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Genetic study of stock structure in North Pacific sei whales

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ABSTRACT

The Institute of Cetacean Research has conducted genetic analyses of stock structure in North Pacific sei whales based on historical genetic samples, and samples collected more recently by the IWC-POWER (biopsy samples) and JARPNII program. Results have contributed to the *in-depth assessment* of North Pacific sei whales conducted by the IWC Scientific Committee. This paper presents details of the research area, samples, genetic approaches, and main results of the genetic analyses of stock structure of sei whales in the pelagic area of the North Pacific. Results of the genetic analyses are consistent with the hypothesis of a single stock of sei whales in the pelagic area of the North Pacific surveyed (140°E–130°W).

INTRODUCTION

Sei whale, *Balaenoptera borealis*, (Figure 1) is a large baleen whale species inhabiting all of the major open oceans (Horwood, 1987; Rice, 1998). Sei whales live up to 60 years of age and their maximum body length is 20 m. Sei whales migrate from winter breeding grounds in low latitude to high latitudinal areas in summer, where they feed on various prey species (e.g. Nemoto, 1959; Nemoto and Kawamura, 1977).

This species was one of the major targets of commercial whaling worldwide (Horwood, 1987). In the North Pacific the main period of exploitation was from 1963 to 1973 (Allen, 1980), and they were caught in waters off California, Canada, Japan, Kamchatka and the Kuril Islands by pelagic fleets (Horwood, 2018).

Delineation of the stock structure of the species and the estimation of abundance by stock are key information required for management and conservation. A pioneering work on stock structure of North Pacific sei whales was carried out by Wada and Numachi (1991) using three polymorphic allozyme loci. The study reported no evidence of temporal and spatial genetic heterogeneity in samples collected in the area east of 160°E, suggesting the existence of only a single stock in the area.

The most comprehensive genetic study was performed by Kanda *et al.* (2009) using 17 microsatellite DNA loci and 487bp of mitochondrial DNA (mtDNA) control region sequences. The study used a total of 790 specimens collected during the past commercial whaling in 1972–1973 and during the JARPNII surveys in 2002–2007. Results of the study showed no evidence of significant genetic differences in areas between 140°E and 135°W of the North Pacific.

Subsequently, Kanda *et al.* (2013) examined not only spatial but also temporal genetic differences of the North Pacific sei whales. The study showed no evidence of temporal (40 years apart between recent and past samples) and spatial (the research area divided into western (140°–170°E), central (170°E–150°W) and eastern (150°–130°W) areas) genetic differences. Results were consistent with those found by Kanda *et al.* (2009). Furthermore, Kanda *et al.* (2015a) considered that spatial genetic differentiation should be tested using samples

Figure 1. North Pacific sei whale (*Balaenoptera borealis*).

collected in the same year in order to eliminate temporal negative biases. Consequently, they examined genetic variations at 16 microsatellite loci using only samples collected during summer in 2010, 2011 and 2012. Again, the results of the study failed to find evidence of multiple stocks of sei whales in the North Pacific.

This paper presents details of the research area, samples, genetic approaches, and main results of the genetic analyses of stock structure of sei whales in the pelagic area of the North Pacific, updated after the work by Kanda *et al.* (2015a). This paper also provides information on some basic concepts of population genetic statistics.

MATERIALS AND METHODS

Research area

The genetic analyses were conducted based on three sampling areas in the pelagic region of the North Pacific: western (140°–150°E), central (150°E–180°) and eastern (180°–130°W) areas (Figure 2). Genetic samples were collected from whales taken during past commercial whaling operations as well during more recent surveys of the JARPNII research program. Biopsy samples obtained during the IWC-Pacific Ocean Whale and Ecosystem Research (IWC-POWER) were also used (see details below).

Samples and laboratory procedures

Samples and DNA extraction

A total of 1,748 genetic samples of sei whales obtained during past commercial whaling in 1972–1973 (n=312), the JARPNII in 2002–2016 (n=1,354) and the IWC-POWER (biopsy samples) in 2010–2013 (n=82) was subjected to DNA extraction (Table 1).

