

STOCK STRUCTURE OF BOWHEAD WHALES (*BALAENA MYSTICETUS*) INFERRED FROM MITOCHONDRIAL DNA SEQUENCES

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INTRODUCTION

The Bowhead whale is one of 16 baleen whale species and lives mostly in the Arctic and subarctic waters. The International Whaling Commission (IWC) recognizes four populations of this species: the Okhotsk (OKS) Sea stock, the Bering-Chukchi-Beaufort (BCB) Seas stock, the East Canada-West Greenland (ECWG) stock, and the East Greenland-Svalbard-Barents (EGSB) Sea stock; and conserves and manages these populations to protect them against overhunting. This ongoing study aims to continuously monitor the BCB, ECWG, and OKS populations (with focus on BCB Seas stock) to determine their genetic variation and distribution levels, and if any unique genetic variants have been hunted. The BCB Seas samples were donated by Alaskan Natives who hunt these whales for subsistence. Additionally, fewer samples from the ECWG and OKS Sea stocks were obtained from whales hunted in Canada and sloughed skin. This study investigates the population structure of these whales by the sequencing of three loci within the mitochondrial genome: the cytochrome B (cytb) gene, the NADH dehydrogenase 1 (ND1) gene, and the hyper-variable Region 1 (HVR1) of the control region. The sequencing data from these three genes were analyzed via construction of a haplotype network, neutrality tests, analysis of molecular variant (AMOVA) tests, and fixation index (Fst) calculations. The study, which has been ongoing for over 3 decades, has data points for over 500 individuals; in this analysis, 429 samples with the cytb, ND1, and HVR1 mitochondrial loci were compiled.

METHODS

- Mitochondrial DNA (mtDNA) was extracted from the tissue samples, then amplified and sequenced. Sequence lengths of the HVR1, cytb, and ND1 loci were 397bp, 1140bp, and 957bp, respectively.
- A haplotype network was established using PopArt. Population neutrality tests and Fst and AMOVA estimates were performed using Arlequin.
- Migration estimates were calculated based upon the formula $M = \frac{1}{2} (1/Fst - 1)$

Samples Used for Analysis

BCB stock: 383 samples
Canada stock: 39 samples
Okhotsk stock: 7 samples

RESULTS & DISCUSSION

- By increasing the number of samples sequenced for the 3 mtDNA loci, we found high levels of genetic diversity in the BCB stock.
- We recovered a total of 141 unique haplotypes. Each stock had unique variants; shared haplotypes were found among BCB/OKS and BCB/ECWG.
- The haplotype network (Figure 1) depicts the relationships among the recovered haplotypes. Most haplotypes are unique to the BCB stock, but there are shared haplotypes among BCB/ECWG and BCB/OKS. These results indicate that the BCB stock is highly genetically diverse, which is expected due to BCB stock's large population size (>16,000 individuals). This diversity has also been found and predicted by previous studies.
- Neutrality tests (Table 1) show recent expansion of the BCB and ECWG stocks, indicated by the negative, significant values found for these stocks.
- Fst estimates (Table 2) show low levels of differentiation of the BCB-ECWG stock, and strong differentiation of the OKS from both stocks.
- An initial AMOVA test was performed using all individuals of the 3 stocks (Table 3). The results showed significant structuring and diversity among the groups.
- To further investigate the source of genetic diversity, we performed another AMOVA test, dividing the BCB population into 2 groups: Barrow Spring and Barrow Fall. This test included these 2 groups, plus the ECWG and OKS stocks (Table 4). Results showed less significant variation due to the splitting of the BCB stock.
- To determine if the Barrow Spring and Barrow Fall groups should be considered one cohesive group, we performed an additional AMOVA test between only these 2 sub-groups (Table 5). The values were found to be non-significant, indicating the lack of sub-structuring in the BCB stock.

Table 1. Neutrality tests. P-values are in parentheses. Significant values are those with p<0.05.

