

Genetic diversity in Eastern Canadian and Western Greenland bowhead whales (*Balaena mysticetus*)

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ABSTRACT

We determined the genetic structure of bowhead whales from the Baffin Bay- Davis Strait (Disko Bay, Cumberland Sound, and Pelly Bay) and the Hudson Bay-Foxe Basin stocks (Repulse Bay and Foxe-Basin) through sequence analyses of a 453 bp stretch of the control region of the mitochondrial DNA. The Hudson Bay-Foxe Basin stock is characterized by a lower number of haplotypes than the Baffin Bay-Davis Strait stock. There was a slight but significant genetic differentiation of the two stocks in terms of F_{ST} . However, there was no differentiation between the Hudson Bay-Foxe Basin stock and the bowhead whales collected from Cumberland Sound, an area within the range of the Baffin Bay-Davis Strait-stock.

INTRODUCTION

Bowhead whales (*Balaena mysticetus*) congregate predictably at several coastal locations in the Canadian eastern Arctic and in West Greenland at different seasons. In 1977, a two-stock hypothesis for bowhead whales occupying eastern Canadian and western Greenlandic waters was adopted as the working model of the IWC (1978). The stocks came to be identified as the Baffin Bay-Davis Strait (BB-DS) or “Baffin Bay stock” and the Hudson Bay-Foxe Basin (HB-FB) or “Hudson Bay stock” and have been treated as separate populations since (see Mitchell and Reeves 1981; Cosens and Innes 2000; Finley 1990, 2001). Satellite tracking studies have demonstrated that bowhead whales wintering off the west coast of Greenland can spend the summer in the eastern Canadian Arctic and move to Hudson Strait for the subsequent winter (Heide-Jørgensen et al. 2003, 2006). Similarly, bowhead whales are found in large concentrations at specific locations in Foxe Basin, Northern Hudson Bay, in fjords along the east coast of Baffin Island and in the Canadian high Arctic during summer. In winter they tend to congregate in the Hudson Strait, at the mouth of Cumberland Sound, along West Greenland and in the North Water.

Given that bowhead whales are subject to a limited hunt in Nunavut, Northern Canada, and that a quota was recently given by IWC to the West Greenland wintering aggregation (IWC 2009), it is timely to re-assess the population structure of these whales in light of new information that has been collected. Heide-Jørgensen et al. (2006) suggested that the bowhead whales summering in eastern Canada and wintering in West Greenland might consist of a single population. Based on satellite tracking studies of bowhead whales tagged in West Greenland, they argued that bowhead whales are capable of travelling long distances in relatively short periods of time and suggested that there was no reason why whales should be restricted to relative small portions of the total potential range in eastern Canada and Greenland waters. They also noted that there was little geographical separation between individuals belonging to the two putative stocks. Heide-Jørgensen et al. (2010) argued that based on patterns of sexual aggregations, bowhead whales summering in the eastern Canadian Arctic and wintering off the west coast of Greenland must belong to one population. Wiig et al. (2010) found three genetically confirmed re-identifications between localities that crossed the putative stock boundary between the Foxe Basin-Hudson Bay stock and the Davis Strait-Baffin Bay stock suggesting that this is not a valid stock delineation. In this paper we study the genetic differentiation between the Baffin Bay-Davis Strait and the Hudson Bay-Foxe Basin stocks as well as the sampling localities within these stocks.

MATERIAL AND METHODS

In this study we collected 710 skin biopsies from bowhead whales by use of crossbows with biopsy darts at several localities in the Eastern Canadian Arctic and in the Disko Bay, West Greenland, between 1995 and 2009 (Wiig et al. 2010). An additional seven samples were collected from the subsistence hunt in Canada and West Greenland. The majority of samples were collected during field operations where bowhead whales were instrumented with satellite transmitters, but in some communities (Pelly Bay, Repulse Bay, Disko Bay) biopsies were collected by local hunters. The majority of samples in Disko Bay were collected between April and May, and the samples from Nunavut, Canada, were collected between July and September. All samples were stored in salt saturated, 20% DMSO and kept frozen at 20 degrees Celsius until analysis in the laboratories.

Total DNA was extracted from the skin biopsy samples using commercially available DNA extraction kits such as DNeasy® (Qiagen), E.Z.N.A. Tissue DNA kit (Omega Bio-tek), or GenElute™ (Sigma-Aldrich). Molecular sex determination followed basically a PCR-based approach as described by Bérubé and Palsbøll (1996).

