Stock structure of sei whales in the North Pacific as revealed by microsatellite and mitochondrial DNA analyses

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ABSTRACT
We analyzed genetic variation at 17 microsatellite loci and 487 bp of mitochondrial DNA (mtDNA) control region sequences in samples of sei whales, *Balaenoptera borealis*, in order to describe their stock structure in the western North Pacific. The samples we used consisted of 489 whales collected during JARPNII surveys from 2002 to 2007 in the area between 143°E and 170°E. In order to explore their stock structure in a wider geographic area, we also analyzed 301 whales (298 for the mtDNA analyses) collected from the 1972 and 1973 commercial whaling conducted at the North Pacific from 165°E to 139°W. Due to the condition of the DNA extracted from the archived blood samples, however, we failed to analyze all of the 17 loci but did analyze 14 loci for the individuals in the commercial whaling samples. All of the microsatellite loci analyzed were polymorphic over the samples, and both markers showed a substantial level of genetic diversity within the JARPNII and commercial whaling samples. In order to look for any evidence of genetic differences among the samples, we conducted conventional hypothesis testing and a Bayesian clustering approach. All of the tests we conducted found no evidence of genetic differences within as well as between the JARPNII and commercial whaling samples. Both females and males showed the same pattern of the stock structure. Phylogenetic analysis of the mtDNA haplotypes did not show any spatially or temporally unique clusters. This study indicated that the open water of the North Pacific was mainly occupied by the individuals from a single stock of sei whales.

KEY WORDS: SEI WHALE, MICROSATELLITE, MTDNA, STOCK STRUCTURE, JARPNII, COMMERCIAL WHALING, NORTH PACIFIC

INTRODUCTION

Sei whale, *Balaenoptera borealis*, is a large baleen whale species inhabiting in all of the major open oceans (Horwood, 1987; Rice, 1998). Sei whales live up to 60 years in age and the maximum size can reach to as large as 20m. Sei whales feed on copepods, Euphausiids, and fish. Sei whales migrate seasonally from winter breeding grounds in low latitude to summer feeding grounds in high latitude. This migration pattern of sei whales in conjunction with other geographic, biological, and ecological factors resulted in spatial and temporal separations of the sei whale stocks (Harwood, 1987; Wada and Numachi, 1991).

Sei whales were one of the major targets of commercial whaling worldwide (Horwood, 1987). In order to investigate current status of whale stocks in given oceanic regions, the Scientific Committee of the International Whaling Commission conducts 'in-depth assessments' of the target species. Among all of the required information, understanding of stock structure in the region is essential for successful in-depth assessment. Past studies, however, have provided very limited information of the stock structure of the North Pacific sei whales, and thus no conclusive evidence has been presented (reviewed by Donovan, 1991). In regard to genetics, a pioneer work carried out by Wada and Numachi (1991) reported no evidence of temporal and spatial genetic heterogeneity in samples of the North Pacific sei whales collected in the area east of 160°E, suggesting existence of only a single stock in the area. Because their conclusion was based on only three polymorphic allozyme loci, lack of the genetic heterogeneity in the samples collected from such a wide area could have been due to low resolution power of the genetic marker.

Since 2002, JARPN phase II (JARPNII) has started to take sei whales to study feeding ecology and fisheries interaction in this area and to acquire information useful to effective management of this species.

Kanda *et al*. (2006) utilized genetic variations at 17 hypervariable microsatellite markers in the 2002 and 2003 JARPNII samples of sei whales from the western North Pacific for describing their genetic characteristics. Genetic analysis of a total of 89 whales found high level of genetic diversity within the samples, while no evidence of genetic heterogeneity between the samples. Even with highly variable microsatellite markers, Kanda *et al*. (2006) also reached the same conclusion to Wada and Numachi (1991). The sizes and geographic origins of the samples were still rather limited, however.

The primary objective of this study was to better understand the stock structure of sei whales in the North Pacific by analyzing the samples collected during the JARPNII surveys from 2002 to 2007 using the microsatellite as well as mtDNA markers. This study is the first one that applied mtDNA analyses to the North Pacific sei whale. We also analyzed the archived samples of sei whales collected during past commercial whaling operated in 1972 and 1973 (165°E to 139°W) to cover a much wider area of the North Pacific than have previously done.

MATERIALS AND METHODS

Samples

JARPNII samples (N=489) of sei whales were collected from the western North Pacific off Japanese coast from 2002 to 2007 (Table 1 and Fig. 1). Sampling dates and locations of scientific surveys slightly differed year by year depending on the sampling plan of a given year. Details of offshore part of JARPNII survey can be found in Tamura *et al*. (2009).

