Population Genetics of Bowhead Whales (*Balaena mysticetus*) in the Western Arctic

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ABSTRACT. Bowhead whales (*Balaena mysticetus*) in the Bering, Chukchi, and Beaufort seas experienced a severe reduction as a result of commercial whaling in the 19th century. Since the cessation of commercial whaling, the population has recovered to a size that is approaching pre-whaling estimates. Inupiat and Yupik communities in northern and western Alaska hunt these Western Arctic (WA) bowheads along their migratory path during spring and fall. This hunting is regulated by the International Whaling Commission. Recent but preliminary analysis of available genetic data (207 whales and 10 microsatellite markers) raised the question of the presence of multiple, genetically distinct populations within the WA bowheads. Here we re-examined this question on the basis of a study of 414 whales and 22 newly developed microsatellite loci. We identified widespread departures from Hardy-Weinberg equilibrium; however, we were unable to detect significant evidence of multiple genetic populations within the WA bowheads that could explain this Hardy-Weinberg disequilibrium, particularly when compared to the strength of evidence for differentiation between WA bowheads and other populations from distant regions such as the Okhotsk Sea and eastern Canada. There was conclusive evidence of genetic differentiation among the three regions. The statistical rejection of panmixia within the WA improves our understanding of bowhead whale biology, and the lack of evidence for multiple populations within the WA enables risk-averse management of aboriginal hunting of Western Arctic bowhead whales.

Key words: population structure, temporal substructure, Hardy-Weinberg disequilibrium, genetic bottleneck, conservation, bowhead whale, Bering, Beaufort, Chukchi, genetic analysis, population differentiation, Alaska

RÉSUMÉ. La population de baleines boréales (*Balaena mysticetus*) des mers de Béring, de Tchoukotka et de Beaufort a enregistré un grave déclin en raison de la pêche commerciale à la baleine au XIXᵉ siècle. Depuis que la pêche commerciale à la baleine a cessé, la population de baleines boréales a connu un certain essor au point où elle approche maintenant les estimations de la taille qu'elle avait avant la pêche commerciale à la baleine. Les collectivités Inupiat et Yupik du nord et de l'ouest de l'Alaska chassent les baleines boréales de l'ouest de l'Arctique le long de leur voie de migration au printemps et à l'automne. La chasse est réglementée par l'International Whaling Commission. Des analyses récentes, bien que préliminaires, des données génétiques disponibles (207 baleines et 10 marqueurs microsatellites) ont soulevé la question de la présence de multiples populations génétiquement distinctes au sein de la population de baleines boréales de l'ouest de l'Alaska. Ici, nous avons réexaminé cette question en fonction de l'étude de 414 baleines et de 22 locis microsatellites nouvellement mis au point. Nous avons remarqué d'importantes déviations de l'équilibre de Hardy-Weinberg; toutefois, nous n'avons pas pu trouver de preuve significative de populations génétiques multiples au sein des baleines boréales de l'ouest de l'Alaska qui pourrait expliquer ce déséquilibre de Hardy-Weinberg, plus particulièrement en comparaison avec la force de la preuve de différenciation entre les baleines boréales de l'ouest de l'Arctique et d'autres populations de régions distantes telles que la mer d'Okhotsk et l'est du Canada. Il y avait des preuves concluantes de différenciation génétique entre les trois régions. Le rejet statistique de la panmixie au sein de l'ouest de l'Arctique améliore notre compréhension de la biologie des baleines boréales, et le manque de preuves de populations multiples dans l'ouest de l'Arctique donne lieu à la gestion de l'aversion au risque de la chasse à la baleine boréale de l'ouest de l'Arctique par les Autochtones.

Mots clés : structure de la population, sous-structure temporelle, déséquilibre de Hardy-Weinberg, étranglement génétique, conservation, baleine boréale, Béring, Beaufort, Tchoukotka, analyse génétique, différenciation de la population, Alaska

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INTRODUCTION

The bowhead whale (*Balaena mysticetus*) is a large baleen whale that inhabits the Arctic Ocean and surrounding areas. Recent studies have led to the recognition of four geographically separated populations of bowhead whales in (i) the Western Arctic, (ii) the Okhotsk Sea, (iii) the eastern Canadian Arctic, and (iv) areas around Svalbard (Spitsbergen) Island in the North Atlantic (Rugh et al., 2003; Heide-Jørgensen et al., 2006). Whereas earlier concepts of bowhead population structure were based primarily on indirect evidence, such as migration patterns and geographic distribution, our current understanding is based not only on such indirect evidence, but also on empirical evidence from satellite tracking, stable isotope analysis, and population genetic analyses (Moore and Reeves, 1993; Rugh et al., 2003; LeDuc et al., 2005; Heide-Jørgensen et al., 2006; Borge et al., 2007).

All four bowhead populations were subjected to extensive commercial hunting, which began in the 16th to the 19th century, depending on the population (Burns et al., 1993). During this period bowhead populations were severely reduced or nearly extirpated. The historical period of commercial whaling of Western Arctic (WA) bowheads (1848–1914) was distinguished by a very strong spatio-temporal pattern of exploitation, some age-selective hunting, and a severe reduction of the population, perhaps to 1000 or fewer animals (Bockstoce and Botkin, 1983; Bockstoce and Burns, 1993). The cessation of the commercial hunt was caused in part by the decline of population sizes to levels that made whaling economically unviable (Bockstoce and Botkin, 1983; Burns et al., 1993). Today, the only remaining sizable populations of bowheads inhabit the Western Arctic region (George et al., 2004b) and the eastern Canadian Arctic (Heide-Jørgensen et al., 2007).

