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Genetic study on JARPNII and IWC-POWER samples of sei whales collected widely from the North Pacific at the same time of the year

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ABSTRACT

We used microsatellite DNA markers to analyze samples of sei whales collected widely from the North Pacific at the same time of the year in order to test spatial genetic heterogeneity in this ocean basin. Although we have been reporting results of the genetic studies on the North Pacific sei whales to previous IWC/SC meetings, this study is the first to utilize temporally similar (collected at the same year), yet geographically very different, samples (covered west-end to east-end of the North Pacific). This study used samples collected from the northwestern (JARPNII), northcentral (POWER), and northeastern (POWER) areas of the North Pacific in the same summer seasons in 2010, 2011 and 2012. No evidences of significant genetic differences between the samples from JARPNII and POWER in each of the three years, were found. Each yearly sample was then combined as JARPNII as well as POWER samples, respectively. No significant genetic differences were detected between these two samples. We used genotypic profiles of each whale in the POWER biopsy samples to find any cases of matching to the individuals in the JARPNII samples, no matching was found at all. In conclusion, this study failed to demonstrate evidence of multiple stocks of sei whales in the North Pacific.

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

INTRODUCTION

The most comprehensive studies conducted so far with regard to the stock structure of the North Pacific sei whales were those presented at the previous JARPNII Review workshop in 2009 (Kanda *et al*. 2009) as well as presented at the recent IWC/SC (Kanda *et al*. 2013). These studies, using multiple sets of microsatellite DNA loci markers, examined the sei whales samples collected from almost the entire range of North Pacific.

Kanda *et al*. (2009), on the one hand, analyzed genetic variation at 17 microsatellite DNA loci and 487 bp of mitochondrial DNA (mtDNA) control region sequences in the JARPNII samples (N=489) from 2002 to 2007 in the area between 143°E and 170°E as well as in the commercial whaling samples (N=301) from 1972 and 1973 conducted in the area between 165°E and 139°W. The results indicated no evidence of genetic differences within as well as between the JARPNII and commercial whaling samples. Both females and males showed same pattern of the stock structure. Sequencing and phylogenetic analysis of the mtDNA control region also showed no evidence of the genetic heterogeneity in the JARPNII samples as well as no spatially or temporally unique phylogenetic clusters.

Kanda *et al*. (2013), on the other hand, examined genetic variations at 14 microsatellite DNA loci in the North Pacific sei whales' biopsy samples obtained from the IWC-POWER (the International Whaling Commission/Pacific Ocean Whale and Ecosystem Research; hereafter POWER) 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), and these obtained data was analyzed with those in Kanda *et al*. (2009). This study allowed the authors to detect temporal (40 years apart between the POWER and commercial whaling data) and spatial (143°E to 135°W area divided into western, central and eastern) genetic differences of the North Pacific sei whales. Similarly to Kanda *et al*. (2009), the results showed no evidence of the temporal genetic differences between the recent POWER and past commercial whaling samples collected from the same area and no evidence of the spatial genetic differences among the western, central and eastern samples.

One drawback to these two studies was that there was no direct comparison among samples collected at the same time of the year from the different areas over the North Pacific. Considering that sei whales conduct seasonal migration from their breeding ground to feeding ground every year, development of stock structure hypothesis

should test the genetic differentiation in the samples collected in the same year that eliminate temporal negative biases. If no genetic difference is found, it should strongly indicate no evidence of multiple stocks in the area. This study thus looked at genetic variation at the microsatellite DNA loci to analyze the JARPNII and POWER samples collected from the same time of years in 2010, 2011 and 2012, respectively.

MATERIALS AND METHODS

Samples

JARPNII samples (N=295) of sei whales from 2010 to 2012 were collected from the western North Pacific off Japanese coast (Table 1 and Fig. 1). Although sampling dates and locations of the surveys slightly differed year by year depending on the sampling plan of a given year, it was fixed to the northwestern North Pacific (37°N-42°N, 151°E-169°E). Skin biopsy samples of sei whales were obtained during the POWER surveys. POWER survey covered 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). Table 1 summarizes collection information of the samples and Fig.1 shows the individual positions.

DNA extraction

Total DNA from each of the whales was extracted from 0.05 g of skin tissue in the JARPNII samples and of skin biopsy in the POWER samples 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).

Microsatellite analysis

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). Although alleles were visualized using Cartographer software specifically designed for the BaseStation, 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.

