Mitochondrial DNA analysis on stock structure in the western North Pacific common minke whales

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ABSTRACT
Genetic variation at the mtDNA control region in the western North Pacific common minke whales was analyzed to examine the plausibility of four stock structure scenarios adopted at the final stage of the *Implementation Simulation Trials (ISTs)* process under the Revised Management Procedure by the IWC Scientific Committee in 2003. A total of 1,639 whales collected during JARPN and JARPNII surveys from 1994 to 2007 in the area from the Japanese coast to the offshore waters (to 170°E) on the Pacific side, was used for the analyses. The samples from 2002 to 2007 (n= 1,124) were not used during the previous *ISTs* process. Heterogeneity tests were based on the randomized chi-square test. The only source of significant mtDNA heterogeneity was found in sub-area 7W in a 2007. Additional analysis in this sub-area and year suggested that the source of heterogeneity was due to the occurrence of J stock animals. Our updated analysis based on a larger data set confirmed that only two stocks distribute in the western North Pacific, the O and J stocks. This study provides no evidences for the occurrence of a W stock in offshore waters or additional structure within the O stock in the Pacific side of Japan. These results are consistent with the results found by the microsatellite and morphometric analyses. Therefore results from the genetic and non-genetic approaches support the single O stock scenario in the Pacific side of Japan (Scenario B).

KEY WORDS: COMMON MINKE WHALE, MTDNA, STOCK STRUCTURE, JARPN, JARPNII, NORTH PACIFIC

INTRODUCTION

The *Implementation Simulation Trials* (*ISTs*) for North Pacific common minke whale under the Revised Management Procedure (RMP) was completed during the 2003 International Whaling Commission Scientific Committee (IWC SC) meeting. At the final stage of the *ISTs* process the SC adopted the following four stock structure baseline scenarios (IWC, 2004):

(1) Baseline A: three-stock scenario (‘J’, ‘O’, ‘W’) with the ‘W’ stock found only in part of sub-area 9 and only sporadically.
(2) Baseline B: two stock scenario (‘J’ and ‘O’) with no W stock as a limiting case of Baseline A.
(3) Baseline C：four-stock scenario overall, with ‘O_W’, ‘O_E’ and ‘W’ to the east of Japan. Boundaries are fixed at 147°E and 157°E and there is no mixing between the stocks.
(4) Baseline D：three-stock scenario (‘J’, ‘O’, ‘W’), with ‘O’ and ‘W’ mixing over 147°E and 162°E, O being dominant to the west and W to the east.

Unfortunately the SC did not examine plausibility of the stock structure scenarios. In order to get agreement among SC members it gave the same ‘high’ plausibility to the four scenarios (IWC, 2004).

In this study the plausibility of the four stock structure scenarios is examined through genetic analysis based on mitochondrial DNA (mtDNA) and samples collected by JARPN and JARPNII from 1994 to 2007. The samples from 2002 to 2007 were not used during the previous *IST*s process.

MATERIALS AND METHODS

Samples

Table 1 shows the number of samples used in the present mtDNA analysis by year, sub-area and the offshore and coastal components of JARPN II. Details of the surveys design and methodology in the offshore and coastal components of JARPNII were described in Tamura *et al*. (2009) and in Kishiro *et al*. (2009), respectively. During the *IST* specification conducted in 2003, eighteen sub-areas were set for management purpose of the western North Pacific common minke whale (Figure.1). Sub-areas 7, 8 and 9 were divided into western and eastern strata by 147°E, 157°E and 162°E, 7W (140.01°E -147.00°E), 7E (147.01°E -150.00°E), 8W (150.01°E -153.00°E), 8E (153.01°E -157.00°E), 9W (157.01°E -162.00°E), and 9E (162.01°E -170.00°E). The sighting position of common minke whales used in this study is shown in Figure 2. Whales were sampled by JARPN and JARPNII surveys during 1994-2007

Sequencing of the mtDNA control region

Using established protocols (Sambrook *et al.*, 1989), total-cell DNA was extracted from skin tissue samples. The first half of control region of the mitochondrial genome was amplified using the polymerase chain reaction (PCR). In order to amplify an approximately 500 bp of the mtDNA control region, primers light-strand MT4 (Árnason *et al*., 1993) and heavy-strand Dlp5R (*5′*-CCATCgAgATgTCTTATTTAA-ggggAAC-*3′*), were used. 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 377ä and ABI3100 Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacturer. For each sample both strands were sequenced.

