An empirical comparison of SNPs and microsatellites for population structure, assignment, and demographic analyses of bowhead whale populations

Phillip A. Morin[1]*, Frederick I. Archer[1], Victoria L. Pease[1], Brittany L. Hancock-Hanser[1], Kelly M. Robertson[1], Ryan M. Huebinger[2]†, Karen K. Martien[1], John W. Bickham[2], J. Craig George[3], Lianne D. Postma[4], Barbara L. Taylor[1].

[1] Protected Resources Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 8901 La Jolla Shores Dr., La Jolla, CA 92037, USA

[2] Center for the Environment, Purdue University, 503 Northwestern Avenue, West Lafayette, IN 47907-2966, USA

[3] North Slope Borough Department of Wildlife Management, PO Box 69, Barrow, AK 99723, USA

[4] Fisheries and Oceans Canada, Central and Arctic Region, 501 University Cr., Winnipeg, Manitoba, Canada R3T 2N6.

† Current address: University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, 75390-9160.

Keywords: Single Nucleotide Polymorphism, SNP, cetacean, population genetics, genetic markers, microsatellites, Balaena mysticetus

Running title: SNPs for bowhead whale population genetics

***Corresponding author**
Phillip A Morin
Fax: 858-546-7003
Email: phillip.morin@noaa.gov

ABSTRACT

Interest in the genetic composition of bowhead whale stocks has been high due to their extreme historical depletion, differential rates of recovery, the potential effects of climate change, and the need to set appropriate quotas for aboriginal hunts. Extensive studies have been conducted to try to characterize stock structure among designated stocks, and to determine whether multiple isolated populations could have existed and potentially still exist within the Bering-Chukchi-Beaufort (BCB) stock. We present here an analysis of 42 linked and unlinked single nucleotide polymorphisms (SNPs) among 3 bowhead whale stocks and within the BCB stock, and compare results to previously published results of mtDNA control region sequences and 22 microsatellites. We performed tests of population structure (F_{ST}, χ^2, Structure), population assignment, and estimates of effective population size (N_e), and evaluated different numbers of loci and samples to estimate the relative statistical power of SNPs and microsatellites. Results indicate that this number of SNPs provides similar power to microsatellites to detect low levels of differentiation (F_{ST} = 0.005-0.03) between bowhead populations with sample sizes of at least 20 per population. Neither marker performed well for Bayesian analysis of population structure with this low level of differentiation, and microsatellites provided greater precision than this set of SNPs for estimates of N_e and for assignment tests. All three genetic marker types indicate that the BCB stock represents a single population. Microsatellites confirm mtDNA results that indicate differences between age-group cohorts. For microsatellites, differences were found between individuals born before 1949 and those born after 1979.. Although microsatellites will remain an important tool for many population studies, SNPs are continuing to prove valuable as tools for understanding structure and demography of populations, and are likely to prove beneficial for long-term monitoring of bowhead whales.

INTRODUCTION:

Bowhead whales (*Balaena mysticetus*) have been subject to management scrutiny resulting from conservation concerns for a species with on-going aboriginal hunting and recent historical depletion. Although the recovery of the Bering/Chukchi/Beaufort (BCB) stock seems to be a success story (see below), the possibility of inadvertent over-harvest of an unidentified smaller stock needed to be investigated. The question of a possible second small population within the BCB stock was raised by Jorde et al. (2007) based on the significant temporal partitioning of inter-individual microsatellite genetic distances in the fall migration. Several subsequent studies using different genetic markers (microsatellites, Givens *et al*., 2010; mtDNA, LeDuc *et al*., 2008), together with the specifics of bowhead whale biology and the pattern of historical depletion and recovery (Archer *et al*., 2010) concluded that genetic results were consistent with a single stock. We examine the same questions here but build on those datasets by adding a third type of genetic marker: Single Nucleotide Polymorphisms (SNPs). In addition, we have increased sample sizes and distribution, and compare the ability of SNPs and microsatellites to address other questions about bowhead demography.

To interpret genetic data for understanding population structure, we need to consider factors that influence genetic patterns, including life history, recent population dynamics and finally connectivity throughout the species' range far enough back in time to influence current patterns. Bowhead whales are the longest-lived large baleen whale species, with populations found historically throughout the Arctic in waters adjacent to the sea ice edge and in polynyas (persistent areas of open water within the pack ice). They are known to migrate large distances, even through areas covered with >90% sea ice (Krutzikowsky and Mate, 2000). Population ranges likely have expanded and contracted across the Canadian Arctic during periods of warmer and cooler climate (Dyke *et al*., 1996; McLeod *et al*., 1993), and currently isolated populations in the Bering/Chukchi/Beaufort Seas and the eastern Canadian archipelagos have likely experienced gene flow multiple times over the last approximately 10,500 years. Strong fidelity to feeding and breeding grounds also likely played a role in limiting mixing of populations within and between northern seas (Finley, 2001; Rugh *et al*., 2003). Bowheads were commercially hunted starting in the 18th century in the North Atlantic and the 19th century in the North Pacific. By 1918, bowhead whales were commercially extinct, and as few as 1000 individuals (≤10% of original populations) may have remained in each region (Brandon and Wade, 2006; Burns *et al*., 1993; Rugh *et al*., 2003).

Four bowhead stocks are currently recognized by the International Whaling Commission for management purposes, and are assumed to correspond to demographically independent populations. In the North Pacific, the largest stock is the BCB stock, with wintering grounds in the Bering Sea, and summer feeding grounds in the Chukchi and Beaufort Seas. The current population size is estimated at 10,000 - 13,000, and the historical population size has been estimated between approximately 14,000 and 30,000 (Brandon and Wade, 2006; Burns *et al*., 1993; George *et al*., 2004; Rugh *et al*., 2003). In the western North Pacific, a small summer feeding population of a few hundred whales is isolated in the Sea of Okhotsk (SO), with no indication of ongoing gene flow with the BCB stock and unknown wintering grounds (LeDuc *et al*., 2005; Rugh *et al*., 2003). In the North Atlantic, three stocks were historically recognized, but recent studies have suggested that the Davis Strait and Hudson Bay populations of eastern Canada and Greenland are actually one population (henceforth referred to as Eastern Canada (EC)), separate from the Spitsbergen stock in the eastern North Atlantic (Heide-Jorgensen *et al*., 2006). The Spitsbergen stock was most likely the largest bowhead population prior to commercial whaling, but was reduced to probably fewer than 300 animals and has not shown signs of recovery; it may number only in the tens currently (reviewed in Rugh *et al*., 2003). The eastern Canadian stock probably numbered close to 12,000 prior to commercial whaling and is now estimated at approximately 8000 (Heide-Jørgensen *et al*., 2006).

Interest in the genetic composition of bowhead stocks has been high due to the need to set appropriate quotas for aboriginal hunts. Additional subdivision has been proposed based on historical whaling records, traditional knowledge, and geographically dispersed feeding grounds (Rugh *et al*., 2003). Especially within the BCB stock, where aboriginal whaling continues and the population is rebounding, extensive studies have been conducted to try to determine whether multiple isolated populations could have existed and potentially still exist, using a variety of methods including genetics (mitochondrial sequences and nuclear

microsatellites; Givens *et al*., 2010; Jorde *et al*., 2007; LeDuc *et al*., 2008), population modeling (Archer *et al*., 2010), and traditional knowledge (Noongwook *et al*., 2007). Prior to this study, microsatellite genotype data have been used twice to assess population structure in BCB bowheads (Givens *et al*., 2010; Jorde *et al*., 2007). These two studies used mostly non-overlapping sets of microsatellites due to difficulty in scoring and reproducibility of some of the loci from the Jorde et al. (2007) study and a previous study by Rooney et al. (1999), as explained by Givens et al. (2010). This is a critical limitation of microsatellite markers, especially if long time periods will lapse between additions of samples to monitor populations (e.g., Davison and Chiba, 2003; Morin *et al*., 2009b). As it is likely that another decade will elapse before the next major study is completed on bowhead whale population structure, there is good reason to develop a nuclear genetic marker that provides consistent genotypes over time and technologies and has sufficient power to address demographic and population structure questions.

Population genetic analyses using microsatellite loci have been widely applied to studies of population differentiation at levels ranging from low-level population subdivision to phylogeography and taxonomy. Further analyses of demographic parameters have included estimation of effective population size, detection of migration (e.g., assignment tests), and inference of recent bottlenecks. SNP genotyping has recently been introduced as a new tool for population geneticists and molecular ecologists (Brumfield *et al*., 2003; Morin *et al*., 2004; Seddon *et al*., 2005; Seeb *et al*., 2011), but it remains to be seen how appropriate they are for some applications. Despite some significant benefits to using SNPs (e.g., large number of SNPs in most genomes, ease and efficiency of genotyping, simple and low mutation rate; reviewed in Helyar *et al*., 2011; Morin *et al*., 2004), there are questions about whether they provide sufficient statistical power to detect low levels of population structure (e.g., for demographically independent populations with $Nm \geq 1$) without using hundreds or thousands of SNPs. Additionally, their appropriateness for estimating demographic parameters has not been fully evaluated. We previously evaluated statistical power for detecting population structure based on simulated data (Morin *et al*., 2009c), and there is a small but rapidly growing number of empirical studies of population structure using SNPs (e.g., Amend *et al*., 2010; Berlin *et al*., 2008; Freamo *et al*., 2011; Hefti-Gautschi *et al*., 2009; Keller *et al*., 2010; Mesnick *et al*., 2011; Mims *et al*., 2010; Narum *et al*., 2008; Quintela *et al*., 2010). For non-model organisms, there are still few empirical studies directly comparing the utility or power of SNPs relative to microsatellites for detecting population structure (Coates *et al*., 2009; Glover *et al*., 2010; Hess *et al*., 2011; Narum *et al*., 2008; Smith and Seeb, 2008).

Here we present a comparative analysis of 22 microsatellites and 42 SNPs for power to detect low levels of genetic structuring in the bowhead whale (*Balaena mysticetus*). We further evaluate the strengths and limitations of microsatellites and SNPs for a variety of population and demographic analyses, including estimation of effective population size (N_e), assignment tests, and estimation of the number of populations using STRUCTURE (Falush *et al*., 2003; Hubisz *et al*., 2009; Pritchard *et al*., 2000). Finally, we use random resampling of the SNP and microsatellite data sets and population sample sets to estimate the relative power of different numbers of each marker type to detect population structure at the low levels observed between bowhead whale populations, and to test the effect of varying sample sizes on statistical power. The populations included in our study represent two common situations; comparison of large and similarly sized populations, and comparison of large and small populations, where the effects of drift are expected to be different and the proportion of sampled individuals to population size are also very different.

MATERIALS AND METHODS:

Samples

Skin samples were obtained from stranded dead animals, projectile-dart biopsy of live animals in the field and whales taken in the annual subsistence hunts. A complete list of BCB and Okhotsk samples and stratification information are presented in the Supplemental Table 1. Samples were stored frozen in 20% (v/v) dimethyl sulfoxide (DMSO) solution saturated with NaCl until ready for DNA extraction (Amos, 1997; Gemmell and Akiyama, 1996). DNA was extracted from soft tissues using a variety of methods: lithium chloride (Gemmell and Akiyama, 1996), sodium chloride protein precipitation (modified from Miller *et al*., 1988), silica-based filter purification (QIAGEN), and standard phenol/chloroform extraction. DNA was extracted from historical bone and baleen samples as described in Morin et al. (2006).

