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# Minimally destructive DNA extraction from archaeological artefacts made from whale baleen

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ABSTRACT

Here we demonstrate the successful extraction and amplification of target species DNA from artefacts made of whale baleen collected from excavations of past palaeo-Eskimo and Inuit cultures in Greenland. DNA was successfully extracted and amplified from a single baleen bristle of 1.5 cm length dated based on archaeological context to the period of the Saqqaq culture, more than 4000 years ago and following decades of storage at room temperature at the National Museum. The results reveal ancient baleen in archaeological material as a potential source of DNA that can be used for population genetic studies. We conclude that genetic investigation of historical baleen collections can contribute to our knowledge of the prehistoric population genetics of baleen whales, for example by quantifying the impact of modern whaling on the genetic diversity of bowhead whales.

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## 1. Introduction

Archaeological or ethnographic objects that utilise animal or plant tissue can be useful sources of DNA with which to facilitate retrospective genetic monitoring of population dynamics and historical exploitation of a species. For example, DNA extracted from Māori feather cloaks has recently been used to reveal that the brown kiwi population from New Zealand's North Island was the main source of the feathers used in the cloaks (Hartnup et al., 2011). However, ethnographic and archaeological artefacts have a high historical and cultural value, thus making the development of minimally destructive methods for DNA sampling of particular importance (e.g. Pichler et al., 2001).

Whale baleen is one such biological tissue that is both present in culturally important artefacts and also represents a potentially important source of DNA for biological questions. Baleen has been demonstrated as a potential source of DNA from studies extracting DNA from a historical sample (<100 years old; Rosenbaum et al., 1997) and a single ancient sample (4000 years old; Gilbert et al., 2008). However, this is the first study to extract DNA from baleen samples of a diverse range of ages and on archaeological material utilised for making tools and other artefacts. Historically utilised by many different cultures throughout the world (Lauffenburger, 1993), baleen is of particular importance for the indigenous human cultures that inhabit (historically and currently) the Arctic coast, for whom it represents an important material for manufacturing tools and utensils (Grønnow, 1994; Gulløv, 1997). In this regard, as a baleen whale found year-round in Arctic waters, the bowhead whale *Balaena mysticetus* has been the most commonly harvested and utilised species (Allen and Keay, 2006; Vibe, 1967). Bowhead whales were also targeted by commercial whaling between the 16th and 20th centuries (McLeod et al., 2008). Here, we investigate the potential for minimally destructive extraction of DNA from artefacts retrieved from excavations of past palaeo-Eskimo and Inuit cultures in Greenland.

## 2. Materials and methods

Baleen bristles are a keratinous tissue structurally similar to hair (Lauffenburger, 1993; Pfeiffer, 1993). Keratinous tissues, including feather, nail and hair, have been demonstrated as an excellent source of historical or ancient DNA (Bengtsson et al., 2012; Olsen et al., 2012), as the keratin protects the endogenous DNA from contamination from exogenous DNA sources such as bacteria (Gilbert et al., 2004). This study investigates the rate of success of DNA recovery from archaeological material from Disko Bay, Greenland, originating from the three distinct Greenlandic cultures: the Saqqaq culture, which spanned approximately 4500 to 2500 $^{14}$C years before present (yr B.P.), the Greenlandic Dorset

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culture of ca. 2500 to 2000 $^{14}C$ yr B.P. and the Thule culture spanning from 800 $^{14}C$ yr B.P. up to 19th century (Grønnow and Sørensen, 2006). The baleen samples used in this analysis are all dated by their archaeological context.

### 2.1. DNA extraction and purification

Small amounts of baleen were taken from a diverse range of samples from the Ethnographic Collection of the National Museum of Denmark, including material belonging to Qasigiannguit Museum and the Greenland National Museum and Archive. The effects utilised in the study includes artefacts and waste material from middens (Table 1.; Fig. 1). In some cases only a single strand of baleen 1 cm in length was used (Fig. 2).

