Genetic and non-genetic analyses of North Pacific common minke whales under newly defined sub-areas

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ABSTRACT

During the preparatory meeting for the first intersessional workshop of the western North Pacific common minke whale *Implementation Review* held at Tokyo in September, 2010, new sub-areas were defined for the management of minke whales in this area, and three hypotheses for the stock structure of minke whales were proposed. Hypotheses I and II listed two stocks, J and O stocks, around Japan, while Hypothesis III listed four stocks, JW, JE, OW, and OE stocks, around Japan, other than Y stock in the Yellow Sea (Hypotheses II and III). We analyzed genetic as well as non-genetic data obtained from the North Pacific common minke whales collected around Japan in order to describe their stock structure under newly defined sub-areas. The analyzed minke whales were collected during the offshore component of JARPN and JARPNII from 1994 to 2007, during the coastal component of JARPNII from 2002 to 2007, and from bycatches in the set net fishery along the Japanese coast from 2001 to 2007. The genetic data was obtained from analyzing genetic variation at 16 microsatellite loci. Prior to the comparisons among the minke whale samples from different sub-areas, two different kinds of stock identification procedure were used to distinguish the individual whales into the J and O stocks: Bayesian clustering method for the microsatellite genotypic data (Kanda *et al.*, 2009) and mitochondrial DNA haplogroups (AG, AA, GA, GG) based on the sequence variation at the control region of mtDNA (Baker *et al.*, 2000). Under the microsatellite stock identification, no evidence of genetic heterogeneity was observed between the J stock samples from Sea of Japan and the Pacific coast of Japan (1E, 6E, 10E, 2C, 7CS, and 7CN) and among the O stock samples from Pacific side of Japan (2C, 7CS, 7CN, 7W, 7E, 8, and 9). In contrast, under the mtDNA haplogroup identification, evidence of heterogeneity was observed between the samples from Sea of Japan and the Pacific coast of western Japan (6E and 2C) and from the Pacific coast of eastern Japan (7CS and 7CN). The most likely explanation for the observed genetic heterogeneity was, however, due to the incomplete stock ID of the both methods. Under the both identification methods, no heterogeneity was detected between the samples from Sea of Japan and from the Pacific side of western Japan in the J stock (no JW and JE) and between the samples from the coastal and from offshore areas of Pacific Ocean in the O stock (no OW and OE). Therefore, these results were inconsistent with the hypothesis III. Analysis of the non-genetic data, such as fluke color pattern and flipper color pattern, showed the same pattern to that observed from the genetic study. Our results supported the hypotheses I and II.

INTRODUCTION

During the preparatory meeting for the first intersessional workshop of the western North Pacific common minke whale *Implementation Review* held at Tokyo in September, 2010, new sub-areas were defined for the management of minke whales in this area, and three hypotheses for the stock structure of minke whales were proposed (IWC, in prep). Around Japan, the hypotheses I and II listed two stocks, J and O; the J stock mainly distributes in Sea of Japan and the Pacific coast of western Japan, and the O stock in the Pacific coast of eastern Japan to the offshore area of western North Pacific. In contrast, the hypothesis III listed four stocks in the same area, JW, JE, OW, and OE; the JW stock mainly distributes in Sea of Japan, the JE stock along the Pacific side of Japan, the OW stock in the coastal area of the western North Pacific Ocean, and the OE stock in the offshore are of the western North Pacific Ocean.

In order to describe the plausibility of these three hypotheses for the *Implementation Review* of western North Pacific common minke whales, one of the issues to be solved is the stock structure of minke whales in sub-areas 7CS and 7CN: whether the whales are the member of the single stock or are the members of the two stocks mixing at the area. Kanda *et al.* (2010b) analyzed genetic and non-genetic data obtained from the samples of minke whales collected from the areas to demonstrate that minke whales in sub-areas 7CS and 7CN (formerly 7W) belonged to the two different stocks, i.e., J and O stocks. Completion for *Implementation Review*, however, requires resolving the offshore stock structure. The purpose of this paper was thus to conduct analyses of genetic (microsatellites) and non-genetic (fluke color pattern and flipper color pattern) data obtained from the samples from coastal (both sides of Japan) as well as offshore (western North Pacific Ocean) areas to describe the stock structure of minke whales with respect to the consistency to the three hypotheses proposed.