Samples were stratified by temporal, spatial and age-related criteria for analysis. The spatial and temporal strata included samples from multiple years (Supplementary Table 1). As in LeDuc et al. (2008), spatial strata included Barrow (the village on Alaska's North Slope with the largest hunt), the entire North Slope of Alaska (NS), St. Lawrence Island (SLI), Gambell and Savoonga (two villages on SLI), Alaska (NS plus SLI), and Chukotka, Russia. These strata were justified based on hypotheses of spatially separated populations that may be differentially harvested near different villages in Alaska and Russia, and based on hypotheses of different migration timing of populations harvested along the North Slope of Alaska (for details, see LeDuc *et al*., 2008). In addition, this study included strata for geographically separated populations in Okhotsk and eastern Canada (including samples from Hudson Bay and the Davis Straits). This study had a larger BCB population sample size (for SNP data only) than previous studies (Givens *et al*., 2010; LeDuc *et al*., 2005; LeDuc *et al*., 2008; Rooney *et al*., 2001), but the sample set included the same samples as were used for microsatellite analysis (Givens *et al*., 2010) and mtDNA analysis. Additional samples for SNP analysis were collected from aboriginal hunt and live-animal biopsy in two more recent years since the previous data were generated, as well as from historical Barrow and SLI bone and baleen samples from the 1960s and early 1970s. Mostly non-overlapping sets of samples from eastern Canada were used for SNPs (this study) and microsatellites (Givens *et al*., 2010), but samples were from the same geographic and genetic stock. The microsatellite analysis did not include any of the historical samples; these samples were used only in mtDNA (SLI samples) and SNP analysis (all samples). Seasonal temporal strata within Barrow and the NS included spring (S; Apr-Jun) and fall (F; Aug-Oct) hunting seasons, and in SLI they included S (Apr-May) and F (Nov-Jan). For age group temporal comparisons, samples were divided into birth-year strata, based on the year of catch and age estimates from amino acid racemization of lens proteins and baleen growth increments (George *et al*., 1999; LeDuc *et al*., 2008; Lubetkin and Zeh, 2006; Lubetkin *et al*., 2008). Four strata were constructed to include animals born prior to the low point in the population's history (prior to 1918) and in approximately 30-year increments after and including 1918 (i.e., 1918-1949, 1950-1979, and after 1979), plus combinations of the two earliest strata to increase sample size, as in LeDuc et al. (2008).

Genetic data

Bowhead SNPs were ascertained from a range-wide set of samples and assays were previously described (Morin *et al*., 2010). SNP genotypes were generated as described in Morin & McCarthy (2007). Data from 22 microsatellite loci were from Givens et al. (2010), and mtDNA data were described in LeDuc et al. (2008).

Genetic data quality analyses and error rates for the bowhead SNP and microsatellite genotypes and mtDNA sequences have been reported previously (Givens *et al*., 2010; LeDuc *et al*., 2008; Morin *et al*., 2009a). For the remainder of the modern and historical samples added in this study, we determined error rates based on duplicate genotyping of random samples throughout the genotyping process, re-genotyping of 42 samples across all 42 SNP loci after the other genotypes had been generated, and replication of 7 control samples in every SNP assay on each 96-well plate of samples. Historical samples were pre-screened for DNA quantity (Morin *et al*., 2001; Morin *et al*., 2007; Morin and McCarthy, 2007), and SNP genotypes for these samples were replicated at least 5 times. Genotypes were confirmed by the presence of both alleles at least twice for heterozygotes, or at least 3 replications of homozygous genotypes. Twenty-two historical and modern samples that frequently resulted in 3 or fewer called genotypes among replicates, or <30 completed genotypes, were excluded from all further analyses.

Complete SNP genotype sets for all samples were screened for heterozygote deficiency using the exact test in GenePop (Rousset, 2008), and significant deviation from HWE expectations was determined after Bonferroni correction for multiple tests for each marker type and in each population.
The effect of individual sample genotypes on deviations from HWE (which could indicate poor sample quality, possibly resulting in allelic dropout) was examined through jackknife analysis (Morin *et al*., 2009a). Presence of unknown duplicate samples was detected using the program "DropOut" (McKelvey and Schwartz, 2004), which identifies perfect and near-perfect composite genotype matches.

All SNP loci were analyzed for linkage disequilibrium using default settings in the program GenePop v.4.0.1 (Rousset, 2008). SNPs known to be linked (found in the same DNA sequence) were combined into haplotypes (sets of alleles shared on each chromosome for two or more SNPs that are close together) using

the program PHASE (Stephens *et al*., 2001), using one million replicates and a haplotype probability cutoff of 0.5 for inferred haplotypes to reduce bias caused by preferential exclusion of double heterozygote samples. PHASE infers haplotypes even in the absence of genotype data, which can cause a bias in haplotype frequencies among populations. We did replicate analyses of summary statistics using the default PHASE function and by removing genotypes inferred when one or more of the linked genotypes were missing (see Mesnick *et al*., 2011).

SNPs were checked for evidence of divergent selection among populations using the program BayeScan (Foll and Gaggiotti, 2008) based on allele frequency differences among the three stocks, using default MCMC parameters in the program.

Analytical methods

We calculated several divergence metrics for each data set. F_{ST}, G''_{ST} (Meirmans and Hedrick, 2011) and χ^2 were calculated using custom scripts coded in R (R_Development_Core_Team, 2011) by FIA. For all analyses, 1,000 permutations were used to calculate the p-value. F_{ST} and G''_{ST} are both measures of divergence (see results for more details), and χ^2 is a statistical test of significance applied to allele frequency differences between populations.

Estimation of the effective population size was conducted using the program LDNe (Waples and Do, 2008). Default minimum allele frequency cutoffs were used under a random mating model.

We used random re-sampling (without replacement) of subsets of loci (5 or 10 loci) to estimate power of different numbers of SNPs and microsatellites to detect the bowhead stocks. Data were sampled 100 times for genetic analysis for both 5 and 10-locus subsets. Power to detect population structure was quantified as the percentage of analyses of each subset with permutation p-values < 0.05. To examine the effect of sample size on the power to detect stock structure, we also analyzed 1,000 replicates of randomly selected subsamples of 20 and 40 individuals per population. For pairwise tests of each subsample, 500 permutations were conducted in order to estimate p-values.

We used the program STRUCTURE 2.3.1 (Falush *et al*., 2003; Hubisz *et al*., 2009; Pritchard *et al*., 2000) to infer the number of populations and assign samples to putative populations. We used the same parameters Givens *et al*. (2010) used for bowhead whale microsatellite analysis: an admixture model with correlated allele frequencies and a burn-in of 50,000 iterations followed by a run of 1,000,000 iterations. We first ran all analyses without using location information as a prior, as was done by Givens *et al*. (2010), and then repeated all analyses with location as a prior. For the latter analyses, the sampling location of each sample was defined as the population (BCB, eastern Canada, or Okhotsk) from which it was sampled. We investigated models containing $k = 1$ to 5 groups and ran STRUCTURE ten times for each k. We evaluated support for different values of k by calculating both the average log probability of the data (Ln P(D)) and the metric Δk (Evanno *et al*., 2005) for each model. To quantify how well individuals were assigned to populations, we first calculated the mean assignment probabilities for all individuals from each population within a run, and then averaged the mean assignment probability for each population across runs for a given value of k.

We investigated the performance of STRUCTURE as a function of the number of markers used by randomly choosing subsets of the microsatellite markers to include in the analysis. For a given number of markers, we generated ten datasets, each containing a different randomly chosen subset of markers. We used this approach to generate datasets containing five, ten and fifteen microsatellite markers. We did not perform this analysis on the SNP data because power was already so low with the complete set that further reduction in the number of markers would be uninformative.

Individual assignment to two of the putative source populations (BCB and Okhotsk) was investigated using GENECLASS2 (version 2.0) (Piry *et al*., 2004). The Eastern Canada population wasn't included because the SNP and microsatellite samples were not overlapping. We used the combined nuclear dataset comprised of microsatellites and SNPs, as well as each set separately, and the Bayesian assignment criterion of Rannala and Mountain (1997) to calculate the assignment likelihoods for samples from the BCB (N=281) and

Okhotsk (N=49) populations. For all analyses, we set the default frequency for missing alleles at 0.01, and the assignment threshold at the default value of 0.05.

RESULTS:

Samples and genotypes:
The final SNP sample set consisted of 526 samples after removal of poor quality and duplicated samples (see below). Population samples were: BCB=427 (including 49 historical bone and baleen samples), Eastern Canada=49, Okhotsk=50 (Figure 1).

Summary statistics for the SNP loci are given in Table 1. Replicate analysis of the bowhead SNP data indicated a per-allele error rate of 0.07-0.2% in modern samples (depending on which group of replicates was analyzed; see methods), and 1.1% in historical samples (Morin and McCarthy, 2007). We removed 22 samples prior to analysis due to low genotyping success rate (<30 completed genotypes). Thirteen samples were determined to be duplicates of others in the sample set (either repeat sampling of the same individual or matching of a skull sample to a tissue sample from a harvested animal). In the remaining samples, all samples differed by five to fifteen SNP genotypes out of 42.

Among the 25 loci known from sequencing to be physically linked in 12 locus groups, linkage analysis indicated only three cases of significant ($p<0.05$) linkage disequilibrium across all three populations and three cases across two populations (data not shown). No other SNP locus pairs showed significant linkage across all three populations, though five locus pairs were significant across two populations. Given that the linkage was not strong enough to show up across all three population samples, we only inferred phased haplotypes from loci known to be physically linked from our SNP discovery sequencing (Morin *et al*., 2010). Analysis of the phased haplotypes for potential bias due to inference of haplotypes from missing data indicated that 1.6% (103/6324) were inferred by PHASE when one or more of the genotypes was missing. No loci changed from significant to non-significant deviation from HWE expectations (or *vice versa*) when haplotypes were inferred from missing data or removed. We also calculated F_{ST} and χ^2 statistics for the three stock pairwise comparisons. All p values were consistent (>0.05 or <0.05) between SNP data sets (with and without inference from missing data), and F_{ST}'s varied by <0.0003. Based on these results, all further analyses were performed on the phased SNP data with haplotypes inferred from missing genotypes (default method). The resulting set of 29 single- and phased-loci are hereafter referred to as the "phased SNP loci" (locus summary statistics are in Table 1 and allele frequencies are in Table 2).

Analysis of deviations from Hardy-Weinberg equilibrium in each population showed that only one SNP locus deviated significantly from HWE in all three geographic populations (Table 1). That locus (Bmys402) was previously known to be X-linked and therefore was expected to deviate from HW expectations. No other loci deviated significantly from HWE expectations in any of the populations (p value cut-off corrected for multiple tests).

The program BayeScan uses allele frequency differences among populations to infer evidence of diversifying selection. Values between 0.0 and 0.5 are considered to indicate no evidence of selection, 0.5 and 1.0 "substantial", 1.0 – 1.5 "strong", 1.5-2 "very strong" and >2 "decisive" evidence of diversifying selection (Foll and Gaggiotti, 2008). Analysis of the SNP data (42 individual loci) among the 3 stocks indicated that 2 loci (Bmys1R248 and Bmys34M251) exhibited "substantial" evidence of positive selection, with Log_{10} Bayes factors (BF) slightly above 0.5. All other loci were below the Log_{10}BF threshold value of 0.5. All loci were included in subsequent analyses.

Geographic and temporal genetic structure
Bowhead whale population structure was previously examined extensively using mitochondrial DNA (LeDuc *et al*., 2008) and microsatellites (Givens *et al*., 2010). For this study, we compared results based on the within-BCB strata presented in LeDuc et al. (2008) plus inter-stock pairwise comparisons, and re-analyzed the microsatellite data using the same methods and as many overlapping samples as possible between microsatellites and SNPs. We performed standard population differentiation analyses and tests for significance on the phased SNP loci dataset and found that F_{ST} and χ^2 results remained consistent for all spatial comparisons, with significant F_{ST} ranging from about 0.005 to 0.04 among the 3 geographically

separated populations with both microsatellites and SNPs (Table 3). Combining SNPs and microsatellites, which would be expected to increase statistical power over either single data set, did not result in detection of additional population structure within the BCB population (Table 3).

