics (Wright 1931; Nei et al. 1975). As such, an estimation of a population's long-term effective size might reveal important historical and/or ongoing processes in that population. For example, Lehmann et al. (1998) estimated long-term effective population sizes in a species of African mosquito to deduce whether or not the populations were subject to recurring bottlenecks or founder events and what significance this had in helping to understand mosquito ecology. Similar analyses of the BCB bowhead whale stock might prove useful for understanding bowhead whale population history. While mtDNA is more sensitive to population processes than nuclear DNA (Avise et al. 1984; Wilson et al. 1985), an analysis of nuclear genetic variability could prove to be informative. For example, Houlden et al. (1996) found a reduction in microsatellite, or SSR, genetic diversity in a study of a koala population that nearly went extinct at the turn of the century. Their results suggest that SSRs are useful for assessing relatively recent bottleneck events. Therefore, to assess genetic diversity and demographic history in the bowhead whale, a genomic library was constructed and screened for the presence of SSRs. Estimates of heterozygosity and polymorphism information content (PCI) (Nei 1977, 1979; Botstein et al. 1980) were obtained to quantify genetic diversity, and tests of the bottleneck hypothesis in the BCB population were conducted using the methods of Cornuet and Luikart (1996) and Luikart et al. (1998).

Materials and Methods

Muscle tissue from a frozen archived sample (84B1) was obtained from researchers at the North Slope Borough Department of Wildlife Management, Alaska. Approximately 0.25 g of this tissue was frozen in liquid nitrogen and ground in a mortar and pestle. DNA was then extracted according to the method of Sambrook et al. (1989). Following this, 30 μg of DNA was partially digested with the enzyme *Sau3*A1 for 1 h at 37°C and then run on a 0.8% agarose gel to select fragments of between 300 and 700 base pairs. These fragments were excised from the gel with a razor blade and purified with the Qiagen Gel Extraction Kit (Qiagen, Inc.). Fifty nanograms of this digest was then ligated into 25 ng of dephosphorylated pBluescript SK^- plasmid (Stratagene, Inc., LaJolla, CA, USA), previously digested to completion with the enzyme *Bam*H1. Following this, 2 μl of the ligation was transformed into TOP10F′ competent cells (Invitrogen, Inc., Carlsbad, CA, USA) using the heat shock method (Sambrook et al. 1989). Colonies were grown directly on nylon membranes and subsequently soaked in hybridization

Table 1. Novel SSR loci isolated from a bowhead whale size-selected genomic library

Locus	Cloned repeat sequence	Annealing temp. (°C)	5′–3′ primer sequence[a]
TexVet10	$(TG)_{10}$	45	5′-TAACACATCCATCACC-3′ 5′-GAAACTTGCTAAGAGAGT-3′
TexVet11	$(TG)_{14}$	54	5′-AAACCCATGTCCCCTGCATTGG-3′ 5′-CATCTGCATTCCTTACGAACAGTG-3′
TexVet12	$(TG)_{16}(GA)_9$	48	5′-TAACTATGGCATCAGTAGG-3′ 5′-TCCTGGGTTAGCAGTGTTC-3′
TexVet13	$(TG)_{13}$	53	5′-CAACTAACACCCGATGCAGCCA-3′ 5′-GCCCTTTAGCAGTGAAAGTGCAGAG-3′
TexVet14	$(TG)_{16}$	50	5′-GCACTCACAGGACCATAA-3′ 5′-GCTGACTCTTTCTTTGGG-3′
TexVet15	$(TA)_{21}(CA)_9(TA)_3(TG)_3(TA)_2TAAA(TA)_5$	50	5′-GCTGGGGGAAAACAGACATTAAAC-3′ 5′-CAAGGACCTACTGTATAGCACAGGGAAT-3′
TexVet16	$(CA)_{10}$	52	5′-CACAGCAGTGAAAGAGCCGAGT-3′ 5′-CGAGCTTCCTGAGAGAACTTTGTT-3′
TexVet17	$(TC)_4TGTAAAATATTTA(CA)_{22}$	46	5′-CATGAATCAATTTCTTAAA-3′ 5′-GGGAGCTTTGTATTTGT-3′
TexVet18	$(TG)_{14}$	46	5′-TCAGATGAGGAGGGA-3′ 5′-CACGTCATACACACAAAA-3′
TexVet19	$(CA)_6GA(CA)_{21}$	54	5′-CTGATTCCACACGTCTTTCATGC-3′ 5′-GGATTGGCAGCAGGACAATAATG-3′
TexVet20	$(TG)_{15}$	55	5′-CTTGTGGCATCCTTGTCTGGTTTTG-3′ 5′-GGAAGAGGCGAGGGTTTTGAATTG-3′

