Genetic identity and genotype matching of southern right whales (*Eubalaena australis*) from Mainland New Zealand and the Auckland Islands

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ABSTRACT

It remains uncertain as to whether the NZ subantarctic and Mainland NZ represent two relatively isolated stocks with different histories of exploitation and recovery or a single stock with a poorly understood pattern of migratory habitat use. A third hypothesis, that the Mainland NZ population was extirpated and subsequently recolonised by a range expansion from the NZ subantarctic, is also possible. Here we construct DNA profiles for southern right whales sampled around the NZ Mainland (n=31) and NZ subantarctic (n=934) by genotyping of microsatellite loci (up to 14 loci, average 12.7 loci), sequencing of the mtDNA control region (minimum of 500bp) and sex identification. Four matches between the NZ subantarctic and Mainland NZ were identified, the first time any such match between the two areas has been documented. These results, and other available data, are most consistent with either the one stock or recolonsiation hypotheses.

KEY WORDS: SOUTHERN RIGHT WHALE, *EUBALAENA AUSTRALIS*, NEW ZEALAND, GENETICS, DNA FINGERPRINTING, MOVEMENTS

INTRODUCTION

Southern right whales (*Eubalaena australis)* were once widespread throughout oceans of the Southern Hemisphere, with an estimated 60,000-100,000 whales distributed among 13 whaling grounds (IWC 2001). Commercial whaling in the 19th and early 20th centuries killed at least 150,000 southern right whales and drove the species to as few as 90 reproductive females worldwide in 1920 (IWC 2001; Jackson et al. 2009a; Jackson et al. 2009b). The scale and duration of the hunt was such that some former whaling grounds, such as the Chile-Peru and southeast Australia populations, show little to no signs of recovery (Kemper et al. 1997; Reilly et al. 2008). The only populations reported to show significant rates of increase are the Argentinean, South African and Southwest Australian populations, which are currently thought to number 2,000-3,000 whales each (increase rate of 6% - 8% per annum; Bannister 2008; Best et al. 2001, 2005; Burnell 2008; Cooke et al. 2001). Based on aerial surveys and photo-identification studies, the southern Brazil population is also recovering. However, the estimated growth rate (14% per annum) is not biologically plausible, possibly indicating emigration from the neighbouring Argentinean population (Groch et al. 2005).

In New Zealand (NZ), this pattern of spatially variable recovery is replayed on a regional scale. Prior to commercial whaling, it is estimated the number of southern right whales in NZ waters was more than 27,000 (Jackson et al. 2009a). Commercial whaling in the early 19th century killed up to 35,000 southern right whales in NZ and the population was reduced to as few as 25 mature females (Jackson et al. 2009a). In the aftermath of this hunt, no southern right whale was seen around the North and South Islands of NZ (hereafter referred to as the Mainland) for over 35 years (1928 - 1963; Gaskin 1964). Subsequently, only a small number of southern right whales were sighted around the Mainland each year between 1976 and 2003 (Patenaude 2003; Patenaude 2005). In contrast, a seasonal aggregation of right whales is found in the subantarctic Auckland and Campbell Islands during the austral winter (Patenaude et al. 1998). This NZ subantarctic population has been the focus of two sets of winter surveys; the first during the winters of 1995-1998 (Patenaude 2002; Patenaude & Baker 2001; Patenaude et al. 1998) and the second during the winters of 2006-2009 (Childerhouse & Dunshea 2008;

Childerhouse et al. 2006; Dunshea et al. 2007). On the basis of mark-recapture analysis of photo-identification records, the population was estimated to number 936 whales in 1998 (95% C.I 740-1140; Patenaude 2002). No estimate is yet available from the second set of surveys.

It remains uncertain as to whether the NZ subantarctic and Mainland NZ represent two relatively isolated stocks with different histories of exploitation and recovery or a single stock with a poorly understood pattern of migratory habitat use. The hypothesis that the NZ subantarctic and Mainland NZ represent one stock is supported by recent genetic evidence, based on mtDNA control region haplotype frequencies, that suggests the two areas are not significantly differentiated (Alexander et al. 2008; Patenaude & Harcourt 2006).

