Microsatellite DNA analysis of sei whales obtained from the 2010-2012 IWC-POWER

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ABSTRACT
Genetic variations at 14 microsatellite loci were examined in the North Pacific sei whales' biopsy samples obtained from the IWC-POWER (the International Whaling Commission/Pacific Ocean Whale and Ecosystem Research) cruises that surveyed 173°E - 172°W area of the central North Pacific in 2010 (N=13), 170°W - 150°W area of the central North Pacific in 2011 (N=29), and 150°W - 135°W area of the eastern North Pacific in 2012 (N=35). All of the areas were north of 40°N. The observed POWER data was then analyzed with previously reported genetic data of the JARPNII samples (N=489) collected from the western North Pacific between 143°E and 170°E in 2002-2007 and the commercial whaling samples collected from the central North Pacific between 180° and 150°W in 1972-1973 (N=57) and from the eastern North Pacific between 150°W and 139°W in 1973 (N=64). Analyses of these samples allowed us to detect temporal (40 years apart) and spatial (143°E to 135°W area divided into western, central, and eastern) genetic differences of the North Pacific sei whales. Our results showed 1) very similar level of genetic diversities among the POWER, JARPNII and commercial whaling samples, 2) no evidence of the genetic differences among the three POWER samples, 3) no evidence of the temporal genetic differences between the recent POWER and past commercial whaling samples collected from the same area, 4) no evidence of the spatial genetic differences among the western, central, and eastern samples. This study supports our previous view that the open waters of the North Pacific were occupied by the individuals from a single stock of sei whales.

KEYWORDS: SEI WHALE, MICROSATELLITE, STOCK STRUCTURE, POWER, JARPNII, COMMERCIAL WHALING, NORTH PACIFIC

INTRODUCTION

The IWC/SC starts 'in-depth assessment' of sei whales, *Balaenoptera borealis*, from 65SC to investigate the current status of stocks of this species 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, Kanda *et al.* (2009) analyzed samples from the 2002-2007 JARPNII (143°E to 170°E) and 1972-1973 commercial whaling (165°E to 139°W) using microsatellite as well as mitochondrial DNA markers, 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.

Recently, using microsatellite DNA markers, we analyzed samples of the North Pacific sei whales collected from the IWC-POWER (the International Whaling Commission/Pacific Ocean Whale and Ecosystem Research) surveyed the area between 173°E -172°W in 2010, between 170°W -150°W in 2011, and between 150°W -135°W in 2012(Matsuoka *et al*., 2011; 2012; 2013). In this paper, we compared the data from the POWER samples to those from the JARPNII and commercial whaling samples in Kanda *et al.* (2009) to investigate temporal and spatial genetic differences among the North Pacific sei whales.

MATERIALS AND METHODS

Samples

DNA were extracted from the biopsy samples obtained from the IWC-POWER (the International Whaling Commission/Pacific Ocean Whale and Ecosystem Research) cruises that surveyed the area north of 40°N from 173°E to 172°W in 2010 (10POWER; N=13), from 170°W to 150°W in 2011 (11POWER; N=29), and from 150°W to 135°W in 2012 (12POWER; N=35) (Matsuoka *et al*., 2011, 2012, 2013). Genetic data of the sei whales from the JARPNII and commercial whaling samples were used to test whether or not there was any evidence of genetic differences among the whales from the North Pacific from 143°E to 139°W. The JARPNII sample (N=489) was obtained at the area between 143°E and 170°E from 2002 to 2007. The commercial whaling samples were obtained at the central area between 180° and 150°W in 1972/73 (CWh-central; N=57) and eastern area between 150°W and 139°W in 1973 (CWh-eastern; N=64) between 150°W and 139°W. For details of these reference samples, please see Kanda *et al*., 2009. Table 1 summarizes collection information of the samples and Fig.1 shows the individual positions.

Table 1. Collection information of the POWER, JARPNII, past commercial whaling samples used for the current data analyses. N=sample size.