[a] The forward primer sequence is given first, and the reverse is listed directly below.

buffer (6× SSC, 0.5% SDS, 5× Denhardt's reagent) for 1 h at 65°C to bind DNA and remove extraneous cellular debris from the membranes. Subsequently, five SSR probes were labeled with [γ-^{32}P]dATP and were allowed to hybridize concomitantly to filters: $(CA)_n$, $(CAG)_n$, $(AAT)_{10}$, $(GAAA)_n$, and $(GATA)_n$. Hybridization to DNA-bound membranes took place for 1 h at 65°C immediately followed by 18 h at 37°C.

A total of 33 positives was identified of 3000 recombinant colonies screened. These clones were sequenced on an ABI 377 automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA, USA) using the manufacturer's suggested protocols. A total of 21 SSR-containing clones was found. Unfortunately, 10 loci were located too close to the plasmid insertion site to allow an oligonucleotide PCR primer to be designed. Therefore, a total of 11 loci was chosen for further study. Primers were designed for each locus using the computer application Mac Vector 5.0 (International Biotechnologies, Inc.). The genotypes of 108 individuals were determined via PCR amplification from whale tissue (skin, liver, kidney, or muscle) obtained from the Department of Wildlife Management, North Slope Borough, Alaska. These samples were collected from Alaska BCB bowhead whales harvested by Alaska Eskimo hunters under the auspices of the International Whaling Commission. A listing of the samples used in this study is given by Rooney (1998). Samples were either preserved in 70 or 100% ethanol, frozen, or preserved in a solution of 20% dimethyl sulfoxide, saturated NaCl. DNA was isolated from samples according to standard protocols (Hillis et al. 1996). PCR reactions were designed according to standard protocol (Hillis et al. 1996) in 25-μl volumes where only one primer was labeled with [γ-^{32}P]dATP. For PCR amplification, a touchdown procedure was used (Hillis et al. 1996), implementing three segments. The first lasted five cycles and annealed at 10° above the locus-specific annealing temperature. The second also lasted five cycles and annealed at 5° above the locus-specific annealing temperature. The final segment lasted 25 cycles and used the locus-specific annealing temperature listed in Table 1 for bowhead isolated SSRs or used an annealing temperature of 45°C for SSRs originally isolated in bottlenose dolphins (see below). The following thermocycling parameters were used: 96°C for 2 min, followed by touchdown segmental cycles of 94°C for 20 s, annealing temperature for 30 s, and 72°C for 45 s. PCR products were then run on 6% acrylamide/7 *M* urea/1× TBE gels in 1× TBE running buffer. A total of 20 markers was scored, which included the 11 new loci described in this study plus 9 loci that were isolated from a *Tursiops truncatus* library (Rooney et al. 1999). If an individual failed to amplify, reamplification was attempted at an annealing temperature of 40°C and an increased magnesium concentration (2.5 m*M*).

Polymorphism information content (PIC) (Nei 1977, 1979; Botstein et al. 1980) and unbiased heterozygosity (Nei 1987) were estimated for each locus. Fisher's exact test, as implemented in the computer application GENEPOP 3.0 (Raymond and Rousset 1995), was used to determine if the population was in Hardy–Weinberg equilibrium. In addition, exact tests of heterozygote deficiency and excess were conducted (Rousset and Raymond 1995). The program LINKDIS (Black and Krafsur 1985; Garnier-Gere and Dillmann 1992), as implemented in GENEPOP 3.0 (Raymond and Rousset 1995), was used to examine gametic phase disequilibrium among SSR loci. This program estimates correlation coefficients according to Weir (1979). Long-term effective population size was calculated from estimates of unbiased (expected) heterozygosity under both the infinite-alleles (IAM) and the stepwise mutation (SMM) models (Nei 1987; Lehman et al. 1998) as shown below:

$$\text{IAM:} \quad N_e = H/(4\mu - 1 - H)$$
$$\text{SMM:} \quad N_e = \{[1/(1-H)]^2 - 1\}/8\mu$$

where N_e is the effective population size, H is the mean heterozygosity, and μ is the mutation rate per locus. A value of 2.05×10^{-4} was chosen for the mutation rate, as this represents the average value of three mammalian species: mouse (Deitrich et al. 1992), human (Weber and Wong 1993), and pig (Johansson et al. 1992; Ellegren 1995). Finally, the bottleneck hypothesis was investigated using the program BOTTLENECK 1.2.01 (Cornuet and Luikart 1996). These methods test for the departure from mutation drift equilibrium based on *hetero-*

