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Genetic Approach to Species Identification of Whale Bones from South Georgia Island Whaling Stations

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ABSTRACT

Skeletal remains of the great baleen whales killed during the onset of 20th century commercial whaling in the Southern Ocean lie scattered across the shores and abandoned whaling stations of the sub-Antarctic island of South Georgia. These bones provide testament to the once prolific cetacean populations that were exploited at the first Southern Ocean commercial whaling stations. Here we present a species identification of whale bones collected between 2006 and 2007 from abandoned whaling stations at South Georgia. The maternally inherited mitochondrial DNA (mtDNA) control region (300-500 bp) sequence was used to identify the bone samples to species using the web-based program DNA Surveillance. Of the 281 available bone samples, 232 provided DNA of sufficient quality for species identification; 162 humpback whale, 48 fin whale, 19 blue whale, 1 sei whale, 1 southern right whale and 1 elephant seal. The prominence of humpback, fin and blue whale bones in the sample correspond to the catch record of the whaling industry from South Georgia Island.

INTRODUCTION

In 1903, Norwegian Captain C. F. Larsen discovered the pristine whale populations in the surrounding waters of South Georgia Island. This sub-Antarctic island provided the perfect landscape for the whaling industry with flat shorelines, safe harbors and access to fresh water (Tonnessen and Johnsen 1982). The first commercial whaling station in the Southern Ocean was established at Grytviken, South Georgia, in 1904 (Headland 1984). The initial factories were floating factories, large converted ships anchored in the sheltered harbors with access to fresh water. Small steam propelled boats caught whales with explosive harpoons and towed the carcasses back to the harbor to be processed by the floating factories. A total of 13 floating factories and 6 land-based whaling stations operated at South Georgia Island between 1904 and 1965 (Headland 1984).

At the conclusion of the 61 year commercial whaling industry on South Georgia Island, 175,250 whales had been caught and processed at the land based and floating factory whaling stations (Tonnessen and Johnsen 1982). During the first 10 years of the whaling industry on South Georgia, humpback whales were the prominent species caught, accounting for 80% of the total catch (Tonnessen and Johnsen 1982). However, by 1915, humpback whales in the surrounding waters of South Georgia had been hunted to commercial extinction. Blue whales followed the same fate and by 1936 had also disappeared from the South Georgian waters. A total of 26,754 humpback whales and 41,515 blue whales were processed at the island of South Georgia (Headland 1984). Other prominent species in the catch record included fin whales

(87,555), sei whales (15,128), and sperm whales (3,716) (Fig. 1). By 1965, whale populations in the surrounding waters of South Georgia Island had been driven to commercial extinction (Headland 1984).

The same devastating effects of the commercial whaling industry at South Georgia Island were seen throughout the Southern Hemisphere oceans during the 20th century. Between 1904 and 1980, the commercial whaling industry in the Southern Hemisphere killed approximately 2 million whales; this included over 200,000 humpbacks, 725,000 fin whales and 350,000 blue whales (Baker and Clapham 2002). Once prolific cetacean populations were driven to dangerously low abundances, and for several, any sign of recovery is still debated today (Moore et al. 1999; Clapham et al. 2007).

OBJECTIVES

From records of the first years of oil production in the South Georgia whaling industry, there is evidence that a large percentage of the whale was wasted during processing at the floating factories. On average, only a third of the possible oil yield obtained, with the rest of the whale carcass was discarded into the harbor waters (Tonnessen and Johnsen 1982). This has resulted in bones scattered across the shores of the now abandoned whaling stations. The ability to extract DNA from ancient material allows these remains to be used to provide a snapshot of the abundance and genetic diversity of the pre-whaling cetacean populations surrounding South Georgia (Lindqvist et al. 2009). To fulfill the initial phase of this project, we provide species identification from the extracted DNA of 232 bone samples collected from shorelines of South Georgia to compare with the catch record from South Georgia Island. For our analyses, we chose the mitochondrial DNA (mtDNA) control region because of its power in species identification (Ross et al. 2003) and the potential for judging loss of haplotype diversity by comparison to sequences from contemporary samples (LeDuc et al. 2007; Olavarria et al. 2007).

METHODS

Sample Collection. Bones samples (n=281) were collected in association with the British Antarctic Survey (BAS) from whaling stations on South Georgia Island. At time of collection, nether species nor anatomy were recognizable from the bones. Therefore, bones were selected based on density and size. Although during sample selection no precaution was taken to avoid replicate bone samples, over the past century, the thousands of whale carcasses released into the harbors, have been broken up by the currents in the harbors, potentially moving individual bones large distances.

