

## DNA profile of a sixteenth century western North Atlantic right whale (*Eubalaena glacialis*)

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**Abstract** Low levels of genetic variability identified within the North Atlantic right whale (*Eubalaena glacialis*), when compared to the Southern right whale (*E. australis*) and other large whales, have been suggested to result from population reductions due to whaling. Previous genetic analysis of 218 whale bones from sixteenth century Basque whaling sites in the western North Atlantic revealed only a single right whale bone. We determined the genotypes of 27 microsatellite loci using DNA isolated from this bone. All alleles from the historic specimen occur in the extant western North Atlantic population and both the probability of identity of the specimen and the number of heterozygous loci are similar to that in the extant population. Assessments of how genetically different the historical population might have been suggest genetic characteristics have not changed substantially over four centuries of whaling.

**Keywords** *Eubalaena glacialis* · Whaling · Species recovery · Population history · Genetic diversity

### Introduction

Despite more than 70 years of international legal protection, the North Atlantic right whale remains one of the world's most endangered large whale species, and an

estimated 350 individuals remain (Kraus et al. 2001). The history of this species in the western North Atlantic has been viewed in the context of a population that was severely reduced by more than 800 years of whaling (Aguilar 1986), with a substantial part of the reduction attributed to sixteenth century Basque whaling in the Strait of Belle Isle (e.g. Gaskin 1991; Greene et al. 2003), followed by over 250 years of American whaling (Reeves et al. 1999, 2007), which may have reduced the population to as few as 60 individuals by the early 1900s (a conclusion for which there is little supporting data; Mitchell 1975; Kenney 1992; Reeves et al. 1992; Schaeff et al. 1997; Reeves 2001). Additionally, the low levels of genetic variability found in this species when compared to the Southern right whale (*E. australis*) (Schaeff et al. 1997; Malik et al. 2000; Schaeff 2002; Waldick et al. 2002) and to other large whales (Schaeff 2002) have been thought to result from population reductions due to whaling (e.g. Palumbi and Roman 2006).

Although exploitation certainly reduced this species throughout its range (e.g. Reeves et al. 1999, 2007), recent data suggest that the observed low genetic variation in the present population may pre-date whaling activities (Waldick et al. 2002; Rastogi et al. 2004; McLeod et al. 2008). Genetic assessment of the contemporary right whale population using nuclear microsatellite markers concluded that a genetic bottleneck has not occurred in the last 200 years (coinciding with the peak of American whaling) and that 800 years of erosion of alleles due to harvesting may be responsible for the low variability in this species (Waldick et al. 2002). In addition, recent genetic analysis of 218 whale bones found throughout the range of known sixteenth to seventeenth century Basque sites in the western North Atlantic identified only a single North Atlantic right whale specimen (McLeod et al. 2008), while the remaining

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specimens were from bowhead whales (*Balaena mysticetus*). These data are not consistent with the suggestion that Basque whaling activities were responsible for what was previously thought to be the largest reduction of the right whale population. Combined, these data refute the current suggestions on the history of this species, and instead indicate that the major decline in this species occurred prior to whaling and that the pre-Basque population of right whales in the western North Atlantic was much smaller than has been assumed.

To further investigate genetic characteristics of the sixteenth century right whale population, we have genotyped this only known sixteenth century *E. glacialis* bone specimen from the western North Atlantic at 27 of the 35 microsatellite loci typed in the contemporary population (Waldick et al. 1999; Frasier et al. 2006, 2007a). Typically, when populations are known or hypothesized to have gone through major long-term declines, analyses of historic samples that pre-date the decline are expected to reveal “new” alleles that have been lost from the contemporary population, and higher levels of variability in the historic population (Groombridge et al. 2000). In contrast, if the population did not lose a large amount of genetic diversity, the historical profile would likely appear more similar to profiles of individuals present in the population today. Here we assess the levels of variability in this historical specimen and the implications on the history of the North Atlantic right whale.

## Materials and methods

Bone core shavings were collected from the single right whale humerus (Rastogi et al. 2004) found during the 1978–1985 marine excavation of a sixteenth century Basque galleon, at Red Bay, Labrador (Grenier et al. 2007). Species designation of this sample has been described previously (Cumbaa 1986; Rastogi et al. 2004).