Archived blood samples of sei whales collected during past commercial whaling in 1972 and 1973 were obtained for comparison (Table 1). These individuals in the samples were captured in the area approximately from 165°E to 139°W (Fig. 1).

DNA extraction

Total DNA from each of the whales was extracted from 0.05 g of skin tissue in the JARPNII samples and from blood tissues in the commercial whaling 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 17 sets of primers, none of which was designed specifically from sei whales: 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). Primer sequences and PCR cycling profiles generally followed those of the original authors. The extracted DNAs from the individuals in the commercial whaling samples appeared not to be in as good condition as the JARPNII ones, maximum of the 14 loci (EV1, EV21, EV94, EV104, GT011, GT23, GT211, GT271, GT310, GT575, GATA28, GATA53, GGAA520, and DlrFCB17) per individual were analyzed. Minimum number of the loci per individual in the commercial whaling sample was 9.

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.

mtDNA analysis

The first half of the mtDNA control region was amplified through the polymerase chain reaction (PCR). Approximately 500bp of mtDNA region containing control region were amplified using the following primer set: light-strand MT4 (Árnason *et al*., 1993) and heavy-strand Dlp 5R (*5'*-CCATCgAgATgTCTTATTTAAggggAAC-*3'*). PCR products were purified by MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed with the same primers, using BigDye terminator cycle sequence Kit (Applied Biosystems, Inc). The cycle sequencing products were purified by AutoSeq G-50 spin Columns (Pharmacia Biotech). The labeled sequencing fragments were resolved by electrophoresis through a 5% denaturing polyacrylamide matrix on an ABI 377ä or ABI3100 Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacture. For each sample both strands were sequenced.

Data analysis

For the microsatellites, 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. For the mtDNA, the number of haplotypes and haplotype diversity were calculated following Nei (1987). The nucleotide diversity (Nei, 1987: equation 10.5) and its standard error for population sampling and stochastic processes were calculated from the pair-wise differences between the mtDNA sequences using the Kimura's 2- parameter adjustment (Kimura, 1980).

Conventional hypothesis testing procedure was conducted using heterogeneity test in microsatellite allele and mtDNA haplotype frequencies among the samples, respectively. 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 for the microsatellites. When simultaneous multiple tests were conducted, Rice (1989) correction for the multiple tests was performed. The randomized chi-square test of independence (Roff and Bentzen, 1989) was used to investigate the temporal/spatial differentiation of mtDNA variation. In each test a total of 10,000 permutations of the original data were performed. Tests were conducted for all samples combined as well for males and females separately. A p-value smaller than 0.05 was used as a criterion to reject the null hypothesis of panmixia.

The Bayesian clustering approach with the microsatellite data was conducted using the software STRUCTURE 2.0 (Pritchard *et al*., 2000) to determine the most likely number of genetically distinct stocks present in our samples. The program is a model-based clustering method for inferring stock structure (K, the number of stocks in the model) using multilocus genotype data with and without information on sampling locations. STRUCTURE allowed us to analyze the samples without choosing sample units that did not necessarily correspond to real biological stock boundaries. Posterior probabilities for K were estimating from three independent runs for each value of K from one to three with only genetic information. These data were calculated based on burn-in period of 10,000 iterations and runs of 100,000 iterations. The ancestry model we used for the simulation was the admixture model, which assumes individuals may have mixed ancestry. The allele frequency model used was the correlated allele frequencies model, which assumes frequencies in the different stocks are likely to be similar due to migration or shared ancestry.

Phylogenetic analysis was conducted for the mtDNA haplotypes. Sequences the haplotypes were aligned initially using Sequence Navigator (Applied Biosystems, Inc). The aligned sequences were then corrected by eye for minor inconsistencies. Phylogenetic reconstruction of sequences was made using the neighbor-joining method implemented in the computer package PHYLIP version 3.5c (Felsenstein, 1993). Genetic distance among haplotypes were estimated using the program DNADIST of the PHYLIP based on Kimura's 2- parameter model (Kimura, 1980) with an observed transition:transversion ratio of 1:1. The tree was rooted using a homologous sequence from a North Pacific Bryde's whale (*B. edeni*) collected from the JARPNII. In addition to that, sequences of coastal Bryde's whales (Eden's whale and Omura's whalestranded to the Japanese coast were included as outgroups (see also Wada et al., 2003). Addition of these three outgroup whales allowed us to ensure that our samples consisted only of sei whales. Alignment with the out-group sequences resulted in total of 497 bp for the analysis. The bootstrap values were obtained by generating 1000 random samples, for which distance matrices were also computed (Kimura 2-parameter model; Kimura, 1980). Finally a majority-rule consensus phylogeny was calculated from the resultant 1000 phylogenies. The obtained tree was visualized using the software TreeView PPC (Page 1996).