Total genomic DNA was extracted from 0.05 g of skin tissue, preserved in 99% ethanol at room temperature or stored frozen at −20°C, using the standard phenol-chloroform method (Sambrook *et al.*, 1989) or using Gentra Puregene kits (QIAGEN). Extracted DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

MtDNA sequencing

See an explanation of mtDNA sequencing analyses in Taguchi *et al.* (2017).

Approximately 500 base pairs of partial control region were amplified by the polymerase chain reaction (PCR) using a set of primers MT4 (Árnason *et al.*, 1993) and Dlp 5R (5′-CCA TCG AGA TGT CTT ATT TAA GGG GAA C-3′). PCR was performed with an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C and 30 seconds at 72°C, with a final extension step at 72°C for 10 minutes. PCR products were purified using MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed using BigDye terminator cycle sequence Kit (Applied Biosystems) and the PCR primers, following the protocols of the manufacturer. The cycle sequencing products were purified using AutoSeq G-50 spin Columns (Pharmacia Biotech). The labeled sequencing fragments from tissue samples collected until 2004, during 2005–2010, and

Table 1

Number of genetic samples of North Pacific sei whales used in this study. Sample size for the present data analyses is shown in parenthesis (mtDNA sequences, microsatellite genotypes).

Sample source	Sampling area		
	Western	Central	Eastern
Past commercial whaling		181 (175, 177)	131 (128, 121)
JARPNII	30 (30, 30)	1324 (1322, 1323)	
IWC-POWER		3 (3, 3)	79 (75, 75)
Total	30 (30, 30)	1508 (1500, 1503)	210 (203, 196)

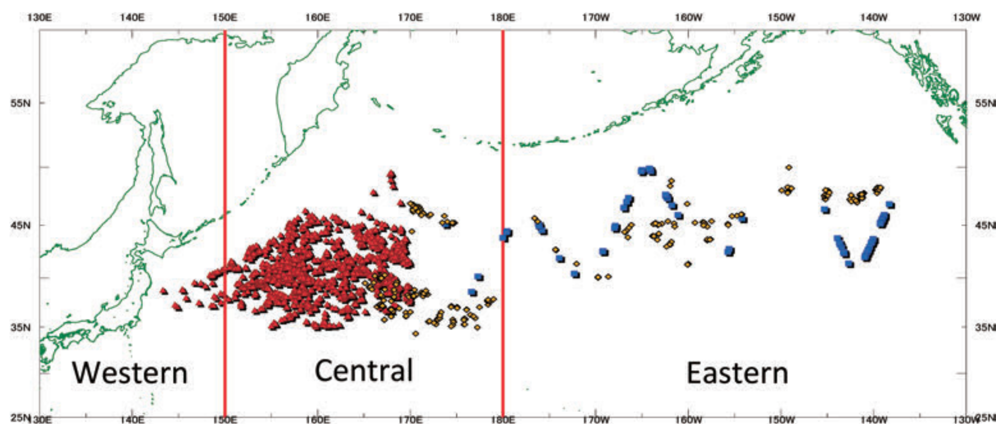

Figure 2. Sampling position of sei whales used in the genetic analyses. Color indicates sample source: yellow: past commercial whaling; red: JARPNII; and blue: IWC-POWER.

from 2011 were resolved using an ABI PRISM 377, ABI PRISM 3100 and ABI3500 Genetic Analyzers (Applied Biosystems), respectively.

Microsatellite DNA genotyping
See an explanation of microsatellite DNA analyses in Taguchi *et al.* (2017).

All individuals sampled in JARPNII and IWC-POWER surveys were genotyped at 17 nuclear microsatellite loci: EV1, EV14, 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). The past commercial whaling samples were genotyped at 15 microsatellite loci (EV14 and GATA417 were not used). Primer sequences and PCR cycling profiles generally followed those of the original authors. The multiplex PCR amplifications were performed in 15 µl reaction mixtures containing 10–100 ng of DNA, 5 pmole of each primer, 0.625 units of Ex Taq DNA polymerase (Takara Shuzo), 2mM of each dNTP, and 10x reaction buffer containing 20mM $MgCl_2$ (Takara Shuzo), with 94°C for 2 minutes, followed by 30 cycles at 94°C for 20 seconds/54–61°C for 45 seconds/72°C for 1 minute, and a post-cycling extension at 72°C for 10 minutes.

