Long-distance longitudinal migration of southern right whales suspected from mtDNA and microsatellite DNA analysis on JARPA and JARPAII biopsy samples

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ABSTRACT
A total of 70 southern right whale biopsy samples obtained from Areas IIIE (2), IV (63), and V (5) during JARPA and JARPAII surveys up to the 2009/10 season were genotyped at 14 microsatellite DNA loci and were sequenced for 430 bp of the mitochondrial DNA (mtDNA) control region in order to describe genetic characteristics of the species in the Antarctic feeding ground. In two of three cases of duplicate sampling, the second samples were collected later year (four years, and eight years) in the same area (IV), suggesting site fidelity to the feeding ground. Paternity analysis for a single calf-mother pair sample detected no potential father of the calf. After exclusion of some of these samples, 66 samples were used to estimate genetic indices. The levels of genetic diversity observed from both markers were comparable to those reported from other southern right whale genetic studies. A total of eight haplotypes were generated from 21 segregation sites and these haplotypes were phylogenetically separated into two clades. On the basis of the sequence variations within the 275 bp consensus region, these eight haplotypes were then compared to the 37 haplotypes reported in Patenaude *et al*. (2007) that used the samples from the Indo-Atlantic (Argentina and South Africa) and the Indo-Pacific (Southern Australia and New Zealand) basins. The comparison revealed that our two clades were same as their A and W clades, each of the eight haplotypes matched to one of their haplotypes, and three were same as their Indo-Atlantic specific haplotypes. These results suggest that some southern right whales operate much longer-distance seasonal migration between their feeding and breeding grounds than we had previously thought and thus whales from multiple stocks migrate to our research area in the Antarctic feeding ground.

Our genetic study is the first to describe the genetic characteristics of southern right whales feeding in the Antarctic and the first to discover the possibility of southern right whales' long-distance feeding migration from the Indo-Atlantic basin to the Indo-Pacific basin that results in the mixing of multiple stocks in the Antarctic. Past genetic studies, in contrast, demonstrated whales' restricted seasonal migration within the same basin probably due to the use of the samples only from coastal areas. Our study implies that southern right whales require different management strategies on an area by area basis even within the same basin for their long-term persistence. This study demonstrated one of the significant contributions of non-lethal part of the comprehensive large-scale JARPAII to acquire valuable information for effective management of large whales in Antarctic.

KEYWORDS: ANTARCTIC, BIOPSY SAMPLING, FEEDING GROUNDS, GENETICS, MIGRATION, SITE FIDELITY, SOUTHERN RIGHT WHALE

INTRODUCTION

The distributions of southern right whales are restricted in the Southern Ocean (Rozenbaum *et al*., 2000). Existence of genetically distinct stocks has been recognized in several areas of the ocean (Patenaude *et al*., 2007), and distributions of these stocks can be separated into two major basins: Indo-Atlantic, such as Argentina and South Africa, and Indo-Pacific, such as Australia and New Zealand. General migration patterns of southern right whales between winter breeding/calving and summer feeding grounds appear to be north-south (see detail information in IWC, 2001). Such latitudinal movement patterns associated with the whales' ability of site fidelity result in accumulation of the regional stock differentiation between as well as within the two major basins as shown by the past genetic studies (e.g., Patenaude *et al*., 2007). In addition to that, strong structuring in a maternally inherited mitochondrial DNA (mtDNA) marker with limited differentiation in biparentally inherited microsatellite DNA markers observed among samples from the local regions in South Pacific suggested female philopatry and male dispersal (Carroll *et al*., 2011).

Stock structure and phylogenetic relationships of southern right whales on their calving/feeding grounds was investigated by Patenaude *et al*. (2007) using mtDNA sequencing analysis. The authors analyzed 146 individuals sampled on two winter calving (New Zealand and Southwest Australia) and one summer feeding (south of Western Australia) grounds in the Indo-Pacific basin and on two winter calving (South Africa and Argentina) and one summer feeding (South Georgia) grounds in the Indo-Atlantic basin. A total of 37 unique mtDNA haplotypes were found and these haplotypes were clearly separated phylogenetically into two distinct clades (Crades A and W). Although the haplotypes from the A and W clades were found in the both ocean basins, their frequencies quite differed between as well as within the ocean basins, indicating very limited female mediated gene flow even among the local regions. Only one of the 37 haplotypes was shared between the two ocean basins and the other 36 were basin specific.

In this paper, we analyzed the biopsy samples obtained from southern right whales in the Antarctic Ocean (mostly from Area IV) during the JARPA and JARPAII surveys up to 2009/10 season using both mtDNA and microsatellite DNA markers in order to describe their genetic characteristics. We compared our results to those in Patenaude *et al*. (2007) and Carroll *et al*. (2011) to better understand the genetic characteristics and feeding migration pattern of the species in the Antarctic feeding ground.