MATERIALS AND METHODS

Samples of minke whales used were those collected during JARPN/JARPNII offshore surveys from 1994 to 2007 and JARPNII coastal surveys (Sanriku and Kushiro) from 2002 to 2007. Minke whales that were bycaught on set net fishery conducted along the Japanese coast from 2001 to 2007 were also used (bycatches). Details of these samples can be found in Kanda *et al.* (2009a) and references therein.

Two different approaches were used to identify stock origins of individual whales in the samples. Firstly, the Bayesian clustering approach was conducted with the genetic data at the 16 microsatellite loci obtained from the total samples of minke whales around Japan (N=2542) using the STRUCTURE version 2.0 (Pritchard *et al.*, 2000) to determine the most likely number of genetically distinct stocks present in our samples and to assign each of the individuals to the inferred stocks (Kanda *et al.*, 2009a). Membership probability of over 90 % was used as a criterion for individual assignment. Details of the analysis can be found in Kanda *et al.* (2009a). Appearance of the individuals identified as neither the J stock nor O stock (9% of the total samples) was mostly due to low resolution power of the markers because the 16 microsatellite markers we used were very informative, but not diagnostic to the stocks (Kanda *et al.*, 2010a; see also Kanda *et al.*, 2010b). Secondly, mitochondrial DNA (mtDNA) haplogroups was used to separate the individuals into the four genetic groups, namely AG, AA, GA, and GG, based on mtDNA sequences at two specific positions at control region as described in Baker *et al.* (2000). Then, the samples were compared using the microsatellite data. Although the mtDNA haplogroup stock identification is not diagnostic, it was indicated that the AG haplogoup was the O stock and the AA, GA, and GG haplogroups were the J stock. Details of the mtDNA analysis are described in Park *et al.* (2010). Sample sizes are shown in Table 1 by new subareas, haplogroup ID, and microsatellite ID.

Conventional hypothesis testing procedure using heterogeneity test in frequencies of the microsatellite alleles among samples was performed in the GENEPOP 4.0 (Rousset, 2008). Statistical significance was determined using the chi-square value obtained from summing the negative logarithm of p-values over the 16 microsatellite loci (Sokal and Rohlf, 1995). F_{ST} was also calculated using the software FSTAT 2.9.3 (Goudet, 1995). The samples with less than 5 individuals were excluded from the genetic divergence analyses.

Genealogy of mtDNA haplotypes was inferred using the maximum likelihood method employed in the DNAML program of PHYLIP (Felsenstein 1993) with 10 multiple jumbles after 500 bootstrap replications. The tree was rooted using a homologous sequence from a Antarctic minke whale (*B.bonaerensis*). The obtained tree was visualized using TreeView PPC (Page 1996).

We again looked for heterogeneity in flipper color and fluke color patterns in the minke whales samples as described in Kanda *et al.* (2010b). Kato *et al.* (1992) first reported that flipper coloration was a valuable trait for the stock identity of North Pacific common minke whales. Nagatsuka (2010) then found that size of the white band of the flipper was different between the individuals assigned as the J and O stocks sampled from 2007 JARPNII coastal and offshore samples. The size of the white band was calculated as the size of the white band over the total size of the flipper (%). Furthermore, Nagatuska (2008) showed that minke whales had different black and white patterns on the underside of their flukes, and separated the sampled whales to three different fluke color types (Fig. 1). Nagatsuka (2010) then found the differences in frequencies of these three types between the individuals assigned as J and O stocks sampled during 2007 JARPNII coastal and offshore surveys. In this paper, distribution of these two traits was examined within new sub-areas (7CS, 7SN, 7W, 8, and 9). No sample was available from 7E in the 2007 survey.

