Genetic analyses (mtDNA and microsatellites) of Okhotsk and Bering/Chukchi/Beaufort Seas populations of bowhead whales

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ABSTRACT

Both North Pacific populations of bowhead whales (*Balaena mysticetus*) underwent heavy exploitation by commercial whalers in the 19th century, but their reduction in numbers was unequal and their contemporary population sizes differ by an order of magnitude. To investigate the genetic divergence of the different populations, tissue samples of bowhead whales representing the Okhotsk Sea (OS) population (25 samples) and the Bering/Chukchi/Beaufort Seas (BCBS) population (29 samples) were used to generate mtDNA control region sequences and genotypes for three microsatellite loci. There were 20 haplotypes represented in the contemporary BCBS samples and four in the OS samples, three of which were shared with the BCBS samples. The BCBS samples had a much greater haplotypic diversity (0.93) than the OS samples (0.61). Analyses of both types of data revealed significant genetic differences between the two populations, indicating that the populations represent discrete gene pools.

KEYWORDS: BOWHEAD WHALE; GENETICS; CONSERVATION; OKHOTSK SEA; BERING SEA; CHUKCHI SEA; BEAUFORT SEA

INTRODUCTION

Within decades of the discovery of North Pacific populations of bowhead whales (*Balaena mysticetus)* by commercial whalers in the 19th century, they had undergone a significant reduction in their numbers. The Bering/Chukchi/Beaufort Seas (BCBS) population was reduced to a quarter or a fifth of pre-exploitation levels (Woodby and Botkin, 1993), but has since substantially recovered (George *et al.*, 2002) and is still harvested in a subsistence hunt by Alaskan Eskimos. The Okhotsk Sea (OS) population was probably reduced by an even greater proportion and has shown little recovery since the cessation of commercial whaling, and is not hunted. One recent estimate for the BCBS population is 10,020 animals (George *et al.*, 2002) while the less-studied OS population is thought to number just a few hundred (Woodby and Botkin, 1993). Based on a genetic mark-recapture analysis, MacLean (2002) estimated the minimum population size for OS bowheads to be 247. Presently, the stocks are geographically isolated, separated by the Kamchatka peninsula, and there is no evidence that animals currently move between the regions.

The present allopatry of the two populations may not be static over large time scales, fluctuating instead with changing climatic factors. Moore and Reeves (1993) reviewed the distribution and movement of bowhead whales, suggesting that they are widely distributed along the boundary of the ice front in winter. The assumption that the distributions of ice and bowhead whales are closely tied led Dyke *et al.* (1996) to use the distribution of radiocarbon-dated bowhead whale subfossils to track changes in sea ice distributions over the last 10,500 years for the Canadian Arctic. Some of the distributional changes they inferred were abrupt and substantial. Although comparable paleontological data for the North Pacific are lacking, parallel changes in ice cover likely occurred for that region as well. Even in modern times, the extent of sea ice shows considerable interannual variability, extending in heavy ice years to the tip of the Kamchatka Peninsula (Niebauer and Schell, 1993). Indeed, Brueggeman (1982) included reports from 19th century commercial whalers of bowhead whales sighted along the eastern Kamchatka coast. While it is conceivable that some BCBS whales could follow the winter ice edge south in the western Bering Sea during heavy ice years and overlap with the OS population, there is no evidence that this has occurred recently. However, even if contact has not occurred in modern times, Overpeck *et al.*'s (1997) study of the 'Little Ice Age' of the last 400 years traces a pattern of cold periods in the Arctic up until the early 19th century. If 'heavy' ice years during this 'Little Ice Age' were more frequent or more extensive than modern records indicate, there may have been greater opportunity for contact in the not too distant past.

In light of the uncertainty regarding how recently contact between the two populations occurred, this paper investigates the degree to which the populations have diverged. With recent separation, genetic differentiation between them would be expected to be minimal. With contact in the more distant past, dependent on even larger-scale climatic change, stock discreteness should be more apparent and stable through time.