MATERIALS AND METHODS

Samples

Table 1 shows the number of southern right whale biopsy samples used in this study that were separated by IWC management areas. A total of 70 samples was obtained from JARPA and JARPAII surveys conducted from 1993/94 to 2009/10 seasons.

DNA extraction

In regard to our DNA data quality control under the IWC guidelines, see Kanda *et al.* (2014). Total DNA was extracted from 0.05 g of a frozen biopsy skin tissue using either the protocol of Sambrook *et al*. (1989) or GENTRA PUREGENE DNA extraction kit (QIAGEN). Extracted DNA was stored in the TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Microsatellite analysis

Genetic variation at microsatellite loci were analyzed using 14 sets of primers: EV1, EV14, EV21, EV37, EV94 (Valsecchi and Amos, 1996), GT23, GT211, GT310 (Bérubé *et al*., 2000), GATA28 (Palsbøll *et al*., 1997), DlrFCB17 (Buchanan *et al*., 1996), TR2F3, TR3G2, TR2G5, TR3F2 (Frasier *et al*., 2006). TR2F3, TR3G2, TR2G5, and TR3F2 were designed specifically from right whales (Frasier *et al*., 2006). Amplified products were run on a 6% polyacrylamide denaturating gel using a BaseStation 100 DNA fragment analyzer (Bio-Rad), and then sizes of visualized alleles were determined manually in relation to the internal size standard (Genescan 400HD, Life Technologies) as well as the Southern right whale microsatellites of known size that were rerun on each gel.

mtDNA analysis

Sequence of 430 bp of the mtDNA control region was amplified using a set of primers: MT4 (Árnason *et al*. 1993) and Dlp 5R (5'-CCA TCG AGA TGT CTT ATT TAA GGG GAA C-3'). PCR products were cycle sequenced for purification with the same primers using the AmpliTaq FS Sequencing Kit (Perkin-Elmer). After the purification, the cycle-sequenced products were direct-sequenced on either an ABI 3100 or 3500 Automated DNA Sequencers (Life Technologies) following the manufacture's protocols. Both light and heavy strands were sequenced for each sample. Observed sequences were aligned using the Sequence Navigator computer program (Life Technologies).

Sex determination

Sex of the whales was determined through co-amplification of SRY locus located on the Y chromosome and GT23, which is slight modification from Abe *et al*. (2001). With this combination of loci, males show amplified products of both SRY and GT23 loci, while females show only GT23.

Genetic diversity analysis

For the microsatellite analysis, MICRO-CHECKER (van Oosterhout *et al*., 2004) was used to check for null alleles and reading/typing errors. The number of alleles per locus and expected heterozygosity per locus was calculated using FSTAT 2.9.3 (Goudet, 1995). Statistical tests for the deviations from expected Hardy-Weinberg genotypic proportions were conducted using GENEPOP 4.0 (Rousset, 2008). Paternity analysis was conducted

using CERVUS (Marshall *et al*., 1998). For the mtDNA analysis, the number of haplotypes per sample, haplotype diversity, and nucleotide diversity were calculated based on Nei (1987).

Phylogenetic analysis
Phylogenetic relationship of mtDNA haplotypes was resolved using neighbour-joining method in PHYLIP (Felsenstein, 1993) after 1000 bootstraps. The tree was rooted using a homologous sequence from a North Pacific right whale obtained from JARPNII. The obtained tree was visualized using TreeView PPC (Page 1996).

RESULTS AND DISCUSSION
Among three cases of duplicate sampling, the second samples of the two cases were collected years later at the same area the first samples were obtained (Table 2). One male was collected first in 1998 and was re-sampled four years later, while one female was collected first in 2000 and was re-sampled eight years later in 2008. These matches were also ascertained during our photo-identification practice (Matsuoka and Pastene, 2014). These findings indicate and support the previous knowledge that southern right whales have site fidelity to their feeding ground. A single calf and mother pair was used for paternity analysis, but no potential father was detected in the samples.

After exclusion of the second individuals from the three cases of the duplicated samples and one calf, a total of 66 samples were used for further analyses at the group level. The biopsy samples from Areas III and V were grouped with those from Area IV because their sampling sites were very close to the boundary with Area IV and the small numbers prevented us from conducting heterogeneity tests among the different areas.