It has been argued that it is not appropriate to compare F_{ST} values between groups of markers with different levels of heterozygosity (Hedrick, 2005; but see Hubisz *et al*., 2009), so we computed Meirman and Hedrick's (2011) unbiased G''_{ST} to account for different levels of heterozygosity. F_{ST} values were very similar between SNPs and microsatellites, but G''_{ST} was lower than F_{ST} for BCB vs. eastern Canada and higher for Okhotsk vs. Eastern Canada and BCB (Table 3). $G"_{ST}$ is supposed to be relatively unaffected by heterozygosity and therefore can be used more appropriately to compare results from markers with different numbers of alleles such as microsatellites and SNPs. For our data from the same populations, levels of differentiation are expected to be small due to historical connectivity. We would expect $G"_{ST}$ and F_{ST} to be similar if heterozygosity was the only difference. In contrast, estimates of $G"_{ST}$ differ by up to 3-fold between marker sets for the same strata comparisons. Two evaluations of published data have also shown that there remains a positive relationship between intra-population heterozygosity and G'_{ST} (a close analog of $G"_{ST}$) (Heller and Siegismund, 2009; Meirmans and Hedrick, 2011), and multiple possible explanations for these differences have been discussed (Balloux and Lugon-Moulin, 2002; Meirmans and Hedrick, 2011; Ryman and Leimar, 2009). Based on the recommendations of Meirmans and Hedrick, we believe that F_{ST} remains a good measure of differentiation for SNPs, while $G"_{ST}$ may better reflect historical demographic patterns inferred from microsatellites, though precision should be estimated using a bootstrap method due to potentially high variance among loci (Meirmans and Hedrick, 2011).

Although there are limitations to using F_{ST} to estimate the amount of gene flow, we do so here in order to get a rough idea of the level of gene flow that is consistent with our F_{ST} estimates. If we use Wright's formula ($F_{ST} = 1/(4Nm + 1)$, and assuming that the effective population size (N) of each is either 400 (estimated from genetic data; see below) or 9100 (breeding adults, 65% of current population), generation time = 52.3 (Taylor *et al*., 2007), and using the average F_{ST} of 0.0065, we get Nm = 38.2, m = 0.1 - 0.004 migrants/generation and annual dispersal rate = 0.002 – $8e^{-5}$ between BCB and EC. Thus, although genetic differentiation as measured by F_{ST} is low, gene flow is also very low (essentially zero) from the perspective of management of aboriginal hunts (Taylor, 1997).

Age structure comparisons that were statistically significant with mitochondrial DNA and microsatellites were not detected with SNP data, but $\chi 2$ analysis indicated significant differentiation in microsatellites and combined microsatellite and SNP genotypes between the older (born before 1950) and younger (born after 1979) animals, in agreement with mtDNA results (Table 3), and in agreement of the "generational gene shift" hypothesis that suggested that "the historical population dynamics of the BCB bowhead whales – extreme reduction followed by rapid recovery – have led to changes in haplotype frequencies across generations, at least between those generations on either side of the population's nadir" (LeDuc *et al*., 2008).

Demographic analyses:
Estimates of N_e varied significantly between data sets, with SNPs providing higher estimates and wider confidence intervals than microsatellites (Table 4). The combination of SNPs and microsatellites provided similar estimates of N_e (405 and 137 in BCB and Okhotsk, respectively) to microsatellites alone (357, 112), with a narrower 95% confidence interval (CI) for BCB and approximately equivalent CI for Okhotsk.

Statistical power and assignment:
Given the relatively large and uneven sample sizes among populations, we randomly sub-sampled the three population samples and conducted standard tests of differentiation. Results indicate that power remained high, and estimates of F_{ST} remained consistent when sample sizes of 40 or 20 were used, though the smallest F_{ST} measure (between BCB and Eastern Canada) was not significant with only 20 samples per population for both SNPs and microsatellites (Table 5). There was no bias in the estimated F_{ST} value with smaller sample sizes, though the variance increased as expected (data not shown), reducing precision. We then randomly resampled 40 samples from each population and investigated power with 5 and 10 randomly selected genetic markers. With 10 loci, both SNPs and microsatellites continued to perform equally; with 5 loci, only the comparison using SNPs to detect the smallest F_{ST} value (BCB vs. EC) was non-significant,

while it remained significant for 5 microsatellites (Table 5). Our results indicate that a reasonable number of samples (e.g., 40) and ≥10 SNPs should be adequate for detection of population structure at these levels of differentiation.

Assignment of samples to their population of origin using the assignment test program GeneClass2 is shown in supplementary Table 2. GeneClass2 considers self-assignment to be correct if the probability is >50% (for 2 populations), but since assignment of unknown samples would be considered strongly supported only at a higher level, we also calculated the portion of individuals correctly assigned when the probability was >90%. Our 22 microsatellites were able to correctly assign almost all samples (>98%) under both criteria, but the 29 phased SNP loci correctly assigned only about 92% and 72% of the samples, respectively. When both marker types were combined, correct assignment increased to greater than 99% under both criteria. To see what portion of microsatellites would result in approximately the same assignment power as our 29 phased SNP loci, we sub-sampled smaller numbers of microsatellites randomly and performed the assignment of the same samples. Ten random samples of five of the 22 microsatellites resulted in a mean of 90.4% (range 86.4-95.2%) of samples assigned correctly with ≥50% probability, and a mean of 74.1% (60.3-88.5%) assigned with ≥90% probability.

When we used STRUCTURE to analyze data from all of the microsatellite markers and did not use sampling location as a prior, the model with the highest average Ln P(D) was the one with five groups (k = 5), while Δk was highest when k = 2 (Supplementary Table 3). It is worth noting, as observed in the previous analysis of the microsatellite data (Givens *et al*., 2010), that the authors of STRUCTURE indicated that the method for statistical inference for the number of populations (k) tends to overestimate the likely value of k (Pritchard *et al*., 2000), and Δk is a better measure of the number of populations when Ln P(D) steadily increases with increasing k (Evanno *et al*., 2005; though see Waples and Gaggiotti, 2006). Givens et al. (2010) also determined that k = 2 best represented the microsatellite data from the three bowhead stocks (BCB, Okhosk, eastern Canada).

STRUCTURE was able to distinguish the Okhotsk population from a combined BCB and Eastern Canada group, with mean assignment probabilities of approximately 96% of BCB and Eastern Canada samples one group and 96% of Okhotsk samples to the other group when k = 2. When k = 3, Okhotsk had a mean assignment probability of 93% to group 3, while BCB and Eastern Canada were both split nearly evenly between groups 1 and 2, indicating that these two populations could not be distinguished (Table 6). When we analyzed the SNP data without using sampling location as a prior, the average Ln P(D) was maximized when k = 1, while Δk was highest for k = 3 (Supplementary Table 3). When k = 2, BCB and Eastern Canada assigned nearly equally to both groups, while Okhotsk had a 62.7% mean assignment probability to one of the groups. When k = 3, Okhotsk had 64.3% assignment to group 3, while the assignments for BCB and Eastern Canada were spread across all three groups (Table 6).

The model containing two groups (k = 2) had the highest Ln P(D) and highest Δk in both datasets when sampling location was used as a prior. With the microsatellite data set and k = 2, BCB and Eastern Canada had mean assignment probabilities to group one of over 99%, while Okhotsk had a 97.7% mean assignment to group two. With SNPs, BCB and Eastern Canada had mean assignments to group one of 96% and 85.6%, respectively, while Okhotsk had a mean assignment to group two of 89.5%. Inclusion of sampling location as a prior improved the ability of STRUCTURE to separate BCB and Eastern Canada. When the model with three groups (k = 3) was evaluated with the microsatellite data set, the BCB samples assigned predominantly to group one (98.1%), Eastern Canada to group two (66.4%), and Okhotsk to group three (96.5%) (Table 6). The three populations were also identified in the SNP dataset, though mean assignments to their respective groups were lower (BCB=84.6%, EC=58.1%, Okhotsk=83.2%; Table 6).

When sampling location is not used as a prior, increasing the number of microsatellite markers resulted in a steady increase in the method's ability to distinguish Okhotsk from BCB and Eastern Canada, with results ranging from no ability to distinguish populations with five markers to near perfect separation with 22 markers (Figure 2; Table 6). When sampling location was used as a prior, changing the number of microsatellites had little effect, as all subsets of markers resulted in correct assignment. Regardless of the

use of sampling location, the performance of the method with the full SNP dataset was intermediate to the performance with five and ten microsatellites (Table 6).

DISCUSSION:

Knowing what level of population structure that one needs to detect is critical to evaluating both the probability of success and the power to reject the hypothesized structure when significant levels of differentiation are not detected (type-II error) (Morin and Dizon, 2009; Morin *et al*., 2009c; Taylor *et al*., 2009). For conservation planning, we would want to detect rates of dispersal that result in demographically independent populations (DIPs), such that an extirpated population would not be recolonized via immigration from other areas on a timescale relevant for management (tens of years). Detection of genetic structure depends on several factors, including number and type of genetic loci, number of samples from each population, and the level of differentiation, which is in turn dependent on effective population size, generation time, and dispersal rate.

This study represents a thorough comparison of the use of SNPs vs microsatellites and mitochondrial DNA to detect low-level population structure in bowhead whales, with a focus on establishing baseline data for monitoring after significant demographic changes due to historical whaling, and in the face of climate change. We have used a combination of 42 linked and unlinked SNPs, inferred the phased haplotypes of linked SNPs, and then used the resulting 29 phased SNP locus data set to compare directly to results obtained using 22 highly polymorphic microsatellites. We expected that microsatellites would provide higher power to detect population differences because they have a much larger number of independent alleles (Kalinowski, 2002), but simulations indicated that even this relatively small number of independent SNP loci would provide sufficient power to detect fine-scale population structure (Morin *et al*., 2009c). Our results confirmed that SNP analysis detected significant geographic genetic differences among bowhead whale populations, even when $F_{ST} < 0.01$. As mentioned previously, this low level of differentiation between the BCB and EC populations results from relatively high abundance coupled with demographically trivial annual dispersal rates (roughly $5.2e^{-5}$). This estimated level of gene flow is consistent with these two populations breeding in different ocean basins. Thus, readers should be cognizant that the magnitude of differentiation (effect size) for the comparisons of these populations is expected to be greater than for populations with higher gene flow that is still demographically trivial. With the low level of differentiation between the BCB and EC populations, SNPs proved surprisingly powerful for detecting population differences between these demographically independent populations even with reduced sample sizes.

Our analysis of age cohorts necessarily used smaller sample sizes, with the oldest cohort numbering only 5 and 7 for microsatellites and SNPs, respectively. With these small sample sizes, SNPs were unable to detect the significant differences found with mtDNA and microsatellites, highlighting again the important relationship between sample number and number of markers (or alleles) (Kalinowski, 2002; Krawczak, 1999; Morin *et al*., 2009c), as well as potential effects of smaller marker N_e (mtDNA) and higher mutation rates (mtDNA and microsatellites). In this case, where addition of samples is unlikely, use of microsatellites or more SNPs is warranted. One way to increase the sample size for these old animals is to use historical samples (bone and baleen) if age can be estimated from museum records. Such samples can be genotyped with SNPs with high accuracy (Morin and McCarthy, 2007), but not with microsatellites.