DNA sequence and microsatellite data are used to investigate genetic differentiation between the BCBS and OS populations of bowhead whales, and its implications for population management. Depending on the collection of comparable data from populations of bowhead whales in other areas (Eastern Canadian Arctic, Davis Strait, Spitsbergen), this may be an important first step in understanding the population structure of bowhead whales from all parts of their range.

MATERIALS AND METHODS

Okhotsk Sea bowhead whale samples were taken as biopsies from live whales in August 1995 (14 samples) and in August 1996 (11 samples). All were taken off the

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Ukurunru Cape of the Tugurskiy Peninsula, Russia (Brownell *et al.*, 1997). Tissue samples from the BCBS stock were all taken from animals killed in subsistence hunts in Alaska in 1992 (11 samples) and 1996 (18 samples). One of the 1992 subsistence hunt animals was taken in Nuiqsut, Alaska; the rest of the Alaskan samples came from Barrow. Seventeen of the Barrow samples were also used in a study by Rooney *et al.* (1998; 2001). In all of the above cases, skin samples were preserved in a saturated salt solution with 20% (v/v) DMSO (dimethyl sulphoxide), and kept there until processing. However, the BCBS samples were initially frozen and later transferred to the salt/DMSO solution.

Extraction of DNA from skin samples followed standard protocols as given in Sambrook *et al.* (1989). A 397 base pair (bp) region of the mitochondrial d-loop gene (5' end) was amplified and sequenced according to the methods given in O'Corry-Crowe *et al.* (1997). The primers used for amplification and sequencing were L15964 (5' −CCT CCC TAA GAC TCA AGG −3') and H16498 (5' −CCT GAA GTA AGA ACC AGA TG −3'), the latter from Rosel *et al.* (1994). One additional bowhead whale sequence was obtained from GenBank (accession number 72197) and was published in Árnason *et al.* (1993); the sample originated from Barrow, AK (Árnason and Best, 1991).

Microsatellite data were generated from the biopsy and subsistence harvest samples according to the methods given in Palsbøll *et al.* (1997); the GenBank sample was excluded from this part of the study. Microsatellite primers to EV1 and EV104 are from Valsecchi and Amos (1996) and GATA028 is from Palsbøll *et al.* (1997). One of the Russian samples failed to amplify for EV1. This locus was considered missing data for this individual in subsequent analyses.

Phylogenetic analyses of the sequence data from all samples using neighbour-joining methodology (from uncorrected *p*-distances) and maximum parsimony (MP) were conducted using PAUP 4.062a (Swofford, 1993). Additional analyses were performed using the programs GenePop 3.1b (Raymond and Rousset, 1995) and Arlequin 1.1 (Schneider *et al.*, 1997). The GenBank sequence was not included in the GenePop and Arlequin analyses, in order to keep the sequence and microsatellite sample sets directly comparable. Specific statistics are described in the table legends.

RESULTS AND DISCUSSION

For the d-loop data, the 55 bowhead whale sequences in the entire dataset contained 34 variable sites defining 22 haplotypes (Table 1). Seventeen sites were phylogenetically informative. Exclusive of the GenBank sequence, the 29 Barrow samples represented 20 haplotypes, and the 25 Okhotsk Sea biopsy samples included four haplotypes. Three haplotypes were shared between the two regions, leaving only one haplotype unique to the Okhotsk Sea samples. One must exercise caution in concluding that that one haplotype is unique to the OS population. Given the limited sampling, and the high diversity and large size of the BCBS population, it is plausible that that haplotype also occurs in the BCBS population, but has yet to be sampled. The low diversity in the OS samples is more telling in that there are probably many fewer unsampled haplotypes still extant in that population. In comparison to other small, endangered populations of baleen whales, the four haplotypes found in OS bowhead whales are much less than the 10 reported for western gray whales, *Eschrichtius robustus* (LeDuc *et al.*, 2002) and are more on a par with the five reported for North Atlantic right whales, *Eubalaena glacialis* (Malik *et al.*, 2000). There were no indels (insertions/deletions) among the contemporary Pacific Ocean samples, although the GenBank sequence, in comparison, had an extra base at bp 154.