DNA extraction. The bones were drilled and bone powder of each sample was shipped to the Cetacean Conservation and Genetics Laboratory (CCGL) at Hatfield Marine Science Center (HMSC) of Oregon State University (OSU) in Newport, OR. An ancient DNA laboratory was established for the processing of the bones in an isolated location equipped with materials that had never been exposed to modern cetacean DNA. DNA was extracted using a modified silica column based procedure (Qiagen DNeasy). A set of 11 samples were run with a negative control. To precaution against contamination the entire extraction region was cleaned with a 50% bleach solution between extractions.

mitochondrial DNA sequencing. The mtDNA control region was amplified using a standard sequencing protocol. The reaction mix consisted of 10X Buffer, $MgCl_2$, BSA (Bovine Serum Albumin), forward and reserve primers, dNTPs, Plat taq and 5 µL of template DNA. Forward primer M13Dlp1.5 (5'TGTAAAACGACAGCCAGTTCACCCAAAGCTGRARTTCTA 3') and reverse primers Dlp4 (5'

GCGGGWTRYTGRTTTCACG3') or Dlp5 (5' CCATCGWGATGTCTTATTTAAGRGGAA 3') were used to amplify a 300-500 base pair of the 5` end of the control region. The reaction was run using the following thermocycle profile protocol: annealing temperature of 94°C for 3 minutes and 30 cycles of denaturing at 94°C for 30 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 60 seconds followed by a final extension step of 72°C for 10 minutes. PCR products were run out on a 1.6% agarose gel, stained with ethidium bromide (EtBr) and exposed to Ultra Violet (UV) light to verify amplification. For samples where no band was present, an additional round of PCR was carried out using the same reaction conditions but 5 µl 1:10 dilution of the PCR product from the first round of amplification.

The PCR products were sequenced using Sanger sequencing methods. Samples were prepared for sequencing using SAPEX (Amersham Biosciences), consisting of a shrimp alkaline phosphotase (SAP) and exonuclease 1 (EX) which removed excess dNTPs and primers from PCR. Sequencing followed using a 1/8 dilution of BigDye Dye Terminator Chemistry v3.1 (Applied Biosystems Inc.) and manufacturer's specifications (Applied Biosystems Inc.) Sequences were edited using Sequencher v 4.9 (Gene Codes Corporation). A subset of the samples was independently amplified and reversed sequenced to validate the mtDNA sequence.

Species Identification. Species were identified from the mtDNA control region sequence using the web-based program DNA Surveillance (http://www.cebl.auckland.ac.nz:9000/) (Ross et al. 2003). The submitted mtDNA control region sequence is identified to species using a phylogenetic approach involving a curated database of all recognized cetacean species (Witness for the Whale, version 4.3). For sequences where the species was not identified using DNA surveillance (e.g., not a cetacean), we conducted a BLAST (Basic Local Alignment Search Tool) search of GenBank.

RESULTS

DNA was extracted and amplified mtDNA control region product was sequenced for 232 of the 281 bone samples. Amplified mtDNA sequences of approximately 300 base pairs allowed for a positive species identification of the whale bone. A subset of the samples was independently reversed sequence to verify the species identification. One error was detected in the independent sequencing due either to a handling error or a cross-contamination. No evidence of external contamination was detected.

Of the whale bones, 162 were identified as humpback whale bone, 48 identified as fin whale bone, 19 identified as blue whale bone, 1 identified as sei whale bone, 1 identified as southern right whale bone, and 1 identified as an elephant seal bone. The majority of the whale bones were identified as humpback whale. Initial comparisons of sequence variation indicate that the 162 humpback samples represent 66 haplotypes, the 48 fin whale samples represent 34 haplotypes and the 19 blue whale samples represent 16 haplotypes. Some of these haplotypes are not found in modern surveys of mtDNA control region diversity in contemporary populations of these species (LeDuc et al. 2007; Olavarria et al. 2007).

CONCLUSIONS

Standard 'ancient DNA' extraction technique enabled the amplification and sequencing of 300-500 base pairs of the mtDNA control region used for positive species identification of 86% of the collected whale

bones. No external contamination was detected and only one internal error was revealed by re-sequencing experiments to date.

During species identification, over 60% of the bones collected from whaling stations at South Georgia were identified as humpback whale. The remaining 40% of the bones were primarily identified as blue or fin whale bone. During the onset of the commercial whaling industry on South Georgia Island, the first species heavily exploited was the humpback whale (Headland 1984). In these early whaling years, captured whales were being processed in the floating factories. After 1913, there was an increase in the number of land-based whaling stations (Headland 1984). The bone samples in this study were collected from the shores near the whaling stations. Compared with whaling history, the species composition of the bone samples appears to be representative of this early period prior to the establishment of the land-based stations. Blue and fin whales were also heavily exploited from the surrounding waters of South Georgia (Fig. 1) and were seen at high frequency in the bone sample.