All laboratory work on unamplified DNA was carried out in a designated clean lab, set up specifically for ancient DNA analyses. Blank DNA extractions and PCRs were incorporated to monitor for contamination. No modern whale DNA was present in the same building. Workflow conformed to ancient DNA protocols, i.e. individuals did not return to the clean lab on the same day following working in post-PCR areas. All post-PCR laboratory work on amplified DNA was conducted in a separate laboratory facility. The baleen was washed in 10% dilution of commercial bleach solution (final bleach concentration ca. 0.5%) and then rinsed in molecular grade water. Each baleen sample was then cut in to smaller fragments and placed in a sterile 1.5 ml tube. The baleen fragment was then immersed in 1.0 ml of a buffer containing 10 mM Tris, 10 mM NaCl, 5 mM CaCl, 2.5 mM EDTA, 2% SDS, 1 mM DTT and 0.1 ml of proteinase K. This was then incubated with agitation for 24 h at 55 °C. The samples were then centrifuged for 5 min at maximum speed to pellet any remaining solid and the supernatant was transferred into a 15 ml Centricon microconcentrator (30kD filter). Once the solution had concentrated down to approximately 100 μl above the filter, this was pipetted into a Qiagen (Valencia, CA) 'Qiaquick' spin column, 500 μl of Qiagen PN buffer was added and mixed before being spun at 6,000 g for 1 min. The column was subsequently washed with Qiagen PE buffer and the purified genomic DNA was eluted in 50 μl of Qiagen EB buffer. For every three samples, we included one blank extraction to monitor for contamination.

### 2.2. PCR

To explore the condition of the extracted DNA we initially attempted to amplify a region of the mitochondrial DNA control region (mtDNA) using previously published primers (297F: 5-CCGCTCCA TTAGATCACGAG-3; dip5R: 5-CCATCGWGATGTCTTATTTAAGRGGAA-3; Borge et al., 2007), resulting in a 200 base pair (bp) amplicon. Each 25 μl PCR contained 5 μl of DNA extract, 1× PCR buffer, 1 mM $MgCl_2$, 1 μM of each primer, 0.2 mM mixed dNTPs and 0.2 μl AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). PCR amplifications were performed using an MJ Thermocycler with a 4 min activation step at 95 °C, followed by 50 cycles of 95 °C for 20 s, 54 °C for 20 s, 72 °C for 20 s, followed by a final extension period of 72 °C for 7 min. For every three samples, we included one blank PCR using $ddH_2O$ to monitor for contamination. Following visualisation on agarose gels, any amplified PCR products were purified using MinElute PCR purification kits (Qiagen). Purified products were sequenced in both directions using the PCR primers and ABI sequencing chemistry by the Macrogen commercial sequencing service (Macrogen, Seoul, S. Korea). To guard against the incorporation of erroneous data derived from DNA damage or contamination, the PCR amplification and sequencing process was replicated twice for each sample. The samples for which the 200 bp mtDNA fragment failed to amplify were included in a PCR amplification with the above conditions, but with primers designed to amplify an 80 bp amplicon of mtDNA: Bow80-F(5-CAGGGATCC CTCTTCTCGCA-3) and Bow80-R(5-GACATCTGGTTCTTACTTCAG-3). These PCR-products were subsequently cloned using the Topo TA system (Invitrogen, Carlsbad, CA), and insert containing colonies were PCR amplified following the manufacturers' guidance, after which 8-16 clones were sequenced for each sample (Macrogen). DNA sequences were subsequently edited and aligned by eye and checked against reference DNA sequences in GenBank using the BLAST algorithm implemented in MEGA 5 (Tamura et al., 2011).

## 3. Results

For the 19 samples tested, we successfully amplified a 200 bp amplicon of mtDNA from nine (Table 1). We were able to amplify an 80 bp fragment for 5 of the 10 samples for which we were unable to amplify the 200 bp amplicon (Table 1). There was complete agreement between the forward and reverse sequence and

**Table 1**
A list of artefacts used in this study, their location of culture of origin, and results of efforts to extract and amplify DNA from them. The Saqqaq culture spanned approximately 4500 to 2500 $^{14}C$ yr B.P.; the Greenlandic Dorset culture spanned approximately 2500 to 2000 $^{14}C$ yr B.P. and the Thule culture spanned from 800 $^{14}C$ yr B.P. up to 19th century. Unworked baleen exhibited no evidence of human manipulation, baleen strips were elongated pieced which showed clear evidence of having been cut by humans, refuse baleen were small offcut pieces that show clear evidence of having been cut by humans.