The level of genetic diversity of the samples was represented as an average number of alleles per locus and an average expected heterozygosity in microsatellites, and the total number of haplotypes in the samples and expected haplotype diversity in mtDNA (Table 3). Comparing these values to those reported in Patenaude *et al*. (2007) and Carroll *et al*. (2011), only the microsatellite heterozygosity was a little lower in this study than in the previous studies and the other genetic indices were similar to each other. It is thought that the lower heterozygosity observed in this study is probably due to our marker selection.

Twenty one variable sites were found along the 430 bp of the control region of mtDNA, generating eight unique haplotypes (SR1-SR8). Phylogenetic tree showed that these eight haplotypes were clearly separated into two clades (Fig. 1). The first (SR2) and the second (SR4) most frequent haplotypes belonged to the different clades. These eight haplotypes were then compared to the 37 haplotypes reported in Patenaude *et al*. (2007) to see any haplotype matches on the basis of the sequence variations within the 275 bp consensus region (Table 4). For this purpose, some of the 37 sequences were retrieved from Genbank, and other ones were reconstructed using the information from their paper (Table2, page 150) for the variable sites and BakHapA (Genbank accession number JN097593) as a template for non-variable sites. The number of segregation sites used was 33, of which 16 were variable in both studies and 17 were variable only in Patenaude *et al*. (2007). All of our eight haplotypes had matches to their study (Table 4). These matches also indicated that our two clades were the same as their A and W clades.

Patenaude *et al*. (2007) described that, although the haplotypes from the A and W clades were found in the two different ocean basins (Indo-Atlantic and Indo-Pacific), only one haplotype (BakHapE in their study that matched to SR3 in this study) was shared between the two basins and all of the remaining 36 haplotypes were basin specific. Their results thus indicated a very limited genetic exchange between the Indo-Atlantic and Indo-Pacific basins and corresponded to the field and genetic observations that demonstrated latitudinal migration within local regions (IWC, 2001). The haplotype matches between the two studies showed that approximately 82% (SR2+SR4+ SR6+SR8) to 95% (SR2+SR4+SR6+SR8+SR3), depending on how we allocate SR3, of our samples were matched to their Indo-Pacific haplotypes. This indicates that most, if not all, southern right whales conduct north-south seasonal migration as expected from the past observations (IWC, 2001).

The results of the haplotype matches between the two studies add one more interesting and important interpretation. Three (SR1, SR5, and SR7) of our eight haplotypes were matched to their Indo-Atlantic haplotypes. Two samples from Area III were the Indo-Atlantic types. Our study thus suggests that some southern right whales migrate much longer distant than we had previously thought. Although it is difficult to determine how frequent this type of migration is occurring, the substantial genetic differentiation between the local regions within the same basin revealed by past studies (Patenaude *et al*. 2007; Carroll *et al*. 2011) suggests that the whales from the Indo-Atlantic basin should migrate back to the same basin. Southern right whales might have very strict site fidelity to the breeding and feeding grounds irrespective of its in-between distance. Furthemore, our study discloses that whales from multiple stocks migrate to our research area at the Antarctic feeding ground.

It is important to note that the above discussion was based on the variations at the 275bp consensus sequences of the mtDNA control region. In addition, according to Carroll *et al.* (2011), their analysis of longer sequences separated PorHap4 in Patenaude et al. *et al*. (2007) (SR1 in this study) into two different haplotyepes, PatHap4.1 and PatHap4.2 (Genbank accession numbers JN097601 and JN097602), and these haplotypes were actually found in the Pacific. Their frequencies were quite different as PatHap4.2 (12 out of 551 individuals) was about 10 times more frequent than PatHap4.1 (1/551) was in the same subantarctic New Zealand population, and SR1 was matched to rare PatHap4.1 based on the comparison of 417 bp sequences (data not shown). Because of its rare appearance, it may be premature to declare this haplotype (SR1) is no longer Indo-Atlantic type.

Our genetic study is the first to describe the genetic characteristics of southern right whales feeding in the Antarctic and the first to discover the possibility of southern right whales' long-distance feeding migration from the Indo-Atlantic basin to the Indo-Pacific basin that results in mixing of multiple stocks in the Antarctic. This is in contrast to the past genetic studies that demonstrated whales' restricted migration within the same basin probably due to the use of the samples from coastal areas. This study implies that southern right whales require different management strategies on an area by area basis even within the same basin for their long-term persistence. This study demonstrated one of the significant contributions of non-lethal part of the comprehensive large-scale JARPAII to acquire valuable information for effective management of large whales in Antarctic.

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Table 1.The number of Southern right whales used in this study by areas (Female, Male).

IIIE	IV	V
2 (1, 1)	63 (28, 35)	5 (1, 4)

Table 2. Sampling dates and locations of matched individuals.

