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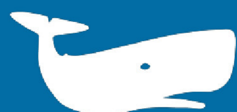

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A note on the genetic diversity of sperm whales in the western North Pacific as revealed by mitochondrial and microsatellite DNA

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ABSTRACT
Genetic variation at 15 microsatellite DNA loci and mitochondrial DNA (mtDNA) control region sequences (338bp) was examined in sperm whales collected during JARPNII from 2000 to 2013 in order to examine the effectiveness of these genetic markers for studies of stock structure in this species. Analyses of mtDNA and microsatellite markers in a total of 56 sperm whales (16 males; 40 females) confirmed that these genetic markers were variable enough to explore stock structure of sperm whales. The overall heterozygosity over 15 loci was 0.730 while the nucleotide and haplotype diversity were 0.0038 and 0.7188, respectively. Statistical tests found no evidence of deviation from the expected Hardy-Weinberg genotypic proportion at all of the 15 microsatellite loci. At this point, no signal of multiple stocks of sperm whale in the western North Pacific off Japan was detected.

INTRODUCTION

Identification of the genetic structure of stocks over a species' geographic range is of primary importance for effective stock management. The objective of the stock structure part of JARPNII survey for sperm whales is to obtain information useful for describing stock structure of sperm whales inhabiting the western North Pacific. The information is also helpful for future In-depth Assessment (IA) of this species by the International Whaling Commission Scientific Committee (IWC SC).

As an initial step to accomplish the objective, microsatellite and mitochondrial (mt) DNA was used on samples collected by JARPNII to examine genetic diversity and the effectiveness of these genetic markers for studies on stock structure of the species. This is an update of the study started by Kanda *et al.* (2009).

MATERIALS AND METHODS

Samples and DNA extraction

A total of 56 sperm whales (16 males; 40 females) used in this study were collected during JARPNII surveys from 2000 to 2013 in the western North Pacific (Figure 1).

The IWC guidelines for DNA data quality (IWC, 2009) were followed as much as possible (see Kanda *et al.*, 2014). Skin tissues were stored in vials filled with 95% ethanol in –70°C until DNA extraction. Total DNA was extracted from 0.05g of skin tissue using the protocol of Sambrook *et al.* (1989). Extracted DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Microsatellite analysis

Microsatellite polymorphisms were analyzed using 15 loci: EV1, EV5, EV94, EV104 (Valsecchi and Amos 1996), GT011 (Bérubé *et al.* 1998), GT23, GT211, GT271, GT509, GT575 (Bérubé *et al.* 2000), GATA28, GATA98, GATA417 (Palsbøll *et al.* 1997), DlrFCB14, and DlrFCB17 (Buchanan *et al.* 1996). Only EV1 and EV5 were designed from a sperm whale. DlrFcB14 and DlrFcB17 were designed from a beluga whale, while all other 11 loci were from a humpback whale. Primer sequences and PCR cycling profiles followed those of the original authors. PCR amplifications followed the manufacturer's instructions for the use of Ex *Taq* DNA polymerase (Takara Shuzo). Amplified products were electrophoresed on a 6% polyacrylamide denaturating gel using a BaseStation 100 DNA fragment

analyzer (Bio-Rad), and alleles were sized manually in relation to an internal size standard (Genescan 400HD, Applied Biosystems) and to sperm whale microsatellites of known size that were included on each gel.

The number of alleles per locus, expected heterozygosity per locus and inbreeding coefficient 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). The False Discovery Rate (FDR) approach (Benjamini and Yekutieli, 2001) was used for adjustment of *p*-value in case of multiple comparisons.

mtDNA analysis

The first half of the mtDNA control region was amplified through the polymerase chain reaction (PCR) using the following primer set: heavy-strand P2 (5'-GAAGAGGGATCCCTGCCAAGCGG-*3'*; Hori *et al*., unpublished) and light-strand MT4 (Árnason *et al*., 1993). PCR products were purified by MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed with the same primers, using BigDye terminator cycle sequence Kit (Applied Biosystems, Inc). The cycle sequencing products were purified by AutoSeq G-50 spin Columns (Pharmacia Biotech). The labeled sequencing fragments were resolved by electrophoresis through a 5% denaturing polyacrylamide matrix on an ABI 377™ or ABI3100 Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacturers. For each sample both strands were sequenced.