RESULTS

Microsatellite identification approach

We first examined heterogeneity among the J stock samples from different sub-areas (1E, 6E, 10E, 2C, 7CS, 7CN) from Sea of Japan side to the Pacific coast of Japan (Table 2). No evidence of heterogeneity was found among the six samples. We then examined heterogeneity among the O stock samples from different sub-areas (2C, 7CS, 7CN, 7W, 7E, 8, and 9) in the coastal and offshore areas of Pacific side of Japan (Table 3). No evidence of heterogeneity was detected among the seven samples. F_{ST} values were very small for the comparisons.

mtDNA haplogroup identification approach

According to Baker *et al.* (2000), we treated the samples belonging to the AG haplogroup as the O stock and those to the AA, GA, and GG haplogroups as the J stock. First, we examined heterogeneity among the J stock samples (AA, GA, and GG haplogroups combined within each) from different sub-areas (1E, 6E, 10E, 2C, 7CS, 7CN) from Sea of Japan side to the Pacific coast of Japan (Table 4), and detected heterogeneity among them. Pair-wise comparisons indicated the heterogeneity was due to the genetic difference between the samples from 6E and from 7CS and 7CS as well as from 2C and from 7CS and 7CN. F_{ST} values were small for each of the comparisons.

Then, we examined heterogeneity among the O stock samples (AG haplogroup) from different sub-areas (2C, 7CS, 7CN, 7W, 7E, 8, and 9) in the coastal and offshore areas of Pacific side of Japan (Table 5). Evidence of heterogeneity was found, and pair-wise comparisons indicated that the heterogeneity was due only to the 2C sample. No evidence of heterogeneity was found among the 7CS, 7CN, 7W, 7E, 8, and 9 samples.

Most likely explanation for genetic heterogeneity detected

We looked for the possible factor for the heterogeneity we found between the 6E and 2C samples and the 7CS and 7CN samples in the mtDNA haplogroup identification. Table 6 shows concordance of mtDNA haplogroups with the microsatellite identification in the samples from sub-areas 6E, 2C, 7CS, and 7CN. In the AG haplogoup that was designated as O stock, proportion of the microsatellite J stock was higher in the 6E and 2C samples than in the 7CS and 7CN. Similarly, in the AA, GA, and GG haplogroups that were designated as J stock, proportion of the microsatellite O stock in the AA haplogroup was higher in the 7CS and 7CN samples than in the 6E and 2C samples. Phylogenetic analysis of the 188 mtDNA we found within the all samples showed that only the GA and GG haplogroups might be useable as stock identification markers (Fig. 2). Although there are a few exceptions, most of the haplotypes in these two haplogroups clustered independently and were rarely found from the individuals collected from sub-areas 8 and 9. We concluded that the genetic heterogeneity we observed was most likely due to the incomplete stock identification and did not indicate the possibility of the third stock other than J and O around Japan.

Size of white band of the flipper and fluke color pattern

The average size of the white band and fluke color was compared between the individuals collected from the different sub-areas in the 2007 surveys in conjunction with the microsatellite stock identification (Table 7). Although both traits appeared to show the difference between J and O stocks, no clear difference was found among the O stock samples from different sub-areas

CONCLUSION

This paper presented the results of the analyses using both genetic and non-genetic data to describe stock structure of North Pacific minke whales under newly defined sub-areas.

During the preparatory meeting for the first intersessional workshop of the western North Pacific common minke whale *Implementation Review* in September, 2010, new sub-areas were defined for the management of minke whales in this area, and three hypotheses for the stock structure of minke whales were proposed. Distribution of stocks in each of the hypotheses was also given under the new sub-areas during the meeting. Around Japan, the hypotheses I and II proposed existence of the two stocks, J and O: the J stock mainly distributes in Sea of Japan and the Pacific side of western Japan (1E, 2C, 6E, 10E), the O stock mainly distribute pacific side of eastern Japan (7W, 7E, 8, and 9), and both stocks mix along the Pacific coast of Japan (2C(occasionally), 7CS, 7CS, and 11). In contrast, the hypothesis III proposed existence of the four stocks, JW, JE, OW, and OE: the JW stock distributes in Sea of Japan (1E, 6E, and

10E), the JE stock distributes along the Pacific side of Japan (2C), the OW stock distributes in the coastal area of the Pacific side of eastern Japan (7CS, 7CN and 7W), the OE stock distributes in the offshore area of the Pacific side of eastern Japan (7W, 7E, 8 and 9), the JE and OW mix in 7CS and 7CN, and the OW and OE mix in 7W.