Data analysis

In regard to our DNA data quality control under the IWC guidelines, see Kanda *et al.* (2014). 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).

Matching exercises between the individuals from JARPNII and POWER samples were conducted using a computer program CERVUS (Marshall *et al*., 1998).

RESULTS AND DISCUSSION

The POWER survey changed its research area from the northcentral North Pacific in 2011 to the northeastern North Pacific in 2012 with no overlapping in contrast to the JARPNII surveys which were largely operated at the same northwestern North Pacific area every years, so the comparison between the POWER and JARPNII

samples were geographically different among the three years (Table 1 and Fig.1). The comparison between the samples collected in 2012 was geographically most apart from each other (northwestern vs northeastern).

The sampling dates of the two surveys were also different from each other with an overlap in August (Table 1). Some may argue that this time lag cause duplicate sampling resulting in underestimation of the level of the genetic differences between the samples from the two surveys. Such duplicate samplings were impossible in each of the years because the samples from the early-operated JARPNII surveys were taken lethally. The JARPNII samples may have contained individuals biopsied by the non-lethal POWER surveys in the previous years. Although we attempted to match the whales in these samples using their genotypic profiles, we were not able to find any matching.

All of the 16 microsatellite loci were polymorphic in the POWER and JARPNII samples (Table 2). Although the number of the alleles per locus per samples was different among the samples, other genetic indices such as allelic richness and expected heterozygosity were very similar to each other. The differences in the number of alleles were most likely due to the differences in sample sizes among the samples. Therefore, no spatial difference in the level of the genetic diversity was observed among the POWER and JARPNII samples.

Firstly, we looked at the genetic differences between the JARPNII and POWER samples collected at the same years. No evidence of the genetic differences was found at each of the 16 loci, as well as all the loci combined, between the two in each of the collected years (Table 3). The values of F_{ST} at all three comparisons were not significantly different from zero. These results indicated that, within each year, the individuals in these two samples came from a genetically same group of sei whales.

We thus combined the POWER and JARPNII samples collected at the same years into one as 2010, 2011 and 2012 samples, respectively. Secondly, we tested for the deviation from the expected Hardy-Weinberg genotypic distributions within each of the three samples (Table 4). None of the 16 microsatellite DNA loci as well as all loci combined showed significant p-values.

Thirdly, we conducted statistically tests among the three samples to see if these samples were originated from genetically different groups of sei whales. Again, no evidence of the genetic difference was detected at each of the 16 loci, as well as all the loci combined, among the three samples (Table 5). The value of F_{ST} at this comparison was low (0.001) and was not significantly different from zero. In addition to that, no evidence of the deviation from the expected Hardy-Weinberg genotypic distributions was detected at each of the loci as well as all loci combined in the sample all three combined (Table 5).

We have been presenting the results of several genetic analyses using the temporally as well as geographically variable samples of the North Pacific sei whales to the IWC/SC (e.g., Kanda *et al*., 2009, 2013). One task remained is that we have never compared the samples collected from the different areas at the same time of year. Evidence of or no evidence of genetic differences among the samples collected at the different years could be due to the temporal genetic effects within the same stock. The analysis presented in this paper thus filled up the empty space in the series of our genetic studies on the North Pacific sei whales. The results from the genetic heterogeneity tests between the two spatially different samples (JARPNII and POWER) showed no evidence of the genetic differences between the two samples. Some may argue that such result can be obtained when two genetically different stocks mix each other widely over the North Pacific with an equal proportion. The results from the Hardy-Weinberg tests in this study denied such possibility because no evidence of the deviation from the Hardy Weinberg equilibrium was detected in the combined samples. All of our genetic studies so far were unable to demonstrate evidence of multiple stocks of sei whales in the North Pacific.

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Table 1. Collection information of the POWER and JARPNII 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					
10NP	2010	June - August	100	38°N-44°N	145°E-168°E
11NP	2011	June - August	95	37°N-45°N	149°E-167°E
12NP	2012	June-July	100	37°N-42°N	151°E-169°E

Table 2. The number of alleles (A), allelic richness, and expected heterozygosity (He) at the 16 microsatellite loci in the POWER and JARPNII samples of sei whales collected in 2010 to 2012.