	BCB	ECWG (CAN)	OKS
Tajima's D	-1.41237 (0.036)	-1.47608 (0.0500)	1.53700 (0.95)
Fu's FS	-23.98149 (0.00)	-24.93650 (0.00)	-0.99561 (0.189)

Table 2. Fst values. P-values are in parentheses. Significant values are those with p<0.05.

	BCB (N=383)	CAN (N=39)	OKS (N=7)
BCB	-		
CAN	0.01048 (0.09)	-	
OKS	0.13019 (0.010)	0.14835 (0.018)	-

Table 3. AMOVA results using 3 groups: all BCB samples (N=383), all CAN samples (N=39), and all OKS samples (N=7).

Source of Variation	Degrees of freedom	% variation	Fixation index	p-value
Among population	2	3.29	Fst= 0.03290	0.00782
Within population	426	96.71		

Table 4. AMOVA results using 4 groups: Barrow Spring (N=111), Barrow Fall (N=155), CAN (N=39), and OKS (N=7).

Source of Variation	Degrees of freedom	% variation	Fixation index	p-value
Among population	3	0.75	Fst= 0.00753	0.10557
Within population	306	99.25		

Table 5. AMOVA results using 2 groups: Barrow Spring (N=111) and Barrow Fall (N=155).

Source of Variation	Degrees of freedom	% variation	Fixation index	p-value
Among population	1	-0.54	Fst= -0.00536	0.95992
Within population	264	100.54		

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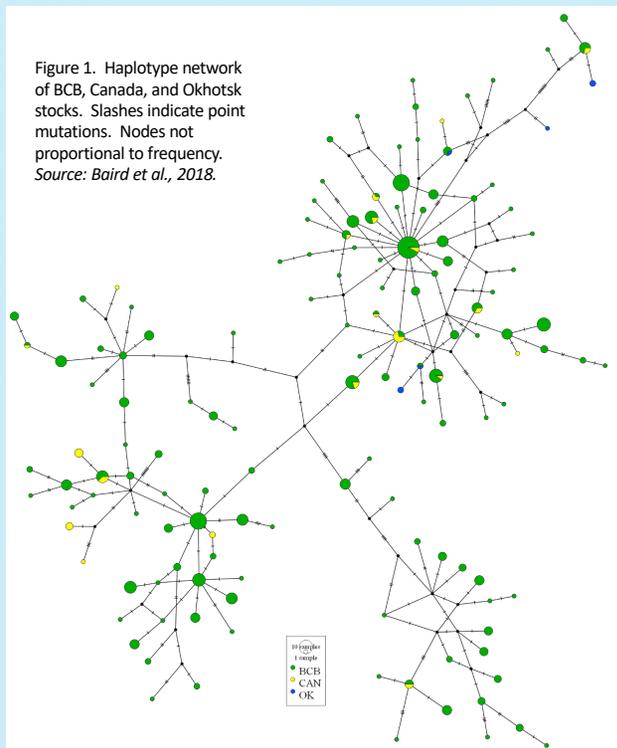


Figure 1. Haplotype network of BCB, Canada, and Okhotsk stocks. Slashes indicate point mutations. Nodes not proportional to frequency. Source: Baird et al., 2018.

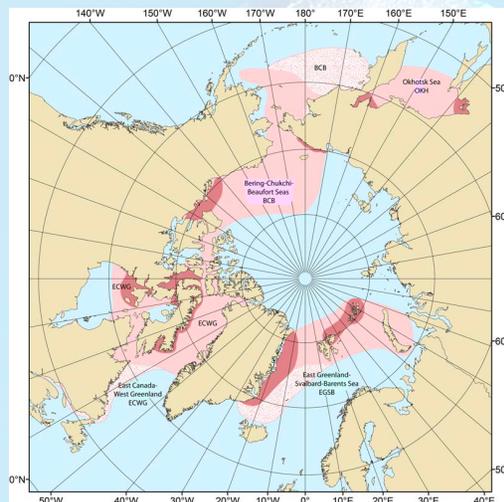


Figure 2. Present-day and historical ranges of bowhead stocks. Source: Map by John Citta as shown in Baird & Bickham, 2021.