Table 2. Genetic diversity and effective population size

Locus	No. of alleles	Exact test	Heterozygosity	PIC	N_e (SMM)	N_e (IAM)
TexVet1	2	HW[a]	0.0510	0.0494	67	66
TexVet2	4	HW	0.5028	0.4662	1,857	1,233
TexVet6	2	Deficiency[b]	0.3173	0.2655	698	567
TexVet7	9	HW	0.6053	0.5525	3,304	1,870
TexVet9	3	HW	0.2332	0.2155	427	371
TexVet11	4	HW	0.6157	0.5565	3,518	1,953
TexVet12	2	HW	0.0185	0.0183	23	23
TexVet13	6	HW	0.6142	0.5626	3,486	1,941
TexVet14	8	Deficiency[b]	0.7631	0.7257	10,250	3,927
TexVet15	5	Deficiency[b]	0.5812	0.5356	2,867	1,693
TexVet16	6	HW	0.4991	0.4514	1,821	1,215
TexVet17	11	HW	0.8067	0.7787	15,716	5,091
TexVet18	4	Deficiency[b]	0.5928	0.5128	3,067	1,775
TexVet19	6	Excess[b]	0.7879	0.7507	12,939	4,529
TexVet20	7	Deficiency[b]	0.6737	0.6336	5,118	2,518
Average			0.5707	0.4717	4,344	1,918

[a] Hardy–Weinberg equilibrium.
[b] $p < 0.05$.

zygosity (not heterozygote) excess or deficiency. Also, using BOTTLENECK 1.2.01, the allele frequency distribution of the SSR loci was examined for a mode shift (Luikart et al. 1998), which may indicate if a recent genetic bottleneck has occurred. In cases where multiple tests were performed and independence could not be established, a tablewide Bonferroni correction was implemented using an α of 0.05 (Rice 1989; Weir 1996). Individuals that failed to amplify were excluded from the analysis.

Results

Clones containing SSRs were identified at a ratio of 1:143 recombinants. These recombinants contained between 300 to 700 base pairs of bowhead whale genomic DNA. This suggests that an SSR can be found at every 43 to 100 kb in the bowhead whale genome. Studies of humans (Weber and May 1989), pigs (Johansson et al. 1992; Ellegren 1995), and dogs (Ostrander et al. 1992) report a similar frequency of occurrence. Of the nine loci that were originally isolated from a bottlenose dolphin genomic library, two did not amplify in any individual and two were monomorphic. Nevertheless, moderate levels of heterozygosity were displayed overall (Table 2), although PIC values were slightly lower in comparison.

Gametic phase disequilibrium was significant in 24 pairwise comparisons of a total of 105 (Table 3). This was greater than expected by chance as evaluated under a binomial distribution with a success rate of 0.05 ($p < 0.05$). However, gametic phase disequilibrium was not found to be significant on a tablewide level. On a locus-specific basis, five loci showed evidence of heterozygote deficiency (Table 2). This is also greater than what is expected by chance as evaluated under a binomial distribution with a success rate of 0.05 ($p < 0.05$). However, Hardy–Weinberg equilibrium could not be rejected on a tablewide level ($p < 1$). Equation 3 of Brookfield (1996) was used to estimate the frequency of null alleles (r) at loci displaying a heterozygote deficiency, assuming that the presence of null alleles is the true cause of a significant heterozygote deficiency. The frequency of null alleles (r) was found to be 0.539 for Tex Vet6, 0.089 for Tex Vet14, 0.206 for Tex Vet15, 0.348 for Tex Vet18, and 0.035 for Tex Vet20. Long-term effective size estimates were larger for the SMM relative to the IAM (Table 2) as expected. The average long-term effective population size estimate for SSR loci under the SMM (4344 ± 4640) was higher than under the IAM (1918 ± 1500).