The WA population winters in the Bering Sea near the marginal sea ice edge. In spring, the bowheads migrate north and east to their primary summer feeding grounds in the Canadian Beaufort Sea. Mating occurs during the early portion of the spring migration. In the fall, the whales follow a westerly migration along the Beaufort coast towards the Chukotka Peninsula, where they turn south towards the Bering Strait and subsequently return to their wintering grounds in the Bering Sea. Figure 1 shows the range of these whales and the villages near which hunting (and genetic sampling) occurs. A more thorough description of the migration is given by Rugh et al. (2003) and Moore and Reeves (1993). WA bowheads have been hunted by aboriginal communities on the North Slope of Alaska, along the Chukotka Peninsula, and in the Bering Sea for more than 2000 years (Stoker and Krupnik, 1993). The WA population has recovered sufficiently from commercial whaling to support hunting, and it remains the only population that is currently harvested annually. (However, Canada and Greenland may soon establish an allowance for hunting of eastern Canadian bowheads.) A quota for the WA harvest is set in accordance with International Whaling Commission (IWC)
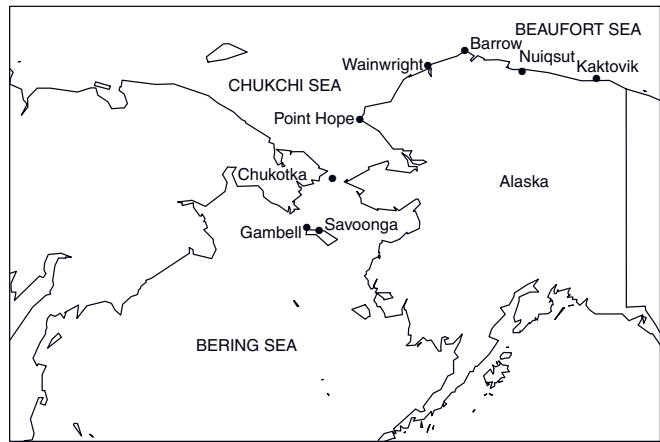

FIG. 1. Map showing the locations of the villages from which samples of Western Arctic bowheads were obtained for genetic analyses (Table 1). The dot in the Bering Strait represents Little Diomede. Samples were also taken from several small hunting villages on the Chukotkan Peninsula (not shown). All samples were from animals harvested in the subsistence hunt sanctioned by the International Whaling Commission and provided by the whaling captains of the respective villages.

decisions. In order to provide proper management advice, the IWC considers multiple sources of data, simulation-tested modeling, and other evidence to estimate sustainable quotas (IWC, 2003). Evaluation of population structure hypotheses and estimates of genetic diversity within and among populations is critical for the IWC to establish sustainable management policies.

Consideration of genetic patterns of bowheads must take into account the important connection between bowhead biology and the history of hunting in the Western Arctic. Bowhead whales are one of the longest-lived species of mammals (George et al., 1999). For example, five of 84 landed bowheads aged using aspartic acid racemization were more than 100 years old, with the oldest estimated to be 178 years old (Rosa et al., 2004). Because of the longevity and low reproductive rate of bowheads (Rugh et al., 1992; Koski et al., 1993), the effects of commercial whaling could have left a persisting genetic imprint. However, previous tests have found no statistically significant evidence of genetic bottlenecks in WA bowheads (Rooney et al., 1999a, 2001; Hunter, 2005).

Evidence of other deviations from genetic homogeneity among WA bowheads has been reported from earlier analyses of available genetic data from 207 whales (Givens et al., 2004; Pastene et al., 2004; Jorde et al., 2007). The findings of Jorde et al. (2007) indicated that bowheads traveling 5–11 days apart while migrating past Barrow in the fall of each year were significantly less genetically similar than those traveling at other temporal separations. This pattern of genetic dissimilarity suggested that there could be two genetically differentiated populations migrating past Barrow in the fall, with slightly offset migration timing. Traditional knowledge and hunter observations also noted variations in spring migratory patterns by bowheads around St. Lawrence Island (Noongwook et al., 2007). Preliminary genetic analyses (Givens et al., 2004) revealed evidence that some genetic

TABLE 1. Number of WA bowhead samples used for analysis categorized by harvest season and village. No seasonal data were provided for the eastern Canadian Arctic and Sea of Okhotsk samples.

	Samples with Microsatellite Data	Spring	Fall
Western Arctic Locations			
Barrow	231	108	123
Chukotka	15	3	12
Gambell	9	5	4
Kaktovik	15	0	15
Little Diomede	1	1	0
Nuiqsut	5	0	5
Point Hope	6	6	0
Savoonga	16	6	10
Wainwright	7	7	0
Western Arctic Total	305	136	169
Outgroups			
Eastern Canadian Arctic: Igoolik, Canada	47		
Sea of Okhotsk	62		
All Population Total	414		

loci were not in Hardy-Weinberg equilibrium; that is, allele frequencies differed from what would be expected under a set of assumptions regarding an idealized population, one of these being that the samples represent a single, well-mixed population. The analyses also showed significant differences between animals harvested at St. Lawrence Island (SLI) and at Barrow (n = 11 and 177, respectively; 11 markers; p = 0.004), even though SLI animals are thought to be among those that migrate past Barrow (Moore and Reeves, 1993; Rugh et al., 2003). Pastene et al. (2004) found significant differences in mtDNA haplotype frequency between bowheads harvested at Barrow in spring and fall. The finding of genetic differences associated with migratory patterns around SLI and the significant temporal genetic patterns at Barrow contributed to the development of hypotheses that the WA population might consist of multiple, genetically differentiated subpopulations. The observed migratory variation and preliminary genetic results described above motivated the hypotheses that we investigate here, using a more comprehensive data set.

Population structure studies by Jorde et al. (2007), Pastene et al. (2004), and Givens et al. (2004) were based on genetic data produced by members of the United States Scientific Delegation to the IWC (Hunter, 2005) and consisted of 10 microsatellite loci developed by Rooney et al. (1999a, b), Valsecchi and Amos (1996), and Palsbøll et al. (1997). We considered these data to be preliminary, given the small sample sizes of whales harvested at villages other than Barrow and the evidence of scoring difficulties with some of the loci (Bickham et al., 2004). The data presented in this paper include 22 new loci (Huebinger et al., 2008) with better scoring properties, as well as a larger number of whales.

Resolution of population structure questions (to the extent possible) using these data was critical for the IWC's 2007 estimation of safe hunting quotas because risk-averse resource management would have been difficult to provide if the number and identity of populations hunted was

uncertain. One key purpose of our work has been to test the hypothesis that the genetic patterns observed in analyses of the earlier data resulted from the presence of multiple genetically differentiated subpopulations within the WA population. Here we present an analysis of 22 microsatellite loci from 414 bowheads, including samples from the WA population and two other populations (in the Okhotsk Sea and eastern Canada) that were included in the analysis as outgroup populations—which proved to be critical to this analysis. Cross-population comparisons provided an important perspective from which to interpret our findings.

