

Genetic characteristics of sperm whales sampled during JARPNII from 2000 to 2007 as revealed by mitochondrial DNA and microsatellite analyses

Naohisa Kanda, Mutsuo Goto and Luis A. Pastene

The Institute of Cetacean Research, 4-5 Toyomi-cho, Chuo-ku, Tokyo 104-0055, Japan

Contact email:kanda@cetacean.jp

ABSTRACT

We analyzed genetic variations at 15 microsatellite DNA loci and mitochondrial DNA (mtDNA) control region sequences in sample of sperm whales collected during JARPNII from 2000 to 2007 in order to examine the effectiveness of these genetic markers for stock structure study of the species. Analyses of mtDNA and microsatellite markers in the total of 45 sperm whales demonstrated that these genetic markers were variable enough to explore stock structure of sperm whales. Statistical tests found no evidence of deviation from the expected Hardy-Weinberg genotypic proportion at all of the 15 microsatellite loci. At this point, we did not detect any signals of multiple stocks in the western North Pacific off Japan from the JARPNII samples.

KEY WORDS: SPERM WHALE, MICROSATELLITE, JARPNII, NORTH PACIFIC

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 surely helpful for future CA of this species in the IWC. As an initial step to accomplish the objective, we examined genetic variation at mtDNA and microsatellites to examine effectiveness of these genetic markers to explore genetic characteristics of the species.

MATERIALS AND METHODS

Samples and DNA extraction

A total of 45 sperm whales used in this analysis were collected during JARPNII surveys from 2000 to 2007 in the western North Pacific (Table 1; Fig. 1). Details of offshore component of JARPNII survey can be found in Tamura *et al.* (2009).

Skin tissues of research takes were stored in vials filled with 95% ethanol in -70°C until DNA extraction. Total DNA was extracted from 0.05 g 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).

mtDNA analysis

The first half of the mtDNA control region was amplified through the polymerase chain reaction (PCR). Approximately 350bp of mtDNA region containing control region were amplified 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 \ddot{a} or ABI3100 Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacture. 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

Do not cite without written permission from the authors

the Kimura's 2-parameter adjustment (Kimura, 1980).

Microsatellite analysis

Microsatellite polymorphisms were analyzed using 15 sets of primers: EV1, EV5, EV94, EV104 (Valsecchi & 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, *Physeter macrocephalus*. DlrFCB14 and DlrFCB17 were designed from a beluga whale, *Delphinapterus leucas*, while all other 11 sets of primers were from a humpback whale, *Megaptera novaeanglia*. 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 denaturing 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 microsatellite alleles and expected heterozygosity per locus was calculated using the software FSTAT 2.9.3 (Goudet, 1995). Statistical tests for deviations from the expected Hardy-Weinberg genotypic proportions were conducted using the software GENEPOP 4.0 (Rousset, 2008). When simultaneous multiple tests were conducted, Rice (1989) correction for the multiple tests was performed.

RESULTS AND DISCUSSION

The use of mtDNA and hypervariable microsatellite markers provides us great opportunity to acquire enough genetic variation to describe genetic stock structure. Especially for the microsatellite analysis in this study, substantial level of genetic variation was observed and the number of loci analyzed was doubled compared to seven in the previous report (Kanda *et al.*, 2002). We believe these genetic markers are effective for the study of sperm whales' stock structure in the western North Pacific.

mtDNA

Sequence variations at 347bp of the mtDNA control region in the North Pacific sperm whales resulted in 11 unique haplotypes. Haplotype diversity was 0.700 and nucleotide diversity was 0.0038 (standard error=0.00069).

Lyrholm *et al.* (1996) reported that haplotype and nucleotide diversities for the 300bp of the mtDNA control region, which was nearly homologous to our present study, among the 37 sperm whales collected from the world-wide oceanic areas (North Atlantic n=10, North Pacific n=16, and Southern Hemisphere n=11). Thirteen haplotypes were identified and the haplotype diversity was 0.74. Nucleotide diversity was 0.0038 (SE=0.00059). The level of haplotype diversity in our sample of the North Pacific sperm whales was similar to that in the sample of the sperm whales world-wide (Lyrholm *et al.* 1996) as well as that reported in the samples of other baleen whales, such as common minke whales (Goto *et al.*, 2009), sei whales (Kanda *et al.*, 2009a) and Bryde's whales (Kanda *et al.*, 2009b) in the North Pacific. Contrary, although the level of nucleotide diversity estimated in our study was similar to that in Lyrholm *et al.* (1996) it was much lower than that reported from the other large baleen whales. Common minke whales (Goto *et al.*, 2009) and sei whales (Kanda *et al.*, 2009a) in the North Pacific showed roughly two-fold higher values and Bryde's whales (Kanda *et al.*, 2009b) showed approximately three-fold higher value. 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.