Locus	10POWER			11POWER			12POWER		
	A	AR	He	A	AR	He	A	AR	He
EV21	5	5.0	0.712	6	5.3	0.691	5	4.7	0.650
GGAA520	7	7.0	0.856	7	5.7	0.772	7	5.4	0.786
GATA98	6	6.0	0.763	6	5.8	0.780	6	5.3	0.753
GT211	2	2.0	0.269	3	3.0	0.398	3	2.8	0.327
GATA53	3	3.0	0.535	3	3.0	0.518	3	3.0	0.530
EV1	10	10.0	0.859	10	8.1	0.834	13	9.4	0.824
EV94	5	5.0	0.724	6	5.1	0.729	6	5.2	0.713
GT23	6	6.0	0.353	7	5.6	0.639	8	5.4	0.610
GT575	4	4.0	0.587	5	3.8	0.578	4	3.5	0.560
GATA417	5	5.0	0.715	7	6.1	0.820	8	6.1	0.777
GT310	3	3.0	0.429	3	3.0	0.443	3	3.0	0.530
EV104	5	5.0	0.692	5	4.7	0.711	6	5.0	0.703
GATA28	8	8.0	0.859	8	7.0	0.826	9	7.5	0.825
GT271	3	3.0	0.282	3	2.3	0.132	2	1.9	0.109
GT011	3	3.0	0.596	4	3.4	0.489	4	3.3	0.450
DlrFCB17	9	9.0	0.894	15	11.0	0.872	14	10.0	0.858
Average	5.3	5.3	0.633	6.1	5.2	0.640	6.3	5.1	0.625

Table 2. continued.

Locus	10NP			11NP			12NP		
	A	AR	He	A	AR	He	A	AR	He
EV21	6	4.7	0.688	6	4.8	0.662	6	4.9	0.661
GGAA520	8	6.5	0.811	8	6.6	0.814	8	6.5	0.806
GATA98	6	5.1	0.718	7	5.6	0.744	7	5.4	0.735
GT211	3	2.6	0.293	3	2.6	0.285	5	3.1	0.300
GATA53	3	3.0	0.561	3	3.0	0.488	3	3.0	0.526
EV1	12	8.0	0.816	13	8.6	0.835	14	8.2	0.815
EV94	6	5.1	0.703	6	4.7	0.682	7	5.2	0.729
GT23	9	5.4	0.639	9	5.7	0.640	10	5.1	0.589
GT575	5	3.5	0.575	4	3.5	0.607	5	3.6	0.581
GATA417	7	5.7	0.792	8	5.9	0.792	6	5.6	0.793
GT310	3	2.9	0.516	3	3.0	0.485	3	2.9	0.470
EV104	6	5.0	0.734	7	4.8	0.724	7	4.7	0.710
GATA28	10	7.1	0.808	10	6.9	0.798	9	7.1	0.834
GT271	4	2.3	0.167	3	2.3	0.177	3	2.3	0.159
GT011	4	3.3	0.453	3	2.9	0.438	4	3.4	0.442
DlrFCB17	18	9.8	0.862	16	9.9	0.863	16	10.1	0.880
Average	6.9	5.0	0.634	6.8	5.0	0.627	7.1	5.1	0.627

Table 3. Results (p-values) of the heterogeneity tests and F_{ST} between the POWER and JARPNII samples by years.

Locus	10POWER x 10NP	11POWER x 11NP	12POWER x 12NP
EV21	0.950	0.346	0.837
GGAA520	0.064	0.205	0.401
GATA98	0.703	0.596	0.527
GT211	0.621	0.213	0.694
GATA53	0.873	0.900	1.000
EV1	0.019	0.053	0.229
EV94	0.154	0.754	0.910
GT23	0.066	0.607	0.832
GT575	0.863	0.150	0.978
GATA417	0.266	0.811	0.309
GT310	0.541	0.655	0.678
EV104	0.634	0.595	0.875
GATA28	0.724	0.918	0.166
GT271	0.291	0.831	0.340
GT011	0.182	0.255	0.057
DlrFCB17	0.272	0.303	0.994
All loci	0.188	0.628	0.889
F_{ST}	0.011	-0.0002	-0.004

Table 4. Results (p-values) of the test for the deviation from the Hardy-Weinberg expected genotypic proportions in the POWER and JARPNII samples combined by years.