Data analysis

The evolutionary distance between two nucleotide sequences was calculated according to Kimura's two parameters method (Kimura, 1980). The degree of genetic diversity within each locality was estimated using the nucleotide diversity (Nei, 1987). The randomized chi-square Test of Independence (Roff and Bentzen, 1989) was used to investigate the temporal/spatial differentiation of mtDNA variation. In each test a total of 10,000 permutations of the original data were performed. A P-value smaller than 0.05 was used as a criterion to reject the null hypothesis of panmixia.

The following steps were conducted: a) analysis of genetic differences between the coastal and offshore samples collected in the same year from sub-area 7W; b) analysis of genetic differences among whales collected in different years from a same sub-area; and c) analysis of genetic differences among whales grouped according to the geographic divisions defining the four stock structure baseline scenarios used in previous *IST*s.

Assignment for J or O types

Analyses were conducted with and without suspected J stock animals in the case of sub-area 7W. Individual assignment to O and J stocks was based on analysis of microsatellite and the computer program STRUCTURE (Kanda *et al.*, 2009a).

RESULTS

Genetic diversity within samples

Sequence variations in a 487bp segment of the mtDNA control region resulted in 123 unique haplotypes in the total sample of 1,632 whales. Forty-one polymorphic sites were detected, 37 of which were transitions, three transversion and one insertion/deletion event..

Table 2 shows the nucleotide (π) and haplotype (H) diversities in each sample. Nucleotide diversities and its standard error of JARPN (1994-1999), JARPNII offshore component (2000-2007) and JARPNII coastal component (2002-2007) were 0.0079 (0.0002), 0.0081(0.0002) and 0.0089 (0.0002), respectively. Haplotype diversities were 0.9522, 0.9529 and 0.9624, respectively. For both indices these values are higher in the JARPNII coastal whales. For nucleotide diversity the difference was statistically significant between JARPN and JARPNII coastal component.

Genetic divergence between samples

Genetic differences between offshore and coastal samples in sub-area 7W

Table 3 shows the results of the heterogeneity test for the comparison between samples taken in 7W by coastal and offshore components of JARPN II, by year and with and without suspected J stock animals. None of the comparison showed significant mtDNA differences except the 2007 samples. We conducted the heterogeneity test for this year excluding suspected J stock animals. In this case no significant differences were found.. Therefore significant differences found were most likely due to the inclusion of whales from the J stock in the sample. For the subsequent analyses, we conducted the heterogeneity tests with and without suspected J stock animals in the 7W sample.

Yearly genetic differences within sub-areas

Table 4 shows the results of the heterogeneity test for yearly differentiation in each sub-area. In some sub-areas, years with less than 10 individuals were omitted. No significant yearly differences were found in the sub-areas. In subsequent analyses samples from different years were combined in the sub-areas.

Plausibility of hypotheses

Explanation of the four stock structure hypotheses used in previous *ISTs* was given above.

Baseline A

In order to test the heterogeneity within sub-area 9, we compared the samples collected in the western and eastern sides of sub-area 9, by year. No significant differences were found. Further there was no significant difference between western and eastern sides of sub-area 9 using total samples (Table 5).

Baseline B.

We examined the genetic differences among all sub-areas (7W, 7E, 8W, 8E, 9W and 9E). No significant differences were found among all sub-areas with (P= 0.0595) and without (P=0.6106) suspected J stock animals in the sub-area 7W.

Baseline C

Table 6 shows the results of heterogeneity test for samples divided according to this scenario (e.g. samples divided by the longitudinal boundaries at 147°E and 157°E). First heterogeneity test was conducted among samples from 7E, 8W and 8E, and no significant differences were found. Therefore these samples were combined into one as 7E-8E for the following analyses. No significant differences were found among samples from 7W, 7E-8E and 9W-9E with and without suspected J stock animals in the 7W samples.

Baseline D

We examined the genetic differences among three groups divided by the longitudinal boundaries at 147°E and 162°E. First heterogeneity test was conducted among samples from 7E, 8W, 8E and 9W, and no significant differences were found. These samples were combined into one as 7E-9W for the following analyses. We then conducted the heterogeneity tests among 7W, 7E-9W, and 9E with and without suspected J stock individuals in the 7W sample (7Wx7E-9Wx9E. 7Wx7E-9Wx9E*, Table 7). There are no significant differences among these samples with and without suspected J stock individuals in the 7W sample

DISCUSSION

Level of genetic diversity

Genetic diversity at the mtDNA control region in common minke whales used in this study was similar to that of other large baleen whales in the North Pacific, such as Bryde's (Kanda *et al*., 2009c) and sei whales (Kanda *et al.*, 2009d). Diversities in the common minke whale in the JARPNII coastal component samples are slightly higher than in the other samples. This could be caused by the occurrence of J stock

animals distributed around the coastal area in the Pacific side of Japan descried by Kanda *et al*.(2009a).