| Sample no. | Context | Artefact | Dating | DNA amplified | GenBank accession number |
|---|---|---|---|---|---|
| Qa.257 | Qajaa | Baleen, unworked | Saqqaq or Dorset | 80 bp | |
| Qa.A39 | Qajaa | Baleen, unworked | Saqqaq or Dorset | 200 bp | JX174095 |
| Qa.F284 | Qajaa | Baleen, unworked | Saqqaq or Dorset | – | |
| L6.995 | Igdlutalik | Baleen cup | Thule culture | 80 bp | |
| L6.1329 | Igdlutalik | Snow beater | Thule culture | 80 bp | |
| L8.1737 | Sermermiut | Baleen wrapped with string | Thule culture | 80 bp | |
| L8.2151 | Sermermiut | Baleen with knots | Thule culture | 200 bp | JX174096 |
| L6.3581 | Qerqertaq | Baleen with holes | Thule culture | – | |
| L8.1176 | Sermermiut | Baleen knot. | Thule culture | 80 bp | |
| L8.1236 | Sermermiut | Baleen ring with knots | Thule culture | 200 bp | JX174097 |
| L8.2489 | Sermermiut | Baleen strip with hole | Thule culture | – | |
| L8.3737 | Sermermiut | Baleen strip | Dorset culture | 200 bp | JX174098 |
| L8.4120 | Sermermiut | Baleen strip | Saqqaq culture | – | |
| Qt 90 20/19: 122 | Qeqertasussuk | Baleen (refuse) | Saqqaq culture | 200 bp | JX174099 |
| Qt 90 19/19: 147 | Qeqertasussuk | Baleen (refuse) | Saqqaq culture | – | |
| Qt 90 20/19: 119 | Qeqertasussuk | Baleen (refuse) | Saqqaq culture | 200 bp | JX174100 |
| Qt 90 26/21,5: 11 | Qeqertasussuk | Baleen (refuse) | Saqqaq culture | 200 bp | JX174101 |
| Qt 90 25/21: 37 | Qeqertasussuk | Baleen (refuse) | Saqqaq culture | 200 bp | JX174102 |
| Qt 90 26/21,5: 29 | Qeqertasussuk | Baleen (refuse) | Saqqaq culture | 200 bp | JX174103 |

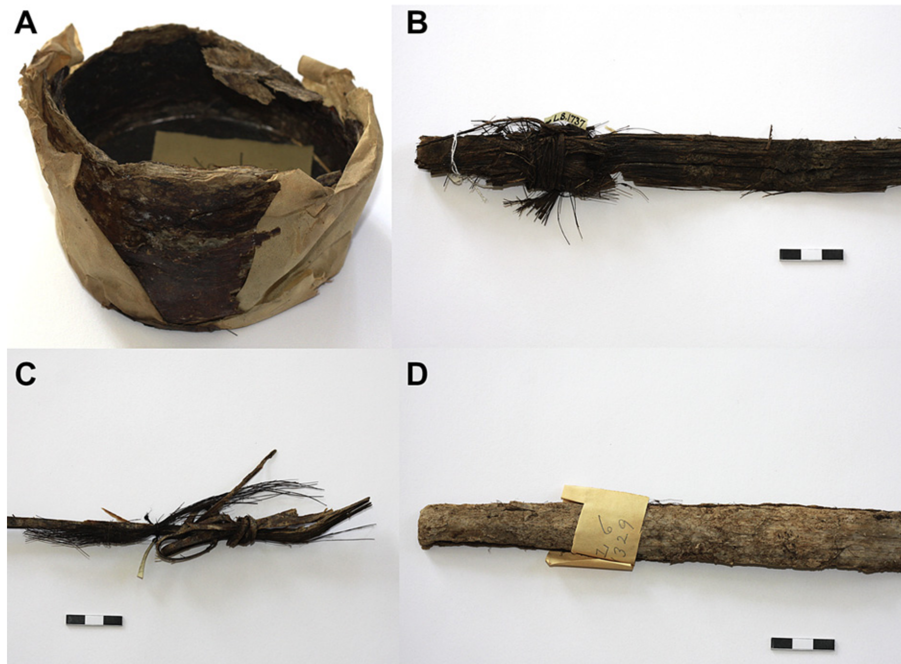


**Fig. 1.** A selection of artefacts sampled in this study: (A) a baleen cup *L6.995*, (B) Baleen wrapped with string *L8. 1737*, (C) Baleen with knots L8 2151, (D) Snow beater *L6 1329*.

between PCR replicates and all cloned sequences for each sample included in this study. All amplified fragments were confirmed as bowhead whale sequences by BLAST search. No DNA was amplified from the negative controls, indicating that there was no contamination during DNA extraction or PCR set up. Comparing the success rate of DNA amplification between samples of different ages and preservation showed little evidence of an effect due to the age of the sample over the timescales investigated here, but an apparent influence of anthropogenic utilisation on the success rate of amplification (Table 1).