RESULTS

Genetic diversity

Microsatellite. All 17 microsatellite loci were successfully amplified and were polymorphic in the JARPNII samples of sei whales (Table 2). Total number of alleles at the loci in all the samples combined ranged from three at GATA53 to 23 at EV14 with an average of 8.9. Expected heterozygosity ranged from 0.133 at GT271 to 0.872 at DlrFCB17 with an average of 0.634. None of the 17 loci showed significant deviation from the expected Hardy-Weinberg genotypic proportions after correction for the simultaneous multiple tests.

Due to the poor DNA conditions extracted from the archived blood samples, we failed to analyze all

of the 17 loci but maximum of 14 for the individuals in the commercial whaling samples (Table 2). Total number of alleles at the loci in all the samples combined ranged from three at GATA53 and GT271 to 20 at DlrFCB17 with an average of 8.1. Expected heterozygosity ranged from 0.129 at GT271 to 0.889 at DlrFCB17 with an average of 0.605. None of the 14 loci showed significant deviation from the expected Hardy-Weinberg genotypic proportions after correction for the simultaneous multiple tests.
mtDNA. Sequence variations at 487bp of the mtDNA control region resulted in 97 haplotypes in the total of 787 sei whales used in the present study. Forty-seven polymorphic sites were detected, 44 of which were transitions, two transversion and one insertion/deletion events. In the JARPNII samples, 81 haplotypes were found and 43 of them were unique to the samples. In the commercial whaling samples, 54 haplotypes were detected and 16 of them were unique to the samples. The number of shared haplotypes between the JARPNII and commercial samples was 38. The proportion of the individuals that had the shared haplotypes between the JARPNII and commercial whaling samples was 85.2% in the JARPNII and 93.2 % in the commercial samples, respectively.

Haplotype diversities were 0.929 in the JARPNII and 0.915 in the past commercial whaling samples. Estimated nucleotide diversities were 0.0078 (standard error 0.0001) and 0.0080 (standard error 0.0002) for JARPNII and past commercial whaling, respectively.

Genetic divergence

JARPNII. We divided the JARPNII samples into three subsamples on the basis of the individuals' sighting sites: 143.00°E-155.00°E, 155.01°E -165.00°E, 165.01°E -170.00°E (Table 1), and then conducted statistical tests using both genetic markers for all and female and male separately. These longitudinal sections were chosen arbitrary but not biologically. First, we looked for evidence of genetic differences among the samples collected from the different years in the same collection area for all and female and male separately. No evidence of genetic differences was detected after the correction for multiple tests in the both genetic markers (Table 3), so that we combined the year samples into one. Then, we looked for evidence of genetic differences among the samples collected from the different areas. Again, no evidence of the genetic differences was detected in the both genetic markers for each of the three cases after the correction for multiple tests (Table 3).
Commercial. We divided the commercial whaling samples into three subsamples on the basis of the individuals' collection sites: 165.00°E-180.00°, 180.00°-150.01°W, 150.00°W -139.00°W (Table 1). We chose these geographic groups because we found a gap between the sampling locations of the groups (Fig. 1). Because of the small sample size, we combined the 1972 and 1973 samples without conducting statistical tests. Tests were conducted for all and female and male separately. No evidence of genetic differences was detected among the three subsamples collected from the different longitudinal areas for all the three cases in the both genetic markers (Table 4).
JARPNII vs commercial. In order to compare the samples of the two different sampling origins that were analyzed with the different number of the loci, we used genetic data of the same 14 microsatellite loci analyzed for the both samples. First, we looked for evidence of the genetic differences between the JARPNII and commercial whaling samples collected from similar longitudinal area (165.01°E -170.00°E and 165.00°E -180.00°) for the three cases (all and female and male separately). No evidence of genetic differences was detected between the two samples for all the cases after the correction for multiple tests in both genetic markers (Table 5). For the subsequent analysis, we combined these two samples into one. Next, we conducted heterogeneity tests among the five longitudinal samples (143.00°E-155.00°E, 155.01°E -165.00°E, 165.00°E-180.00°, 180.00°-150.01°W, 150.00°W -139.00°W) for all and female and male separately. No evidence of genetic differences was detected among the five samples after the correction for multiple tests in the both genetic markers (Table 6). Finally, we conducted heterogeneity tests between the two samples divided at 180° for all and female and male separately. We separated the samples at 180° because it was raised as a potential stock boundary according to Donovan (1991). Again, no evidence of the genetic differences was detected between the two samples after the correction for multiple tests for the three cases in the both genetic markers (Table 6).

Bayesian clustering analysis conducted without information on geographic sampling origins presented the lowest likelihood probability at K = 3 and the highest at K = 1 (Table 7).