Table 1
Variable sites; reference numbers from beginning of 5' end of the d-loop light strand.

Haplotype	111111122222222222333333 13588901112440225556677892258 99 56674581137413627458562851013601	Freq. BCBS	Freq. OS
A	AGCCCTCCCGCCGAGACGGTTCAATCGGCTCG	1	
B	AACCCCCCCGCCAAGACGGTTCAATTGGCTCG	1	
C	AACCTCCCCGCTAATACAGCTTAATTAGCTCG	1	3
D	AACCCTCCCGCCGAGACGGTTCAATCGGCTCG	5	13
E	AACCCCCCCGCCAAGACGGTTCAATCGGCTCG	1	
F	AACCCTCCCGCCGAGACGGTTCAATTGGCTCG	1	
G	AACCCTCCCGTCAAGATGGTTCAATCGGCTCG	1	
H	AACACTCCCGCCAAGATAGCTTAACCGGCCTG	2	
I	AACCCTCCCGCCAAGATATTTCAGTTGGCTCG	1	
J	AACCCTCCCGCTAAGACAGCTTAATTAGCCTG	3	
K	AACCCTCCCGCCGAGACGGTTCAATCGGTTCG	2	8
L	AACCCTCCCGCTAAGACAGCTTAATTAGCCCG		1
M	AACCCTCTTATCAGGACAGCTTAATCGGCCTG	1	
N	AACCCTCCCGCCAAGACGGTTCAATCGGCTCG	2	
O	AACCCTCCCGCCGAGACGGTTCAATTGGTTCG	1	
P	AACCCTCCCGCCAGGACGGCTTAACCGGCCCG	1	
Q	AACCCTCCCGCCAAGGTGGTTCAATTGGCTCG	1	
R	AACCCTCCCGCCGAGACGGTTCAGTCGGCTCG	1	
S	AACCCTCCCGCCGAGACGGTCCAATCGACTCG	1	
T	AACCCTCCCGCCAAGACATTTCAATTGGCTCG	1	
U	AATCCTTCCGCCGAGACGGTTCAATCGGCCCG	1	
GenBank	GACCCTCCCGCCGAGACGGTTCGATCGGCTCA		

The 80 MP trees had similar topologies to the unrooted neighbour-joining tree (Fig. 1), although the MP consensus tree showed less resolution. In any case, there was no geographic concordance with the topology of any of the trees. Diversity statistics for the sequences are given in Table 2 (exclusive of the GenBank sequence). The most notable difference is the lower haplotypic diversity shown by the OS samples (0.61) *versus* the BCBS samples (0.93), reflecting not only the fact that the OS sample set contained far fewer haplotypes than BCB, but also displayed a much greater skew in their frequency distribution (see Table 1 for haplotype frequencies). This pattern is consistent with a smaller historical population size in the Okhotsk Sea and the loss of haplotypes through genetic drift. However, a severe bottleneck of a historically large population could also result in low diversity. Given the uncertainty of estimating the pre-exploitation size of the OS population (Woodby and Botkin, 1993), it is not possible to determine which scenario (small historical population *versus* bottleneck) is more likely to have occurred with the present data. However, it is likely that additional haplotypic diversity was lost when 133 bowhead whales were killed from this already small population in 1968 (Doroshenko, 2000). The high haplotypic diversity value for the BCBS population is consistent with Rooney *et al.*'s (1999; 2001) conclusion that this population did not undergo a genetic bottleneck. Although the presence of only four haplotypes among the 25 OS samples resulted in the low haplotypic diversity calculated for that population, these four haplotypes were not particularly closely related to each other (Fig. 1). As a result, the phylogenetic analysis of the sequence data reveals little about relationships between the two populations.