The number of mtDNA haplotypes and haplotype diversity were calculated following Nei (1987). The nucleotide diversity (Nei, 1987: equation 10.5) and its standard error for population sampling and stochastic processes were calculated from the pairwise differences between the mtDNA sequences using the Kimura's 2- parameter adjustment (Kimura, 1980).

RESULTS AND DISCUSSION

Given the level of genetic variation found, the genetic markers used are effective for future studies of sperm whales' stock structure in the western North Pacific.

Microsatellites

All the 15 microsatellite loci were polymorphic (Table 1). The total number of alleles at each locus ranged from three at GT575, GATA41 and GATA28 to 18 at DlrFCB17, with an average of 8.7. The average expected heterozygosity at each locus ranged from 0.480 at GT23 to 0.900 at DlrFCB17, with an average of 0.730.

The level of microsatellite genetic diversity of the sperm whales used in this study was similar to that of other oceanic areas. Lyrholm *et al*. (1999) analyzed sperm whales from the North Atlantic, North Pacific, and Southern Hemisphere using nine microsatellite loci, three (EV1, EV5, GATA28) of which were same as those in this study. Lyrholm *et al*. (1999) showed that the level of genetic diversity in these oceanic samples were similar to each other with the average allele numbers of 10.0 and average heterozygosity of 0.720-0.730. These values were similar to those found in the present study.

Some of the loci showed low levels of genetic diversity in the JARPNII sample. Possibility of null alleles was excluded because no signal of such case was detected (e.g. Hardy-Weinberg genotypic proportions). The negative effect on genetic diversity of population level appeared not to be a reason because the low diversity was not consistent over all of the 15 loci.

All of the 15 loci showed no evidence of significant deviation from the expected Hardy-Weinberg genotypic proportions (Table 1). At this point, no signal of multiple stocks in the western North Pacific sperm whale was detected.

mtDNA

Sequence variations at 338bp of the mtDNA control region resulted in 11 unique haplotypes. Haplotype diversity was 0.719 and nucleotide diversity was 0.0038 (SE=0.0006) (Table 2).

The levels of mtDNA diversity (nucleotide diversity and haplotype diversity) found for western North

Pacific in the present study were similar to those reported by Lyrholm *et al*. (1996) for sperm whales worldwide, using a similar segment of the mtDNA control region and similar sample size.

On the other hand the levels of mtDNA diversity was much lower than those reported for North Pacific common minke whale of the 'O' stock (Pastene *et al*., 2016a: SC/F16/JR40), Bryde's whale (Pastene *et al*., 2016b: SC/F16/JR44) and sei whale (Pastene *et al*., 2016c: SC/F16/JR46). Low nucleotide diversity appeared to be a characteristic of sperm whales, and Lyrholm *et al.* (1996) described two alternative explanations for the low nucleotide diversity in the sperm whales: 1) the highly biased substitution pattern, with most substitutions occurring as repeated transition at a few hot spot, and 2) genetic bottleneck in population size.

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Table 1. Number of alleles detected (A), expected heterozygosity (H_E), the results of test for deviation from the expected Hardy-Weinberg genotypic proportion (HW) and inbreeding coefficient (F_{IS})at each of the 15 microsatellite loci for western North Pacific sperm whale (n=56, 16 males; 40 females).