Locus	2010(POWER+NP)	2011 (POWER+NP)	2012 (POWER+NP)
EV21	0.692	0.741	0.974
GGAA520	0.732	0.528	0.265
GATA98	0.260	0.221	0.062
GT211	0.123	1.000	0.488
GATA53	0.189	0.528	0.891
EV1	0.559	0.728	0.649
EV94	0.803	0.171	0.810
GT23	0.186	0.768	0.875
GT575	0.373	0.173	0.347
GATA417	0.946	0.401	0.430
GT310	0.872	0.955	0.768
EV104	0.394	0.848	0.865
GATA28	0.704	0.670	0.888
GT271	0.678	0.405	1.000
GT011	0.056	0.263	0.746
DlrFCB17	0.959	0.058	0.107
All loci	0.664	0.689	0.922

Table 5. Results (p-values) of the heterogeneity tests and F_{ST} among the three yearly samples (POWER and JARPNII combined for each year) and of the test for the deviation from the Hardy-Weinberg expected genotypic proportions in the sample all POWER and JARPNII combined.

Locus	2010x2011x2012	HW for all combined
EV21	0.570	0.755
GGAA520	0.125	0.343
GATA98	0.693	0.122
GT211	0.482	0.676
GATA53	0.169	0.095
EV1	0.823	0.647
EV94	0.801	0.393
GT23	0.713	0.806
GT575	0.020	0.081
GATA417	0.783	0.789
GT310	0.303	0.948
EV104	0.231	0.840
GATA28	0.993	0.975
GT271	0.947	1.000
GT011	0.062	0.663
DlrFCB17	0.249	0.734
All loci	0.343	0.878
F_{ST}	0.001	

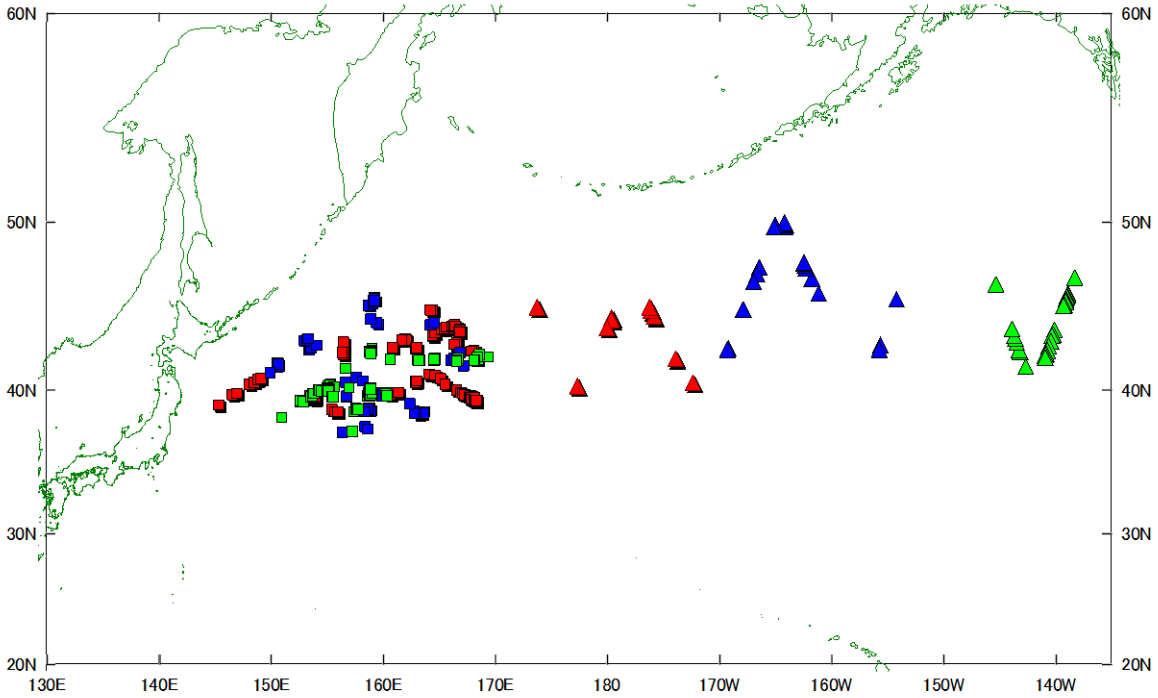

Fig.1. Sampling locations of sei whales in the North Pacific. Square: JARPNII, Triangle: POWER; red: 2010, blue: 2011, green: 2012.