Both the IAM and the SMM were applied when the computer program BOTTLENECK was used to test if the SSR loci showed a departure from mutation-drift equilibrium. Under the IAM, results indicated that a deviation from mutation-drift equilibrium is not upheld using either the sign test ($p > 0.25$) or the Wilcoxon test ($p > 0.35$; two-tailed). Under the SMM, results indicated that there was a heterozygosity deficiency resulting from too many alleles, using either the sign test ($p = 0.00054$) or the Wilcoxon test ($p = 0.00021$; two-tailed). Specifically, the expected number of loci displaying heterozygosity excess (8.62) is substantially greater than the observed number of loci displaying heterozygosity excess (2) and substantially less than the observed number of loci displaying a heterozygosity deficiency (13). The results of the standardized differences test are not reported because 20 polymorphic loci are required for this test (Cornuet and Luikart 1996), although the results under both models are in agreement with those of the other tests. Finally, an analysis of allele frequency distribution (Fig. 1) revealed an L-shaped distribution, which is expected for a population that has not experienced a recent bottleneck that affected genetic variability (Luikart et al. 1998).

Table 3. Correlation coefficients resulting from tests of gametic phase disequilibrium

	TexVet1	TexVet2	TexVet6	TexVet7	TexVet9	TexVet11	TexVet12
TexVet1	—	0.04523	0.036690[a]	0.07746	0.04103	0.05133	0.44231[a]
TexVet2		—	0.13241	0.09275	0.12671	0.10843	0.06202
TexVet6			—	0.10870	0.14337	0.03727	0.06706
TexVet7				—	0.08846	0.07464	0.04067
TexVet9					—	0.10096	0.15052
TexVet11						—	0.07628
TexVet12							—
TexVet13							
TexVet14							
TexVet15							
TexVet16							
TexVet17							
TexVet18							
TexVet19							
TexVet20							

Table 3. Extended

	TexVet13	TexVet14	TexVet15	TexVet16	TexVet17	TexVet18	TexVet19	TexVet20
TexVet1	0.04831	0.07387	0.09973	0.07393	0.10863	0.09984	0.09630	0.08411
TexVet2	0.07527	0.07995	0.10188	0.09312	0.11991	0.06973	0.07637	0.08401
TexVet6	0.11607	0.15374[a]	0.06312	0.10731[b]	0.11550	0.05107	0.08025	0.12606[b]
TexVet7	0.08824[b]	0.10676[a]	0.10045	0.10519[a]	0.09438	0.08395[b]	0.10571[a]	0.07874
TexVet9	0.10257	0.12581[a]	0.16827	0.09950	0.07838	0.09469	0.09983	0.08058
TexVet11	0.08573	0.06618	0.10335	0.06883	0.08562	0.10108	0.09483	0.08081
TexVet12	0.04129	0.06365	0.09292	0.04242	0.26605[a]	0.02282	0.06275	0.06989
TexVet13	—	0.09293	0.13339[a]	0.09705[a]	0.09293	0.08775[a]	0.10651	0.09381
TexVet14		—	0.21139[a]	0.10207[b]	0.15211[a]	0.09452	0.09333	0.07849
TexVet15			—	0.25956[a]	0.10335	0.10780	0.09914	0.07926
TexVet16				—	0.10415	0.12906[a]	0.12800[a]	0.07993
TexVet17					—	0.08747	0.08435	0.08097
TexVet18						—	0.10351[a]	0.10597[a]
TexVet19							—	0.10310[b]
TexVet20								—

[a] $p < 0.01$.
[b] $p < 0.05$.

Discussion

Lande (1988) indicated that one must make the distinction between demographic and genetic bottlenecks, where the former does not result in a reduction of effective size or loss of genetic variability, unlike the latter. Our main objective was to determine if the BCB stock underwent a genetic bottleneck in response to the decrease in total population size at the beginning of this century. If lack of variability is to be used as evidence for a bottleneck, then variation at the same loci in other populations that did not experience a bottleneck might be compared to the presumed bottleneck population. This would be done in order to evaluate lack of variation in reference to background heterozygosity (e.g., Houlden et al. 1996). Unfortunately, this is not possible for bowhead whales because such reference populations do not exist. Regardless, it is a somewhat precarious approach for investigating the likelihood of a putative bottleneck. It would require the assumption that variability is the same at loci examined in nonbottlenecked reference populations and the prebottleneck BCB population. It would also require that the demographic history in both bottlenecked and nonbottlenecked populations was equivalent over the course of these populations' histories.