Microsatellites

All of the 15 microsatellite loci, 13 of which were designed from other whale species, were polymorphic (Table 2). The total number of alleles at each locus ranged from three to 17 with an average of 8.3. Average expected heterozygosity at each locus ranged from 0.498 to 0.902 with an average of 0.724.

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 sets of microsatellite primers, three (EV1, EV5, GATA28) of which were same as those in this study. Lyrholm *et al.* (1999) showed that the level of the genetic diversity of these oceanic samples were similar to each other with the average allele numbers of 10.0 and average heterozygosity of 0.72-0.73. The sperm whale sample from the Japanese coast had the average

Do not cite without written permission from the authors

allele numbers of 7.0 and average heterozygosity of 0.73. Some of the loci showed low levels of genetic diversity in our sample. Possibility of null alleles was excluded because no signal of such case was detected (e.g. Hardy-Weinberg genotypic proportions). Unlike one of the possible factors for the mtDNA low diversity, the negative effect on genetic diversity at a 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 deviation from the expected Hardy-Weinberg genotypic proportions (Table 2). At this point, we did not detect any signals of multiple stocks in the western North Pacific off Japan from the JARPNII samples.

ACKNOWLEDGMENTS

We gratefully acknowledge the researchers and crew members that participated in JARPNII survey. We also thank H. Oikawa and S. Azumi (ICR Ayukawa Laboratory) who collaborated in the process of DNA extraction.

REFERENCES

- Árnason, Ú., Gullberg, A., and Widegten, B. 1993. Cetacean mitochondrial DNA control region: Sequences of all extant baleen whales and two sperm whale species. *Mol. Biol. Evol.* 10:960-970.
- Buchanan, F.C., Friesen, M.K., Littlejohn, R.P. and Clayton, J.A. 1996. Microsatellites from beluga whale *Delphinapterus leucas*. *Mol. Ecol.* 5: 571-575
- Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo-di-Sciara, G., Sears, R., Sigurjónsson, J., Urban-Ramirez, J., and Palsbøll, P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus, 1758); analysis of mitochondrial and nuclear loci. *Mol. Ecol.* 7:585-599.
- Goudet, J. 1995. FSTAT, version 1.2: a computer program to calculate F-statistics. *J. Hered.* 86:485-486.
- Goto, M., Kanda, N., Kishiro, T., Yoshida, H., Kato, H. and Pastene, L.A. 2009. Mitochondrial DNA analysis on stock structure in the western North Pacific common minke whales. Paper SC/J09/JR29 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 10pp.
- Kanda, N., Goto, M., and Pastene, L.A. 2002. Genetic analysis of sperm whales sampled during JARPNII in 2000 and 2001. Paper SC/54/O17 Appendix 11 presented to the Scientific Committee of the International Whaling Commission. (unpublished).
- Kanda, N., Goto, M. and Pastene, L.A. 2009a. Stock structure of Bryde's whales in the western North Pacific as revealed by microsatellite and mitochondrial DNA analyses. Paper SC/J09/JR31 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 8pp.
- Kanda, N., Goto, M., Yoshida, H. and Pastene, L.A. 2009b. Stock structure of sei whales in the North Pacific as revealed by microsatellite and mitochondrial DNA analyses. Paper SC/J09/JR32 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 14pp.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111-120.
- Nei, M. 1987. *Molecular Evolutionary Genetics*. New York: Columbia University Press.
- Lyrholm, T., Leimar, O., and Gyllensten, U. 1996. Low diversity and biased substitution patterns in the mitochondrial DNA control region of sperm whales: Implications for estimates of time since common ancestry. *Mol. Biol. Evol.* 13:1318-1326.
- Lyrholm, T., O. Leimar, B. Johanneson, and U. Gyllensten. 1999. Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proc. R. Soc. Lond. B* 266:347-354.
- Palsbøll, P. J., Bérubé, M., Larsen, A.H. and Jørgensen, H. 1997. Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. *Mol. Ecol.* 6: 893-895.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.
- Rousset, F. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molec. Ecol. Resources* 8:103-106.
- Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular cloning: A laboratory manual. 2nd Ed.*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Tamura, T., Matsuoka, K., and Fujise, Y. 2009. Methodology and survey procedure under the JARPN II - offshore component- with special emphasis on whale sampling procedures. Paper SC/J09/JR4 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 16pp.
- Valsecchi, E. and Amos, W. 1996. Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* 5: 151-156.