In the preparatory meeting, Kanda *et al.* (2010b) presented evidence of the heterogeneity in distribution pattern by the distance from coast line, body length, seasonal distribution, fluke color pattern, and flipper color pattern between the individuals assigned as the J and O stocks collected from the Pacific side of eastern Japan (140-147°E; formerly 7W, now 7CN, 7CS, 7W) in order to demonstrate that the area was a mix zone of the two genetically different stocks. A stock can be defined as a group of individuals sharing a common gene pool maintained by random mating. If these two genetically separated groups of the individuals based on the clustering method had belonged to a single stock, it was hard to believe that they also clearly showed such differences by size, pattern of seasonal migration, and pattern of distribution within a relatively narrow area. Evidence of the deviation from the expected Hardy-Weinberg genotypic proportion at the microsatellite loci was also detected in the samples with homozygous excess, supporting the mix of the two stocks.

Because Kanda *et al.* (2010b) restricted the analyses to the samples from 7CS, 7CN, and 7W, this paper attempted to examine the genetic relationships among the samples from other sub-areas with respect to the data consistency to the three hypotheses. For the comparison among sub-areas, we separated the individuals into the two main stocks, J and O, using two different kinds of genetic identification: Bayesian clustering method based on genetic variation at the 16 microsatellite loci (i.e., Kanda *et al.* 2009) and mtDNA haplogroups based on sequence variation at the control region of mtDNA (Baker *et al.* 2000). Genetic heterogeneity was detected between the samples from 6E and 2C and from 7CS and 7CN only under the mtDNA haplogroup identification. The reason for the heterogeneity was because incomplete stock identification did not allow us to fully separate samples to J and O stocks for examining structure within them. Under the both identification methods, no heterogeneity was detected between the samples from Sea of Japan and from the Pacific side of western Japan in the J stock (no JW and JE) and between the samples from the coastal and from offshore areas of Pacific Ocean in the O stock (no OW and OE). Therefore, the results were inconsistent with the hypothesis III.

Detail look of the samples from these areas strongly suggested that the observed heterogeneity resulted from the mixing of the two stocks. The results of this paper in conjunction with those from the past stock structure studies using either genetic or non-genetic data (Hatanaka and Miyashita, 1997; Hakamada, 2009; Hatanaka *et al.* 2010; Kanda *et al.*, 2010b; Pastene *et al.* 2010) indicated existence of only J stock from Sea of Japan to the Pacific side of western Japan (1E, 6E, 10E, and 2C) and of only O stock in the Pacific Ocean (7W, 7E,, 8, and 9) with the two mixing at the coastal area of the Pacific side (7CS and 7CS) to Okhotsk Sea (11), supporting the hypotheses I and II.

ACKNOWLEDGEMENTS

We thank researchers and crewmembers participating in the JARPN and JARPNII surveys for their effort in collecting the samples used in this study.

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Table 1. Sample sizes by sub-areas and by stock identification methods.

	1E	2C	6E	7CS	7CN	7W	7E	8	9	10E	11
Total	22	180	392	437	598	70	48	224	466	9	96
microsatellite J	20	141	366	89	106	1	0	0	2	7	38
microsatellite O	1	24	3	288	438	64	46	200	419	0	49
micro. unidentified	1	15	23	60	54	5	2	24	45	2	9
mtDNA AG (O)	3	34	41	324	461	67	40	210	450	3	58
mtDNA AA (J)	5	22	49	21	34	1	3	8	10	3	5
mtDNA GA (J)	9	66	191	54	63	1	4	3	5	1	19
mtDNA GG (J)	5	58	111	38	39	1	1	2	1	2	14

Table 2. Results (p-values) of the heterogeneity tests and F_{ST} among the J stock samples collected from different sub-areas.

J stock	p value	F_{ST}
1E x 6E x 10E x 2C x 7CS x 7CN	0.594	-0.001 (-0.001, -0.000)

Table 3. Results (p-values) of the heterogeneity tests and F_{ST} among the O stock samples collected from different sub-areas.

O stock	p value	F_{ST}
2C x 7CS x 7CN x 7W x 7E x 8 x 9	0.294	-0.000 (-0.000, 0.001)

Table 4. Results (p-values) of the heterogeneity tests and F_{ST} among the mtDNA haplogroups collected from different sub-areas. J stock is the samples from the AA, GA, and GG haplogroups.