A 453 bp stretch of the mitochondrial control region, corresponding to position 15 473–15 925 in the complete mitochondrial genome of the bowhead whale (Arnason et al. 1993, GenBank Accession no. AP006472) was amplified as described by Borge et al. (2007). The obtained PCR products were purified using 10x diluted exoSAP-IT (USB Corporation) and subsequently sequenced according to the instructions of the BigDye 1.1 sequencing kit (Applied Bioscience). The obtained nucleotide sequences were aligned and edited with the software sequencher 4.1 (GeneCodes).

Individual genotypes were determined for four to eight microsatellite loci (Bmy26, Bmy29, Bmy33, Bmy38, Bmy41, Bmy42, Bmy53, and Bmy58) according to Huebinger et al. (2008).

Within- and between-year recaptures were identified according to three main arguments. First, re-identified individuals must have the same sex, and second, they must also have the same mitochondrial haplotype. Once, the first sorting of individuals was done according to these criteria, re-captures were identified through microsatellite genotypes. It was decided that recaptured individuals must be verified by at least 4 microsatellite loci.

The quality of the obtained molecular data was assessed by a number of control experiments. The molecular sexing was compared between the two labs at the Natural History Museum, University of Oslo, Norway, and the Fisheries and Oceans Canada, Winnipeg, Manitoba, Canada for ~240 samples, and no incongruence was detected. We therefore conclude that the error rate for the molecular sexing is less than 0.5%. For the quality assessment of the mitochondrial control region sequences 48 biopsies were re-extracted and re-sequenced. No sequence differences were determined in comparison with the originally obtained sequences. Accordingly, the error rate for the DNA sequencing is below 2%. For seven microsatellite loci (Bmy26, Bmy29, Bmy33, Bmy41, Bmy42, Bmy53, and Bmy58) a total of 460 genotypes were determined on independent extractions, which yielded 5 different genotypes, i.e. an error rate for the microsatellite genotyping of slightly above 1%, given that at least one of the disaccoring genotypes is the correct one.

Estimates of genetic diversity, molecular variance, and population differentiation for the final dataset were obtained using the computer programs Arlequin Ver 2.000 (Schneider et al. 2000).

RESULTS

Molecular sexing, sequencing of the mitochondrial control region, and microsatellite genotyping was successful for 647 samples, 437 from the Baffin Bay-Davis Strait stock and 200 from the Hudson Bay-Foxe Basin stock. A total of 107 recaptures were detected within and between sampling years (Wiig et al. 2010) and removed from the dataset. After cleaning the dataset for subsequent analysis consisted of 543 samples (Baffin Bay-Davis Strait stock: Disko Bay N = 272, Cumberland Sound N = 67, Pelly Bay N = 7; Hudson Bay-Foxe Basin stock: Repulse Bay N = 13, Foxe Basin N = 184).

For the Baffin Bay-Davis Strait stock a total of 50 mitochondrial haplotypes were detected, out of which 17 (34.0%) were detected in only one individual. For the Hudson Bay-Foxe Basin stock a total of 30 mitochondrial haplotypes were detected, out of which 8 (26.7%) were detected in only one individual. Twenty-five haplotypes are shared between the Baffin Bay-Davis Strait and the Hudson Bay-Foxe Basin stocks. Further details on haplotype diversity are summarized in Table 1 for both stocks as well as for the collecting sites Disko Bay (West Greenland), Pelly Bay, Pangnirtung (Cumberland Sound), Repulse Bay, and Igloolik (Foxe Basin) separately.

Haplotype DB4/EC2 is the most common one at all sampling sites with an average frequency of 0.215 in the Baffin Bay-Davis Strait stock and 0.332 in the Hudson Bay-Foxe Basin stock. This particular haplotype is the same one that was found most common for the Holocene Spitsbergen stock (Borge et al. 2007) and the extant

BCB stock (Rooney *et al.* 2001). With respect to the high number of mitochondrial haplotypes in bowhead whales, there are 15 further haplotypes that deserve attention, since they occur with relatively high frequencies (> 0.03) in at least one stock/sampling site. These are listed in Table 2 along with the respective frequencies.

The genetic differentiation between the Baffin Bay-Davis Strait and the Hudson Bay-Foxe Basin stocks as well as the sampling localities Disko Bay, Pelly bay, Cumberland Sound, Repulse bay, and Foxe Basin were estimated in terms of F_{ST} values (Table 3). There is a slight genetic differentiation between the Hudson Bay-Foxe Basin stock and the Foxe Basin subsample and the Baffin Bay-Davis Strait stock and the Disko Bay subsample ($P < 0.05$). No genetic differentiation was found between the Cumberland Sound subsample (Baffin Bay-Davis Strait stock) and either the Foxe Basin subsample or the pooled Hudson Bay-Foxe Basin sample.