However, the two stock hypothesis is consistent with apparent difference in recovery between the regions (Patenaude 2002; Patenaude 2003). The timing of historical migratory arrivals at the different whaling grounds also supports this theory (Dawbin 1986; Richards 2002). Based on the analysis of whaling ship logbooks and historical texts, southern rights wintered in bays and inlets around the Mainland from April/May to October, in contrast, the whales arrived as early as February in the subantarctic Auckland and Campbell Islands (Dawbin 1986; Jackson et al. 2009a; McNab 1913; Richards 2002). The two stock hypothesis is also consistent with a lack of documented movement between the two areas, based on photo-identification studies and previous genetic comparisons (Childerhouse 2009; Patenaude 2003; Patenaude & Harcourt 2004).

A third hypothesis, that the Mainland NZ population was extirpated and the region was subsequently recolonised by a range expansion from the NZ subantarctic, is also plausible. However, to differentiate this hypothesis from the one stock hypothesis would require the analysis of historical DNA samples from Mainland whales that are currently unavailable.

Here we individually identify southern right whales sampled around the Mainland (2003-2007, n=31) and the subantarctic Auckland Islands (1995-1998 and 2006-2008, n=934) by the construction of DNA profiles. Each profile consists of microsatellite genotypes (up to 14 loci), mitochondrial control region haplotype (minimum 500bp) and genetically identified sex. We compare these data to document possible movement between the two regions.

METHODS

Biopsy sample collection, DNA extraction, genetic sex determination and mtDNA haplotype identification

Biopsy samples were collected from southern right whales at Port Ross, Auckland Islands, under Department of Conservation (DOC) Marine Mammal Research permit and University of Auckland Animal Ethics Committee approved protocol (to C.S. Baker) during winter surveys between 2006-2008 (n=580; Table 1) and stored in ethanol on location until transfer to -80°C at the University of Auckland (Childerhouse & Dunshea 2008; Childerhouse et al. 2006; Dunshea et al. 2007). Sample collection during the 1995-1998 Auckland Islands winter fields surveys has been described previously (n=354; Baker et al. 1999; Patenaude 2002). Biopsy samples were collected opportunistically by DOC staff from around the Mainland from 2003-2007 and transferred to the University of Auckland while stored in ethanol (n=31; for a complete list of sampling locations see Alexander et al. 2008).

Total genomic DNA was extracted from skin samples using standard proteinase K digestion and phenol/chloroform methods (Sambrook et al. 1989), as modified by Baker et al. (1994). The samples collected from the Auckland Islands during the 1995, 1996, 1997, 1998 and 2006 field seasons had previously been extracted (N. Patenaude, M. Vant and G. Dunshea), as had the Mainland samples (Alexander et al. 2008). The sex of the samples was genetically determined using primers that amplify a 224 base pair (bp) fragment of the sex-determining regions (SRY) on the Y chromosome of males (Gilson et al. 1998) and an approximately 440bp fragment of the ZFX/ZFY region, present in both males and females (Aasen & Medrano 1990). Sex had previously been determined for Auckland Islands samples 1995-1998 (Carroll 2006; Patenaude 2002) and the Mainland samples (Alexander et al. 2008). As previously described, the mtDNA control region was amplified and sequenced for the first 500bp (Alexander et al. 2008; Carroll 2006).

Microsatellite genotyping

A total of 22 microsatellite loci were selected from the literature and were trialled in a pilot study of 24 southern right whale samples. From these, 17 loci were chosen for use on the entire dataset, based on consistency of amplification and diversity levels suitable for the purpose of individual identification (GT23 and GT211; Bérubé et al. 2000; TR3G1, TR3G2, TR3F4, and TR3G10; Frasier et al. 2006; GATA28 and GATA98; Palsbøll et al. 1997; EV1, EV37 and EV14; Valsecchi & Amos 1996; RW18, RW26, RW31, RW410, RW48 and RW51; Waldick et al. 1999). PCR products of four to six different loci were co-loaded, and each multiplex (2µL) was

combined with GS500 LIZ size standard (Applied Biosystems), heat treated at 95°C for 5 min, and run on an ABI3730 Genetic Analyser (Applied Biosystems) at Oregon State University or an ABI3130 Genetic Analyser at the University of Auckland.

Microsatellite quality control and matching

Electropherograms were analysed by Genemapper v4.0 (Applied Biosystems), and all automated calling was double checked by eye as suggested by the literature (Bonin et al. 2004; DeWoody et al. 2006). Each amplification and sizing run had internal controls to ensure consistent allele sizing and a negative control to detect contamination. Excel Microsatellite Toolkit (Park 2001) was used to identify basic errors, such as duplicates and data entry errors producing novel alleles or incompletely typed individuals.