J stock	p value	F_{ST}
1E x 6E x 10E x 2C x 7CS x 7CN	***<0.001***	0.001 (0.000, 0.002)
1E x 6E	0.646	-0.0037
1E x 10E	0.868	-0.0027
1E x 2C	0.858	-0.0064
1E x 7CS	0.939	-0.0051
1E x 7CN	0.331	-0.0010
6E x 10E	0.437	0.0003
6E x 2C	0.388	0.0001
6E x 7CS	***<0.001***	0.0014
6E x 7CN	***<0.001***	0.0028
10E x 2C	0.223	0.0011
10E x 7CS	0.904	-0.0028
10E x 7CN	0.546	-0.0005
2C x 7CS	***<0.001***	0.0011
2C x 7CN	***<0.001***	0.0022
7CS x 7CN	0.060	0.0017

Table 5. Results (p-values) of the heterogeneity tests and F_{ST} among the mtDNA haplogroups collected from different sub-areas. O is the samples from the AG haplogroup.

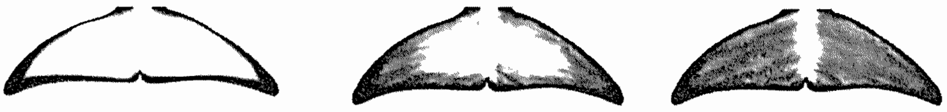
O stock	p value	F_{ST}
2C x 7CS x 7CN x 7W x 7E x 8 x 9	***0.034***	0.000 (-0.000, 0.001)
2C x 7CS	***0.009***	0.0041
2C x 7CN	***0.006***	0.0035
2C x 7W	***0.015***	0.0052
2C x 7E	***0.072***	0.0043
2C x 8	***0.021***	0.0042
2C x 9	***0.006***	0.0046
7CS x 7CN	0.174	0.0005
7CS x 7W	0.105	0.0004
7CS x 7E	0.523	0.0001
7CS x 8	0.219	0.0002
7CS x 9	0.129	0.0004
7CN x 7W	0.099	-0.0002
7CN x 7E	0.734	-0.0007
7CN x 8	0.456	0.0001
7CN x 9	0.308	-0.0002
7W x 7E	0.625	-0.0010
7W x 8	0.574	-0.0003
7W x 9	0.217	-0.0002
7E x 8	0.884	-0.0019
7E x 9	0.589	-0.0005
8 x 9	0.960	-0.0004

Table 6. Concordance of mtDNA haplogroups with the microsatellite identification in the samples from sub-areas 6E、2C, 7CS, and 7CN.

	6E		2C		7CS		7CS	
	J	O	J	O	J	O	J	O
AG (O)	36	2	6	16	7	274	14	406
AA (J)	45	0	20	0	9	9	11	21
GA (J)	178	1	61	0	43	2	45	7
GG (J)	107	4	52	1	30	3	35	4

Table 7. Number of minke whales assigned to J and O stock in each of the sub-areas with respect to size of the white band of the flipper and fluke color pattern.

		Size of flipper white band			Fluke color pattern		
SA	ID	no gray	intermed.	gray	white	intermed.	black
7CS	J	6	2	1	4	5	0
	O	0	4	76	1	25	50
7CN	J	2	6	6	6	6	2
	O	1	8	52	0	29	31
7W	J	0	0	0	0	0	0
	O	0	0	5	0	2	3
8	J	0	0	0	0	0	0
	O	0	1	14	0	1	12
9	J	0	0	0	0	0	0
	O	0	0	5	0	1	0



white fluke
(0-30% black)

intermediate fluke
(31-90% black)

black fluke
(91-100% black)

Fig. 1. Diagram of the fluke color pattern.

Fig. 2. Phylogenetic relationship among 118 mtDNA haplotypes found in the minke whale samples. Antarctic minke whale (SHminke) was used as an outgroup. MI001GAOJ indicates haplotype 1 belonging GA haplogroup. Last two letters indicate geographic information: OJ haplotypes were found from both Sea of Japan and offshore Pacific Ocean, JJ from Sea of Japan but not from offshore Pacific Ocean (SA8 and 9), OO from offshore Pacific Ocean but not from Sea of Japan, and xx only from the coastal area (2C, 7CS and 7CN). Other haplogroups are AG, AA, GG.