DISCUSSION

In this report we present an analysis of the mitochondrial DNA diversity of West Greenland and eastern Canadian Arctic bowhead whales and address the validity of the two stock hypothesis for the Davis Strait-Baffin Bay and the Hudson Bay-Foxe Basin stocks that was adopted as the working model by the IWC (1978). According to this model, the sample localities of Disko Bay, Pelly Bay and Cumberland Sound are within the Davis Strait-Baffin Bay stock range while the localities of Foxe Basin and Repulse Bay are within the Hudson Bay-Foxe Basin stock distribution area. Alternatively, Heide-Jørgensen *et al.* (2006) suggested that the bowhead whales summering in eastern Canada and wintering in West Greenland might consist of a single population.

Based on a dataset consisting of mitochondrial DNA haplotypes for a total of 543 bowhead whales a slight genetic differentiation was observed between the Baffin Bay-Davis Strait and the Hudson Bay-Foxe Basin stocks. At first glance the observed genetic differentiation seems to be in line with the two stock hypothesis. However, it needs to be emphasized that the detected genetic differentiation relates only to a single maternally inherited locus. Furthermore, both stocks show a high haplotype diversity, while the most common haplotypes are shared by the two stocks.

Sample sizes varied considerably among areas with as small sample sizes as seven individuals. The small sample sizes will in turn have implications for the precision and accuracy of our estimates of genetic divergence. However, we do not provide estimates of the uncertainty of our F_{ST} estimates. Such estimates of uncertainty are helpful in order to assess the confidence that can be placed in the estimates of genetic divergence (and these should be complemented with divergence estimates from nuclear loci as well).

Another issue that needs to be addressed is in the management implications of the estimates of genetic divergence. What does a F_{ST} value of 0.01 imply in terms of management and stock issues? Even if all the underlying assumptions for translating estimates of F_{ST} into an estimate of migration rates were valid (which is not known in this case) then an estimate of the effective population sizes is needed to calculate the migration rates (Palsbøll *et al.* 2006).

It is noteworthy that the genetic differentiation between the two stocks is first of all a genetic differentiation between the Hudson Bay-Foxe Basin stock and the bowhead whales sampled in Disko Bay, West Greenland. The bowhead whales sampled in Cumberland Sound show no differentiation from the Hudson Bay-Foxe Basin stock, but are also not differentiated from the Disko Bay individuals. This is congruent with the detection of recaptures of three Hudson Bay-Foxe Basin stock bowhead whales in the Cumberland Sound out of 16 whales recaptured between years (Wiig *et al.* 2010). These recaptures between different stock ranges indicate a significant level of migration between the stocks. Satellite tracking data (Heide-Jørgensen *et al.* 2006) support the notion that migration between the stock ranges is common. Based on the tracking data of bowhead whales tagged in West Greenland, they argued that bowhead whales are capable of travelling long distances in relatively short periods of time and suggested that there was no reason why whales should be restricted to relative small portions of the total potential range in eastern Canada and Greenland waters. In 2009, a further 27 bowhead whales were tagged in Disko Bay. Four of these whales crossed the borders for the spatial delineation of the Hudson Bay-Foxe Basin stock and the Baffin Bay-Davis Strait stock (GINR unpublished data, Wiig *et al.* 2010). It is assumed that the two stocks are separated by the Fury and Hecla Strait in northern Foxe Basin and the Hudson Strait, but these straits formed no barrier to restrict the whales' migration. Similar movement patterns of bowhead whales were also recorded in earlier satellite tracking studies in the area (cf. Heide-Jørgensen *et al.* 2008). In addition, Heide-Jørgensen *et al.* (2010) argued that the most reasonable explanation for the sex-segregation found of genetically sexed bowhead in this area is that bowhead whales summering in the eastern Canadian Arctic and wintering off the west coast of Greenland belong to just one population with those occupying Baffin Bay mainly being adult males and resting females and those in the Prince Regent, Gulf of Boothia, Foxe Basin and north-western Hudson Bay being nursing females, calves and sub-adults.

The results presented here should be viewed as preliminary and additional analyses and measures of confidence, as well as clear criteria of the genetic expectations of the two and one-stock hypothesis are needed before any conclusive and consistent results are to be expected.