Source	Year	Survey period	N	Latitude	Longitude
POWER					
10POWER	2010	July-August	13	40°N-46°N	173°E -172°W
11POWER	2011	August	29	40°N-50°N	170°W -150°W
12POWER	2012	August	35	40°N-53°N	150°W -135°W
JARPNII	2002-2007	June - August	489	35°N -48°N	143°E - 170°E
Commercial					
CWh-central	1972-1973	May - August	57	40°N -50°N	180° -150°W
CWh-eastern	1973	May - August	64	47°N -50°N	150°W -139°W

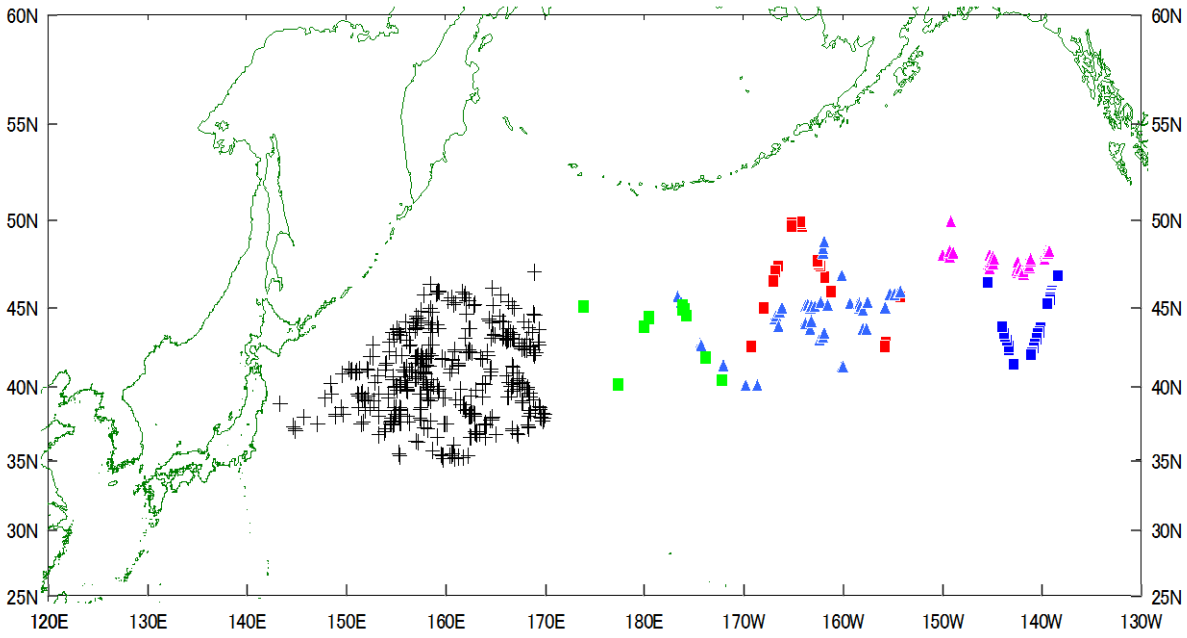

Fig. 1. Sampling locations of sei whales in the North Pacific.
■: 2010POWER, ■: 2011POWER, ■: 2012POWER, ▲: Commercial whaling (CWh-central), ▲: Commercial whaling (CWh-eastern), +: JARPNII (Western).

Microsatellite analysis

Total DNA from each of the whales in the POWER samples 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 14 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, GGAA520 (Palsbøll *et al*., 1997), and DlrFCB17 (Buchanan *et al*., 1996). In regard to PCR amplifications, microsatellite electrophoresis, and sample/data handling, please see Kanda *et al*. (2009).

Data analysis

In regard to our DNA data quality control under the IWC guidelines, see Kanda *et al.* (2010). The number of alleles per locus, allelic richness, 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 whether or not the samples came from a genetically same group of sei whales. If statistically significant allele frequency differences exist, 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. F_{ST} value was calculated using FSTAT 2.9.3 (Goudet, 1995).

RESULTS AND DISCUSSION

No case of matchings between the individuals from the different years' POWER samples was observed.

All of the 14 microsatellite loci were polymorphic in the POWER samples, and the level of the genetic diversities (the number of alleles, allelic richness, and expected heterozygosity) of the POWER samples was similar to that of the JARPNII and commercial whaling samples (Table 2). Therefore, no temporal and spatial difference in the genetic diversity was observed among the POWER, JARPNII, and commercial whaling samples.

Table 2. The number of alleles (A), allelic richness, and expected heterozygosity (He) at the 14 microsatellite loci in the POWER, JARPNII, and commercial whaling (CWh) samples of sei whales.