Previous comparisons of SNPs and microsatellites have typically been limited to one or two types of analysis (e.g., assignment and population structure; Glover *et al*., 2010; Narum *et al*., 2008; Smith and Seeb, 2008) and included at least some ascertainment bias (intentional or due to application of markers ascertained in one population). These studies have taken place on commercially important salmonids, with the goal of finding an optimal set of loci that can assign individuals in a mixed-stock analysis to known populations of origin. In contrast, this study represents a comparison of the use of SNPs vs microsatellites to detect low-level population structure with broadly ascertained and unbiased SNP set, to assign individuals to populations using commonly used methods, and to estimate N_e.

It has previously been suggested that ≥10 times more SNPs might be needed to match the power of microsatellites for some applications such as detecting population structure (Kalinowski, 2002). Subsequent studies have indicated that fewer SNPs are needed, based on either simulated data (Morin *et al*., 2009c) or empirical data (e.g., Coates *et al*., 2009; Narum *et al*., 2008; Ryynanen *et al*., 2007; Smith and Seeb, 2008). In all of these latter cases, a given number of SNPs was compared to a set of microsatellites to determine whether SNPs performed similarly to microsatellites for one or more analyses (e.g., F_{ST} or assignment). As in several of those studies, we obtained nearly identical F_{ST} values and statistical significance with our two sets of markers, but then went on to evaluate how the use of fewer markers or samples randomly selected from our set would perform (rather than based on information content for differentiating samples from specific populations). The results were surprising for some analyses. For divergence (F_{ST}), as few as 10 SNPs or microsatellites (with 40 samples per population) were roughly equal in detecting significant divergence between populations with F_{ST} values ranging from 0.005 to 0.04 (Table 6). This was true both for the comparison of two large and similarly sized populations (BCB and EC), and for the large populations compared to the small population (Okhotsk).

The ability to assign individuals to a given population is particularly useful when organisms are migratory or mix at times during their annual or life cycle. Assignment using SNPs has been evaluated extensively for mixed-stock analysis of salmonids (e.g., Narum *et al*., 2008; Smith and Seeb, 2008), where markers are selected to maximize assignment probability to a set of well-characterized populations. When the number of markers is limiting (as it may be for some time in most species with little genomic information or economic importance), assignment probability must be assessed with the markers available. Our results from the program GeneClass2 indicate that our set of 29 multi-allelic and bi-allelic SNP loci performed nearly as well as 22 microsatellites when the assignment criterion was 50% but performed less well when the assignment criterion was 90%. Nevertheless, these results are encouraging for the use of randomly selected SNPs for assignment tests, as even with the more stringent 90% assignment criterion nearly three-quarters of the samples were still assigned correctly to their population of origin for two populations with a divergence level of $F_{ST} < 0.04$. Improved assignment probability can be achieved by adding SNPs and/or increasing the reference population sample sizes. If assignment tests are the primary objective of a study, however, high probabilities of assignment can be achieved with relatively few microsatellites, but larger numbers of SNPs or highly diagnostic SNPs have also been selected and shown to outperform microsatellites when the SNPs can be pre-selected for assignment to known populations (e.g., Lao *et al*., 2006; Paschou *et al*., 2007; Smith and Seeb, 2008). It is likely that Bowhead whales will start moving between the eastern and western Arctic via the Northwest Passage in the near future as climate change causes decreasing ice coverage. If it becomes important to assign individuals to stock of origin, the current SNP dataset could be supplemented with additional SNPs to improve assignment probability.

A method that is commonly used in population structure studies is inference of structure and assignment of individuals to putative populations without *a priori* stratification of samples, using the Bayesian program STRUCTURE. This program is known to have limited power to detect differentiation of populations when the level of divergence is low (Hubisz *et al*., 2009). We did replicate analyses to determine the most probable number of populations inferred by STRUCTURE from SNP and microsatellite data, and found that in the absence of locality information used as a prior, neither marker type was able to correctly determine the number of populations or assign samples to the two most similar populations (BCB and Eastern Canada). These results are consistent with those found by Givens et al. (2010) for the microsatellite data. Both marker types correctly assigned samples to the small Okhotsk population most of the time, but the rate of correct assignment was 96% with 22 microsatellites and only 63% with 29 phased SNP loci. Adding locality information significantly improved the ability to detect both the number of populations and to correctly assign individuals. However, the ability to correctly assign individuals using SNPs was consistently lower than that achieved using approximately 5-10 microsatellites, indicating that more SNPs will be needed for Bayesian assignment using STRUCTURE and that fewer microsatellites could perform equally as well. For both marker types, STRUCTURE was able to differentiate a small population compared to a large one but was unable to detect the presence of two separate large populations with no current gene flow. Given the highly significant differences using frequency statistics ($p = 0.001$, Table 3) and low annual dispersal rates (roughly $5.2e^{-5}$) between these populations, this empirical case provides strong evidence that negative results from STRUCTURE (i.e., no indication that the BCB and EC are

separate populations) should not be used as evidence against demographic independence of relatively abundant populations.

Previous studies with microsatellites and mtDNA have failed to detect evidence of a genetic bottleneck (Givens *et al*., 2010; Rooney *et al*., 1999; Rooney *et al*., 2001). Although it would have been interesting to compare the power of SNPs and microsatellites to detect a bottleneck, the available methods rely on detection of excess of heterozygosity that would be affected by the ascertainment methods. Given that most SNP ascertainment methods will have some ascertainment bias, especially when the ascertainment sample size is limited or when low-frequency SNPs are excluded, these methods will be biased to indicate a bottleneck even when one has not occurred (G. Luikart and A. Gonçalves da Silva, pers comm.). This leaves us with only the option of inferring changes in effective population size relative to an historical estimate (if that can be known).

Our results indicate that SNPs are likely to have much greater variance in estimates of N_e due to the lower number of pairwise allelic linkage disequilibrium comparisons (Table 4). Based on simulated data, Waples and Do (2010) suggested that about 180 SNPs would be needed to match the same level of precision as 20 microsatellites with 10 alleles each. However, Antao et al. (2011) have recently shown that increasing the number of sampled individuals is more beneficial than increasing the number of loci for two methods of estimating Ne, and that 40 SNPs gave only slightly lower precision than 10 microsatellites. The estimates from our 29 phased SNP loci is somewhat high for the Okhotsk population, which is believed to contain only a few hundred animals and is estimated to have been 3000 – 6500 animals prior to commercial whaling (Woodby and Bodkin, 1993), though the fact that this method is estimating the number of individuals in the parental generation indicates that it is not completely unrealistic. All estimates of N_e are likely to be affected by violations of the assumptions of equilibrium (Archer *et al*., 2010), non-overlapping generations, and random sampling with respect to relatedness. Overlapping generations would likely tend to affect the N_e estimate (up or down depending on species life history and sampling strategy) because the model assumes only one generation in a cohort, and the estimate may be increased by the larger total number of parents in multi-generation sampling or decreased due to nonrandom sampling of family cohorts (Waples, 2006, pers. comm). The N_e/N ratio could be higher than would be expected for a population in equilibrium in the case of a population recovering from severe depletion coupled with a high longevity such that some living individuals were born prior to the bottleneck. Our results support the need for more performance testing of available methods to explore the expected results of using different types of genetic markers.

Some other advantages of SNPs should be considered when selecting markers. One is the type of samples that are likely to be available for genotyping. SNPs can be used to accurately genotype samples of very poor quality, such as historical and noninvasive samples (Morin and McCarthy, 2007). In this study of bowhead whales, we were able to add 49 historical samples to one of our strata from a poorly represented geographic area. A second consideration is the likely need to add data to the study in the future, or by researchers in other laboratories. Microsatellite genotypes have proven difficult to replicate over space and time, or to combine datasets from different labs (Amos *et al*., 2007; Davison and Chiba, 2003; LaHood *et al*., 2002; Morin *et al*., 2009b). In contrast, SNP genotypes are less technology dependent because they represent differences between discrete nucleotides (G, A, T, C), not estimated allele sizes, and can easily be combined from different data sets for analysis without the need for calibration.

This comparative study illustrates that for detecting and monitoring bowhead populations, SNPs provide similar power to microsatellites for all analyses except those with very small sample sizes, and offer the additional benefits of cost-effective genotyping methods, ability to genotype poor-quality and historical samples, and ease of combining data generated over time with changing technologies and in different laboratories. With SNPs, ancestral states of alleles can be known (e.g., by genotyping individuals of related species) for inference of derived alleles in evolutionary studies. Interpretation of population genetic analytical methods is also more straightforward, as traditional F_{ST} measures of differentiation can be used without the need to infer mutation rates and models, or to correct for high levels of heterozygosity. Although microsatellites will remain an important tool for many population studies, SNPs are continuing to prove viable, and in some cases superior, tools for understanding structure and demography of populations.

REFERENCES:

Amend A., Garbelotto M., Fang Z.D. and Keeley S. 2010. Isolation by landscape in populations of a prized edible mushroom *Tricholoma matsutake*. *Conserv Genet* 11:795-802.

Amos W. 1997. Marine mammal tissue sample collection and preservation for genetic analyses. *In*: A.E. Dizon, S.J. Chivers, W.F. Perrin eds.) *Molecular Genetics of Marine Mammals*. Special Publication 3, The Society for Marine Mammalogy, Lawrence, KS. 107-113.

Amos W., Hoffman J.I., Frodsham A., Zhang L., Best S. and Hill V.S. 2007. Automated binning of microsatellite alleles: problems and solutions. *Mol Ecol Notes* 7:10-14.

Antao T., Perez-Figueroa A. and Luikart G. 2011. Early detection of population declines: high power of genetic monitoring using effective population size estimators. *Evolutionary Applications* 4:144-154.

Archer F.I., Martien K.K., Taylor B.L., LeDuc R.G., Ripley B.J., Givens G.H. and George J.C. 2010. A simulation-based approach to evaluating population structure in non-equilibrial populations. *J Cetacean Res Manag* 11:101-113.

Balloux F. and Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. *Mol Ecol* 11:155-165.

Berlin S., Quintela M. and Hoglund J. 2008. A multilocus assay reveals high nucleotide diversity and limited differentiation among Scandinavian willow grouse (*Lagopus lagopus*). *BMC Genetics* 9:89.

Brandon J.R. and Wade P.R. 2006. Assessment of the Bering-Chukchi-Beaufort seas stock of bowhead whales using Bayesian model averaging. *J Cetacean Res Manag* 8:225-239.

Brumfield R.T., Beerli P., Nickerson D.A. and Edwards S.V. 2003. The utility of single nucleotide polymorphisms in inferences of population history. *Trends Ecol Evol* 18:249-255.

Burns J.J., Montague J.J. and Cowles C. eds.). 1993. *The Bowhead Whale*. Society for Marine Mammology, Lawrence, KS.

Coates B.S., Sumerford D.V., Miller N.J., Kim K.S., Sappington T.W., Siegfried B.D. and Lewis L.C. 2009. Comparative performance of single nucleotide polymorphism and microsatellite markers for population genetic analysis. *J Hered* 100:556-564.

Davison A. and Chiba S. 2003. Laboratory temperature variation is a previously unrecognized source of genotyping error during capillary electrophoresis. *Mol Ecol Notes* 3:321-323.

Dyke A.S., Hooper J. and Savelle J.M. 1996. A history of sea ice in the Canadian Arctic Archipelago based on postglacial remains of the bowhead whale (*Balaena mysticetus*). *Arctic* 49:235-255.

Evanno G., Regnaut S. and Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611-2620.

Falush D., Stephens M. and Pritchard J.K. 2003. Inference of population tructure uing multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567-1587.

Finley K.J. 2001. Natural history and conservation of the Greenland whale, or bowhead, in the northwest Atlantic. *Arctic* 54:55-76.