DNA was extracted as per Rastogi et al. (2004). Standard precautions were taken for working with ancient and low template DNA (Cooper and Poinar 2000). Because the historic bone specimen possessed a mitochondrial control region haplotype different from those found in the extant population (haplotype ‘F’; AY821863; Rastogi et al. 2004), the purity of each DNA extraction ( $n = 8$ ) was assessed by amplifying and sequencing 218 bp of this fragment using primers UP098 (Malik et al. 2000) and LP282 (Rastogi et al. 2004). PCR conditions followed that of McLeod et al. (2008) and included 5  $\mu$ l DNA extract. PCR product was purified and sequenced as per McLeod et al. (2008) and then compared to the six known *E. glacialis* haplotypes (Malik et al. 2000; Rastogi et al. 2004) (Genbank Accession numbers AF395039–AF395043, AY821863).

Thirty-five microsatellite loci used to profile the extant North Atlantic right whale population (Frasier et al. 2006,

2007a; Table 1) were initially used to obtain a profile for the historic bone specimen. Amplification differed across loci and therefore PCR cycling and reaction conditions varied accordingly. Two PCR cycling protocols were used: the first was identical to the cycling of McLeod et al. (2008), and the second method was a 50-cycle ‘touchdown’ procedure consisting of an initial denaturation at 94°C for 5 min, 12–20 cycles of 94°C for 30 s, varied annealing temperatures ( $T_a$ ) for 1 min, and 72°C for 1 min. During the initial 12–20 cycles, the annealing temperature was 6–10°C above the determined optimal  $T_a$  and decreased by 1°C every two cycles. When the optimal  $T_a$  was reached, an additional 30 cycles were run, followed by a final extension at 65°C for 45 min. PCR cocktail conditions were similar to that above, with the exception of a 15  $\mu$ l reaction volume, 2–4  $\mu$ l DNA extract, and 0.1–2.5  $\mu$ M of each primer (Table 1).

To account for potential genotyping problems due to low template and allelic dropout (Taberlet et al. 1996), up to six replicate amplifications were conducted for each microsatellite locus to provide 95% confidence ( $P = 0.0156$ ; based on a binomial distribution) that both alleles are detected (under the assumption that the probability of detecting either allele is equal). A locus was designated as heterozygous if each allele was observed at least twice and homozygous if the profile at a particular locus was independently determined as homozygous six times.

Amplified PCR products were electrophoresed and visualized on an ABI PRISM<sup>®</sup> 377 automated DNA sequencer. An allelic ladder Genescan<sup>®</sup> ROX<sup>™</sup> 500 Size Standard was incorporated into the loading buffer for scoring, and each locus was scored manually using the Genescan<sup>®</sup> Analysis Software. The probability of identify statistic ( $P_{ID}$ ; Paetkau and Strobeck 1994) was used as a metric to quantify the similarity of the bone profile to the extant population. The rationale for this approach is that the  $P_{ID}$  is based on allele frequencies, with individuals possessing common alleles having a high  $P_{ID}$  and individuals possessing rare alleles having a low  $P_{ID}$ . Thus, calculating the  $P_{ID}$  of the bone sample, based on the allele frequencies of the contemporary population provides a means to quantify how similar the genetic profile of this historic whale is to the contemporary whales.

## Results

The extent of amplification of microsatellite alleles from the historic bone DNA varied considerably among loci, therefore only 27 of the 35 loci were used to obtain a profile of the historic right whale (Table 1). Seven of these loci were heterozygous and 20 were homozygous in the bone profile, resulting in a profile consisting of 34 different alleles (Table 1). This number of homozygous loci is comparable

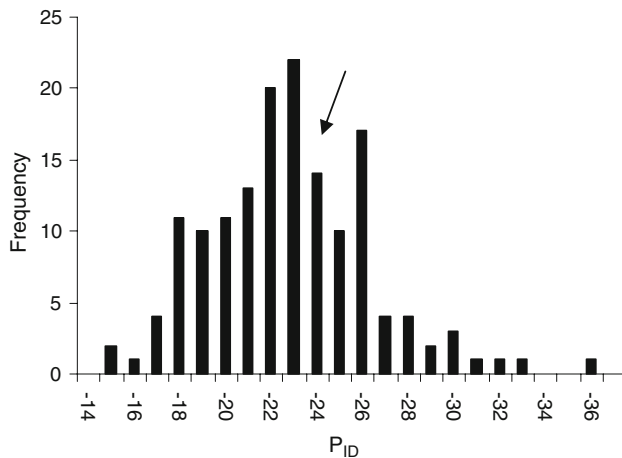
**Table 1** Microsatellite loci and respective PCR conditions used to DNA profile the historic right whale bone