PCR products from tissue samples collected until 2013 were run on a 6% polyacrylamide denaturating gel (Long Ranger) with internal size standard (GENESCAN400HD, Applied Biosystems) 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. The PCR products of samples collected after 2013 were electrophoresed on an ABI 3500 DNA Analyzer (Applied Biosystems), and allele sizes were determined using a 600 LIZ size standard (Applied Biosystems) and GeneMapper v. 4.0 (Applied Biosystems). Microsatellite scores from the latter platform were standardized according to those from the former for each locus.

Data analyses
Three whales re-sampled (two genetic samples obtained from the same individual whale) were excluded from the analysis. Three calves accompanied by their mothers were also excluded from all subsequent analyses, to ensure an independence of the dataset. In addition to this, nine mtDNA sequences data and thirteen microsatellite genotype data sets were also excluded from the present data set due to their low data qualities.

FDR correction (Benjamini and Hochberg, 1995) was used to adjust the significance level for all multiple comparisons in this study.

Considering a long time series of sample collection of over 40 years, annual genetic variations were preliminary examined in each of the sampling areas, which consistently showed no significant genetic differences. Therefore, the genetic data from several years were combined in each sampling area in all subsequent analyses. More detailed descriptions of the temporal analyses were provided in Appendix 7 in Tamura *et al.* (2019).

Genetic diversities
For microsatellite DNA, the departure from Hardy–Weinberg equilibrium (*HWE*: see Box 1) was tested in each locus using the R package ′*HWxtest*′ (Engels, 2009). A global test across loci combining the observed *P*-values in each locus by Fisher′s method was performed using the R package ′*metap*′ (Dewey, 2018). The inbreeding coefficient (F_{IS}; Weir and Cockerham, 1984) in each locus and across loci was estimated using the R package ′*Demerelate*′ (Kraemer and Gerlach, 2017). The number of alleles (*A*) and expected heterozygosity (H_E: see Box 2) in each locus and across loci were estimated using the program ARLEQUIN v. 3.5.1.2 (Excoffier and Lischer, 2010).

For mtDNA, haplotype (*h*) and nucleotide (π) diversities (Nei, 1987) (see Box 2) with sample standard deviations were estimated using the program ARLEQUIN.

Genetic differentiation and structuring
The F_{ST}-like estimates for microsatellites and conventional pairwise F_{ST} for mtDNA (see Box 3) were calculated to measure the genetic differentiation between sampling areas using 10,000 random permutations of the original dataset in the program ARLEQUIN. A probability test implemented in the program GENEPOP (Rousset, 2008) was used to detect the genetic heterogeneity in microsatellite allele frequency among sampling areas. Difference in mtDNA haplotype frequency among sampling areas was also tested using the Monte Carlo simulation-based chi-square test of independence (Roff and Bentzen, 1989) in R.

Bayesian clustering analysis was performed using microsatellite data to infer the most likely number of clusters using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). The analysis was conducted with ten independent runs for K=2–3. All runs were performed with 100,000 Markov chain

Box 1. Hardy–Weinberg equilibrium

The genetic composition of a population is usually described in terms of allele frequency, number of alleles and heterozygosity. Allele and genotype frequencies attain an equilibrium, referred to as the Hardy–Weinberg equilibrium (*HWE*), in the next generation in infinitely large and random mating populations when there are no mutation, immigration or selection. When any of the assumptions underlying *HWE* are violated, then deviations from the equilibrium genotype frequencies will be observed. Thus, the *HWE* are widely used to detect if the population has non-random mating, migration or selection.

For a single locus with two alleles, *HWE* genotype frequencies can be expected according to the relationship:

$$p^2 + 2\mathrm{pq} + q^2 = 1$$

where p and q are the respective allele frequencies; p^2=frequency of one allele homozygotes, q^2=frequency of the other allele homozygotes, and $2pq$=frequency of heterozygotes.

To determine if the observed genotype frequencies deviate from the frequencies expected from the *HWE* relationship statistically, the difference in number of genotypes between them is tested using a χ^2 test.