Foll M. and Gaggiotti O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* 180:977-993.

Freamo H., O'Reilly P., Berg P.R., Lien S. and Boulding E.G. 2011. Outlier SNPs show more genetic structure between two Bay of Fundy metapopulations of Atlantic salmon than do neutral SNPs. *Mol Ecol Resour* 11 Suppl 1:254-267.

Gemmell N.J. and Akiyama S. 1996. An efficient method for the extraction of DNA from vertebrate tissues. *Trends Genet* 12:338-339.

George J.C., Bada J., Zeh J.E., Scott L., Brown S.E., O'Hara T. and Suydam R.S. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. *Can J Zool* 77:571-580.

George J.C., Zeh J.E., Suydam R.S. and Clark C. 2004. Abundance and population trend (1978-2001) of western Arctic bowhead whales (*Balaena mysticetus*) surveyed near Barrow, Alaska. *Mar Mamm Sci* 20:755-773.

Givens G.H., Huebinger R.M., Patton J.C., Postma L.D., Lindsay M., Suydam R.S., George J.C., Matson C.W. and Bickham J.W. 2010. Population Genetics of Bowhead Whales (*Balaena mysticetus*) in the Western Arctic. *Arctic* 63:1-12.

Glover K.A., Hansen M.M., Lien S., Als T.D., Hoyheim B. and Skaala O. 2010. A comparison of SNP and STR loci for delineating population structure and performing individual genetic assignment. *BMC Genetics* 11:2.

Hedrick P.W. 2005. A standardized genetic differentiation measure. *Evolution* 59:1633-1638.

Hefti-Gautschi B., Pfunder M., Jenni L., Keller V. and Ellegren H. 2009. Identification of conservation units in the European Mergus merganser based on nuclear and mitochondrial DNA markers. *Conserv Genet* 10:87-99.

Heide-Jørgensen M.P., Laidre K.L. and Fossette S. 2006. Re-analysis of a re-analysis of a Canadian bowhead survey. International Whaling Commission.

Heide-Jorgensen M.P., Laidre K.L., Jensen M.V., Dueck L. and Postma L.D. 2006. Dissolving stock discreteness with satellite tracking: Bowhead whales in Baffin Bay. *Mar Mamm Sci* 22:34-45.

Heller R. and Siegismund H.R. 2009. Relationship between three measures of genetic differentiation G(ST), D(EST) and G'(ST): how wrong have we been? *Mol Ecol* 18:2080-2083; discussion 2088-2091.

Helyar S.J., Hemmer-Hansen J., Bekkevold D., Taylor M.I., Ogden R., Limborg M.T., Cariani A., Maes G.E., Diopere E., Carvalho G.R. and Nielsen E.E. 2011. Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Mol Ecol Resour* 11 Suppl 1:123-136.

Hess J.E., Matala A.P. and Narum S.R. 2011. Comparison of SNPs and microsatellites for fine-scale application of genetic stock identification of Chinook salmon in the Columbia River Basin. *Mol Ecol Resour* 11 Suppl 1:137-149.

Hubisz M.J., Falush D., Stephens M. and Pritchard J.K. 2009. Inferring weak population structure with the assistance of sample group information. *Mol Ecol Res* 9:1322-1332.

Jorde P.E., Schweder T., Bickham J.W., Givens G.H., Suydam R., Hunter D. and Stenseth N.C. 2007. Detecting genetic structure in migrating bowhead whales off the coast of Barrow, Alaska. *Mol Ecol* 16:1993-2004.

Kalinowski S.T. 2002. How many alleles per locus should be used to estimate genetic distances? *Heredity* 88:62-65.

Keller S.R., Olson M.S., Silim S., Schroeder W. and Tiffin P. 2010. Genomic diversity, population structure, and migration following rapid range expansion in the Balsam poplar, *Populus balsamifera*. *Mol Ecol* 19:1212-1226.

Krawczak M. 1999. Informativity assessment for biallelic single nucleotide polymorphisms. *Electrophoresis* 20:1676-1681.

Krutzikowsky G.K. and Mate B.R. 2000. Dive and surfacing characteristics of bowhead whales (*Balaena mysticetus*) in the Beaufort and Chukchi seas. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 78:1182-1198.

LaHood E.S., Moran P., Olsen J., Grant W.S. and Park L.K. 2002. Microsatellite allele ladders in two species of Pacific salmon: preparation and field-test results. *Mol Ecol Notes* 2:187-190.

Lao O., van Duijn K., Kersbergen P., de Knijff P. and Kayser M. 2006. Proportioning whole-genome single-nucleotide-polymorphism diversity for the identification of geographic population structure and genetic ancestry. *Am J Hum Genet* 78:680-690.

LeDuc R.G., Dizon A.E., Burdin A.M., Blokhin S.A., George J.C. and Brownell Jr R.L. 2005. Genetic analysis (mtDNA and microsatellites) of Okhotsk and Bering/Chukchi/Beaufort Seas populations of bowhead whales. *J Cetacean Res Manag* 7:107-111.

LeDuc R.G., Martien K.K., Morin P.A., Hedrick N.M., Robertson K.M., Taylor B.L., Mugue N.S., Borodin R.G., Zelenina D.A., Litovka D. and George J.C. 2008. Mitochondrial genetic variation in bowhead whales in the western Arctic. *J Cetacean Res Manag* 10:93-97.

Lubetkin S.C. and Zeh J.E. 2006. Deriving age-length relationships for bowhead whales (*Balaena mysticetus*) using a synthesis of age estimation techniques. Anchorage, AK: International Whaling Commission.

Lubetkin S.C., Zeh J.E., Rosa C. and George J.C. 2008. Age estimation for young bowhead whales (*Balaena mysticetus*) using annual baleen growth increments. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 86:525-538.

McKelvey K.S. and Schwartz M.K. 2004. Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. *J Wildl Manage* 68:439-448.

McLeod S.A., Whitmore Jr F.C. and Barnes L.G. 1993. Evolutionary relationships an dclassification. *In*: J.J. Burns, J.J. Montague, C.J. Cowles eds.) *The Bowhead Whale*. Society for Marine Mammalogy, Lawrence, Kansas. 45-70.

Meirmans P.G. and Hedrick P.W. 2011. Assessing population structure: Fst and related measures. *Mol Ecol Res* 11:5-18.

Mesnick S., Taylor B., Archer E.I., Martien K., Escorza Treviño S., Hancock B.L., Moreno Medina S.C., Pease V.L., Robertson K.M., Straley J.M., Baird R., Calambokidis J., Wade P., Burkanov V., Lunsford C.R., Rendell L., Whitehead H. and Morin P.A. 2011. Sperm whale population structure in the eastern North Pacific inferred by the use of single nucleotide polymorphisms (SNPs), microsatellites and mitochondrial DNA. *Mol Ecol Res* 11 (suppl. 1):278-298.

Miller S.A., Dykes D.D. and Polesky H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215.

Mims M.C., Hulsey C.D., Fitzpatrick B.M. and Streelman J.T. 2010. Geography disentangles introgression from ancestral polymorphism in Lake Malawi cichlids. *Mol Ecol* 19:940-951.

Morin P.A., Chambers K.E., Boesch C. and Vigilant L. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (Pan troglodytes verus). *Mol Ecol* 10:1835-1844.

Morin P.A. and Dizon A.E. 2009. Genetics for management. *In*: W.F. Perrin, B. Würsig, J.G.M. Thewissen eds.) *Encyclopedia of Marine Mammals*. Elsevier Inc.

Morin P.A., Hedrick N.M., Robertson K.M. and LeDuc C.A. 2007. Comparative mitochondrial and nuclear quantitative PCR of historical marine mammal tissue, bone, baleen, and tooth samples. *Mol Ecol Notes* 7:404-411.

Morin P.A., LeDuc R.G., Archer F.I., Martien K.K., Huebinger R., Bickham J.W. and Taylor B.L. 2009a. Significant deviations from Hardy-Weinberg equilibrium caused by low levels of microsatellite genotyping errors. *Mol Ecol Res* 9:498-504.

Morin P.A., LeDuc R.G., Robertson K.M., Hedrick N.M., Perrin W.F., Etnier M., Wade P. and Taylor B.L. 2006. Genetic analysis of killer whale (*Orcinus orca*) historical bone and tooth samples to identify western U.S. ecotypes. *Mar Mamm Sci* 22:897-909.

Morin P.A., Luikart G., Wayne R.K. and SNP-Workshop_Grp 2004. SNPs in ecology, evolution and conservation. *Trends Ecol Evol* 19:208-216.

Morin P.A., Manaster C., Mesnick S.L. and Holland R. 2009b. Normalization and binning of historical and multi-source microsatellite data: overcoming the problems of allele size shift with ALLELOGRAM. *Mol Ecol Res* 9:1451-1455.

Morin P.A., Martien K.K. and Taylor B.L. 2009c. Assessing statistical power of SNPs for population structure and conservation studies. *Mol Ecol Res* 9:66-73.

Morin P.A. and McCarthy M. 2007. Highly accurate SNP genotyping from historical and low-quality samples. *Mol Ecol Notes* 7:937-946.

Morin P.A., Pease V.L., Hancock B.L., Robertson K.M., Antolik C.W. and Huebinger R.M. 2010. Characterization of 42 SNP markers for the bowhead whale (*Balaena mysticetus*) for use in discriminating populations. *Mar Mamm Sci* 26:716-732.

Narum S.R., Banks M., Beacham T.D., Bellinger M.R., Campbell M.R., Dekoning J., Elz A., Guthrie C.M., 3rd, Kozfkay C., Miller K.M., Moran P., Phillips R., Seeb L.W., Smith C.T., Warheit K., Young S.F. and Garza J.C. 2008. Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms. *Mol Ecol* 17:3464-3477.

Noongwook G., Huntington H.P., George J.C., Savoonga N.V. and Gambell N.V. 2007. Traditional knowledge of the bowhead whale (Balaena mysticetus) around St. Lawrence Island, Alaska. *Arctic* 60:47-54.

Paschou P., Ziv E., Burchard E.G., Choudhry S., Rodriguez-Cintron W., Mahoney M.W. and Drineas P. 2007. PCA-correlated SNPs for structure identification in worldwide human populations. *PLoS Genet* 3:1672-1686.

Piry S., Alapetite A., Cornuet J., Paetkau D., Baudouin L. and Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *J Hered* 95:536-539.

Pritchard J.K., Stephens M. and Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.

Quintela M., Berlin S., Wang B.A. and Hoglund J. 2010. Genetic diversity and differentiation among *Lagopus lagopus* populations in Scandinavia and Scotland: evolutionary significant units confirmed by SNP markers. *Mol Ecol* 19:2380-2393.

R_Development_Core_Team 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rannala B. and Mountain J.L. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Science U S A* 94:9197-9201.

Rooney A.P., Honeycutt R.L., Davis S.K. and Derr J.N. 1999. Evaluating a putative bottleneck in a population of bowhead whales from patterns of microsatellite diversity and genetic disequilibria. *J Mol Evol* 49:682-690.

Rooney A.P., Honeycutt R.L. and Derr J.N. 2001. Historical population size change of bowhead whales inferred from DNA sequence polymorphism data. *Evolution* 55:1678-1685.

Rousset F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Res* 8:103-106.

Rugh D., DeMaster D., Rooney A., Breiwick J., Shelden K. and Moore S. 2003. A review of bowhead whale (*Balaena mysticetus*) stock identity. *J Cetacean Res Manag* 5:267-279.

Ryman N. and Leimar O. 2009. G(ST) is still a useful measure of genetic differentiation - a comment on Jost's D. *Mol Ecol* 18:2084-2087; discussion 2088-2091.