CERVUS (Kalinowski et al. 2007) and DROPOUT (McKelvey & Schwartz 2005) were used to identify which loci were error-prone and hence should be removed (Waits et al. 2001). DROPOUT's DCH test was used to determine if any loci had a high error rate. CERVUS was then used to investigate how the removal of these loci increased the number of high-quality genotype matches. Based on these analyses three loci were removed from the dataset (GT211, TR3G10 and RW51). Micro-Checker analyses were conducted to detect large allele, null alleles and stutter (van Oosterhout et al. 2004) and the program indicated stutter was problematic at one locus (GATA98), due to the lack of adjacent allele heterozygotes. As this locus was a tetra-nucleotide repeat, and visual inspection showed no evidence of stutter, we considered this unlikely and retained the locus. Micro-Checker analysis also suggested one locus could have null alleles (TR3G1), however, it was retained as it had a comparatively high number of alleles and polymorphic information content, and it is likely null alleles would be a consistent effect. In total, 14 loci were retained and samples that did not amplify at a minimum of 10 were discarded. This dataset was denoted the quality-controlled (QC) dataset. The error rate of the QC database was calculated per allele (Pompanon et al. 2005) using the internal control samples amplified in every PCR reaction.

DNA profiles in the QC dataset were compared using the program CERVUS to identify individuals and replicate samples. Genotypes that matched at nine or more loci were considered strong evidence of a replicate sampling, as the probability of identity ($P_{(ID)}$) and the probability of identity of siblings ($P_{(ID)sib}$) were expected to be sufficiently small to preclude matching by chance in a population of up to a several thousand individuals (Waits et al. 2001). As a check against false exclusion, samples that matched at six to eight loci were genotyped at additional loci to confirm the match. For genotypes that matched at 9 loci (likely matches), but mismatched at one or more loci, mismatching alleles were inspected for errors, the most common of which was allelic dropout (for reviews see Bonin et al.2004; Pompanon et al. 2005). Where possible, the mismatching locus was reamplified for both samples to confirm the match.

RESULTS AND DISCUSSION

QC dataset construction and microsatellite genotyping error rates

The QC criterion of successful amplification at a minimum of 10 of 14 loci (average of 12.7 loci), was met by 840 samples (90%) from the NZ subantarctic; 68 from 1995, 47 in 1996, 57 in 1997, 128 in 1998, 131 in 2006, 214 in 2007 and 195 in 2008. All 31 Mainland NZ samples passed the QC review. Based on the internal control samples, there were 14 single-allele errors in 2152 successfully amplified alleles, giving a per allele error rate of 0.65% (Pompanon et al. 2005).

CERVUS analysis showed that the overall $P_{(ID)}$ and $P_{(ID)sib}$ for the 14 loci in the dataset were 2.44E-18 and 5.8E-07, respectively. For nine matching loci, which we considered strong evidence of identity, the $P_{(ID)}$ ranged between 7.29E-11 and 2.70E-13, and $P_{(ID)sib}$ was between 1.58E-04 and 5.6E-05, depending on the combination of matching loci. Given this low probability, we assumed that matching profiles represented resamplings of the same individual and the loci chosen were able to differentiate first-order siblings.

Identifying unique individuals and migratory return to the NZ subantarctic

Matching of genotypes with CERVUS showed there were 61 unique genotypes sampled in the NZ subantarctic in the 1995 field season, 42 in 1996, 50 in 1997, and 106 in 1998 (Table 1). In total, 232 individuals were sampled during these four winter surveys; 209 in one year, 21 in two years, two in three years and none in all four years. For the second set of field surveys, removal of within-year replicates showed there were 111 unique genotypes sampled in the NZ subantarctic in the 2006 field trip, 165 in 2007, and 157 in 2008. In total, 408 individuals were sampled during these three winter surveys; 383 in one year, 24 in two years and one in all three years.

Between 232 individuals sampled from 1995-1998 and the 408 individuals sampled from 2006-2008 there were 27 genetic matches; 19 females and eight males. Three whales were sampled as calves in the 1995-1998 surveys and subsequently resampled as adults in 2006 or 2007. Furthermore, 15 of the 19 females recaptured were reported as reproductive females; 12 had calves in one year they were sampled and three in both years of sampling. One of these whales was a calf in 1998 and recaptured as a mother in 2006, potentially the first parturition for this whale at age eight years (Best et al. 2001; Cooke et al. 2001). In total, 613 unique genotypes were sampled during seven years of field surveys in the NZ subantarctic, including 286 males and 312 females.