MATERIALS AND METHODS

Tissue samples were collected from 414 individual whales representing three previously described populations of bowheads (Table 1). The majority of samples were obtained from harvested animals, and the rest were obtained via non-lethal skin biopsy. A detailed list of number of samples per sampling location and season is provided in Table 1. The data set includes roughly equal numbers of males and females and a wide distribution of ages. About 75% of the Western Arctic samples were collected at Barrow. We analyzed 24 bowhead-specific microsatellite loci (Huebinger et al., 2008). Genomic DNA was isolated from tissue samples, and PCR amplification was performed in a 25 μl reaction volume using an ABI2700 thermocycler (Perkin-Elmer; Foster City, CA) with approximately 50 ng of genomic DNA as template. Final amplification conditions consisted of 12.5 pmol unlabeled reverse primer, 12.5 pmol fluorescently labeled forward primer, 1.5 mM $MgCl_2$, 200 μM each dNTP, and 0.5 units of *Taq* DNA polymerase (Promega; Madison, WI). The PCR amplification profile was 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, a primer-specific annealing temperature for 30 sec (Huebinger et al., 2008), 72°C for 30 sec, ending with a single extension of 72°C for 10 min. Allele sizes were determined by fragment separation on an ABI3100 DNA Analyzer (Applied Biosystems, Inc; Foster City, CA). Fragment lengths were assigned by the GeneMapper software program (Applied Biosystems, Inc.) using the GeneScan-400 [ROX] size standard. Samples that produced poor quality chromatograms or failed to amplify were reanalyzed. Additionally, a portion of the data set was reanalyzed to calculate a per-allele error rate for the data set. The per-allele error rate was estimated to be between 1% and 2.4%, which is low and similar to some published observed error rates (Morin et al., 2009). Two loci (Bmy38, Bmy44) were excluded from our analyses because of extreme and statistically significant excesses of homozygosity. These two loci, analyzed using the methods of van Oosterhout et al. (2004) and Chakraborty et al. (1992), showed estimates of null allele frequencies that were four and six times as large, respectively, as those observed for the other 22 loci.

The conceptual approach for our statistical analysis of population structure is best described as a weight-of-

evidence approach that accumulates evidence from a variety of analyses. No one of our analysis methods is intended to represent the crux upon which all other results hinge. In the discussion, we examine how the balanced results from our various analyses generally converge toward an overall conclusion.

Version 3.4 of the program GENEPOP (Raymond and Rousset, 1995) was used to test for departures from Hardy-Weinberg equilibrium, including heterozygote deficiency; to test for evidence of linkage disequilibrium; and to compare allele frequencies and genotypic differentiation among various seasonal and spatial groups. Corresponding F-statistics (the fixation index F_{st} and the inbreeding coefficient F_{is}) and their associated confidence intervals were calculated using FSTAT (Goudet, 2005). Confidence intervals for F-statistics were obtained by bootstrapping over loci. The program STRUCTURE (Pritchard et al., 2000; Falush et al., 2003) was also used to identify potential clustering in the data. The admixture model with correlated allele frequencies was chosen for analysis, and 1 000 000 iterations were used for parameter estimation (after discarding 50 000 initial burn-in iterations).

Tests for subtle temporal structuring within the migration of WA bowheads (as suggested by Jorde et al., 2007) were conducted using the method of Givens and Ozaksoy (2007). Their approach computes a measure of pairwise genetic distances between sampled whales and estimates the degree to which such distances are correlated with pairwise covariates, such as those related to the temporal distribution or the ages of whales. To understand the method, consider the outcome of whether or not two alleles sampled entirely at random from the entire data set match (i.e., are identical in state). In an idealized panmictic population, such pairwise allele match outcomes should be independent of whether the alleles originate from the same individual or from different individuals. Likewise, match outcomes should be independent of any other potential covariates. However when sampling occurs from more than one genetically distinct population, allele-matching frequencies are greater for within-whale pairwise allele comparisons than for between-whale comparisons because of the Wahlund effect (Hartl and Clark, 2007). The barriers to panmixia that maintain genetic population differentiation induce within-population genetic correlation. With respect to covariate effects, the argument is similar. Suppose that genetic frequencies co-vary with some other variable. For the sake of argument, consider binning the pairwise outcomes into groups on the basis of pairwise covariate values. Then, by the same reasoning as for the Wahlund effect, a within-group genetic correlation is induced. The Givens and Ozaksoy (2007) method treats covariates continuously rather than binned. It estimates both types of effects using a model that relates pairwise allele matching probabilities to the within/between whale factor and to pairwise covariate values. To the extent that genetic correlations of the above sorts are present in the data, the model can estimate them and provide a Monte Carlo (permutation) basis for hypothesis testing.

TABLE 2. P-values for tests of deviation from Hardy-Weinberg equilibrium and heterozygote deficiency, by locus, for bowhead whale samples from Barrow. Corresponding estimates of the inbreeding coefficient (F_{is}) are also shown .

Locus	HW Equilibrium	Heterozygote Deficiency	F_{is}
Bmy1	0.1634	0.4156	-0.0060
Bmy2	0.9280	0.1566	0.0220
Bmy7	0.5032	0.0778	-0.0260
Bmy8	0.5102	0.2552	0.0250
Bmy10	0.5361	0.1079	0.0270
Bmy11	0.5163	0.7301	-0.0070
Bmy12	0.8508	0.1412	-0.0160
Bmy14	0.3201	0.0164	0.0640
Bmy16	0.4027	0.3117	-0.0220
Bmy18	0.7836	0.1244	0.0190
Bmy19	0.1658	0.4242	0.0230
Bmy26	0.5160	0.3757	0.0100
Bmy33	0.0078	0.4279	0.0150
Bmy36	0.6778	0.4587	0.0000
Bmy41	0.0514	0.0243	0.0070
Bmy42	0.2184	0.0234	0.0850
Bmy49	0.0188	0.3321	0.0120
Bmy53	0.4350	0.0319	0.0010
Bmy54	0.1727	0.0040	0.0540
Bmy55	0.0114	0.0756	0.0360
Bmy57	0.0487	0.0000	0.0500
Bmy58	0.0852	0.4305	-0.0080

The program Bottleneck version 1.2.02 (Cornuet and Luikart, 1996) was used to test for evidence of a genetic bottleneck.