The correspondence of the species identification results to the catch record of South Georgia Island provide validation of the utility of ancient DNA, along with the methods for extraction and amplification used in this study. This work employs methods to utilize a previously untouched resource for the study of pre-whaling cetacean populations. Assessments of contemporary cetacean populations will be enhanced with a better comprehension of historical population abundances and genetic diversity. Not only will this improve modeling accuracy, but will enhance assessments of contemporary population growth rates and recovery (Jackson et al. 2008).

FUTURE WORK

Genetic Diversity. This work will contribute to previous effort to improve understanding of historical whaling records using DNA identification (Rosenbaum et al. 1997; Rosenbaum et al. 2000; Roman and Palumbi 2003; Rastogi et al. 2004; Lindqvist et al. 2009; McLeod et al. 2010). This work is the first step in the analysis of the genetic diversity of pre-whaling cetacean populations from the surrounding waters of South Georgia. Genetic diversity found in the historical bone samples will be compared to genetic diversity of contemporary worldwide populations to gauge a potential loss of genetic diversity due to commercial whaling.

Minimum population abundance. This work will also be supplemented by an analysis of the current Antarctic blue whale genetic diversity through analysis of a dataset of the 1989-2009 IDCR/SOWER Antarctic blue whale biopsy samples (SC/61/019). The number of mtDNA haplotypes found in the contemporary population will be used to estimate the minimum population abundance of the Antarctic blue whale in the Southern Ocean at their lowest abundance due commercial whaling (Carroll 2006; Jackson et al. 2008). During the 20th century, it is estimated that Antarctic blue whales were reduced to less than 1% of their original abundance (Branch et al. 2004). Initial research has indicated a loss in haplotype diversity in addition to population abundance (Branch and Jackson 2008). A revised estimate of the minimum population abundance, along with comparisons of pre - and post-whaling haplotype diversity will help assess the effect of whaling on this once prolific species.

REFERENCES

Baker, C. S. and P. J. Clapham (2002). Marine mammal exploitation: whales and whaling. Causes and consequences of global environmental change. I. Douglas. Chichester, John Wiley & Sons, Ltd. **3:** 446-450.

Branch, T. A. and J. A. Jackson (2008). Minimum bottleneck abundance of Antarctic blue whales based on current mtDNA diversity. Report SC/60/SH10 to the Scientific Committee of the International Whaling Commission

Branch, T. A., K. Matsuoka and T. Miyashita (2004). "Evidence for increase in Antarctic blue whales based on Bayesian modeling." Marine Mammal Science **20**(4): 726-754.

Carroll, E. L. (2006). The demographic and genetic bottleneck of the New Zealand southern right whale. School of Biological Sciences. Auckland, The University of Auckland**:** 131.

Clapham, P. J., A. Aguilar and L. T. Hatch (2007). "Determining spatial and temporal scales for management: lessons from whaling." Marine Mammal Science **24**: 183-201.

Headland, R. (1984). The Island of South Georgia. New York, Cambridge University Press.

Jackson, J. A., N. J. Patenaude, E. L. Carroll and C. S. Baker (2008). "How few whales were there after whaling? Inference from contemporary mtDNA diversity." Molecular Ecology **17**(1): 236-251.

LeDuc, R. G., M. G. Dizon, L. A. Pastene, H. Kato, S. Nishiwaki, C. A. LeDuc and R. L. Brownell Jr. (2007). "Patterns of genetic variation in Southern Hemisphere blue whales and the use of assignment test to detect mixing on the feeding grounds." Journal of Cetacean Research Management **9**(1): 73-80.

Lindqvist, C., A. Probst, A. R. Martin, W. Oystein and L. Bachmann (2009). "Molecular species identification of historical whale remains from South Georgia." Marine Mammal Science **25**(1): 229-238.

McLeod, R. A., M. W. Brown, T. R. Frasier and B. N. White (2010). "DNA profile of a sixteenth century western North Atlantic right whale (*Eubalaena glacialis*)." Conservation Genetics **11**: 339-345.

Moore, M. J., S. D. Berrow, B. A. Jensen, P. Carr, R. Sears, V. J. Rowntree, R. Payne and P. K. Hamilton (1999). "Relative abundance of large whales around South Georgia (1979-1998)." Marine Mammal Science **15**(4): 1287-1302.

Olavarria, C., C. S. Baker, C. Garrigue, M. Poole, N. Hauser, S. Caballero, L. Florez-Gonzalez, M. Brasseur, J. Bannsiter, J. Capella, P. J. Clapham, R. Dodemont, M. Donoghue, C. Jenner, M.-N. Jenner, D. Moro, M. Oremus, D. Paton, H. C. Rosenbaum and K. Russell (2007). "Population structure of South Pacific humpback whales and the origin of the eastern Polynesian breeding grounds." Marine Ecology Progress Series **330**: 257-268.