Microsatellite locus	Allele	Extant frequency	Source	PCR cycling	$T_a$ (°C)	$T_a$ gradient (°C)	Template DNA (μl)
EV1Pm	135	0.61	1	50 Cycle touchdown	55	63–55	2
	137	0.38					
EV37Mn	–	–	1	–	–	–	–
GATA028	172	0.42	2	50 Cycle touchdown	52	62–52	2
	168	0.19					
GT023	119	0.31	3	50 Cycle touchdown	62	66–62	2
	115	0.05					
GT271	102	1.0	3	50 Cycle touchdown	55	65–55	2
IGF	152	0.15	4	50 Cycle touchdown	50	60–50	4
RW18	199	0.67	5	50 Cycle	60	–	2
	195	0.05					
RW2-12	121	0.998	5	50 Cycle touchdown	52	62–52	2
RW2-17	169	1.0	5	50 Cycle touchdown	50	60–50	4
RW2-19	86	0.976	5	50 Cycle	56	–	2
RW25	143	0.94	5	50 Cycle	50	–	2
RW31	–	–	5	–	–	–	–
RW34	111	0.21	5	50 Cycle touchdown	57	67–57	2
	105	0.02					
RW4-17	121	0.42	5	50 Cycle touchdown	60	67–60	2
RW4-5	142	0.98	5	50 Cycle	52	62–52	2
RW48	138	0.77	5	50 cycle	53	–	4
	136	0.11					
SAM25	204	0.30	5	50 Cycle touchdown	53	63–53	2
TR2F3	–	–	6	–	–	–	–
TR2G5	–	–	6	–	–	–	–
TR3A1	306	0.39	6	50 Cycle touchdown	54	64–54	2
	310	0.38					
TR3F2	292	0.996	6	50 Cycle touchdown	58	67–58	2
TR3F7	157	0.953	6	50 Cycle touchdown	53	63–53	4
TR3G1	–	–	6	–	–	–	–
TR3G2	177	0.86	6	50 Cycle	60	–	4
TR3G5	167	0.70	6	50 Cycle	60	–	4
TR3G6	270	0.984	6	50 Cycle touchdown	58	67–58	2
TR3G10	210	1.0	6	50 Cycle touchdown	59	66–59	2
TR3G11	131	1.0	6	50 cycle touchdown	55	65–55	2
TR3G13	–	–	6	–	–	–	–
TR3H4	–	–	6	–	–	–	–
TR3H14	–	–	6	–	–	–	–
TexVet14	85	1.0	7	50 Cycle touchdown	56	66–56	25
TexVet17	202	0.59	7	50 Cycle touchdown	46	56–46	25
TexVet19	165	0.969	7	50 Cycle touchdown	59	65–59	45
TexVet20	166	0.78	7	50 Cycle touchdown	60	67–60	25

Also shown are alleles identified in the historical bone specimen and corresponding frequencies in the extant population. Source reference numbers refer to: 1, Valsecchi and Amos (1996); 2, Palsbøll et al. (1997); 3, Bérubé et al. (2000); 4, Barendse et al. (1994); 5, Waldick et al. (1999); 6, Frasier et al. (2006); and 7, Rooney et al. (1999)

to the extant population, where ~95% of the individuals profiled are homozygous at 16–22 of these loci (Frasier 2005). No novel alleles were found in the bone profile, nor

did it possess any alleles that are currently rare in the extant population (with a frequency < 0.005). Additionally, the  $P_{ID}$  value calculated for the single historic specimen was

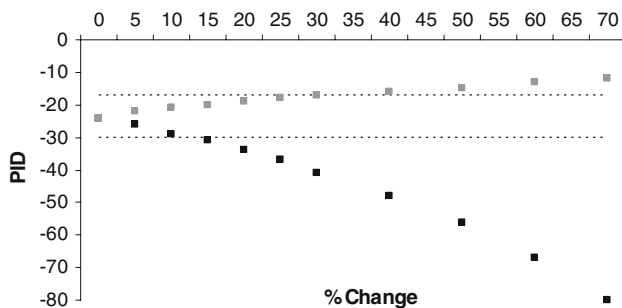


**Fig. 1**  $P_{ID}$  value of the historic right whale specimen (indicated by arrow;  $P_{ID} = 4.8 \times 10^{-24}$ ) as it relates to the distribution of  $P_{ID}$  values for the extant population