Identifying unique individuals for the Mainland NZ and connectivity to the NZ subantarctic

The DNA profiles of the 31 Mainland NZ samples were resolved into 22 unique individuals (Table 1); 12 females and 10 males, including three calves (two males and one female). There were no between-year recaptures in the Mainland NZ dataset and no additional within-year matches were found compared with previously analyses (Alexander et al. 2008).

Between the NZ subantarctic and Mainland NZ datasets, DNA profile matching established there were four matches (Figure 1); one male sampled as a calf in Taunga Waka Bay, East Coast/Hawke's Bay in 2003 and subsequently resampled at the Auckland Islands in 2006; an adult female sampled in Te Wae Wae Bay, Southland in 2003 and resampled at the Auckland Islands in 2006; another adult female sampled in Patea, Wanganui in 2005 and resampled at the Auckland Islands in 2007; and a further adult female sampled Taieri River Mouth, Otago, in 2007 and resampled at the Auckland Islands in 2008.

Conclusion

Here we used DNA profiling to document the first movement of southern right whales between the NZ subantarctic and Mainland NZ. This finding supports previous analyses, based on mtDNA control region haplotypes, that these two regions are not genetically differentiated (Alexander et al. 2008). Consistent with this finding are the results of recent satellite tagging work conducted by researchers from the Australian Antarctic Division. Six implantable satellite transmitters were deployed on southern right whales at the Auckland Islands during the 2009 winter field season and one of these tagged individuals travelled to Fiordland, confirming direct, within-year movement between the two regions (S. Childerhouse pers. comm.).

It is interesting to note that the four matches to the Mainland dataset were from the 2006-2008 NZ subantarctic surveys. Although this could be due to the concurrent years of the survey, it shows the importance of the recent series of field seasons for uncovering connectivity between the two areas and may suggest a recent expansion to the Mainland from the NZ subantarctic breeding population. The lack of genetic matches between the 1995-1998 NZ subantarctic samples and the Mainland dataset is also consistent with the lack of matches between the Mainland (1990-2008) and NZ subantarctic (1995-1998) photo-identification catalogues (Childerhouse 2009; Patenaude 2003). However, no comparison has yet been made between the Mainland catalogue the 2006-2009 NZ subantarctic photo-identification data.

The comparison of DNA profiles between the two NZ subantarctic survey periods showed the long-term migratory return of individual southern right whale calves, males and females. Maternal fidelity of adult whales to breeding grounds has been documented in other southern right whale populations (e.g. Best et al. 2005; Groch et al. 2005), however, to our knowledge this is the first report of natal fidelity to a breeding ground, e.g. the confirmed return of a calf as a mother with her own calf. We believe this indicates maternally-directed fidelity to breeding grounds.