Low levels of heterozygosity have been taken as evidence of bottlenecks in populations known to have undergone severe demographic declines (e.g., Houlden et al. 1996). Following this logic, the relatively high estimates of average heterozygosity observed for SSR loci (Table 2) suggest that the BCB stock maintained a large long-term effective population size and did not experience a genetic bottleneck. Obviously, the accuracy of long-term effective size estimates depends on mutation-drift equilibrium (Nei and Graur 1984; Waples 1991), how well the assumed models fit the evolutionary dynamics of the SSRs under study, and the accuracy of the assumed mutation rate(s) at these SSR loci (Lehmann et

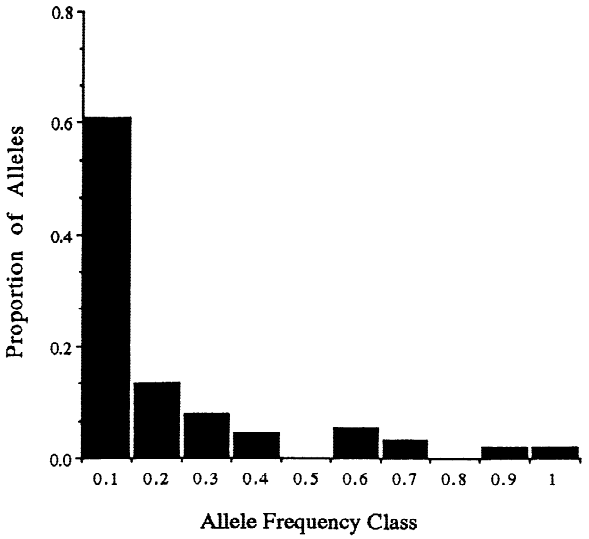

Fig. 1. Allele frequency distribution for all polymorphic SSR loci examined. An L-shaped distribution is obtained (Luikart et al. 1998), suggesting that the bottleneck of the BCB population did not affect genetic variability. The values along the *x* axis represent the maximum value for each respective allele frequency class (e.g., the first class represents alleles with frequencies between 0 and 0.1; the second, between 0.1 and 0.2; etc.).

al. 1998). However, comparisons of heterozygosity per se are not optimal for studying bottlenecks, primarily because a high level of heterozygosity may be maintained following a bottleneck (Nei et al. 1975). Instead, the number of alleles at a given locus is a better indicator of a population bottleneck because they are more sensitive to demographic fluctuations (Nei et al. 1975; Maruyama and Fuerst 1985). This is not to say that heterozygosity is not a useful parameter. Rather, it is limited and should be regarded only as a general descriptor of genetic diversity. Instead, an analysis of allelic diversity in reference to heterozygosity excess is more appropriate for testing if a bottleneck has occurred in a given population (Cornuet and Luikart 1996).

The computer program BOTTLENECK (Cornuet and Luikart 1996) was used to test if the BCB population was in mutation drift equilibrium at the SSR loci examined. This program analyzes genetic polymorphism data in terms of heterozygosity excess or deficiency. If the population were not at mutation drift equilibrium, then either a heterozygosity excess, resulting from a bottleneck or founder event, or a heterozygosity deficiency, resulting from an historical population expansion, is inferred (Cornuet and Luikart 1996). Whether a bottleneck or expansion is inferred depends on the observed number of loci displaying heterozygosity excess and deficiency relative to the expected number of loci displaying heterozygosity excess. Specifically, too few alleles will result in an excess of heterozygosity, which is indicative of a bottleneck or founder event. In the case of the BCB population, the results from Cornuet and Luikart's (1996) tests do not support the hypothesis of a recent bottleneck. Furthermore, an analysis of allele frequency distribution does not reveal evidence of a recent genetic bottleneck either (Fig. 1), as a mode shift from an L-shaped distribution would have resulted in the event that a bottleneck reduced genetic variability in a given population (Luikart et al. 1998).