Ryynanen H.J., Tonteri A., Vasemagi A. and Primmer C.R. 2007. A comparison of biallelic markers and microsatellites for the estimation of population and conservation genetic parameters in Atlantic salmon (*Salmo salar*). *J Hered* 98:692-704.

Seddon J.M., Parker H.G., Ostrander E.A. and Ellegren H. 2005. SNPs in ecological and conservation studies: a test in the Scandinavian wolf population. *Mol Ecol* 14:503-511.

Seeb J.E., Carvalho G., Hauser L., Naish K., Roberts S. and Seeb L.W. 2011. Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. *Mol Ecol Res* 11:1-8.

Smith C.T. and Seeb L.W. 2008. Number of alleles as a predictor of the relative assignment accuracy of STR and SNP baselines for chum salmon. *Trans Am Fish Soc* 137:751-762.

Stephens M., Smith N.J. and Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics* 68:978-989.

Taylor B.L. 1997. Defining "population" to meet management objectives for marine mammals. *In*: A.E. Dizon, S.J. Chivers, W.F. Perrin eds.) *Molecular Genetics of Marine Mammals*. Special Publication 3, The Society for Marine Mammalogy, Lawrence, KS. 49-66.

Taylor B.L., Chivers S.J., Larese J. and Perrin W.F. 2007. Generation length and percent mature estimates for IUCN assessments of cetaceans. Administrative Report, Southwest Fisheries Science Center, 8604 La Jolla Shores Blvd., La Jolla, CA 92038, USA.

Taylor B.L., Martien K.K. and Morin P.A. 2009. Defining Populations & Stocks. *In*: I. Boyd, D. Bowen, S. Iverson eds.) *Marine Mammal Ecology and Conservation: A handbook of techniques*. Oxford University Press, Oxford.

Waples R.S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conserv Genet* 7:167-184.

Waples R.S. and Do C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Mol Ecol Res* 8:753-756.

Waples R.S. and Do C. 2010. Linkage disequilibrium estimates of contemporary *Ne* using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications* 3:244-262.

Waples R.S. and Gaggiotti O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol Ecol* 15:1419-1439.

Woodby D.A. and Bodkin D.B. 1993. Stock sizes prior to commercial whaling. *In*: J.J. Burns, J.J. Montague, C. Cowles eds.) *The Bowhead Whale*. Society for Marine Mammalogy, Lawrence, KS. 387-407.

ACKNOWLEDGEMENTS:
We are grateful to the Southwest Fisheries Science Center, the North Pacific Research Board, and the North Slope Borough for support of this research. We thank Amy van Cise and Carrie Antolik for assistance with SNP genotyping. BCB Bowhead whale DNA samples for genotyping were supplied by The North Slope Borough, and we thank the whaling captains, their crew, and the Eskimo villages that provided invaluable samples, as well as Dave Withrow, National Marine Mammal Lab, NOAA Alaska Fisheries Science Center, and Bob Brownell, Southwest Fisheries Science Center. Museum samples were generously provided by Jim Dines and the Los Angeles County Museum of Natural History. We are grateful to Vicki Pease, Brittany Hancock-Hanser, Kelly Robertson, Amy van Cise, and Caroline Antolik for assistance in generating SNP genotype data, and to Rich Cosgrove for preparing sample map figures. Valuable discussion on earlier versions of the manuscript was provided by Kim Parsons, Robin Waples, Bill Perrin and 5 anonymous reviewers.

FIGURE LEGENDS:

Figure 1: Sampling locations and populations (BCB=squares, OS=circles, EC=triangles) for samples used in this study. The size of the box is proportional to the number of samples from that location. Samples from far eastern Russia that appear to be on land are from the Senyavin Strait.

Figure 2. Mean assignment of individuals to group 1 as a function of the number of microsatellite markers included in the analysis when STRUCTURE is used to define two groups. Results are shown for analyses in which sampling location is not used as a prior (a), and when it is used (b). All analyses were run 10 times. In analyses where a subset of microsatellite markers was used, a different randomly chosen set of markers was used in each replicate.

Tables and Figures:
Figure 1

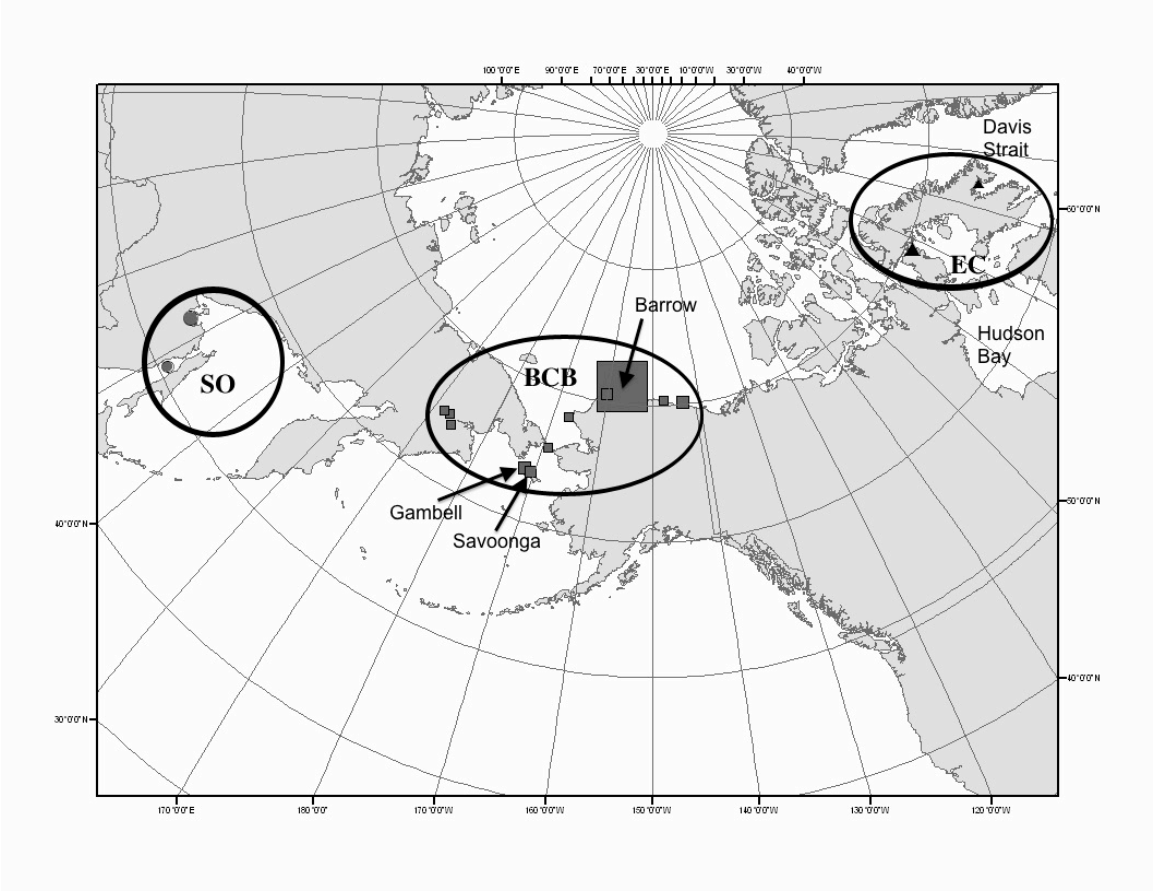
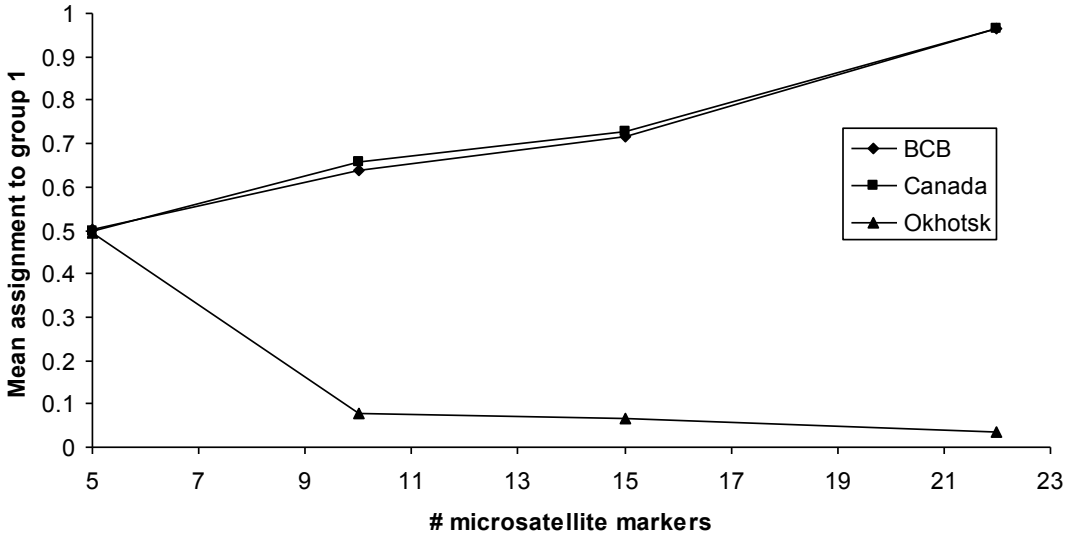


Figure 2

a)


Mean assignment to group 1
microsatellite markers
BCB
Canada
Okhotsk

b)

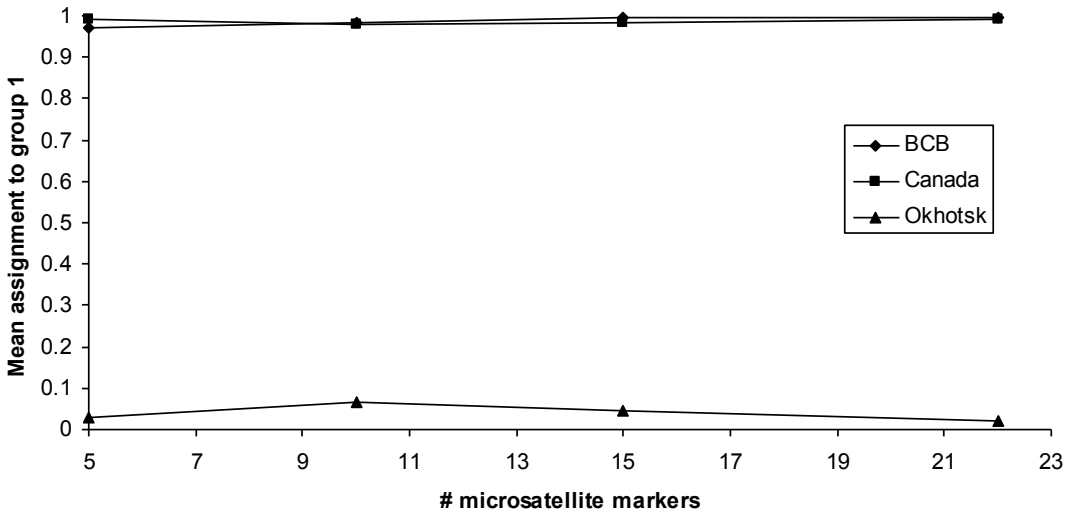

Mean assignment to group 1
microsatellite markers
BCB
Canada
Okhotsk

Table 1: Descriptive statistics of microsatellite and phased SNP loci in the 3 bowhead whale populations. Allelic Richness, observed (Ho) and expected (He) heterozygosity, genetic diversity (θ) and p-value for deviations from Hardy-Weinberg equilibrium. Significance of HWE(p) after Bonferroni correction for multiple tests is indicated in bold. SNP locus names are for phased SNPs when multiple SNPs were used from a single sequenced locus, and include the IUPAC SNP letters and positions as described in Morin et al. 2010.