We used a threshold of 5% ($p \leq 0.05$) for assessing the significance of effects, hypothesis tests, and confidence intervals. Some of our analyses raised the statistical issue of multiple comparisons, i.e., the dilution of statistical significance due to observing some p-values in a large collection to be less than 0.05 by chance alone. Where appropriate, we used the method of Fisher (1935) to control multiple comparisons. In other cases where the concern might arise, the problem is actually moot since the individual findings were non-significant. In a few cases the p-values were so extreme as to preclude concern.

RESULTS

Our results revealed strong departures from Hardy-Weinberg equilibrium among the Barrow samples (Table 2). Six of the 22 loci exhibited heterozygote deficiency at the nominal 0.05 significance level, for an overall p-value of 0.0002 using Fisher's method (Fisher, 1935). Levels of disequilibrium for individuals harvested at Barrow were statistically significant in the fall samples ($p < 0.0001$), but not in the spring samples ($p = 0.20$). No significant heterozygote deficiencies were observed for other spatial strata. Tests for linkage disequilibrium detected 26 of 231 significant comparisons at the nominal 0.05 level. Nineteen of the 22 loci analyzed exhibited between one and six significant linkage associations (Table 3). Analysis using the program Bottleneck gave a non-significant result for the Wilcoxon sign-rank test using 95% single-step mutations, and a variance of 12 as recommended by Piry et al. (1999). Analysis using the

TABLE 3. List of significant linkages per locus. Linkages are listed cumulatively to avoid repeating instances such as, for example, the linkage between Bmy2 and Bmy57.

Locus (Bmy)	Significantly Linked to
2	57, 58
7	11
8	11, 19, 55, 57
10	54, 55
11	14, 41, 42, 55
12	14, 16
14	19, 33
16	18, 19, 57
18	55
19	41
41	54
49	57
53	58
57	58

TABLE 4. F_{st} estimates with accompanying 95% confidence intervals for comparisons between various spatial groups and between possible temporal groups (light- and mid-toned) identified by the STRUCTURE analysis. Estimates significantly different from zero are indicated by asterisks.

Strata	F_{st}	95% Confidence Interval
Canada vs. Okhotsk	0.039*	(0.028, 0.051)
Barrow vs. Okhotsk	0.034*	(0.026, 0.043)
Barrow vs. Canada	0.006*	(0.002, 0.009)
Barrow vs. St. Lawrence Island	0.002	(-0.001, 0.006)
Light- vs. mid-toned WA temporal division	0.000	(-0.001, 0.001)

method of Givens and Ozaksoy (2007) found no age-related pattern to support the conjecture by Taylor et al. (2007) that a genetic imprint was left by the historical period of severe population depletion.

Within the WA population, no significant levels of genotypic differentiation or allele frequency differences were detected between any of the sampling locations of Barrow, Little Diomede, Point Hope, Wainwright, St. Lawrence Island, Kaktovik, and Nuiqsut. In particular, samples from St. Lawrence Island (Gambell and Savoonga pooled) and Barrow were not significantly different ($p = 0.40$). The St. Lawrence Island (SLI) villages of Gambell and Savoonga were not significantly different ($p = 0.34$). This provides additional support for pooling the sampling locations into one geographic sampling location. The absence of significant findings for various SLI comparisons is contrary to a previous preliminary result based on a smaller data set (Givens et al., 2004). Our estimate of F_{st} (fixation index) for the comparison of Barrow and SLI, including the 95% confidence interval, is shown in Table 4.

On a regional scale, genotypic comparisons between the WA and other populations were significant ($p < 0.00001$). Tests for allelic differentiation identified significant differences in allele frequencies for 21 of 22 loci between the Western Arctic and Okhotsk populations and 9 of 21 loci between the WA and the eastern Canadian Arctic populations. These differences resulted in overall significantly different allele frequencies between the WA and Okhotsk populations ($p < 1 \times 10^{-10}$) and between the WA and eastern Canadian Arctic populations ($p < 1 \times 10^{-10}$). Table 4 reports F_{st} estimates for these comparisons.

As another method to examine potential spatial differences within the Western Arctic, the program STRUCTURE was used to analyze the data for differing hypothesized numbers of populations (K = 1, …, 5). The whales were organized into 15 spatial/seasonal groups for the STRUCTURE output plots. In Figure 2, whales within each group were ordered sequentially from left to right according to the Julian date when the whale was taken. Plots for varying numbers of K are shown (Fig. 2).

In tests for temporal structuring within the Western Arctic, comparisons of allele frequencies between the spring and fall harvest were not significant. Tests for temporal structuring within the fall migration using the method of Givens and Ozaksoy (2007) also did not reveal significant patterns. A plot of the estimated pairwise allele match probabilities arranged by pairwise days apart in the fall migration is shown in Figure 3, with 95% joint coverage null probability bands shown with the dotted lines. As Givens and Ozaksoy (2007) explain, the presence of temporal differentiation in the fall migration should be signaled by a time period in which the solid curve representing different-whale allele match probabilities estimated by the model lies outside the null probability bands. Since our estimated match probability function does not fall outside the 95% null probability bands for temporal lag separations greater than zero, the p-value for rejection of the null hypothesis exceeds 0.05, providing no significant evidence for temporal substructure.