Rastogi, T., M. W. Brown, B. A. McLeod, T. R. Frasier, R. Grenier, S. L. Cumbaa, J. Nadaraja and B. N. White (2004). "Genetic analysis of 16th-century whale bones prompts a revision of the impact of Basque whaling on right and bowhead whales in the western North Atlantic." Canadian Journal of Zoology **82**: 1647-1654.

Roman, J. and S. R. Palumbi (2003). "Whales before whaling in the North Atlantic." Science **301**(5632): 508-510.

Rosenbaum, H. C., M. G. Egan, P. J. Clapham, R. L. Brownell Jr. and R. DeSalle (1997). "An effective method for isolating DNA from historical specimens of baleen." Molecular Ecology **6**(7): 677-681.

Rosenbaum, H. C., M. G. Egan, P. J. Clapham, R. L. Brownell Jr., S. Malik, M. W. Brown, B. N. White, P. Walsh and R. Desalle (2000). "Utility of North Atlantic Right Whale Museum Specimens for Assessing Changes in Genetic Diversity." Conservation Biology **14**(6): 1837-1842.

Ross, H. A., G. M. Lento, M. L. Dalebout, M. Goode, G. Ewing, P. McLaren, A. G. Rodrigo, S. Lavery and C. S. Baker (2003). "DNA Surveillance: Web-Based Molecular Identification of Whales, Dolphins, and Porpoises." Journal of Heredity **94**(2): 111-114.

Tonnessen, J. N. and A. O. Johnsen (1982). The History of Modern Whaling. Berkeley and Los Angeles, University of California Press.

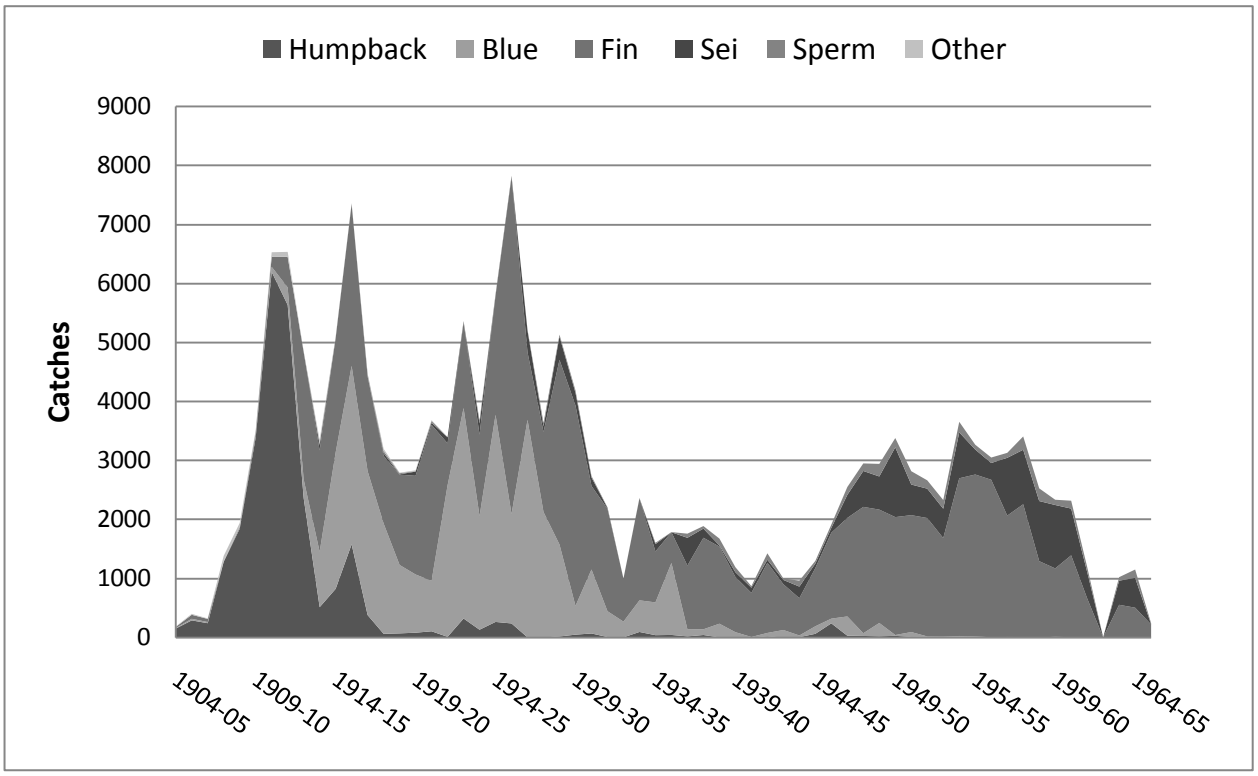

Figure 1. Catch record in total number of whales processed each year for various species at whaling stations during the commercial whaling industry at South Georgia Island (1904-1965) (Headland 1984).