As an example, consider a single microsatellite locus GATA53 with alleles 198 and 202 in ten sei whales (See Table 2.1 in Box 2), hence three genotypes (198/198, 202/198, 202/202) are found. From the observed number of respective genotypes, it is possible to calculate p and q, and to estimate the *HWE* genotype frequencies based on the allele frequencies calculated from the observed genotype frequencies, as shown in Table 1.1.

Table 1.1

Calculation of allele frequencies based on the observed number of genotypes, and the genotype frequencies expected from the *HWE* in a microsatellite locus GATA53 of sei whales.

Genotypes	Observed						Expected	
	Number of genotypes (*O*)	Genotype frequency	Number of alleles		Allele frequency		Genotype frequency	Number of genotypes (*E*)
			198	202	198	202		
198/198	1	1/10 0.1	2×1 2		2/20 0.1		p^2=0.3×0.3 0.09	0.09×10 0.9
202/198	4	4/10 0.4	1×4 4	1×4 4	4/20 0.2	4/20 0.2	$2pq$=2×0.3×0.7 0.42	0.42×10 4.2
202/202	5	5/10 0.5		2×5 10		10/20 0.5	q^2=0.7×0.7 0.49	0.49×10 4.9
Total	10	1.0	6	14	p=0.3	q=0.7	1.00	10.0

Monte Carlo repetitions and 10,000 burn-in length using the admixture model with correlated allele frequencies. The web-based program STRUCTURE HARVESTER (Earl and vonHoldt, 2012) was used to estimate the mean posterior probability of the data (Also see Goto *et al.* (2017) for detailed basic concept of the STRUCTURE analysis).

RESULTS AND DISCUSSION

All 17 microsatellite loci were polymorphic in the entire dataset of 1,729 sei whales from the North Pacific, which ranged from 22 alleles at EV14 to 3 alleles at GATA53 (Table 2). The mtDNA control region sequences of 1,733 sei whales from the North Pacific contained 39 variable nucleotide sites and a single alignment gap defining 84 haplotypes.

Genetic diversities

The H_E at each locus and across loci were not largely different among sampling areas (Table 2), and the estimates across loci ranged from 0.632 in the western area to 0.639 in the eastern area (Table 2). Significant deviations from *HWE* were not observed at any loci and across loci in each sampling area after FDR correction (Table 2), which suggested that North Pacific sei whales in each of the sampling areas derived from a single breeding population.

Regarding mtDNA, both h and π were similar among sampling areas, which ranged from 0.908 in the western area to 0.927 in the eastern area, and from 0.789% in the western area to 0.803% in the central area, respectively (Table 3). These observations were consistent with the

Box 2. Estimating genetic diversity

Genetic diversity is characterized by allele frequencies at each locus, which can be influenced by population size, gene flow, reproductive system, natural selection, genetic mutation or genetic linkage. In natural environment, populations are commonly fragmented into subpopulations in different size partly separated from each other by barriers to migration, and this may change the genetic diversity among populations.

Observed heterozygosity (H_O) is defined as the proportion of individuals heterozygous across a set of loci (h_j), or the proportion of loci for which an individual is heterozygous (h_i). This estimate can be obtained by direct count of heterozygotes, as shown in Table 2.1. Overall estimates are averages across the loci used in the study.

Table 2.1
Sub-set of data used in this study involving seventeen microsatellite loci genotyped in each of ten sei whales.