DISCUSSION

Commercial whaling significantly reduced population sizes in all bowhead populations. The WA population, estimated to have numbered about 9900 to 14 000 individuals prior to commercial harvest (Brandon and Wade, 2006; Punt, 2006), was reduced to perhaps 1000 or fewer individuals by the time commercial whaling ceased in 1914. George et al. (2004b) estimate that the population had recovered to approximately 10 470 in 2001 (with a 95% confidence interval of 8100 to 13 500), and it continues to grow annually at the substantial rate of 3.4% (1.7% to 5.0%). Although the WA population experienced a severe demographic bottleneck, the present analysis is consistent with previous analyses (Rooney et al., 1999a; Hunter, 2005) that were unable to detect the presence of a genetic bottleneck. This is likely because of the short duration of the demographic bottleneck relative to the generation time of the species. Some individuals that were alive during and prior to the demographic bottleneck have survived and are still found in the population. Since there is little evidence of reproductive senescence in bowheads (George et al., 2004a), it is likely that these very old whales are still contributing to the reproduction of the
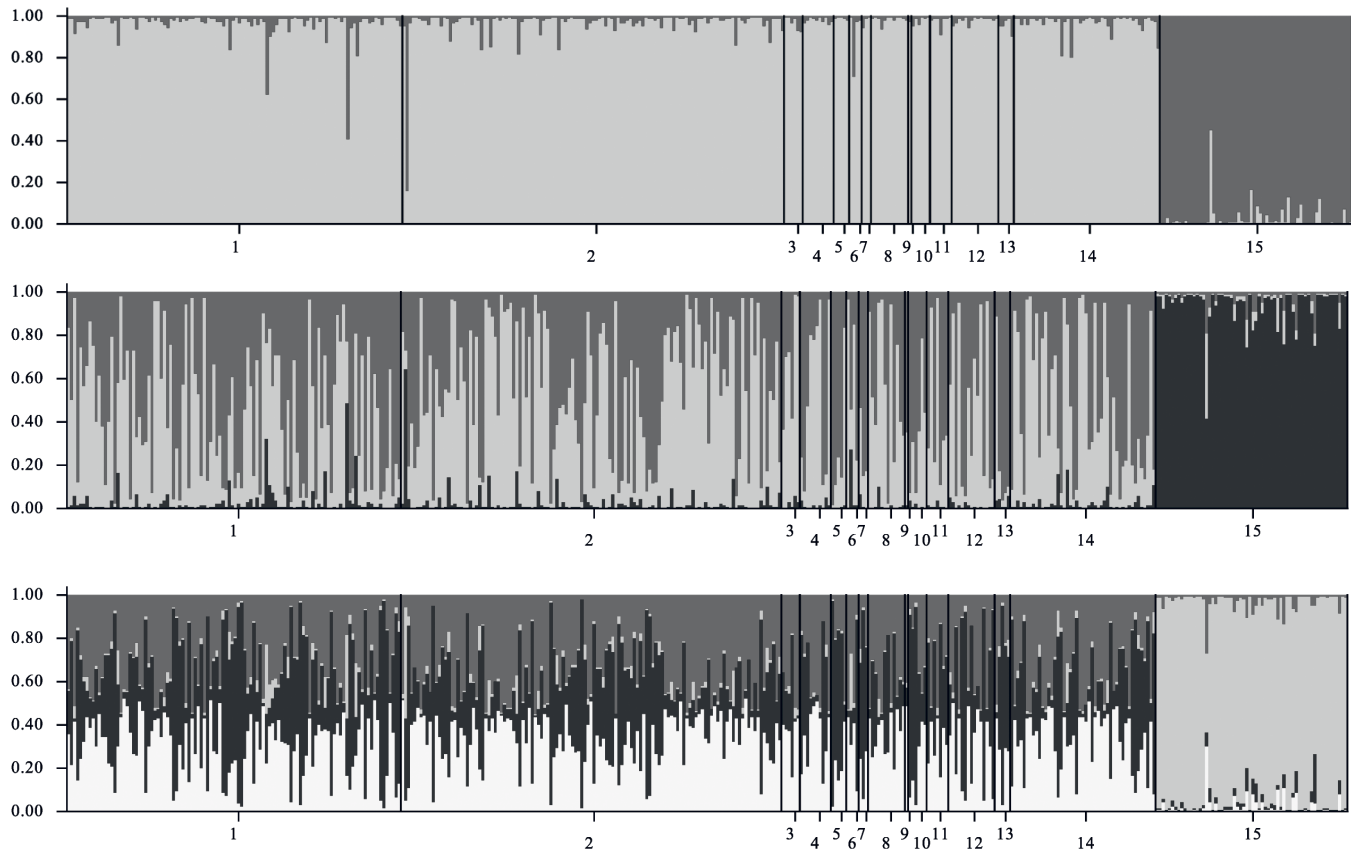
FIG. 2. STRUCTURE clustering results for K = 2, 3, and 4, from top to bottom. Group labels are: 1 = spring Barrow; 2 = fall Barrow; 3 = spring Savoonga; 4 = fall Savoonga; 5 = spring Gambell; 6 = fall Gambell; 7 = spring Chukotka; 8 = fall Chukotka; 9 = (spring) Diomede; 10 = (spring) Point Hope; 11 = spring Wainwright; 12 = (fall) Kaktovik; 13 = (fall) Nuiqsut; 14 = Igoolik, Canada; 15 = Okhotsk. Within each of these 15 groups, whales are ordered sequentially by calendar day from left to right.

population. Archer et al. (in press) show that simulations of WA bowhead population dynamics and biology also fail to yield data sets in which significant genetic bottleneck effects can be detected.

Notwithstanding the apparent absence of a genetic bottleneck, genetic disequilibria have been reported in each of the microsatellite analyses of the WA population, including this study. The genetic disequilibria we found within the WA population could be caused by the mixing of multiple subpopulations after the cessation of commercial whaling; however, it is more likely due to sampling variability, measurement error or age effects such as sampling individuals that were born before the end of commercial whaling and whales born during the population recovery. Taylor et al. (2007) describe several single-population scenarios for which age-correlated genetic differences could result in observed patterns of spatio-temporal genetic variation that might be wrongly interpreted as evidence for multiple populations. The disequilibrium identified within the WA population is not likely the result of a Wahlund effect because if it were, the loci that were out of Hardy-Weinberg equilibrium should exhibit significant heterozygote deficiencies. This was the case only for Bmy57; the other three loci with significant disequilibria (Bmy33, Bmy49, and Bmy55) did not show significant heterozygote deficiencies (Table 2).