Genetic divergence
Overall our study based on a larger data set than used previously failed to find any evidence of significant genetic heterogeneity in sub-areas 7, 8 and 9. The only source of genetic heterogeneity was found in sub-area 7 in 2007. Results of further analysis in this sub-area suggested that such heterogeneity could be due to the occurrence of J stock animals.

These results based on mtDNA and a larger data set are valuable to re-evaluate the plausibility of the four stock structure scenarios defined by the IWC SC in 2003. Results constitute a very strong demonstration that the underlying hypothesis of scenario C, e.g. three stocks with hard boundaries at 147°E and 157°E, has no independent support as there are no genetic evidences for multiple O stocks as proposed by this scenario. Genetic differentiation among stocks is facilitated by some geographical or ecological 'barrier', which acts restricting the gene flow among them. Minke whales in sub-areas 7, 8 and 9 have been described as opportunistic feeders. Distribution of these whales is related to the distribution and dynamics of prey species and the distribution of prey species is defined by oceanographic conditions in the area, which are variable. There is no evidence for ecological barriers at 147°E or 157°E

Common minke whales were taken by the Japan's small-type coastal whaling in sub-area 7W for more than 40 years. The annual catches of minke whales were in the range 200-300 (IWC, 2004). If the occurrence of a small coastal Ow stock, as proposed by the Baseline C stock scenario, is true, such stock could have been extinct under such catch levels. Kawahara (2002) reviewed the small-type whaling and two types of CPUE analyses. Judging from the corrected CPUE1 trend for about 35 years, the drastic decline of abundance seems unlikely. And CPUE2 analysis in 1977-1987 with operation hours by area also does not suggest that O stock has declined. Consequently no considerable decline was detected in either CPUE series. These results are consistent with results of the present genetic analysis and show that the plausibility of Baseline C is low.

Regarding to the Scenario D, results of our genetic analysis are also difficult to reconcile with the underlying hypothesis of scenario D. This hypothesis establishes that O and W stocks mix with each other between 147°E and 162°E. The O stock is predominant to the west and the W stock to the east of this range. Under this scenario only O stock occur between the Japanese coast and 147°E and only W stock from 162° to the east. One of the inconsistencies is that the mtDNA haplotype frequency in samples from sub-area 9E (scenario D assume that only W stock is distributed in this sector) is genetically similar to those in sub-areas 7 and 8 (O stock). Furthermore analysis of microsatellite indicated no significant departure from the Hardy-Weinberg equilibrium in the sector 147°-162°E (Kanda et al., 2009b). A significant departure from equilibrium could indicate a situation of mixing between different stocks as suggested by the scenario D. Scenario D is therefore inconsistent with genetic data.

One of the objectives of the JARPNII was to look for any evidence of existence of the W stock. Past mtDNA studies (e.g., Goto *et al*., 2000) found the genetic heterogeneity in the 9W in some years. In the present study, we conducted haplotypic statistics based on Chi-square test because this statistics showed the better performance in comparison to Hst, Fst, Kst* and PHIst (Taylor and Chivers, 2000). Based on this statistic and a large data set were unable to find significant heterogeneity in sub-area 9W as detected in our previous study. Therefore stock structure under scenario A was not supported by this analysis.

Therefore our updated analysis based on mtDNA and a larger data set confirmed that only two stocks distribute in the western North Pacific, the O and J stocks. This study provides no evidences for the occurrence of a W stock in offshore waters or additional structure within the O stock in the Pacific side of Japan. These results are consistent with the results found by the microsatellite (Kanda et al., 2009b) and morphometric (Hakamada and Bando, 2009) analyses.

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Table 1. Sample sizes used in this study by year, sub-area in offshore (A) and Coastal (B) components.