Microsatellite loci (*j*)	Individual (*i*)										h_j
	SEI-001	SEI-002	SEI-003	SEI-004	SEI-005	SEI-006	SEI-007	SEI-008	SEI-009	SEI-010	
EV21	120124	120120	120120	118120	120126	120122	120120	120124	118122	120124	0.7
GGAA520	217221	221221	217229	225229	225229	217233	213225	221229	217229	221225	0.9
GATA98	102110	094098	098106	102102	102102	094102	098102	102102	094106	098102	0.7
GT211	115115	115115	115117	115117	115115	115115	115115	115115	115119	115121	0.4
EV14	155167	159179	155167	157167	155167	161161	155155	155159	155155	155155	0.6
GATA53	202202	198198	198202	202202	202202	202202	198202	202202	198202	198202	0.4
EV1	130146	130146	144162	146146	148152	146146	148158	148152	146148	130138	0.8
EV94	219221	217217	217219	213221	217225	217219	217219	221223	217219	217221	0.9
GT23	116118	116126	116116	116116	112116	116116	116116	116118	116116	116120	0.5
GT575	140146	138146	146148	138138	138138	138146	138148	146146	138148	138138	0.6
GATA417	220220	224224	212240	216232	216224	212228	220224	224224	212220	216220	0.7
GT310	108110	108108	108110	108108	108110	110110	108108	108108	108108	108114	0.4
EV104	134140	134144	134140	140140	140144	134134	134142	140142	140144	140142	0.8
GATA28	228232	228232	224232	216236	232232	212220	224232	216232	232232	212232	0.8
GT271	096096	096096	096096	096096	096096	094096	094096	094096	096096	096096	0.3
GT011	123127	123123	123123	123123	123129	123129	123123	123127	123123	123123	0.4
DlrFCB17	199203	203207	205221	199215	199207	199203	209215	183205	201203	203211	1.0
h_i	0.765	0.471	0.765	0.471	0.647	0.588	0.647	0.647	0.647	0.765	0.641

Genotypes are represented by 6-digits codes (each allele is coded by 3-digits), and heterozygotes are indicated with red color. In this example, 109 of 170 assayed genotypes are heterozygous (overall H_O=0.641).

Expected heterozygosity (H_E) is defined as the expected proportion of individuals heterozygous from observed allele frequencies, assuming the population is in *HWE* (see Box 1), as shown in Table 2.2. In the case of two alleles at a locus with their respective frequencies of *p* and *q*, the expected heterozygosity $H_E=2pq$. When there are more than two alleles, it is simpler to calculate H_E as follows:

$$H_E = 1 - \sum_{i=1}^{k} p_i^2$$

where p_i is the frequency of the *i*-th allele, and *k* is the number of alleles. H_E is normally used rather than H_O since it is less affected by sampling.

comparable genetic diversities among sampling areas observed in microsatellite DNA.

Genetic differentiations and structuring

Pairwise F_{ST}-like estimates for microsatellites did not show any genetic differentiations between sampling areas (Table 4), which was consistent with the pairwise conventional F_{ST} estimates for mtDNA (Table 4). Heterogeneity tests also showed no differences in microsatellite allele (P=0.679, *d.f.*=34, χ^2=29.69) and mtDNA haplotype

Table 2.2
Allele frequencies and three statistics of genetic diversity, i.e. number of alleles (A), observed (H_O) and expected (H_E) heterozygosity, per microsatellite loci based on the data set in Table 1.1

Microsatellite loci	Alleles (i)										A	H_O	H_E
	allele 1	allele 2	allele 3	allele 4	allele 5	allele 6	allele 7	allele 8	allele 9	allele 10			
EV21	0.10	0.60	0.10	0.15	0.05						5	0.7	0.60
GGAA520	0.05	0.20	0.25	0.20	0.25	0.05					6	0.9	0.79
GATA98	0.15	0.20	0.50	0.10	0.05						5	0.7	0.68
GT211	0.80	0.10	0.05	0.05							4	0.4	0.35
EV14	0.50	0.05	0.10	0.10	0.20	0.05					6	0.6	0.69
GATA53	0.30	0.70									2	0.4	0.42
EV1	0.15	0.05	0.05	0.35	0.20	0.10	0.05	0.05			8	0.8	0.80
EV94	0.05	0.40	0.25	0.20	0.05	0.05					6	0.9	0.73
GT23	0.05	0.75	0.10	0.05	0.05						5	0.5	0.42
GT575	0.50	0.05	0.30	0.15							4	0.6	0.64
GATA417	0.15	0.15	0.25	0.30	0.05	0.05	0.05				7	0.7	0.80
GT310	0.70	0.25	0.05								3	0.4	0.45
EV104	0.30	0.40	0.15	0.15							4	0.8	0.71
GATA28	0.10	0.10	0.05	0.10	0.10	0.50	0.05				7	0.8	0.71
GT271	0.15	0.85									2	0.3	0.26
GT011	0.80	0.10	0.10								3	0.4	0.34
DlrFCB17	0.05	0.20	0.05	0.25	0.10	0.10	0.05	0.05	0.10	0.05	10	1.0	0.86
Means											5.1	0.64	0.60

The averaged statistics over the seventeen loci were shown at the bottom (overall H_E=0.60).