Another explanation for the rejection of Hardy-Weinberg equilibrium (HWE) in our analysis is that apparent disequilibrium within the WA population could be due to a few unusual observations. For many villages, the number of whales sampled was quite small. Although the number of markers was large, it is difficult to draw firm scientific conclusions about differences in allele frequency between villages where few whales were sampled. To investigate the potential effects of small numbers of whales, Morin et al. (2007, 2009) assessed the sensitivity of Hardy-Weinberg equilibrium to errors in the data set. They used the jackknife approach (e.g., Efron and Tibshirani, 1993) to assess the contributions of each whale to Hardy-Weinberg disequilibrium. Each whale was individually removed from the data set, and the p-value for rejection of HWE was recomputed in each case. Each whale's contribution to the overall rejection of HWE in the original data set was measured by the change in p-value from the original result to the result with that whale deleted. Changes in p-values were quantified by the odds ratio. This jackknife analysis identified 35 cases in which removal of a single sample changed a locus from being out of HWE ($p < 0.05$) to being in HWE ($p > 0.05$). In six jackknife instances, removal of a single individual (whale 83B1, 96B11, 99B3, 02B6, 02B16, or 05B7) had a disproportionate effect on the rejection of HWE

(log-odds ratio between jackknife and observed *p*-values > 2.0). Each of these samples was homozygous for a rare allele (an allele with frequency of less than 6%) at the locus under consideration. Additionally, the degree of the difference in the HWE *p*-value between the original and jackknife replicates was directly related to the frequency of this rare allele. These results suggest that observed deviations from HWE are attributable to homozygous rare genotypes at a small number of loci in a very small number of individuals. In nature, such genotypes would most likely result from inbreeding, but the presence of such genotypes in our data set is more likely due to the indistinguishable occurrence of a rare allele/null allele genotype or to laboratory error, such as incorrect scoring. To the extent that these rare alleles in a few influential individuals potentially bias tests for HWE, they may also affect other analyses we describe here.

The level of linkage disequilibrium detected in the data (26 of 231 comparisons were significant, and 19 of 22 loci showed 1–6 significant linkage associations) was higher than expected and is too high to be explained by physical linkage. Factors that have previously been shown to produce linkage disequilibrium include non-random mating, familial relationships in the sampled individuals, factors related to the recent demographic history of the population (e.g., bottleneck), and sampling from multiple populations (Slatkin, 2008; Tenaillon et al., 2008; Wang et al., 2008). Of these, multiple populations and a bottleneck are possible, but not supported by other results here; non-random mating is plausible, assuming a sperm competition model, as we observed a few large males with very large testes (O'Hara et al., 2002); familial relationships among the samples are strongly suspected (Skaug and Givens, 2007).

Whalers from SLI have observed two paths taken by bowhead whales during the spring migration (Noongwook et al., 2007). In the spring, hunters from the village of Savoonga hunt from the southwest side of the island, near Southwest Cape. They report that the whales they hunt approach from the southeast. However, they recognize another group of whales that passes Southwest Cape far offshore and becomes available to hunters from the village of Gambell at the northwest tip of the island. The Gambell hunters confirm these observations, saying that the bowheads they hunt approach Gambell from the southwest, and then head northeast after passing Gambell. Migratory traffic on these two paths is said to be negatively correlated: if the whales are seen in large numbers at Southwest Cape they are unlikely to be available at Gambell at the same time. The hunters do not know whether these two migratory paths represent routes of two distinct groups of whales, or whether they represent alternative routes chosen at various times by various portions of the same population. Considering that both putative groups commingle in the passage between SLI and Chukotka during the early spring migration, some degree of interbreeding seems plausible for two reasons. First, aerial surveys have reported a considerable amount of mating behavior in this region. Second, several researchers (Nerini et al., 1984; Reese et al., 2001; George

TABLE 5. Estimates of the log of P[Data|K] for K = 1,..., 5, using the correlated admixture model in STRUCTURE. The estimates are adjusted by an additive constant of 37513 for clarity.

K	log(P[Data|K])
1	1
2	735
3	836
4	880
5	857

et al., 2004a) have estimated from the size of fetuses collected from harvested whales that mating occurs during this period. No significant genetic difference was detected between animals harvested by the two SLI villages. Additionally, the lack of significant genetic differences between harvest locations throughout the Western Arctic suggests the presence of a single genetic population migrating through this region. In concordance with the non-significant allele frequency differences between Barrow and SLI, we found that the F_{st} value between Barrow and SLI was small, with a 95% confidence interval encompassing zero. By comparison, F_{st} values estimated between separate regional populations are 3–20 times as large as this estimated F_{st} (Table 4).

The results from STRUCTURE analyses require careful consideration. For example, Table 5 shows estimates of P[Data|K] that increase with K (the number of hypothetical populations assumed). This support for larger K cannot simply be ignored because it potentially conflicts with the aforementioned general lack of evidence for allelic differentiation and significant F_{st} values. However, inference is complicated by the limitations of STRUCTURE (e.g., Evanno et al., 2005; Waples and Gaggiotti, 2006; Kaeuffer et al., 2007). Notably, the developers of STRUCTURE indicate that their method for statistical inference for the number of populations (K) can only be viewed as a rough approximation (Pritchard et al., 2000). One consequence is that the model used in the analysis tends to overestimate the likely value of K. If one overlooks this criticism, it is possible to examine estimates of P[Data|K] and therefore the posterior probabilities for K under a discrete uniform prior. Although the P[Data|K] estimates increase with K, the method of Evanno et al. (2005) and the evidence of diminishing returns in Table 5 both support a single WA population. Moreover, Figure 2 also shows that the selection of K = 2, which differentiates the Okhotsk population from the combined WA and eastern Canadian Arctic populations, provides a greatly improved fit over K = 1, but there is little to be gained for K > 2.