A) Offshore

Y	7W	7E	8W	8E	9W	9E	Total
1994					7	14	21
1995					78	22	100
1996	31		1	15			47
1997	1	1	1	30	19	48	100
1998	25	31	44				100
1999	50						50
2000	24				16		40
2001	43	7		21	29		100
2002	60			8	32		100
2003	17	7	21	16	25	14	100
2004	16				42	42	100
2005	32		6	7	19	30	94
2006	36	2	10	28	23	1	100
2007	79		2	13	2	4	100
Total	414	48	86	138	294	178	1158

(B) Coastal

Year	7W
2002	50
2003	50
2004	59
2005	119
2006	95
2007	107
Total	481

Table 2. Nucleotide (π) and haplotype (H) diversities in each sample.

samples		N	π	SE	H
JARPN (1994-1999)		418	0.0079	0.0002	0.9522
JARPNII (2000-2007) Offshore component		734	0.0081	0.0002	0.9529
	2000	40	0.0078	0.0007	0.9321
	2001	100	0.0086	0.0004	0.9489
	2002	100	0.0079	0.0004	0.9535
	2003	100	0.0078	0.0004	0.9491
	2004	100	0.0087	0.0004	0.9594
	2005	94	0.0083	0.0005	0.9520
	2006	100	0.0077	0.0004	0.9582
	2007	100	0.0082	0.0004	0.9590
JARPNII (2002-2007) Coastal component		480	0.0089	0.0002	0.9624
	Kushiro	260	0.0091	0.0003	0.9656
	Sanriku	220	0.0086	0.0003	0.9584
Sum		1632	0.0083	0.0001	0.9561

Table 3. Statistical comparison between offshore and coastal samples in sub-area 7W by year, and with and without suspected J stock animals.

Year	All animals			Without suspected J stock animals		
	N		P	N		P
	Offshore	Coastal		Offshore	Coastal	
2002	60	50	0.1726	56	43	0.2808
2003	17	50	0.1369	16	44	0.1674
2004	16	59	0.2813	13	55	0.2919
2005	32	119	0.2326	29	94	0.2799
2006	36	95	0.3818	34	78	0.3074
2007	79	107	**0.0192**	75	88	0.1238

Bold indicate significant difference.

Table 4. Results of the heterogeneity test for yearly differences within each sub-area

Sub-area	Year	N	P	N*	P*
7W	1996	31	0.3603	30	0.3929
	1998	25		25	
	1999	50		48	
	2000	24		21	
	2001	43		42	
	2002	110		99	
	2003	67		60	
	2004	75		68	
	2005	151		123	
	2006	131		112	
	2007	186		163	
8W	1998	44	0.1807		
	2003	21			
	2006	10			
8E	1996	15	0.3676		
	1997	30			
	2001	21			
	2003	16			
	2006	28			
	2007	13			
9W	1995	78	0.3702		
	1997	19			
	2000	16			
	2001	29			
	2002	32			
	2003	24			
	2004	42			
	2005	19			
	2006	23			
9E	1994	14	0.4097		
	1995	22			
	1997	48			
	2003	14			
	2004	42			
	2005	30			

*suspected J stock individuals were excluded from the 7W sample.

Table 5.Results of the statistical comparison between 9W and 9E by year and total samples.

	Sample size		
	9W	9E	P
1994	7	14	0.5425
1995	78	22	0.1031
1997	19	48	0.8581
2003	25	14	0.0627
2004	42	42	0.3468
2005	19	30	0.4563
Total	292*	175*	0.4194

*:including 2000, 2001 2002, 2006 and 2007 samples

Table 6. Results of the heterogeneity tests for the baseline C.

Combination of samples	P
7E x 8W x 8E	0.3661
9W x 9E	0.4194
7W x 7E-8E x 9W-9E	0.1930
7W x 7E-8E x 9W-9E*	0.8904

*suspected J stock individuals were excluded from the 7W sample.

Table 7.Results of the heterogeneity tests for the baseline D.

Combination of samples	P
7E x 8W x 8E x 9W	0.2342
7W x 9E	0.3568
7W x 9E*	0.5616
7W x 7E-9W x 9E	0.1648
7W x 7E-9W x 9E*	0.7805

*suspected J stock individuals were excluded from the 7W sample.