For haplotypic data such as mitochondrial DNA, a simple estimate of diversity, which is equivalent to H_E for diploid data, called as **haplotype diversity (*H*)** can be also calculated as $H=1-\sum_{i=1}^{k} p_i^2$, where k is the number of haplotypes, and p_i is the frequency of haplotype i (Nei, 1987). In the case of full sequence comparison, **nucleotide diversity (π)**, which is equivalent to H at the nucleotide level, is given by $\pi=\sum_{ij} x_i x_j \pi_{ij}$, where p_{ij} is the proportion of different homologous nucleotide sites between haplotypes i and j, and x_i and x_j are the frequencies of haplotypes i and j, respectively (Nei, 1987).

Box 3. Measuring genetic differentiation

F-statistics, which is first introduced by Wright (1951), is one of important estimators to describe population genetic structure. This statistics partitions overall genetic diversities into components within and among populations, and can be calculated using heterozygosity as the following equations (Nei, 1987):

$$F_{IS} = (h_s - h_i)/h_s$$
$$F_{IT} = (h_t - h_i)/h_t$$
$$F_{ST} = (h_t - h_s)/h_t$$

where h_i is the H_O averaged across all populations, h_s is the H_E averaged across all populations, and h_t is the H_E for the total population. Two of which, i.e. F_{IS} and F_{ST}, are widely used as estimators to describe genetic structure. The F_{IS} (inbreeding coefficient) can be positive indicating a deficiency of heterozygotes when inbreeding or population subdivision occur within populations, while negative values indicating an excess of heterozygotes will be observed when outbreeding occurs within populations. The F_{ST} (fixation index) is a common estimator for genetic differentiation among populations, and varies from 0 (no differentiation between populations) to 1 (fixation of different alleles in populations).

Table 2
Summary statistics for 17 microsatellite loci in North Pacific sei whale in each sampling area.

Microsatellite loci	Western					Central					Eastern				
	n	*A*	H_E	*HWE*	F_{IS}	*n*	*A*	H_E	*HWE*	F_{IS}	*n*	*A*	H_E	*HWE*	F_{IS}
EV21	30	5	0.706	0.667	−0.136	1501	6	0.644	0.867	−0.004	196	6	0.651	0.295	−0.011
GGAA520	30	7	0.789	0.648	−0.014	1499	9	0.802	0.965	0.004	193	9	0.792	0.833	0.059
GATA98	30	5	0.686	0.441	−0.070	1485	7	0.734	0.792	−0.005	159	6	0.748	0.043	0.050
GT211	30	4	0.295	1.000	0.099	1503	6	0.308	0.019	0.016	194	4	0.316	0.124	−0.078
EV14	30	12	0.818	0.625	0.105	1326	22	0.865	0.846	0.001	74	14	0.861	0.862	−0.020
GATA53	30	3	0.413	0.291	−0.050	1500	3	0.494	0.990	0.003	192	3	0.508	0.819	0.015
EV1	30	12	0.814	0.814	0.017	1502	16	0.836	0.583	0.010	196	17	0.835	0.407	0.034
EV94	30	6	0.655	0.222	0.085	1502	8	0.685	0.519	−0.023	196	6	0.702	0.287	−0.032
GT23	30	6	0.554	0.335	−0.146	1503	14	0.606	0.308	0.017	195	11	0.600	0.741	0.008
GT575	30	4	0.603	0.378	−0.108	1503	5	0.593	0.657	0.024	190	6	0.577	0.816	0.061
GATA417	30	7	0.791	0.956	−0.055	1326	9	0.782	0.449	−0.005	75	8	0.785	0.561	0.049
GT310	30	3	0.513	1.000	0.026	1502	5	0.483	0.160	−0.020	196	4	0.483	0.364	−0.077
EV104	30	5	0.743	0.847	−0.078	1500	10	0.726	0.878	0.003	191	8	0.703	0.678	0.017
GATA28	30	9	0.821	0.082	0.027	1503	11	0.812	0.559	0.016	196	10	0.825	0.719	−0.015
GT271	30	3	0.242	1.000	−0.105	1503	4	0.140	0.579	0.006	196	3	0.130	1.000	0.022
GT011	30	4	0.415	0.889	−0.128	1503	6	0.449	0.899	0.006	196	4	0.458	0.751	0.020
DlrFCB17	30	14	0.890	0.310	−0.049	1502	20	0.872	0.106	0.012	188	17	0.882	0.165	0.023
Overall		6.41	0.632	0.939	−0.034		9.47	0.637	0.790	0.003		8.00	0.639	0.753	0.007