	BCB (N = 302)						Okhotsk (N =60)						Eastern Canada (N = 47)					
locus	Num. alleles	Allelic Richness	He	Ho	q	HWE (p)	Num. alleles	Allelic Richness	He	Ho	q	HWE (p)	Num. alleles	Allelic Richness	He	Ho	q	HWE (p)
Microsatellites																		
Bmy1	10	0.033	0.81	0.82	0.61	0.55	8	0.133	0.82	0.77	0.61	0.42	9	0.191	0.82	0.83	0.61	0.72
Bmy2*	11	0.037	0.77	0.75	0.57	0.18	8	0.133	0.77	0.70	0.57	0.13						
Bmy7	13	0.042	0.79	0.81	0.59	0.17	10	0.167	0.87	0.95	0.65	0.98	8	0.170	0.78	0.70	0.58	0.26
Bmy8	16	0.053	0.80	0.79	0.59	0.51	9	0.150	0.81	0.85	0.60	0.90	10	0.217	0.83	0.80	0.62	0.43
Bmy10	22	0.072	0.93	0.90	0.69	0.06	15	0.250	0.89	0.93	0.66	0.80	18	0.383	0.92	0.98	0.68	0.95
Bmy11	14	0.046	0.88	0.87	0.65	0.47	9	0.150	0.79	0.73	0.59	0.36	11	0.239	0.88	0.91	0.66	0.71
Bmy12	27	0.088	0.92	0.93	0.69	0.36	11	0.183	0.84	0.87	0.63	0.43	16	0.364	0.90	0.98	0.67	0.97
Bmy14	6	0.020	0.56	0.52	0.41	0.02	3	0.050	0.48	0.42	0.35	0.21	6	0.128	0.58	0.57	0.44	0.52
Bmy16	8	0.026	0.77	0.79	0.57	0.91	6	0.102	0.76	0.80	0.57	0.86	7	0.149	0.76	0.74	0.57	**0.00**
Bmy18	17	0.056	0.90	0.88	0.67	0.10	9	0.150	0.76	0.77	0.57	0.52	14	0.298	0.89	0.96	0.66	0.91
Bmy19	16	0.053	0.87	0.86	0.65	0.12	12	0.200	0.83	0.80	0.62	**0.00**	10	0.213	0.87	0.89	0.65	0.73
Bmy26	22	0.073	0.93	0.91	0.69	0.07	16	0.281	0.89	0.88	0.67	0.02	18	0.383	0.91	0.98	0.68	1.00
Bmy33	12	0.039	0.81	0.80	0.60	0.60	8	0.133	0.80	0.72	0.60	0.19	8	0.170	0.78	0.83	0.58	0.66
Bmy36	28	0.092	0.94	0.94	0.70	0.69	21	0.350	0.89	0.88	0.66	0.60	19	0.413	0.94	0.96	0.70	0.67
Bmy41	22	0.072	0.91	0.90	0.68	0.09	11	0.186	0.83	0.85	0.62	0.64	19	0.404	0.91	0.87	0.68	0.42
Bmy42	11	0.036	0.79	0.74	0.59	0.07	7	0.117	0.82	0.85	0.61	0.83	11	0.234	0.85	0.79	0.63	0.19
Bmy49	24	0.078	0.90	0.91	0.67	0.39	14	0.233	0.89	0.92	0.66	0.76	19	0.404	0.88	0.83	0.65	0.30
Bmy53	17	0.056	0.88	0.88	0.65	0.39	10	0.167	0.79	0.75	0.59	0.24	16	0.340	0.90	0.89	0.67	0.44
Bmy54	8	0.026	0.71	0.70	0.53	0.05	6	0.100	0.76	0.77	0.56	0.69	7	0.149	0.77	0.77	0.57	0.22
Bmy55	6	0.020	0.71	0.69	0.53	0.06	6	0.100	0.53	0.53	0.40	0.69	6	0.128	0.72	0.66	0.54	0.23
Bmy57	9	0.030	0.61	0.59	0.45	**0.00**	5	0.083	0.56	0.55	0.42	0.41	8	0.170	0.65	0.64	0.49	0.61
Bmy58	27	0.089	0.93	0.93	0.69	0.54	14	0.255	0.90	0.95	0.67	0.59	21	0.447	0.93	0.89	0.69	0.49
Average	15.73	0.05	0.82	0.81	0.61		9.91	0.17	0.79	0.78	0.59		12.43	0.27	0.83	0.83	0.62	
SNPs	BCB (N=427)						Okhotsk (N=50)						Eastern Canada (N=49)					
BmC5R700_Y910	3	0.007	0.36	0.37	0.27	0.73	3	0.060	0.37	0.36	0.28	0.33	3	0.063	0.48	0.52	0.36	0.79
BmCATR205_R212	4	0.009	0.37	0.37	0.28	0.67	3	0.060	0.60	0.72	0.45	0.98	4	0.082	0.35	0.37	0.26	0.52
BmCHYY286_R417	4	0.009	0.62	0.63	0.46	0.54	3	0.060	0.47	0.50	0.35	0.40	3	0.061	0.56	0.55	0.42	0.44
BmCOL3A1Y82	2	0.006	0.35	0.30	0.26	0.01	2	0.048	0.31	0.29	0.23	0.45	2	0.041	0.39	0.49	0.29	0.99
BmCSF2S320	2	0.005	0.50	0.53	0.37	0.94	2	0.040	0.47	0.56	0.35	0.96	2	0.041	0.50	0.61	0.37	0.97
BmEDN1Y91	2	0.005	0.38	0.41	0.29	0.94	2	0.040	0.34	0.42	0.25	1.00	2	0.041	0.43	0.45	0.32	0.74

BmFESY136	2	0.005	0.36	0.37	0.27	0.74	2	0.040	0.23	0.26	0.17	1.00	2	0.041	0.38	0.39	0.29	0.66
BmMPOR184_R284	3	0.007	0.61	0.57	0.46	0.05	3	0.063	0.59	0.69	0.44	0.82	4	0.085	0.64	0.60	0.47	0.31
BmPMKS494	2	0.005	0.45	0.42	0.34	0.13	2	0.041	0.37	0.37	0.28	0.59	2	0.041	0.40	0.43	0.30	0.79
Bmys108D91	2	0.005	0.29	0.29	0.22	0.55	2	0.040	0.50	0.46	0.37	0.39	2	0.043	0.30	0.32	0.22	0.83
Bmys1R248	2	0.005	0.01	0.01	0.01	1.00	2	0.040	0.26	0.22	0.19	0.28	1	0.020	0.00	0.00	0.00	NA
Bmys28Y154_R162	4	0.009	0.56	0.55	0.42	0.58	3	0.060	0.50	0.54	0.37	0.39	3	0.061	0.59	0.49	0.44	0.07
Bmys31Y94	2	0.006	0.25	0.27	0.19	0.97	2	0.040	0.23	0.14	0.17	0.02	2	0.041	0.22	0.20	0.16	0.53
Bmys34M251	2	0.005	0.10	0.10	0.07	0.65	2	0.040	0.24	0.28	0.18	1.00	2	0.043	0.04	0.04	0.03	1.00
Bmys368R272	2	0.005	0.28	0.30	0.21	0.91	2	0.040	0.30	0.36	0.22	1.00	2	0.041	0.39	0.49	0.29	0.99
Bmys382Y279	2	0.005	0.18	0.17	0.14	0.06	2	0.040	0.10	0.10	0.07	1.00	2	0.041	0.12	0.08	0.09	0.15
Bmys387R245_R361	3	0.007	0.34	0.35	0.25	0.28	3	0.060	0.46	0.40	0.34	0.17	3	0.061	0.44	0.39	0.33	0.07
Bmys395Y158	2	0.005	0.50	0.50	0.37	0.49	2	0.040	0.50	0.50	0.37	0.61	2	0.042	0.50	0.54	0.38	0.79
Bmys402M56	2	0.005	0.48	0.24	0.35	**0.00**	2	0.040	0.50	0.20	0.37	**0.00**	2	0.041	0.50	0.16	0.37	**0.00**
Bmys404Y286_K316	4	0.010	0.63	0.63	0.47	0.49	3	0.061	0.66	0.69	0.49	0.77	3	0.061	0.64	0.67	0.48	0.61
Bmys410K107	2	0.005	0.49	0.49	0.36	0.53	2	0.040	0.29	0.30	0.21	0.81	2	0.043	0.50	0.48	0.37	0.52
Bmys412R79_R463	3	0.007	0.43	0.52	0.32	1.00	4	0.080	0.42	0.44	0.31	0.70	3	0.061	0.38	0.41	0.28	0.15
Bmys414R127	2	0.005	0.46	0.46	0.34	0.50	2	0.041	0.29	0.27	0.22	0.43	2	0.041	0.48	0.45	0.36	0.44
Bmys42aK46_aR225_bK232	7	0.018	0.79	0.80	0.59	0.01	7	0.156	0.81	0.78	0.60	0.15	7	0.146	0.81	0.85	0.60	0.87
Bmys43Y237_Y377	4	0.010	0.60	0.60	0.45	0.47	3	0.063	0.53	0.60	0.40	0.91	4	0.082	0.58	0.65	0.43	0.87
Bmys48S269	2	0.005	0.49	0.51	0.36	0.89	2	0.043	0.50	0.40	0.37	0.15	2	0.041	0.50	0.37	0.37	0.05
Bmys60Y148_R260	4	0.009	0.20	0.20	0.15	0.29	4	0.080	0.41	0.34	0.31	0.06	4	0.082	0.33	0.27	0.25	0.01
Bmys92Y230_K271	3	0.007	0.59	0.61	0.44	0.89	4	0.083	0.51	0.65	0.38	0.99	3	0.061	0.44	0.49	0.33	0.72
Bmys96R421	2	0.005	0.35	0.39	0.26	0.99	2	0.040	0.43	0.46	0.32	0.78	2	0.041	0.17	0.18	0.13	1.00
Average	2.76	0.01	0.41	0.41	0.31		2.66	0.05	0.42	0.42	0.31		2.66	0.05	0.42	0.41	0.31	

*Bmy2 was not genotyped in the Eastern Canada population

Table 2: List of SNP loci with number of alleles from inferred haplotypes where >1 SNP is present in a locus.

