Stock identity of sei whales in the central North Pacific based on microsatellite analysis of biopsy samples obtained from IWC/Japan joint cetacean sighting survey in 2010

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ABSTRACT
Genetic variations at 16 microsatellite loci were analyzed for the central North Pacific sei whales (13 biopsies) obtained during the 1st annual IWC/Japan joint cetacean sighting survey cruise conducted at the area between 173°E -172°W in 2010. All of the loci analyzed were polymorphic with substantial level of genetic diversity in the sample. Genetic differences between this sample and the past JARPNII samples (N=489) obtained in the western North Pacific at the area between 143°E and 170°E from 2002 to 2007 as well as commercial whaling sample (N=64) obtained in the eastern North Pacific at the area between 150°W and 139°W in 1973 were examined. Due to the condition of the commercial whaling sample, the genetic data of 14 of the 16 loci were used for the comparisons among the three samples. No evidence of genetic differences was detected between the IWC/Japan survey and JARPNII samples as well as among the three samples. This study supported our previous view that the open water of the North Pacific was occupied by the individuals from a single stock of sei whales.

KEYWORDS: SEI WHALE, MICROSATELLITE, STOCK STRUCTURE, IWC/JAPAN JOINT CETACEAN SIGHTING SURVEY, JARPNII, COMMERCIAL WHALING, NORTH PACIFIC

INTRODUCTION

The IWC/SC decided to conduct 'in-depth assessment' of sei whales, *Balaenoptera borealis*, to investigate the current status of stocks in the North Pacific (e.g., IWC, 2010). Among all of the required information, understanding of stock structure in the region is essential for successful in-depth assessment. Due to the limited information on the North Pacific sei whales in the past, no conclusive evidence has been presented for their stock structure (see Donovan, 1991). Recently, using microsatellite as well as mitochondrial DNA markers, Kanda *et al.* (2009) analyzed samples collected during the JARPNII surveys from 2002 to 2007 (143°E to 170°E) and during past commercial whaling operated in 1972 and 1973 (165°E to 139°W), and indicated that the open water of the North Pacific was mainly occupied by the individuals from a single stock of sei whales because no evidence of genetic differences was found among the samples. In this paper, we analyzed sample of the central North Pacific sei whales collected during the 1st annual IWC/Japan joint cetacean sighting survey cruise in 2010 from the area between 173°E -172°W (Matsuoka *et al*., 2011a) and compared to the samples in Kanda *et al.* (2009) to better understand the stock structure of the species in this region.

MATERIALS AND METHODS

Samples

Thirteen biopsies were obtained during the 1st annual IWC/Japan joint cetacean sighting survey cruise conducted at the area between 173°E and 172°W in the summer of 2010 (Matsuoka *et al*., 2011). Genetic data of the sei whales in the 2002 - 2007JARPNII survey samples obtained at the area between 143°E and 170°E and the 1973 commercial whaling sample obtained at the area between 150°W and 139°W were also used in this study for comparison (for details see Kanda *et al*., 2009). Table 1 summarizes collection information of the samples.

Microsatellite analysis

Total DNA from each of the whales was extracted from 0.05 g of skin tissue stored in ethanol using GENTRA PUREGENE DNA extraction kit (QIAGEN). Extracted DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Genetic variation at microsatellite loci were analyzed using 16 sets of primers, none of which was designed specifically from sei whales: EV1, EV21, EV94, EV104 (Valsecchi and Amos, 1996), GT011 (Bérubé *et al*., 1998), GT23, GT211, GT271, GT310, GT575 (Bérubé *et al*., 2000), GATA28, GATA53, GATA98, GATA417, GGAA520 (Palsbøll *et al*., 1997), and DlrFCB17 (Buchanan *et al*., 1996). Primer sequences and PCR cycling profiles generally followed those of the original authors. PCR amplifications were performed in 15μl reaction mixtures containing 10-100ng of DNA, 5 pmole of each primer, 0.625 units of Ex Taq DNA polymerase (Takara Shuzo), and 2mM of each dNTP, and 10x reaction buffer containing 20mM $MgCl_2$ (Takara Shuzo). Amplified products with internal size standard (GENESCAN400HD, Applied Biosystems Japan) were run on a 6% polyacrylamide denaturating gel (Long Ranger) using BaseStation100 DNA fragment analyzer (Bio-Rad). Allelic sizes were determined manually in relation to the internal size standard and sei whale's DNA of known size that were rerun on each gel. In regard to our sample/data handling under DNA data quality control, please see Kanda *et al*. (2010).