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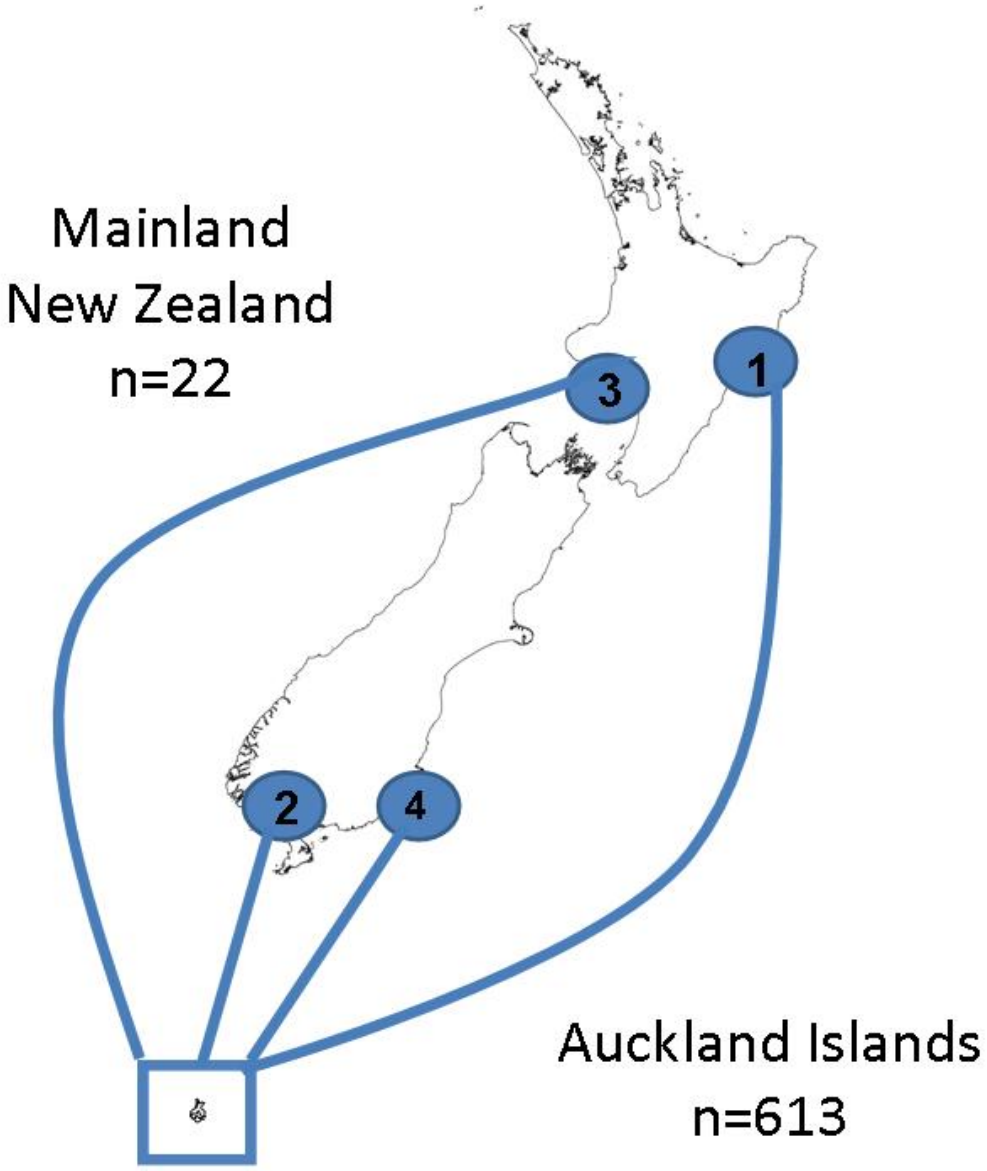
Table 1: The total number of samples collected and unique genotypes (assumed to represent individual whales) collected from southern right whales at the Auckland Islands during winter field surveys 1995 – 1998 and 2006-2008 and around the New Zealand Mainland 2003-2007. Samples QC is the number of samples after quality control. The numbers in parenthesis represent the number of unique genotypes for that sampling period after removal of between- and within-year replicates.

Location	Year	Samples collected	Samples QC	Unique Genotypes	Males	Females	Unknown
Auckland	1995	70	68	61	31	30	0
Islands	1996	51	47	42	22	20	0
	1997	75	57	50	30	20	0
	1998	158	128	106	52	49	5
	SUBTOTAL	354	300	259 (232)	135 (117)	119 (110)	5 (5)
	2006	142	131	111	60	49	2
	2007	234	214	165	72	87	6
	2008	204	195	157	60	95	2
	SUBTOTAL	580	540	433 (408)	192 (177)	231(221)	10 (10)
	TOTAL	934	840	692 (613)	327 (286)	350 (312)	15 (15)

	Year	Samples collected	Samples QC	Unique Genotypes	M	F	U
New	2003	12	12	9	5	4	0
Zealand	2004	1	1	1	1	0	0
Mainland	2005	5	5	3	0	3	0
	2006	8	8	5	2	3	0
	2007	5	5	4	2	2	0
	TOTAL	31	31	22 (22)	10 (10)	12 (12)	0 (0)
	GRAND TOTAL	965	871	714 (632)	337 (295)	362 (322)	15 (15)



Match	Date sampled		Sex	N loci match	PID	PID (sibs)
	Mainland	Auckland Is.				
1	27 July 2003 (calf)	1 August 2006	M	11	5.09E-18	3.83E-06
2	25 August 2003	29 July 2006	F	12	3.34E-15	7.49E-06
3	8 July 2005	22 July 2007	F	13	2.63E-19	1.56E-06
4	13 July 2007	23 July 2008	F	12	7.24E-15	1.14E-05

Figure 1: Location of southern right whales sampled around the New Zealand Mainland that were microsatellite genotype matches to individuals sampled at the Auckland Islands. 1-4 on the map corresponds to the matches in table below, giving addition data on the matches. Sample sizes on figure refer to the number of unique individuals per region.