			Allele frequencies						
SNP assays	No. of SNPs	No. of alleles	1	2	3	4	5	6	7
BmC5R700_Y910	2	3	0.77	0.20	0.04				
BmCATR205_R212	2	4	0.75	0.18	0.08	0.00			
BmCHYY286_R417	2	4	0.29	0.00	0.52	0.19			
BmCOL3A1Y82	1	2	0.23	0.77					
BmCSF2S320	1	2	0.47	0.53					
BmEDN1Y91	1	2	0.26	0.74					
BmFESY136	1	2	0.23	0.78					
BmMPOR184_R284	2	4	0.52	0.19	0.28	0.00			
BmPMKS494	1	2	0.33	0.67					
Bmys108D91	1	2	0.20	0.80					
Bmys1R248	1	2	0.02	0.98					
Bmys28Y154_R162	2	4	0.00	0.08	0.38	0.54			
Bmys31Y94	1	2	0.86	0.14					
Bmys34M251	1	2	0.94	0.06					
Bmys368R272	1	2	0.82	0.18					
Bmys382Y279	1	2	0.09	0.91					
Bmys387R245_R361	2	3	0.15	0.07	0.78				
Bmys395Y158	1	2	0.52	0.48					
Bmys402M56*	1	2	0.58	0.42					
Bmys404Y286_K316	2	4	0.23	0.47	0.00	0.30			
Bmys410K107	1	2	0.60	0.40					
Bmys412R79_R463	2	3	0.05	0.72	0.23				
Bmys414R127	1	2	0.34	0.66					
Bmys42aK46_aR225_bK232	3	7	0.01	0.29	0.24	0.12	0.21	0.10	0.03
Bmys43Y237_Y377	2	4	0.14	0.25	0.04	0.57			
Bmys48S269	1	2	0.57	0.43					
Bmys60Y148_R260	2	4	0.01	0.87	0.03	0.09			
Bmys92Y230_K271	2	3	0.20	0.22	0.58				
Bmys96R421	1	2	0.22	0.78					
Total	42	81							

*X-linked locus

Table 3: Population structure results for spatial, temporal, and age cohort pairwise analyses. Statistically significant results (p<0.05) are shown in bold. Actual F_{ST} and G"$_{ST}$ values are provided, but only p-values for χ^2 test results are shown.

	mtDNA*			SNPs				Microsatellites				Combined SNPs & Microsatellites			
Strata	**n**	**F_{st}**	**c^2 p**	**n**	**F_{st}**	**G"st**	**c^2 p**	**n**	**F_{st}**	**G"st**	**c^2 p**	**n**	**F_{st}**	**G"st**	**c^2 p**
Spatial															
Barrow v SLI	258/52	-0.003	0.493	312/47	0.000	-0.010	0.520	214/23	-0.001	-0.076	0.554	213/23	-0.001	-0.033	0.610
Barrow v Savoonga	258/20	-0.004	0.687	312/19	0.003	-0.021	0.698	214/16	-0.003	-0.116	0.311	213/16	-0.001	-0.045	0.409
Barrow v Gambell	258/32	-0.004	0.597	312/28	-0.001	-0.018	0.500	214/7	0.002	-0.210	0.834	213/7	0.001	-0.097	0.759
Barrow v Chukotka	258/22	-0.009	0.877	312/14	0.003	-0.026	0.183	214/12	-0.002	-0.143	0.909	213/12	-0.002	-0.058	0.878
AK v Chukotka	349/22	-0.008	0.799	413/14	0.003	-0.026	0.138	269/12	-0.002	-0.141	0.849	268/12	-0.002	-0.058	0.839
NS v SLI	297/52	-0.003	0.709	365/47	0.000	-0.010	0.459	245/23	-0.001	-0.073	0.430	244/23	0.000	-0.032	0.484
NS v Savoonga	297/20	-0.002	0.637	365/19	0.003	-0.020	0.625	245/16	-0.002	-0.113	0.245	244/16	-0.001	-0.044	0.284
NS v Gambell	297/32	-0.004	0.563	365/28	0.000	-0.017	0.450	245/7	0.002	-0.210	0.770	244/7	0.002	-0.095	0.708
NS v Chukotka	297/22	-0.009	0.86	365/14	0.004	-0.024	0.148	245/12	-0.003	-0.145	0.884	244/12	-0.002	-0.058	0.875
BCB v Okhotsk†	29/25	**0.062**		427/50	**0.037**	**0.053**	**0.001**	306/60	**0.035**	**0.155**	**0.001**	280/49	**0.034**	**0.069**	**0.001**
BCB v E. Canada†				427/49	**0.008**	**0.004**	**0.001**	306/47	**0.005**	**-0.005**	**0.001**		NA		NA
Okhotsk v E. Canada†				50/49	**0.035**	**0.043**	**0.001**	60/47	**0.039**	**0.145**	**0.001**		NA		NA
Temporal															
Barrow S v F	125/133	0.000	0.546	157/155	-0.001	-0.007	0.920	102/112	0.001	-0.023	0.212	102/111	-0.001	-0.013	0.493
NS S v F	141/154	-0.001	0.421	173/191	-0.001	-0.006	0.864	113/132	0.000	-0.021	0.121	131/113	0.000	-0.011	0.299
SLI S v F	**17/14**	**0.056**	0.154	10/13	-0.020	-0.118	0.948	10/13	-0.007	-0.311	0.863	10/13	-0.012	-0.143	0.948
Age cohort (birth year)															
<1918 v 1918-1949	8/13	-0.010	0.320	7/13	-0.001	-0.101	0.423	5/11	-0.001	-0.411	0.707	5/11	0.000	-0.181	0.526
<1918 v 1950-1979	8/25	-0.013	0.686	7/22	0.007	-0.074	0.294	5/17	-0.011	-0.422	0.988	5/16	-0.002	-0.166	0.920
<1918 v >1979	8/33	0.003	**0.030**	7/26	-0.004	-0.091	0.217	5/23	0.581	-0.351	0.526	5/23	-0.006	-0.167	0.508
1918-1949 v 1950-1979	13/25	-0.010	0.981	13/21	0.004	-0.045	0.287	11/17	0.005	-0.192	0.365	11/16	0.002	-0.091	0.296
1918-1949 v >1979	13/33	0.010	**0.050**	13/26	-0.001	-0.052	0.229	11/23	0.004	-0.180	**0.009**	11/23	0.003	-0.079	**0.011**
1950-1979 v >1979	25/33	0.008	0.088	21/26	0.000	-0.037	0.528	17/23	0.000	-0.151	0.214	16/23	-0.001	-0.069	0.284
<1950 v 1950-1979	21/25	-0.007	0.829	20/21	0.005	-0.033	0.256	16/17	0.001	-0.174	0.725	16/16	0.001	-0.075	0.549
<1950 v >1979	21/33	0.009	**0.009**	20/26	-0.001	-0.040	0.198	16/23	0.002	-0.144	**0.025**	16/23	0.000	-0.066	**0.020**

Table 4: Estimates of effective population size (N_e) based on linkage disequilibrium. The lowest allele frequency = 0.02. n=harmonic mean of sample size (accounting for missing data).

Loci	Population (N)	Ne (95% CI)	# Indep. Alleles
22 Microsatellites	BCB (298)	356.8 (134.1 - ∞)	22743
	EC (46)	214.4 (124.1 - 655.3)	20019
	OK (60)	112.4 (78.8 - 183)	13156
29 phased SNPs	BCB (409)	1592.6 (689 - ∞)	1172
	EC (48)	237.6 (90.3 - ∞)	1005
	OK (49)	5827.5 (165.4 - ∞)	1141
29 SNPs and 22 microsatellites	BCB (277)	404.6 (183.2 - 4001.2)	33380
	OK (48)	137.2 (100.6 - 208.5)	23922

Table 5: Pairwise F_{ST} (G''_{ST}) below diagonal and p-value above diagonal for a) all samples and all loci, b) median of 1000 replicates of 40 random samples per population, c) median of 1000 replicates of 20 random samples per population, d) same as b), but with 10 loci chosen randomly for each replicate, and e) with 5 loci chosen randomly for each replicate. Sample sizes are indicated in parentheses after the population names. The same samples were genotyped for BCB and Okhotsk for both SNPs and microsatellites, but samples from Eastern Canada were non-overlapping, so different samples were used to calculate statistics for microsatellites vs. SNPs. P values were based on 500 permutations.

	SNPs			Microsatellites		
a) (all loci)						
	BCB	E. Canada	Okhotsk	BCB	E. Canada	Okhotsk
BCB (280)	--	**0.001**	**0.001**	--	**0.001**	**0.001**
E. Canada (49)	**0.008 (0.004)**	**--**	**0.001**	**0.005 (-0.005)**	**--**	**0.001**
Okhotsk (49)	**0.037 (0.053)**	**0.035 (0.043)**	**--**	**0.035 (0.155)**	**0.039 (0.145)**	**--**
b) (all loci)						
	BCB	E. Canada	Okhotsk	BCB	E. Canada	Okhotsk
BCB (40)	--	**0.018**	**0.002**	--	**0.006**	**0.002**
E. Canada (40)	**0.008 (-0.008)**	**--**	**0.002**	**0.005 (-0.045)**	**--**	**0.002**
Okhotsk (40)	**0.037 (0.042)**	**0.035 (0.040)**	**--**	**0.035 (0.118)**	**0.039 (0.128)**	**--**
c) (all loci)						
	BCB	E. Canada	Okhotsk	BCB	E. Canada	Okhotsk
BCB (20)	--	0.126	**0.002**	--	0.079	**0.002**
E. Canada (20)	0.007 (-0.031)	--	**0.002**	0.005 (-0.122)	--	**0.002**
Okhotsk (20)	**0.036 (0.020)**	**0.034 (0.018)**	**--**	**0.035 (0.056)**	**0.038 (0.063)**	**--**
d) (10 loci)						
	BCB	E. Canada	Okhotsk	BCB	E. Canada	Okhotsk
BCB (40)	--	0.096	**0.002**	--	**0.034**	**0.002**
E. Canada (40)	0.007 (-0.009)	**--**	**0.002**	**0.005 (-0.05)**	**--**	**0.002**
Okhotsk (40)	**0.035 (0.040)**	**0.032 (0.035)**	**--**	**0.035 (0.117)**	**0.039 (0.127)**	**--**
e) (5 loci)						
	BCB	E. Canada	Okhotsk	BCB	E. Canada	Okhotsk
BCB (40)	--	0.174	**0.004**	--	0.076	**0.002**
E. Canada (40)	0.006 (-0.011)	**--**	**0.004**	0.005 (-0.05)	**--**	**0.002**
Okhotsk (40)	**0.033 (0.036)**	**0.031 (0.033**	**--**	**0.035 (0.116)**	**0.037 (0.126)**	**--**

Table 6. Mean assignment of individuals from the three populations (BCB, Canada, and Okhotsk) to groups when STRUCTURE was used to define three groups (k=3). Analyses were run using all SNP markers, all (22) microsatellite markers, or a randomly chosen subset of microsatellite markers. All analyses were run 10 times. In analyses where a subset of microsatellite markers was used, a different randomly chosen set of markers was used in each replicate. The USELOC parameter controls whether or not sampling location is used as a prior in the analysis (T = used, F = not used).

	Markers Used	Population	STRUCTURE groups 1	2	3
		BCB	0.485618	0.515062	0.032861
	22 msats	Canada	0.482087	0.453198	0.035427
		Okhotsk	0.032298	0.031811	0.931647
		BCB	0.468911	0.44387	0.05621
	15 msats	Canada	0.4636	0.49033	0.060343
		Okhotsk	0.067472	0.065791	0.883445
USELOC=F		BCB	0.42658	0.402079	0.07432
	10 msats	Canada	0.414357	0.436806	0.087465
		Okhotsk	0.159068	0.16114	0.838222
		BCB	0.334964	0.329689	0.32871
	5 msats	Canada	0.33307	0.334206	0.333714
		Okhotsk	0.331966	0.336087	0.33762
		BCB	0.385495	0.288827	0.147302
	SNPs	Canada	0.337129	0.422343	0.210147
		Okhotsk	0.277362	0.288849	0.642555
		BCB	0.981256	0.32996	0.028124
	22 msats	Canada	0.015043	0.664172	0.006614
		Okhotsk	0.003737	0.005891	0.965263
		BCB	0.984195	0.279291	0.039516
	15 msats	Canada	0.005907	0.701821	0.007916
		Okhotsk	0.009917	0.018913	0.952545
USELOC=T		BCB	0.969562	0.247649	0.058404
	10 msats	Canada	0.009906	0.727294	0.01388
		Okhotsk	0.020523	0.025026	0.927694
		BCB	0.734019	0.636036	0.028273
	5 msats	Canada	0.227746	0.32947	0.139339
		Okhotsk	0.038235	0.034485	0.832376
		BCB	0.846025	0.385153	0.086831
	SNPs	Canada	0.122126	0.581027	0.081516
		Okhotsk	0.031839	0.033839	0.831667