In a further exploratory analysis, we used STRUCTURE to identify a putative scenario of two clusters within the Western Arctic. The K = 3 result provides such a scenario (Fig. 2, middle panel). We used this result to explore how WA bowheads might be assigned to groups if multiple clusters were forced. The resulting pattern of estimated ancestries among whales sampled from the fall migration
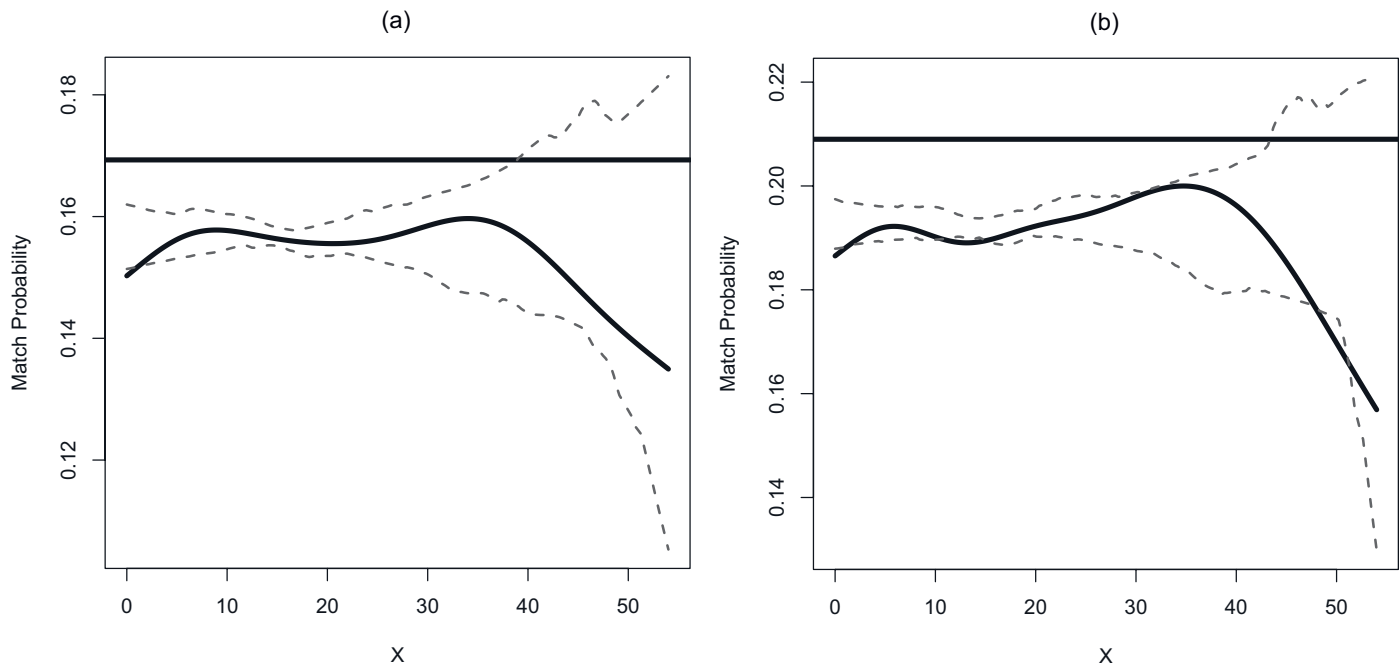
FIG. 3. Results of two analyses to detect temporal genetic differences within the Western Arctic using the method of Givens and Ozaksoy (2007). The flat solid line indicates the estimated homozygosity. The curved solid line represents estimated match probabilities for pairwise comparisons of alleles from individuals harvested and is used to make inference about temporal effects (see text). The x-axis represents the days apart that two individuals were harvested. Dotted lines represent the 95% joint coverage null probability bands, outside of which would lie statistically significant results. Panel (a) uses the 22 loci analyzed in this paper. Panel (b) uses 33 microsatellite loci.

at Barrow could be interpreted as exhibiting a weak degree of temporal pulsing of whales of differing ancestry. These pulses can be seen by noting the wave-like appearance in the middle panel of Figure 2 in which whales assigned to the lightest-toned ancestry alternated with groups assigned to mid-toned ancestry. Such oscillation, if it were real, would be consistent with the findings of Jorde et al. (2007). However, a comparison of WA fall individuals that are assigned to these two clusters according to their predominant estimated ancestry (Table 4) shows no detectable evidence for genetic differentiation between the two forced groups; see Table 4, where F_{st} = 0.000 with 95% confidence interval from -0.001 to 0.001. This result suggests that the potential population subdivision within the Western Arctic represented by STRUCTURE is either smaller than would be indicative of separate breeding subpopulations or spurious.

Application of the method of Givens and Ozaksoy (2007) identified no significant temporal correlation or pulsing pattern with the 22 loci used here (Fig. 3a). A sensitivity analysis was conducted by incorporating 11 more microsatellite loci, including the 10 used by Jorde et al. (2007) and one they set aside to test for the existence of a significant temporal structure with all 33 loci. In the test of all 33 loci, a significant effect was identified (Fig. 3b). The two-week interval detected in this analysis (10–16 days) was longer than that in the results of Jorde et al. (5–11 days), but still somewhat consistent with their hypothesis of pulsing. The different findings from the two data sets suggest that the signal is essentially confined to the 10 loci used by Jorde et al. (2007), which is one important reason why our findings

are not a refutation or contradiction of the Jorde et al. (2007) result.

Note that in Figure 3(b), the average match probability for alleles within the same whale (flat line) is higher than the probability for alleles from two different whales (curved line) during the 5–16 day range discussed above. This finding implies that even after controlling for the effect of any temporal correlation pattern that could be identified by examining capture time differences, there is still evidence of non-specific excess homozygosity in the data. This evidence suggests that the findings of Jorde et al. (2007) are probably not the sole source, and perhaps not the primary source, of the Hardy-Weinberg disequilibrium reported for the WA population.