Additional analyses and measures of confidence are certainly welcome, and clear criteria of the genetic expectations of the two and one-stock hypothesis are needed to arrive at conclusive and consistent identification of stocks and/or management units.

ACKNOWLEDGEMENTS

This study was funded by the Greenland Institute of Natural Resources, the Commission for Scientific Research in Greenland, the National Ocean Partnership Program (US National Science Foundation and Office of Naval Research), the Danish Cooperation for the Environment in the Arctic under the Danish Ministry of Environment and the Department of Fisheries and Oceans. We thank the hunters in Greenland and Canada for assisting with collecting the samples and the University of Copenhagen for providing access to its Arctic Station in Disko Bay.

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Table 1. Genetic diversity of the mitochondrial control region (CR) of bowhead whales from the Baffin Bay-Davis Strait and the Hudson Bay-Foxe Basin stocks based on sequencing of 453 bp

Stock/collecting site	Sample size	Number of haplotypes	Population specific	Nucleotide diversity(\pm SD)	Gene diversity (\pm SD)
Baffin Bay-Davis Strait	346	50	25	0.01207 (0.006)	0.9190 (0.008)
Disko Bay	272	45	21	0.01243 (0.007)	0.9203 (0.009)
Cumberland Sound	67	21	4	0.01046 (0.006)	0.9100 (0.022)
Pelly Bay	7	6	0	0.01261 (0.008)	0.9524 (0.096)
Hudson Bay-Foxe Basin	197	30	5	0.0084 (0.005)	0.8662 (0.019)
Foxe Basin	184	28	3	0.0082 (0.005)	0.8650 (0.020)
Repulse Bay	13	9	0	0.0119 (0.007)	0.9103 (0.068)

Table 2. Frequencies of mitochondrial haplotypes that occur with > 3% in at least one bowhead whale stock/sampling site (with the exception of Pelly Bay and Repulse Bay due to low sample size).

Haplotype	Baffin Bay-Davis Strait	Disko Bay	Cumberland Sound	Pelly Bay	Hudson Bay-Foxe Basin	Foxe Basin	Repulse Bay
DB10/EC28	0.108	0.118	0.077	-	0.04	0.043	-
DB13/EC37	0.029	0.037	-	-	-	-	-
DB17/EC16	0.087	0.081	0.123	-	0.081	0.081	0.077
DB18/EC17	0.032	0.029	0.031	0.143	0.04	0.038	0.077
DB189	0.015	0.011	0.031	-	0.005	-	0.077
DB22/EC9	0.02	0.015	0.047	-	0.015	0.016	-
DB28/EC21	0.026	0.026	0.031	-	0.035	0.038	-
DB3/EC8	0.073	0.074	0.077	-	0.101	0.103	0.077
DB34/EC5	0.038	0.026	0.062	0.286	0.04	0.043	-
DB4/EC2	0.215	0.21	0.246	0.143	0.328	0.33	0.308
DB6/EC34	0.067	0.07	0.046	0.143	0.035	0.038	-
DB67/EC14	0.026	0.022	0.046	-	0.01	0.011	-
DB70/EC12	0.012	0.007	0.031	-	0.01	0.011	-
DB77/EC22	0.032	0.037	0.015	-	0.04	0.032	0.154
DB8/EC6	0.009	0.007	0.015	-	0.035	0.032	0.077
DB9/EC35	0.012	0.011	0.015	-	0.03	0.032	-

Table 3. Number of shared mitochondrial haplotypes between stocks/sampling sites (above diagonal) and genetic differentiation expressed as F_{ST} based on haplotype frequencies (below diagonal)

	Baffin Bay-Davis Strait	Disko Bay	Cumberland Sound	Pelly Bay	Hudson Bay-Foxe Basin	Foxe Basin	Repulse Bay
Baffin Bay-Davis Strait		45	21	6	25	24	7
Disko Bay	-0.00295		16	6	24	23	7
Cumberland Sound	-0.00390	-0.00140		5	17	16	7
Pelly Bay	0.00704	0.01098	0.00635		6	6	2
Hudson Bay-Foxe Basin	0.00885*	0.01054*	0.00074	0.02744		28	9
Foxe Basin	0.00859*	0.01030*	0.00043	0.02687	-0.00514		7
Repulse Bay	0.00704	0.01098	0.00635	0.01618	0.02744	0.02687	

* significant at $p \leq 0.05$