Locus	POWER			JARPNII			CWh		
	A	AR	He	A	AR	He	A	AR	He
EV21	6	6.0	0.675	6	5.7	0.621	6	5.6	0.637
GGAA520	8	8.0	0.792	10	8.6	0.797	9	8.8	0.794
GT211	3	3.0	0.343	6	4.7	0.312	4	3.6	0.306
GATA53	3	3.0	0.520	3	3.0	0.480	3	3.0	0.496
EV1	15	15.0	0.839	15	13.8	0.834	17	15.7	0.838
EV94	6	6.0	0.717	6	6.0	0.684	6	5.9	0.687
GT23	10	10.0	0.587	12	9.0	0.604	10	9.2	0.608
GT575	5	5.0	0.585	5	4.5	0.592	5	4.7	0.579
GT310	3	3.0	0.480	4	3.3	0.511	4	3.6	0.473
EV104	6	6.0	0.700	7	5.7	0.724	7	6.3	0.707
GATA28	9	9.0	0.828	11	9.1	0.810	10	9.6	0.819
GT271	3	3.0	0.147	4	3.1	0.133	3	3.0	0.118
GT011	4	4.0	0.491	4	3.9	0.441	3	3.0	0.434
DlrFCB17	16	16.0	0.871	18	15.6	0.872	17	15.9	0.890
Average	6.9	6.9	0.613	7.9	6.9	0.601	7.4	7.0	0.599

No evidence of the genetic differences was found at each of the 14 loci, as well as all the loci combined, among the three POWER samples (Table 3). F_{ST} was 0.002 among them and it was not significantly different from zero. These three POWER samples were combined into one for the

comparison with the commercial whaling samples (CWh-central and CWh-eastern combined), that were collected from the similar area to the POWER samples in 40 years ago, to see if there were any temporal genetic differences. No evidence of the genetic differences was detected at each of the 14 loci, as well as all the loci combined, between the POWER and commercial whaling samples (Table 3). F_{ST} was negative between the two and it was not significantly different from zero. The result indicated that these old and new samples collected after the elapse of 40 years were genetically similar to each other.

Table 3. Results (p-values) of the heterogeneity tests and F_{ST} among the three POWER samples and between the three POWER and two commercial whaling (CWh) samples.

Locus	10POWER x 11POWER x 12POWER	POWER x CWh
EV21	0.632	0.775
GGAA520	0.245	0.231
GT211	0.472	0.347
GATA53	0.928	0.509
EV1	0.065	0.887
EV94	0.955	0.331
GT23	0.179	0.776
GT575	0.314	0.757
GT310	0.415	0.942
EV104	0.916	0.223
GATA28	0.897	0.359
GT271	0.337	0.844
GT011	0.252	0.270
DlrFCB17	0.607	0.560
All loci	0.641	0.875
F_{ST}	0.002	negative

In order to examine genetic differences among samples by areas, each of the POWER and commercial whaling samples were again treated separately as central (10POWER, 11POWER, and CWh-central from 180° - 150°W) and eastern (12POWER and CWh-eastern from 150°W -135°W) samples. No evidence of the genetic differences was detected at each of the 14 loci, as well as all the loci combined, among the three central samples (Table 4). F_{ST} was 0.001 among them and it was not significantly different from zero. These samples were combined into one as a single central sample for the further analysis. Similarly, no evidence of the genetic differences was detected at each of the 14 loci, as well as all the loci combined, between the two eastern samples (Table 4). F_{ST} was negative between the two and it was not significantly different from zero. These samples were combined into one as a single eastern sample for the further analysis.

No evidence of genetic differences was detected at each of the 14 loci, as well as all the loci combined, among the western, central and eastern samples (Table 4). F_{ST} was almost 0 among the three samples and it was not significantly different from zero. When all of the samples were combined into one, none of the 14 loci showed significant deviation from the expected Hardy-Weinberg genotypic proportions after correction for the simultaneous multiple tests (data not shown). This result indicated that the individuals utilizing the North Pacific from west end to east end (143°E to 139°W) as their feeding ground belonged to the genetically same group of sei whales. These results support our previous conclusion that the offshore open waters of the North Pacific are occupied by the individuals of the single stock of sei whales.

Table 4. Results (p-values) of the heterogeneity tests and F_{ST} among the POWER (10-12POWER), JARPNII (Western), and commercial whaling (CWh-central, eastern) samples by areas.

Locus	Central North Pacific	Eastern North Pacific	North Pacific
	10POWER x 11POWER x CWh-central	12POWER x CWh-eastern	Western x Central x Eastern
EV21	0.585	0.965	0.178
GGAA520	0.161	0.808	0.723
GT211	0.396	0.531	0.558
GATA53	0.887	0.643	0.741
EV1	0.317	0.646	0.434
EV94	0.833	0.392	0.652
GT23	0.535	0.392	0.496
GT575	0.878	0.806	0.088
GT310	0.537	0.819	0.197
EV104	0.630	0.662	0.240
GATA28	0.926	0.195	0.621
GT271	0.761	1.000	0.084
GT011	0.469	0.125	0.588
DlrFCB17	0.230	0.946	0.586
All loci	0.918	0.959	0.424
F_{ST}	0.001	negative	<0.001

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