## 4. Discussion

The cold and dry Arctic environment is optimal for long-term preservation of the DNA in keratinous tissue (Gilbert et al., 2007, 2008), enhancing the potential for extraction of ancient DNA. Our study demonstrates that this is the case for baleen, even in samples up to several thousand years in age. The age of the sample, over the timescales investigated in this study, did not appear to influence the survival of DNA within the baleen. There was an approximately equal success rate of amplification of the longer 200 bp DNA fragment for samples from each of the different cultures. The overall success rate of DNA amplification from baleen samples from bowhead whales was approximately 75%, compared with a success rate of over 95% for bone samples of bowhead whales from the Arctic spanning a similar age range (Borge et al., 2007; McLeod et al., 2012). Although the number of samples used in this study was too low to be conclusive, there was some possible indication that human utilisation decreases the success rate of amplifying larger DNA fragments (>80 bp) of DNA. Baleen artefacts that had been worked could have been subject to stresses such as heat, hydrolysis from contact with water, or microbial activity due to longer periods above freezing temperatures, escalating the process of fragmenting DNA and thereby decreasing the success rate of the DNA amplification. However, the amplification of an 80 bp fragment of mtDNA was relatively consistent in both worked and unworked objects, demonstrating the potential use of such worked archaeological artefacts as data sources for ancient DNA population genetics studies.

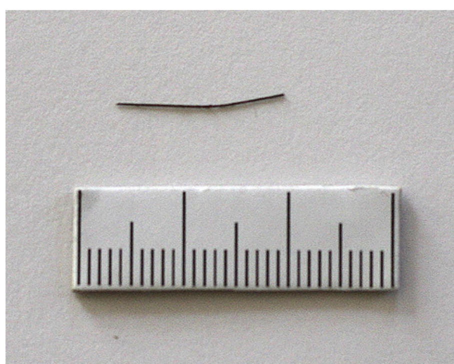

**Fig. 2.** An example of the typical amount of baleen sampled from each artefact from which DNA was extracted. Large markers on scale bar indicate 1 cm.

All successfully extracted and amplified DNA was confirmed as originating from bowhead whale. However, additional baleen whale species, such as humpback, minke, fin, sei and blue whale feed in Greenlandic waters during summer (Clapham et al., 1999; Laidre et al., 2010) and it seems likely that their baleen would have been utilised as well. Through broader geographic sampling it can be determined if and to what extent and where these species were exploited. The impact of commercial whaling on baleen whales is of great interest to conservation biologists who need to determine the baseline population sizes to set targets for assessing the status of population recovery. This has traditionally been done by the inspection of historic logbooks of whaling vessels (Reeves and Smith, 2002). However, using DNA can also provide an estimate of effective population size (Roman and Palumbi, 2003) and ancient DNA can be used to track changes in effective population sizes through time (see Foote et al., 2012 for a review). Therefore we predict that the field of bioarchaeology will have an important role to play in answering some critical conservation questions and our findings here suggest that the investigations into baleen whale

population dynamics could benefit from genetic investigations of ethnographic material from Greenland.

## 5. Conclusions

Ancient >2500 $^{14}$C (yr B.P.) baleen sampled from artefacts from prehistoric paleo-Eskimo and Inuit middens and settlements can contain well preserved DNA. The method published here has been successful in the extraction and amplification of DNA from small quantities of degraded and ancient baleen samples. This DNA can first of all determine the species of origin and therefore provide insights in to the hunting habits of these cultures, but can also be used to estimate historic population dynamics of the whales prior to, during and post-commercial whaling. Therefore the findings of this study suggest that ethnographic artefacts constructed from baleen can potentially provide important clues for estimating the impact of hunting and utilisation of artic baleen whales, especially the genetic legacy of commercial whaling.

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