The interpretation of results from bottleneck tests using SSR polymorphism data from the BCB stock are dependent on the assumed model, supporting either mutation-drift equilibrium (IAM) or an historical population expansion (SMM). Many SSR loci appear to fit a variant of the SMM (Di Rienzo et al. 1994), perhaps owing to the fact that the mutational processes of SSRs are complex and yet to be fully understood. Nevertheless, if the SMM best describes the evolutionary dynamics of most SSR loci (Shriver et al. 1993; Valdes et al. 1993; Weber and Wong 1993; Jin et al. 1996; Chakraborty et al. 1997), then analyses assuming this model are more accurate than those assuming the IAM. Evidence exists suggesting that an historical expansion might have occurred in the BCB population, following the subdivision of a potentially larger population of bowhead whales. Approximately 8500 years ago, the M'Clintock Channel sea-ice plug formed and subsequently cut off the BCB population from the Davis Strait population (Dyke et al. 1996). If these populations were connected through migration, a lowered effective size within the BCB may have resulted subsequent to the formation of the sea-ice plug. Sometime afterward, growth of the effective population size could have been initiated, leaving a signature of population expansion in the genetic polymorphism data. In fact, the results of an analysis of mtDNA control region polymorphism also point to the occurrence of an historical expansion in the BCB population (Rooney, in preparation).

Although gametic phase disequilibrium could be rejected on a tablewide level, it was found that several pairs of SSR loci were in gametic phase disequilibrium (Table 3). The probability of observing this pattern is greater than by chance alone ($p < 0.05$), and it is unlikely that size homoplasy (Estoup et al. 1995) could account for multiple loci. Nevertheless, several factors could be potentially responsible for observed gametic phase disequilibrium in pairwise comparisons. First, the BCB population may exhibit population subdivision. However, this process would have resulted in a significant tablewide probability, which was not observed. Furthermore, we sampled only whales that migrate past Point Barrow, and evidence of geographical substructuring in these whales is lacking based on previous ecological studies focused on migratory behavior and demography (Moore and Reeves 1993). Familial substructuring could cause population subdivision. Although bowheads appear to be solitary during their migration, they remain in contact with each other across long distances through vocalizations that are apparently communicative (Würsig

and Clark 1993). Individuals which are in contact with one another form core migratory groups known as "pulses," and it is possible that these pulses are established along kin lines, thus subdividing the population into family groups. Unfortunately, there is no evidence to support this hypothesis in bowhead whales. Despite these explanations, if population subdivision is occurring through either geographic or kin substructuring, one also would expect to see Hardy–Weinberg disequilibrium reflected by a significant tablewide probability, and this did not result. Second, strong drift effects could be active as a consequence of a bottleneck and thereby produce genetic disequilibria. However, there is no genetic evidence from the SSR data for a bottleneck, as described previously. Furthermore, a study of mtDNA variation in the BCB stock (Rooney 1998; Rooney et al. submitted for publication) also argues against a genetic bottleneck. Third, selection may act to produce this pattern if the loci in question are hitchhiking to a gene or functional region under selection (Slatkin 1995). Clearly, more evidence is needed to confirm this. Fourth, the SSR loci in disequilibrium may be syntenic groups. Ultimately, mapping studies are needed to confirm whether synteny (i.e., linkage) is the true cause of gametic phase disequilibrium in the affected SSR loci. Finally, the loci demonstrating gametic phase disequilibrium may contain null alleles, or alleles that are actually present but fail to be detected, for example, due to mutations in the priming sites. It was found that three of the five loci (Tex Vet6, -15, and -18) had substantially large *r* values (values larger than 0.1). However, this does not necessarily prove that null alleles are the true cause of heterozygote deficiency because the method used (equation 3 of Brookfield 1996) assumes that the presence of null alleles is the actual cause of a statistically significant heterozygote deficiency. But the deviation of the expected frequency of heterozygotes from the observed, which is used to estimate *r* (Brookfield 1996), might be caused by factors other than null alleles in any given situation. Therefore, alternative primer designs and possibly sequencing studies are needed to prove whether null alleles are a problem or not. In light of this discussion, the latter three explanations of heterozygote deficiency are preferred, although at this time one cannot be considered better than the others.

Conclusions

The results of this study indicate that the commercial whaling bottleneck of the BCB stock did not affect genetic variability in this population of bowhead whales. This is in agreement with the conclusions from a study of mtDNA polymorphism in the BCB stock (Rooney et al., submitted for publication), where evidence of bottleneck effects on genetic variability could not be found. If it can be assumed that the SMM generally applies to the loci used in this study, an historical expansion of the BCB stock can be inferred, perhaps having initiated some time subsequent to the formation of the M'Clintock Channel sea-ice plug, approximately 8500 years ago (Dyke et al. 1996). Finally, while we suspect that null alleles may be the true cause of the statistically significant heterozygote deficiency at five SSR loci, it remains to be proven whether null alleles are actually responsible for this pattern or if it is attributable to some other process.

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