Table 3
Summary statistics for mtDNA control region in North Pacific sei whale in each sampling area.

	Western					Central					Eastern				
mtDNA	*n*	*h*	S.D.	*π* (%)	S.D.	*n*	*h*	S.D.	*π* (%)	S.D.	*n*	*h*	S.D.	*π* (%)	S.D.
	30	0.908	0.036	0.789	0.454	1500	0.925	0.004	0.803	0.446	203	0.927	0.010	0.797	0.445

Table 4
Pairwise F_{ST} between sampling areas. Upper and below diagonals indicate results from microsatellite and mtDNA.

Sampling area	Western	Central	Eastern
Western		−0.0002	−0.0010
Central	−0.0007		−0.0014
Eastern	0.0004	0.0002	

(P=0.699, χ^2=142.09) frequencies among sampling areas.

The clustering patterns in each *K* estimated by the program STRUCTURE also did not infer a distinct genetic structuring of this species (Figure 3). These results suggested absence of genetic structure among sei whales distributed in the pelagic area of the North Pacific investigated in this study, which was also supported by the fact that genetic diversities were not different among sampling areas in both markers, and there were no departures from *HWE* in each of sampling areas.

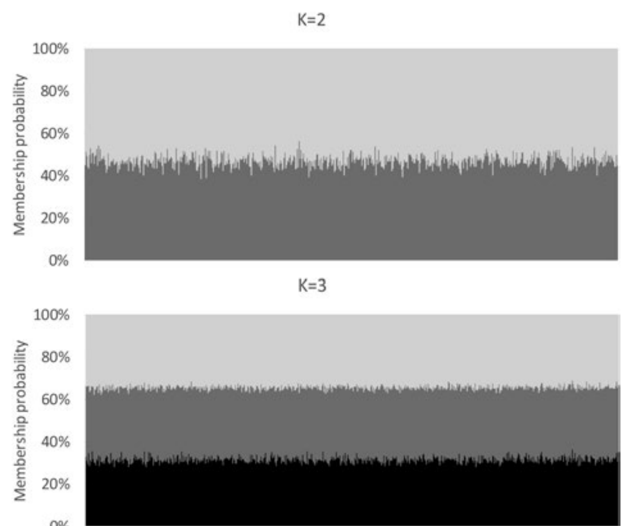

Figure 3. Bar plot of membership probabilities in STRUCTURE analyses at *K*=2–3 for North Pacific sei whales. Each individual is characterized by a thin vertical line, which is divided into *K* colored segments on the basis of the individual's membership fractions in *K* clusters.

CONCLUSIONS

The present pairwise F_{ST} estimates and heterogeneity tests did not show any significant genetic differentiations among the three sampling areas for both genetic markers. This was also supported by the STRUCTURE analysis suggesting a lack of genetic structure of this species. These findings implied that the North Pacific sei whale consists of a single breeding population at least in the pelagic areas surveyed. This inference was consistent with the comparable genetic diversities, i.e. h, π, H_E, among sampling areas as well as the results from the tests of *HWE* and F_{IS} estimates in this study. Kanda *et al.* (2015b) suggested that a single stock of sei whale occurs in the entire North Pacific based on genetic and non-genetic evidence. However, this study did not covered the coastal areas in both sides of the North Pacific. Additional genetic samples from the coastal areas are required to confirm this hypothesis.

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