Data analysis

In regard to our DNA data quality control under the IWC guidelines, see Kanda *et al.* (2010). The computer program 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). When simultaneous multiple tests were conducted, Rice (1989) correction for the multiple tests was performed.

Conventional hypothesis testing procedure was conducted using heterogeneity test in microsatellite allele frequencies among samples. Our null hypothesis to be tested is if the samples came from a genetically same group of sei whales. If a statistically significant allele frequencies differences exist, then it could indicate these samples came from genetically different stocks of sei whales. Probability test (or Fisher's exact test) implemented in GENEPOP 4.0 (Rousset, 2008) was used to conduct the heterogeneity tests. When simultaneous multiple tests were conducted, Rice (1989) correction for the multiple tests was performed.

RESULTS AND DISCUSSION

All 16 microsatellite loci were successfully amplified and were polymorphic in the IWC/Japan joint cetacean sighting survey cruise samples (Table 2). The sample showed the level of genetic diversity similar to the past JARPNII and commercial whaling samples (Table 2; Kanda *et al*., 2009). None of the 16 loci in the sample showed significant deviation from the expected Hardy-Weinberg genotypic proportions after correction for the simultaneous multiple tests (data no shown).

No evidence of genetic differences was found between the IWC/Japan joint survey and the past JARPNII samples at each of the 16 loci after correction for the simultaneous multiple tests as well as all the loci combined (Table 3). F_{ST} was 0.007 between them and it was not significantly different from zero. We then examined the genetic differences between these samples and the 1973 commercial whaling one using the genetic data obtained from 14 of the 16 loci (Table 3). These samples thus covered the wide area in the North Pacific from 35°N -50°N/143°E - 139°W (Fig. 1). No evidence of genetic differences was again detected among the three samples at each of the loci as well as all the loci combined. F_{ST} was almost 0 among the three samples and it was not significantly different from zero. As similar to the past JARPNII samples (Kanda *et al*. 2009), the most recent 2010 sample showed no evidence of genetic differences to the sample from the 150°W-139°W area. The results support our previous conclusion that the offshore open water of the North Pacific was occupied mainly by the individuals of the single stock of sei whales.

Statistical power of the present tests might be low due to the small sample size of the IWC/Japan joint survey sample. That should not discredit the significance of this paper, however. This paper is still very informative because of the area covered. It was the first time to obtain the sample of sei whales in the central North Pacific from 173°E to 172°W after the end of commercial whaling era.

According to its future plan, the IWC/Japan joint cetacean sighting survey cruise will cover further eastern area comparable to a principal part of the past commercial whaling grounds (Kato *et al*., 2011). Additions of samples from the future IWC/Japan joint surveys as well as future JARPNII ones will thus not only increase the statistical power of our analyses but further extend area covered. The currently planned in-depth assessment of this species will not be successfully conducted without considering new information obtained from the future surveys.