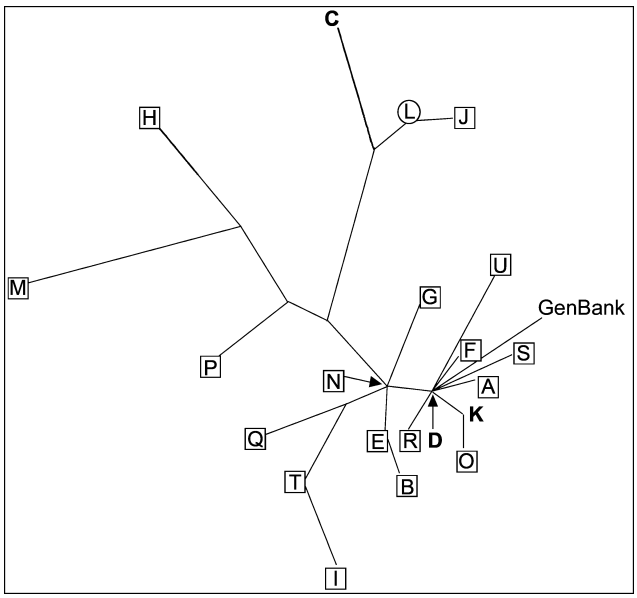

Fig. 1. Neighbour-joining tree determined using uncorrected *p*-distances among all haplotypes. Haplotypes in squares are those unique to the BCBS samples; those in circles are unique to the OS samples. Haplotypes in bold are shared between populations.

For the microsatellite data, locus EV104 exhibited a total of six alleles, EV1 had seven and GATA028 eight. Two of the alleles for EV104 were unique to the BCBS samples, as was one of the GATA028 alleles. EV1 contained a single allele that was unique to the OS samples. All other alleles for all loci were shared between the two populations. Frequencies of alleles and of homozygotes and heterozygotes are given in Table 3. The AMOVA and g-statistic analyses of the data showed small but significant differences between the populations for both microsatellite and sequence data (Tables 4 and 5). The microsatellite results are noteworthy because significant differences were found even though the use of only three loci would have made the power to detect those differences relatively low. These differences indicate that the two populations should be considered genetically and demographically separate for management purposes; gene flow between them is negligible at most. The results also seem to parallel those for gray whales (LeDuc *et al.*, 2002), another North Pacific species with a large eastern population showing high diversity and a small western population with considerably lower diversity.

The fact that the OS biopsy samples were taken from the same locality on successive days each year and in successive years, led to some concern about the possibility of replicate sampling of individuals introducing some bias into the data. However, none of the OS samples had identical genotypes for the three microsatellite loci examined, indicating that no animals were resampled.

Overall, the significance of the genetic differences is consistent with a lack of any appreciable recent gene flow between the two populations, but the small degree of those differences does not preclude the possibility that their most recent contact was in the not too distant past. Given the reduced population sizes, especially for the OS population, an increased rate of genetic drift in the last century could

Table 2

MtDNA genetic diversity by population. Values ± SD; n = 29 for the BCBS sample, and n = 25 for the OS one. For both populations, 398 nucleotide sites were sequenced. No insertions or deletions were observed. Nucleotide diversity is the mean number of pairwise nucleotide differences per observed nucleotide sites (Schneider *et al.*, 1997). Haplotype diversity: $h = 1 - \sum x_i^2$, where x_i is the frequency of the i_{th} haplotype (Nei, 1987).

Population	Number of transitions	Number of transversions	Nucleotide diversity	Haplotype diversity (h)
BCBS	26	3	0.015 ± 0.008	0.9275 ± 0.003
OS	11	1	0.008 ± 0.005	0.6112 ± 0.009

Table 3

Allelic frequencies and observed and expected numbers of homozygotes and heterozygotes (Raymond and Rousset, 1995) by population by microsatellite locus. n = 29 for the BCBS sample, and n = 25 for the OS sample. The null hypothesis of random union of gametes could not be rejected when tested against excess numbers of heterozygotes or excess numbers of homozygotes (Raymond and Rousset, 1995). "AA"= homozygotes; "Aa" = heterozygotes.