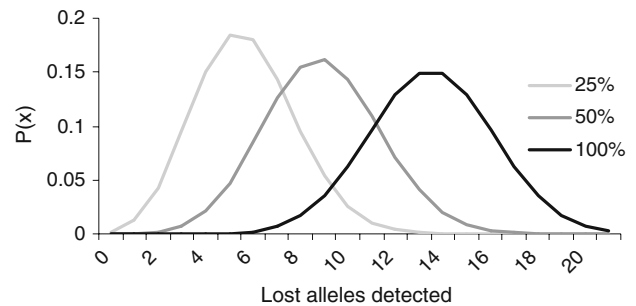
$4.8 \times 10^{-24}$  and this value fell near the centre of the distribution of  $P_{ID}$  values for the extant population (Fig. 1). Combined, these data indicate that the genetic profile of this historic whale is very similar to those found in the contemporary population.

To provide a context for interpretation of these results, several analyses were conducted based on different hypotheses of population history, and based on different methods of quantifying variation. First, to evaluate how different the historical allele frequencies could have been, while still maintaining the  $P_{ID}$  of the bone profile within 95% of the extant distribution, the frequencies of all alleles identified within the bone were varied (5–70%) and the distribution was recalculated. Results indicate that all allele frequencies could have decreased by only 12%, and increased by 30% to maintain the bone  $P_{ID}$  within the extant distribution (Fig. 2).

To investigate the potential to detect ‘lost’ alleles, hypothetical historic allele distributions were created as if the allelic diversity in the historical population was 25, 50,



**Fig. 2**  $P_{ID}$  values of the bone specimen as all allele frequencies identified in the specimen decrease (black squares) or increase (gray squares) by varied percentages (% change) of their initial frequency. Dashed lines indicate the 95% range of  $P_{ID}$  values for the extant population ( $1.00E-17$  to  $1.00E-30$ )



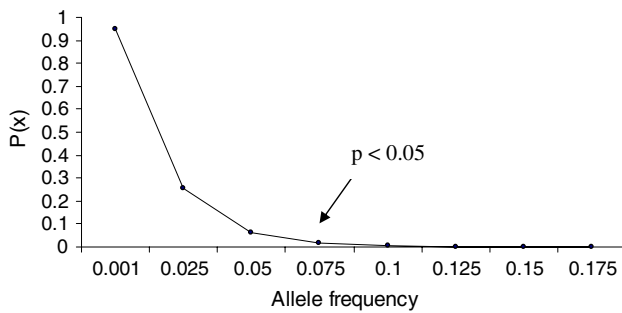
**Fig. 3** Binomial distributions of the expected number of ‘lost’ alleles that would be detected if historical allelic diversity was 25, 50 and 100% greater than it is today

and 100% greater than the extant population. The binomial distribution was used to calculate the probability of detecting lost alleles under these scenarios, where  $P$  was equal to the percentage of lost alleles (0.21, 0.33, 0.5), and  $q = 1 - P$  (the percentage of retained alleles) (0.79, 0.66, 0.5) and the current allelic diversity at these 27 loci is 4.63. Results indicate that if diversity was greater by 25, 50, and 100%, then on average 5–7, 8–10 and 12–15 lost alleles should have been identified when assessing 27 loci (Fig. 3). The probability of identifying 0 new alleles was  $P < 0.005$  in all cases, with the probability falling largely outside of the binomial distribution (Fig. 3). This suggests that if allelic diversity was greater historically, it was not likely more than one allele per locus ( $\sim 25\%$  greater than the present).

The probability of not detecting any lost alleles if each locus historically had one additional allele (and therefore an allelic diversity of 5.63) was also plotted using the binomial distribution, with 54 sampling events (2 alleles at each of 27 loci) and all lost alleles present at the same frequency (ranging from 0.001 to 0.175). This analysis indicated that it is unlikely that all loci had an additional allele with a frequency greater than 0.075 or it would have been detected ( $P < 0.05$ ; Fig. 4). Therefore, if allelic diversity was greater historically, alleles could only have been present at very low frequencies, and would have been even lower if there was  $>1$  additional allele per locus.