Microsatellite loci	A	H_E	HW	F_{IS}
GT509	12	0,81	0,256	0,035
GT271	14	0,88	0,154	-0,052
EV1	9	0,57	0,994	-0,191
EV5	9	0,72	0,079	-0,036
GT211	6	0,77	0,701	-0,070
GT575	3	0,56	0,113	-0,256
DlrFCB14	11	0,84	0,048	0,063
GT23	4	0,48	0,283	0,142
DlrFCB17	18	0,90	0,304	0,024
EV94	11	0,76	0,554	0,015
GT011	13	0,86	0,739	0,007
GATA41	3	0,60	0,809	0,042
EV104	5	0,69	0,118	0,101
GATA98	9	0,85	0,144	-0,009
GATA28	3	0,63	0,535	-0,052
Overall	8,67	0,73	0,121	-0,012

Table 2. Results of mtDNA diversity for western North Pacific sperm whale (n=56, 16 males; 40 females).

Number of haplotypes	Nucleotide diversity (SE)	Haplotype diversity
11	0.0038 (0.0006)	0.7188

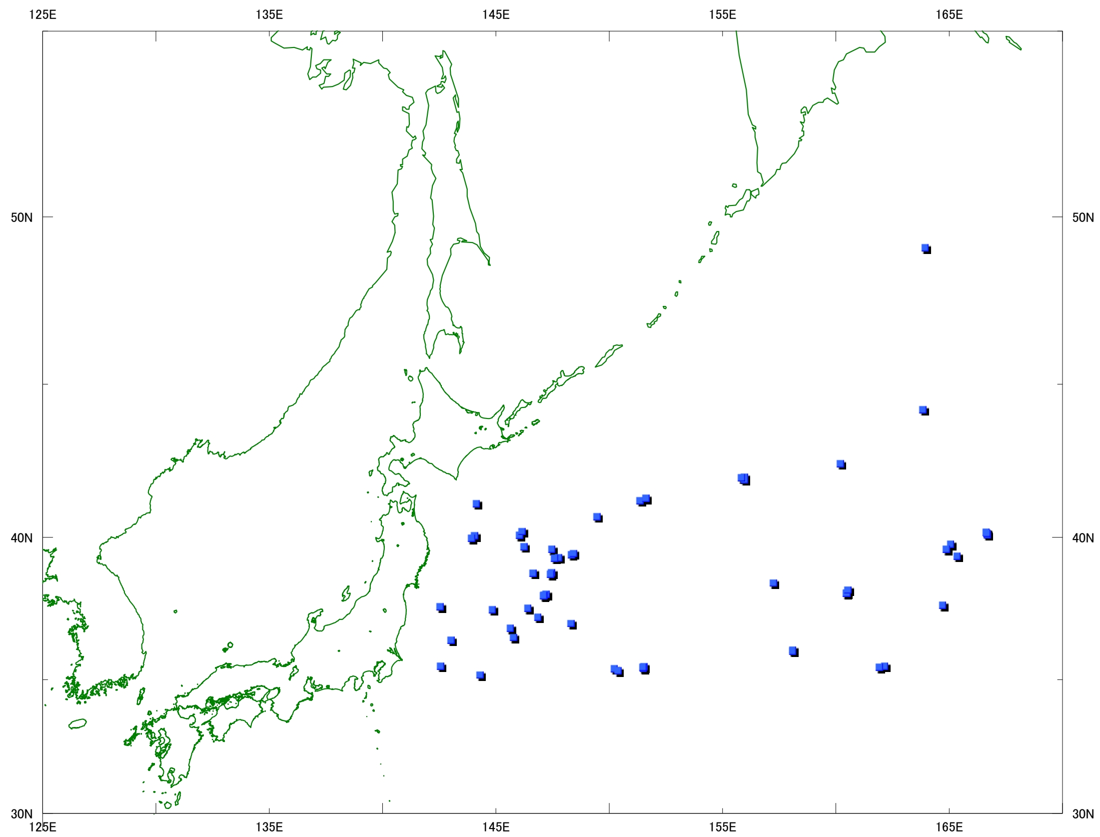

Figure 1. Positions of first sighting of sperm whales collected during the JARPNII from 2000 to 2016 (n= 56, 16 males; 40 females).