	Sampling	Lat.	Long.	Cap.→recap.
Male	1998/1/15	6255S	10028E	IV→IV, 4 yrs later
	2002/2/15	6436S	9215E	
Female	2000/2/10	6435S	11431E	IV→IV, 8yrs later
	2008/3/1	6454S	12617E	

Table 3. Genetic indices based on variations at microsatellite DNA and mtDNA markers.

		Microsatellite DNA				mtDNA		
	N*	#loci	#allele	He	HW	bp	#HP	h
Total	66	14	6.9	0.651	n.s	430	8	0.767
Female	27	14	6.3	0.643	n.s	430	6	0.795
Male	39	14	6.5	0.655	n.s	430	6	0.754

*mtDNA:Total=65, Female=27, male=38

Table 4. Reference to Patenaude *et al*. (2007)

This study		Patenaude *et al*. (2007)		
Haplotype	n	Haplotype matched	Basin found	phylogeny
SR1	1	PorHap4	Indo-Atlantic	Clade A
SR2	24	BakHapA	Indo-Pacific	
SR5	1	PorHap11	Indo-Atlantic	
SR6	12	BakHapB	Indo-Pacific	
SR3	9	BakHapE	Shared	Clade W
SR4	15	BackHapC	Indo-Pacific	
SR7	1	PorHap16	Indo-Atlantic	
SR8	2	BakHapD	Indo-Pacific	

Indo-Pacific: Southwest Australia calving, New Zealand sub-Antarctic calving, Southwest Australia feeding grounds.

Indo-Atlantic: Argentina calving, South Africa calving, South Georgia feeding grounds.

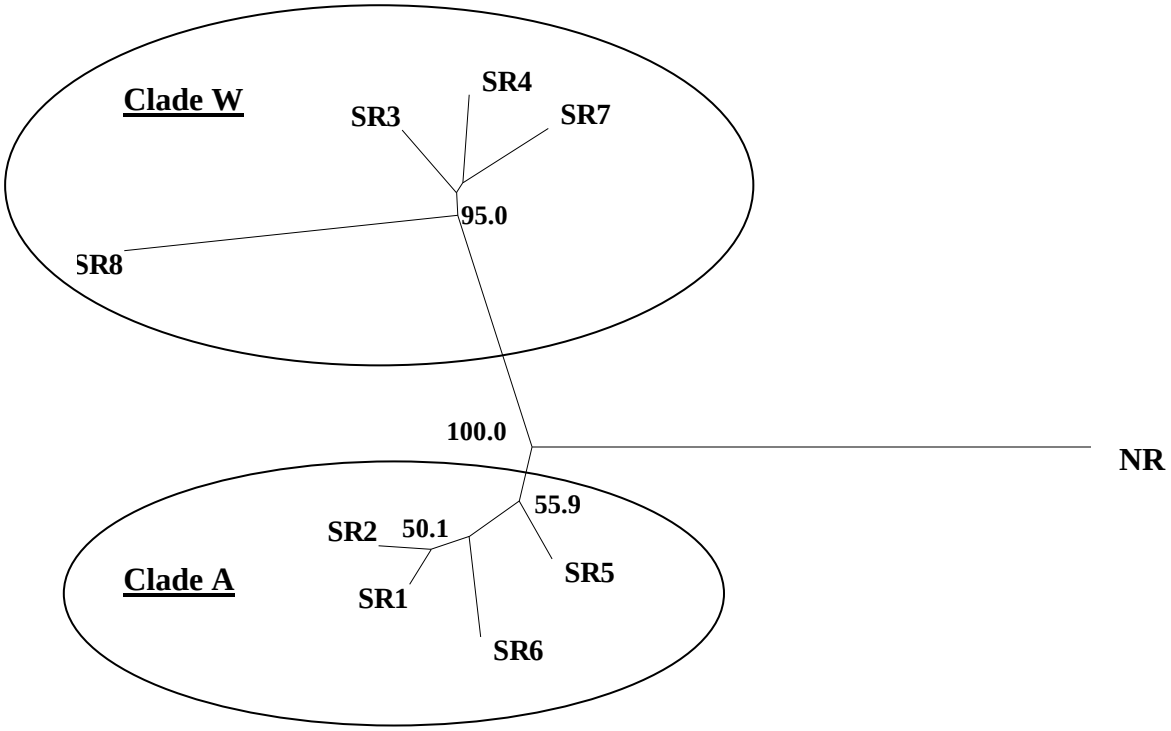

Figure 1. Phylogenetic relationship of southern right whale mtDNA haplotypes (SR1-SR8).
The tree was rooted using a homologous sequence from a North Pacific right whales. Only bootstrap values (%) over 50% from 1000 replicates are given. Clade A, W from Patenaude *et al*. (2007).