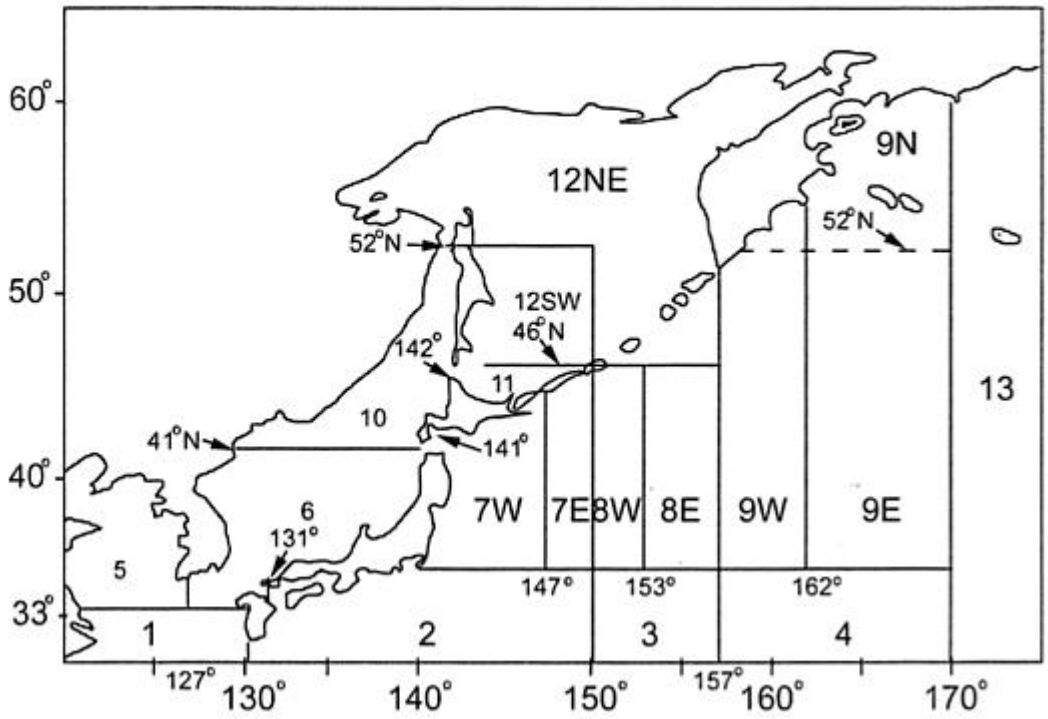

Fig. 1. The 18 sub-areas used for the Implementation Simulation Trials for North Pacific minke whales.

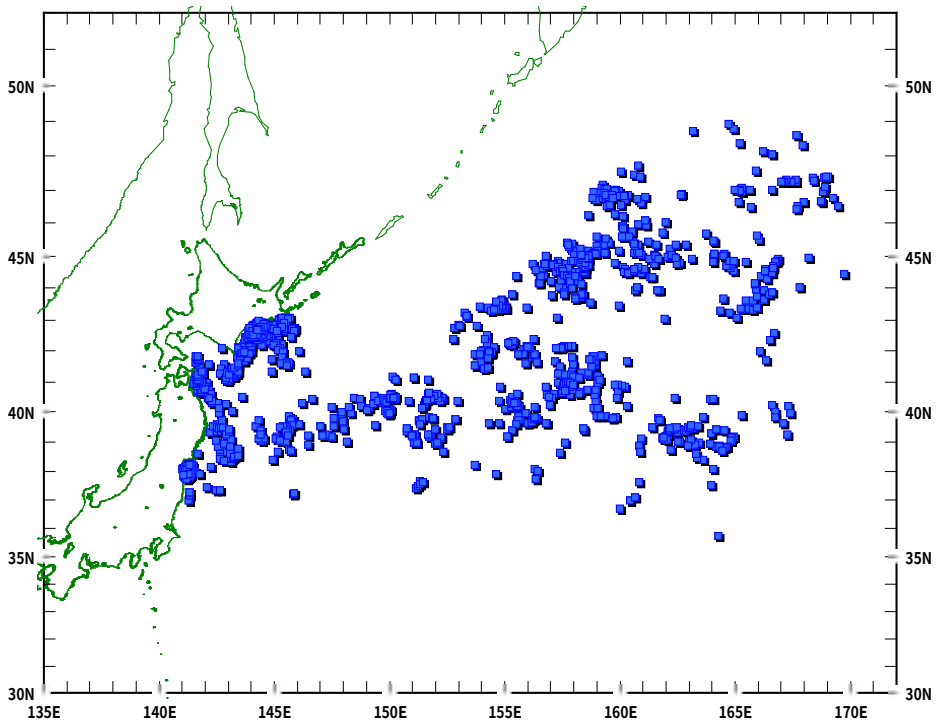

Fig.2. Geographic distribution of sighting position of common minke whale used in this study taken by JARPN and JARPNII surveys during 1994-2007.