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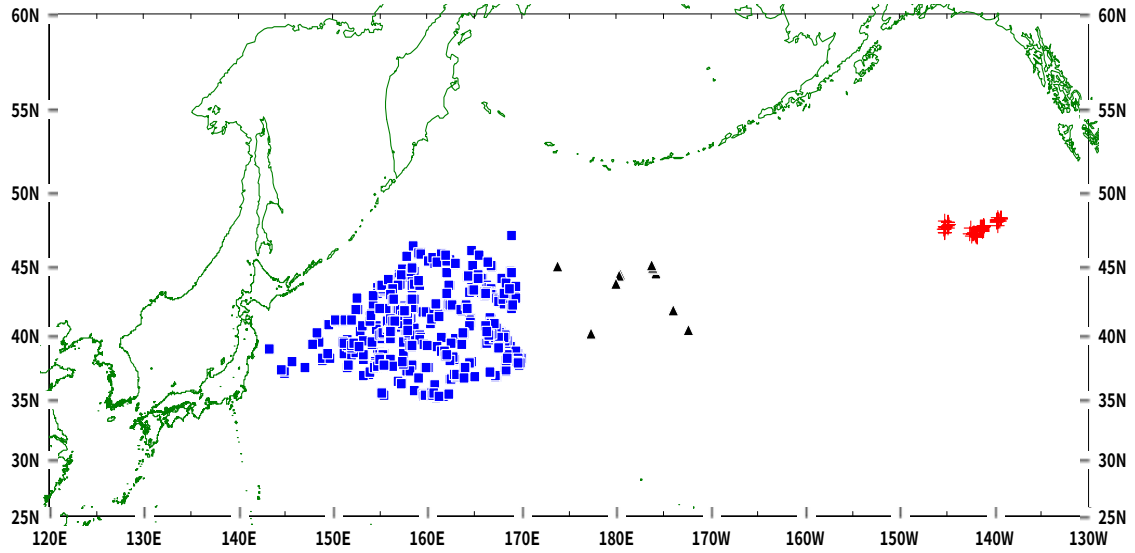

Fig. 1. Sampling locations of sei whales in the North Pacific.
■:JARPNII, ▲: IWC/Japan joint cetacean sighting survey cruise, +: commercial whaling.

Table 1. Collection information of the samples obtained from JARPNII, 1st annual IWC/Japan joint cetacean sighting survey cruise, and past commercial whaling. N=sample size.

Source	Year	Survey period	N	Latitude	Longitude
IWC/Japan	2010	July-August	13	40°N-46°N	173°E -172°W
JARPNII	2002-2007	June - August	489	35°N -48°N	143°E - 170°E
Commercial	1973	May - August	64	47°N -50°N	150°W -139°W

Table 2. The number of alleles (A) and expected heterozygosity (He) at the microsatellite loci in the JARPNII, 1st annual IWC/Japan joint cetacean sighting survey cruise, and commercial whaling samples of sei whales. n.s. = not significant, n.a.= not available.

	IWC/Japan		JARPNII		Commercial	
Locus	A	He	A	He	A	He
EV21	5	0.712	6	0.621	6	0.616
GGAA520	7	0.856	10	0.797	8	0.792
GATA98	6	0.763	7	0.733	n.a.	n.a.
GT211	2	0.269	6	0.312	3	0.305
GATA53	3	0.535	3	0.48	3	0.520
EV1	10	0.859	15	0.834	15	0.847
EV94	5	0.724	6	0.684	5	0.696
GT23	6	0.353	12	0.604	8	0.564
GT575	4	0.587	5	0.592	4	0.572
GATA417	5	0.715	8	0.774	n.a.	n.a.
GT310	3	0.429	4	0.511	4	0.485
EV104	5	0.692	7	0.724	6	0.700
GATA28	8	0.859	11	0.81	9	0.832
GT271	3	0.282	4	0.133	2	0.104
GT011	3	0.596	4	0.441	3	0.415
DlrFCB17	9	0.894	18	0.872	16	0.885
Average*	5.3	0.633	7.9	0.620	6.6	0.595

*16 loci for JARPNII and IWC/Japan, 14 loci for Commercial.

Table 3. Results (p-values) of the heterogeneity tests and F_{ST} among the JARPNII, 1st annual IWC/Japan joint cetacean sighting survey cruise, and commercial whaling samples of sei whales.

Locus	JARPNII x IWC/Japan	JARPNII x IWC/Japan x Commercial
EV21	0.633	0.847
GGAA520	0.117	0.357
GATA98	0.860	n.a.
GT211	0.483	0.505
GATA53	0.826	0.920
EV1	0.250	0.419
EV94	0.244	0.279
GT23	0.154	0.481
GT575	0.758	0.664
GATA417	0.320	n.a.
GT310	0.545	0.247
EV104	0.863	0.469
GATA28	0.652	0.496
GT271	0.327	0.174
GT011	0.249	0.333
DlrFCB17	0.444	0.548
All	0.644	0.727
F_{ST}	0.007	0.000