## Discussion

We present the DNA profile from a sixteenth century right whale bone consisting of 27 of the 35 microsatellite loci used to profile the contemporary population of western North Atlantic right whales. Loci of this profile are not linked (Frasier et al. 2006), thus the profile represents 27 independent sampling events from the historic population. This represents the survival of 34 alleles through four centuries of right whale history. Although the small sample size limits the interpretations that can be made from the



**Fig. 4** Probability of detecting at least one ‘lost’ allele within a profile if every locus historically had one additional allele. Probability of detection is plotted with all alleles at the same frequency (recalculated at frequencies ranging from 0.001 to 0.175)

results, the data are contrary to those expected based on previous suggestions of population history and indicate that the genetic characteristics of the pre-Basque exploitation historical population in the western North Atlantic were not substantially different from those of the contemporary population. No ‘lost’ (alleles present in the historical population but absent from the extant population) were found and the level of heterozygosity identified in the profile is consistent with the level of heterozygosity within individuals in the contemporary population. Finally, the probability of identity value ( $P_{ID}$ ) calculated from the profile of this specimen places it directly within the distribution of  $P_{ID}$  values for the extant population. These results would not have been expected if the historical population had lost a significant amount of genetic variability as a result of whaling. It is possible that the historical population of right whales in the western North Atlantic were more genetically similar to the population today than has previously been thought. We asked the question: how genetically different might the historical population have been while still obtaining this result?

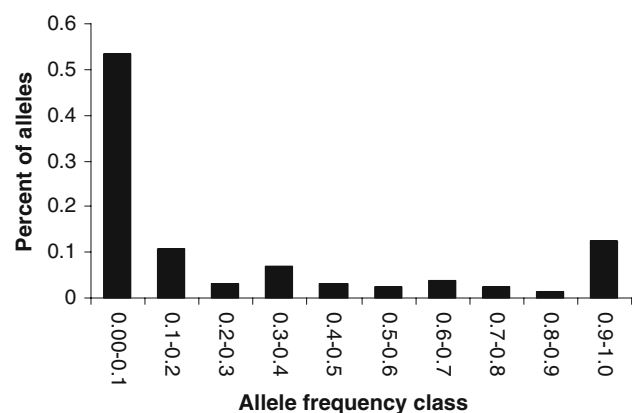
Using the data obtained from profiling the historical specimen, we were able to evaluate to what degree the historical population might have been genetically different than the population today. Together, the results are not consistent with the idea that the sixteenth century population of right whales in the western North Atlantic had levels of genetic diversity substantially higher than found today.

Both the genetic profile and the  $P_{ID}$  value of the profile support an emerging view of the history of this species in the western North Atlantic. Recent data from both the extant population (Waldick et al. 2002), and historic specimens (Rosenbaum et al. 2000; Rastogi et al. 2004, this study), suggest that a large reduction in genetic variability has not occurred over the past 440 years. To date, the only data presented to support the hypothesis of a loss of genetic variability (as a result of whaling) has been the small number of low frequency alleles identified in the western North Atlantic population (Waldick et al. 2002). However,

recent data shows that the majority of the alleles identified in the extant population (69%) have frequencies <0.1 and 40% have frequencies <0.05 (Frasier et al. 2007a), resulting in a L-shaped allele frequency distribution (Fig. 5). This L-shaped allele frequency distribution would not be expected if this species has gone through a genetic bottleneck in the last 40–80 generations (~800–1,600 years; Luikart et al. 1998). This difference in results with those of Waldick et al. (2002) is likely due to the small sample size used in the previous study ( $n = 30$ ). Although this type of analysis has little power to identify the occurrence of a bottleneck event, we update the data here to illustrate that the data previously presented as support of the hypothesis that diversity has decreased as a result of whaling is not accurate.

It is becoming increasingly evident that western North Atlantic right whales may not have numbered 12,000–15,000 individuals during the sixteenth century as has been suggested (Gaskin 1991). Molecular analysis of over 200 whale bones from the majority of all identified Basque whaling sites in the western North Atlantic indicate that bowhead whales, and not right whales, were the principal target in the region (Frasier et al. 2007b; McLeod et al. 2008). Genetic analyses suggest that the sixteenth century population was genetically not substantially different from the extant population. Together these findings do not support the idea that the population suffered a major population reduction due to Basque whaling.

The results presented both here and previously (Rastogi et al. 2004; McLeod et al. 2008) illustrate the importance of incorporating data obtained from historical specimens in interpreting the history and the current status of species. For the North Atlantic right whale, such data have rewritten the history of the species, and provided a new context in which the current status and recovery potential need to be considered and addressed.



**Fig. 5** Distribution of allele frequency class categories in the extant population of western North Atlantic right whales at the 27 microsatellite loci used to profile the historic bone specimen

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