Do not cite without written permission from the authors

Table 1. Sperm whales analyzed in this study.

	Survey year							
	2000	2001	2002	2003	2004	2005	2006	2007
N	5	8	5	10	3	5	6	3

Table 2. Number of alleles detected (A), expected heterozygosity (He), the results of test for deviation from the expected Hardy-Weinberg genotypic proportion at each of the 15 microsatellite loci (HW), haplotype number, haplotype diversity, and nucleotide diversity with its standard error at mtDNA analyzed in the samples (N=45) of sperm whales collected during JARPNI.

Microsatellites	A	He	HW
DlrFCB14	11	0.838	n.s.
DlrFCB17	17	0.902	n.s.
EV1	9	0.562	n.s.
EV5	8	0.708	n.s.
EV94	11	0.762	n.s.
EV104	5	0.674	n.s.
GATA28	3	0.635	n.s.
GATA98	9	0.849	n.s.
GATA417	3	0.575	n.s.
GT23	4	0.498	n.s.
GT011	11	0.867	n.s.
GT211	6	0.776	n.s.
GT271	14	0.883	n.s.
GT509	10	0.776	n.s.
GT575	3	0.559	n.s.
mtDNA			
Haplotype number	11		
Haplotype diversity	0.7000		
Nucleotide diversity (standard error)	0.0038 (0.0009)		

n.s. = non significant

Do not cite without written permission from the authors

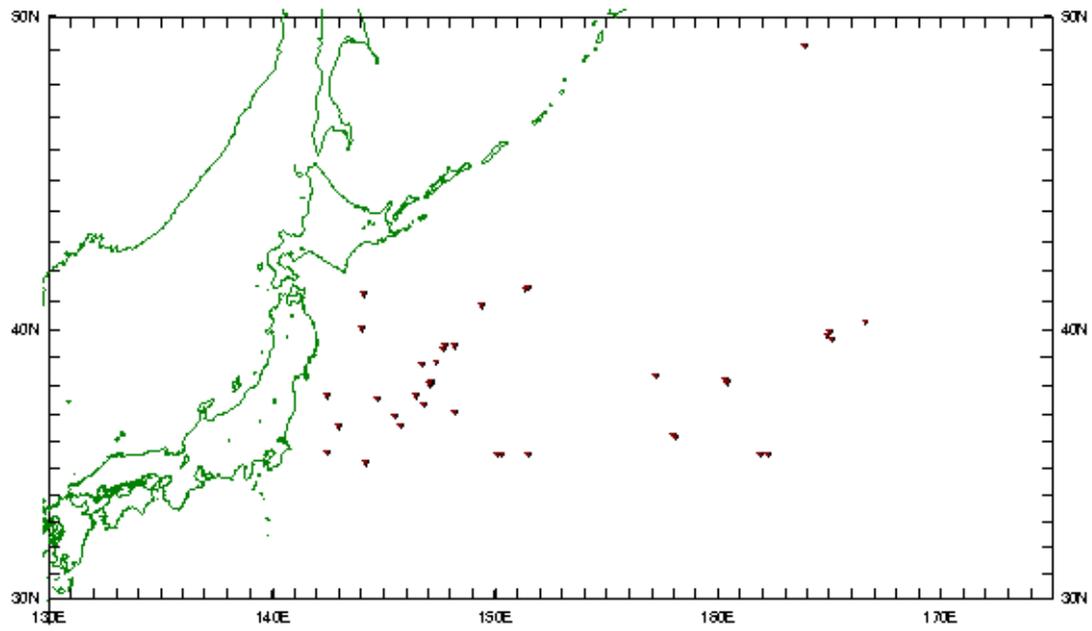


Fig. 1 Sighting positions of sperm whales collected during the JARPNII from 2000 to 2007.