	Alleles							
EV1	1	2	3	4	5	6	7	
BCBS	0.224	0.138	0.086	0.224	0.310	0.000	0.017	
OS	0.146	0.104	0.167	0.167	0.292	0.083	0.042	
	"AA" BCBS		"Aa" BCBS		"AA" OS		"Aa" OS	
Expected	6.1		22.9		3.9		20.1	
Observed	4		25		6		18	
EV104	1	2	3	4	5	6		
BCBS	0.190	0.190	0.155	0.293	0.138	0.034		
OS	0.000	0.400	0.100	0.440	0.060	0.000		
	"AA" BCBS		"Aa" BCBS		"AA" OS		"Aa" OS	
Expected	5.5		23.5		8.9		16.1	
Observed	7		22		9		16	
GATA028	1	2	3	4	5	6	7	8
BCBS	0.241	0.155	0.069	0.121	0.034	0.103	0.241	0.034
OS	0.140	0.140	0.040	0.320	0.000	0.140	0.180	0.040
	"AA" BCBS		"Aa" BCBS		"AA" OS		"Aa" OS	
Expected	4.6		24.4		4.5		20.5	
Observed	2		27		6		19	

Table 4

Population differentiation based on mtDNA. Analysis of variance of pairwise mtDNA distances (i.e., number of nucleotide differences; Excoffier *et al.*, 1992) between and among individuals. The significance of measured fixation index, F_{st}, is obtained by permuting individuals among populations to determine the probability of obtaining, by chance, an F_{st} value greater or equal to the observed value; 1,000 permutations were used.

Source of variation	d.f.	Sum of squares	Variance components	Percent variation
Among populations	1	6.35	0.15	6.21
Within populations	52	118.94	2.29	93.79
Total	53	125.30	2.44	

Fixation index F_{st} = 0.062; significance P = 0.026

Table 5

Population differentiation based on three microsatellite loci. A. H_o = *allelic* distribution is identical across BCBS and OS populations (Fisher exact test; Raymond and Rousset, 1995), or B. H_o = *genotypic* distribution is identical across BCBS and OS populations (log-likelihood [G] based exact test; Raymond and Rousset, 1995).

A. Allelic differentiation
$\chi^2 = 21.5$
d.f. = 6
$P = 0.0018$
B. Genotypic differentiation
$\chi^2 = 20.6$
d.f. = 6
$P = 0.0021$

have enhanced a pre-existing level of differentiation. However, the present data are inadequate to evaluate this possibility.

In any analysis of this sort, conclusions drawn about population differentiation are limited by the sampling regime that was employed. When only a single locality is sampled for each population, any substructure or site fidelity within populations could introduce a sampling bias. This is likely not a factor for the BCBS samples, as the sampling locality is along a migration route, by which the vast majority of the population passes en-route to their feeding grounds. The OS samples, on the other hand, were collected from a single locality on the feeding grounds, and as such may not be as representative of the population. However, the structure and/or site fidelity in this population would have to be highly developed for any sampling bias to account for the observed genetic differentiation and differences in diversity. Increased sampling from more areas within the OS is obviously desirable to mitigate these concerns. It would also provide the basis for a better mark-recapture estimate of population size; the estimate of MacLean (2002) was based on only one between-year and one within-year resampling event. For the OS population, a genetic mark/recapture method is probably the most promising method for determining the current population size. This type of study is needed because of the difficulty in conducting photo-identification studies on this population. The OS bowhead whales, at least in our study area, appear to have a low frequency of distinctive markings compared to BCBS bowhead whales. In addition, a modelling study on populations of these sizes and incorporating their historical demographics could establish possible time-frames for recent contact and subsequent genetic drift.

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