It is worth considering whether the differing results from the temporal analyses using 22 and 33 loci could be related to the reliability of scoring. Some sorts of data quality issues can be investigated empirically or controlled, as we describe below. Jorde et al. (2007) also describe their procedure of re-typing and other quality checks. Some data quality concerns are as follows. The earlier 10 loci were chosen opportunistically from the available literature, using markers derived from sperm whales (Valsecchi and Amos, 1996), humpback whales (Palsbøll et al., 1997), and bottlenose dolphins (Rooney et al., 1999b), as well as bowheads (Rooney et al., 1999b). Some of these loci have exhibited strong evidence of null alleles in bowheads. Furthermore, the median scoring failure rate for the 10 original loci (10.7%) was triple the rate for the 22 loci described here. In the present study, all 22 loci were pure CA repeats and the primers were
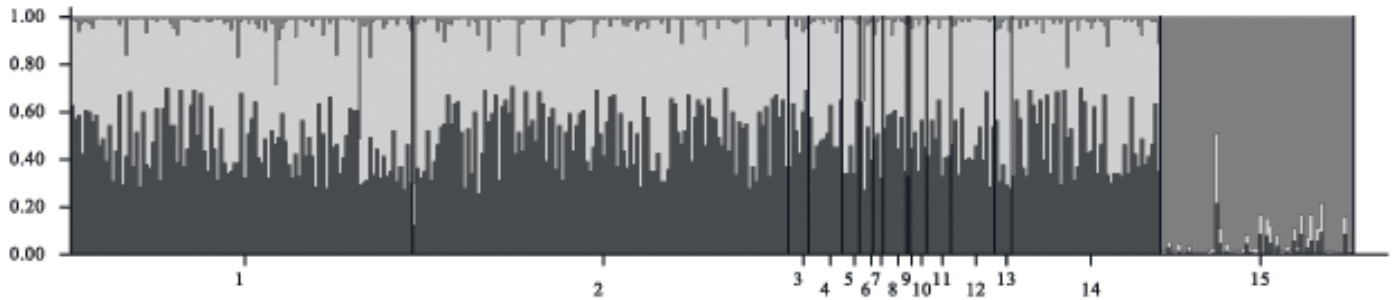
FIG. 4. STRUCTURE results for K = 3 after omitting potentially related individuals and three individuals that demonstrated large influences towards HW disequilibrium. Group labels are the same as in Figure 2.

designed specifically for bowheads and selected on the basis of their ability to amplify consistently with relative strength. Heterozygosity and genetic diversity are much higher in our 22 loci than in the 10 preliminary loci. In our view, these are reasons to view the data set used in this study as qualitatively superior to the previous data set, without implying that the previous data set was inadequate.

Above we described a jackknife procedure that identified within our data set six whales collected at Barrow that had large influences on the non-specific Hardy-Weinberg disequilibrium we have found. Additionally, Skaug and Givens (2007) tentatively identified individuals that were potential close relatives. In a sensitivity analysis, we eliminated from the data set the three whales that contributed most disproportionately to the disequilibrium and one individual from each of the likely related pairs. The purpose of this sensitivity analysis was to test the robustness of our finding of Hardy-Weinberg disequilibrium. After the removal of these individuals, the generic Hardy-Weinberg disequilibrium persisted. When this data set was analyzed using STRUCTURE, the previous weak pattern of pulsing within the WA bowheads was eliminated, showing all WA individuals to be of nearly equally mixed ancestry (Fig. 4). The results of this sensitivity analysis are consistent with the hypothesis that the main sources of genetic variation in the data can be attributed to scoring errors (allelic dropout or mis-amplification) and familial relationships. Moreover, concerns over data quality, excessively influential individuals, and the lack of any reliable supportive evidence from the 22 new loci also lead us to question the presence of any temporal population subdivision.

Expanding our viewpoint beyond the Western Arctic, we found conclusive evidence of genetic differentiation between the Okhotsk Sea, Western Arctic, and eastern Canadian populations. Although expected, this result is nevertheless interesting for several reasons. First, our findings show that the Okhotsk and WA populations are genetically distinct. This distinction may have arisen because whaling activity separated the two groups or left a very small remnant population of bowheads in the Okhotsk Sea. Note also that the historical populations may have co-mingled during the last ice age (> 10 000 yrs BP) while the Bering land bridge existed. Yet study of krill locations (Bockstoce et al., 2005) and other detailed consideration (Moore and Reeves,

1993) find no evidence that bowheads in the Okhotsk move eastward into the Bering Sea. The breeding grounds of WA bowheads are unknown, but they are likely in the spring polynyas and lead systems in the Gulf of Anadyr and within the sea ice boundaries of the Bering Sea. The sea ice boundary and bowhead sightings rarely extend farther south than 58° N (Moore and Reeves, 1993). Thus, WA bowheads appear not to breed in areas sufficiently south to enable mixing between the WA and Okhotsk populations.

On the other end of the WA bowhead range, the continued recession of the polar ice raises the possibility of interchange between the WA and eastern Canadian populations. Rugh et al. (2003) summarized several direct records of exchange between these two populations. Bockstoce and Burns (1993) described two incidents in which commercial whaling irons used in the western North Atlantic fishery were later found in whales taken in the Chukchi Sea. Tomilin (1957) reviewed at least four reports (some as far back as 1643) of European-made harpoons found in bowheads in the Bering or Chukchi seas. Furthermore, a WA bowhead that was satellite-tagged in 2006 was tracked to the north central shore of Banks Island. The distance from Banks Island to the east end of Lancaster Sound, where eastern Canadian bowheads are known to travel in summer (Heide-Jørgensen et al., 2006), is only 400 km or several days' travel. But even if there is any recent geographic co-mingling of individuals from these two populations, our results confirm that the degree of genetic mixing has been extremely small.

Our conclusions could be strengthened by the collection of samples from additional whales and by analysis of mtDNA and other markers. However, our use of a large number of microsatellite markers and a large number of samples at Barrow offers substantial statistical power, and yet scant evidence for population structure in the Western Arctic has been found. Furthermore, Palsbøll et al. (2006) caution that it is important to consider the level of population genetic divergence rather than the statistical rejection of population panmixia in the designation of management units. The WA bowheads, like most populations in nature, are not in Hardy-Weinberg equilibrium. While the finding of disequilibrium is consistent with the possibility of two genetic populations, it is important to interpret these findings in the context of bowhead whale biology within the Western Arctic. Extensive research on historical whaling,

migration, and life history all support a single population of WA bowheads (Rugh et al., 2003). In the present case of large abundance and sparse, broadly distributed hunting, the magnitude of detected genetic differences is small relative to what might trigger a management concern. We have found no evidence for small, genetically distinct subpopulations within the Western Arctic and no convincing evidence that the WA bowheads should be managed as more than one population. The IWC (2008) has agreed and has established sustainable management policies accordingly.

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