Phylogenetic analysis

Fig. 2 shows the neighbor-joining tree of the mtDNA haplotypes of the North Pacific sei whales. Closed circles indicate those nodes for which bootstrap values were above 50% in 1,000 simulations. Some nodes are supported by relatively high bootstrap values. The haplotypes represented in these nodes were distributed in more than one locality. The tree appeared not to be informative on the stock structure

because no spatially or temporally unique cluster was detected.

DISCUSSION

Genetic diversity

Even though all of the microsatellite primers we used were not designed from sei whales, amplifications were successfully accomplished. The levels of allelic diversity and heterozygosity were compatible to those species used for primer design (Buchanan *et al*., 1996; Valsecchi and Amos, 1996; Palsbøll *et al*., 1997; Bérubé *et al*., 1998, 2000) and others reported (Bérubé *et al*., 2000; Kanda *et al*., 2007). Some of the loci, however, showed low levels of genetic diversity in our samples compared to that in the other whale species from different studies. Possibility of null alleles is less likely because no signal of such case was detected (e.g. Hardy-Weinberg genotypic proportions). The negative effect on genetic diversity at a population level is also excluded because the low diversity is not consistent over the 17 loci. The reason could be due to the use of the heterologus loci. The mtDNA diversity within the sei whale samples in this study was also comparable to that of other whale species (Kanda *et al*., 2007; Pastene *et al*., 2007).

In the western North Pacific off Japanese coast, sei whales were subjected to commercial whaling for a long time until 1975 when it was ended (Horwood, 1987). Over a few hundreds of sei whales were caught every year throughout the commercial whaling era (Horwood, 2002). Decline of abundance can cause loss of the genetic diversity (i.e., genetic bottleneck). Kanda *et al*. (2006) statistically tested the effect of genetic bottleneck using the 2002 and 2003 JARPNII samples, but failed to demonstrate that the sei whales in the western North Pacific has been subjected to a large decline in stock size. The JARPNII samples from 2003 to 2007 showed the same level of genetic diversity (Appendix 1). Same level of genetic diversity was also detected from the JARPNII and commercial whaling samples. Although not as quite statistically sophisticated as the previous study, therefore, observation from this simple comparison of the observed levels of the genetic diversity within the JARPNII as well as between the JARPNII and commercial whaling samples supported the previous conclusion. From genetics perspective, past commercial whaling appeared to have no negative effect on the stock and thus the North Pacific sei whales are far from immediate local extinction.

Stock structure

In this study, the sample size for describing stock structure of sei whales in the North Pacific was increased substantially from the previous genetic study (Kanda *et al*., 2006) and geographic covering of the samples was extended considerably to eastward (143°E-139°W) by using the commercial whaling samples in addition to the JARPNII ones. This study is also first one that utilized mtDNA sequencing analysis on the North Pacific sei whales. We then demonstrated that the offshore open water of the North Pacific was occupied mainly by the individuals of the single stock of sei whales. We believe the results of this study provide valuable information not only about the biology of sei whales but also for effective management of this species as a resource in the area.

Addition of the 400 more sei whales from the 2004-2007 JARPNII surveys for the genetic analyses did not change the results observed in Kanda *et al*. (2006) that used only the 2002 and 2003 JARPNII samples. Sequencing analysis of the mtDNA control region also showed no evidence of the genetic heterogeneity in the JARPNII samples. The previous and current genetic studies thus strongly indicated that the JARPNII samples came from a genetically same stock of sei whales in the western North Pacific.

Although total number of the individuals was substantially increased for the JARPNII samples, some could still argue that it might be a little premature to conclude that only a single stock occupies the western North Pacific. This is because the JARPNII research area covered only a small part of sei whales' distribution in the North Pacific. Although it was from the limited data set, Masaki (1976, 1977) proposed the existence of three stocks of sei whales with the stock boundaries at 174°W and 155°W in the North Pacific on the basis of the observations that a frequency distribution of the historical catches and east-west movement observed from mark-recapture studies during past commercial whaling period was not constant over the North Pacific (reviewed in Horwood 1987; but also see Donovan, 1991). Similar to the gaps seen in Fig.1, accumulated number of catches of sei whales over 20 years from 1952 to 1972 was relatively low around 175°W and 150°W compared to other areas. Similar pattern was also observed in a frequency distribution of the number of marked whales crossing a particular longitude. In order to test if this pattern is created by patchy distributions of the genetically different stocks, we also analyzed the archived sei whale sample of past commercial whaling operated in the North Pacific between 165°E-139°W. This addition of the historical sample to the JARPNII sample allowed us to investigate the stock structure of sei whales over the area from 143°E to 139°W. Both conventional

hypothesis testing and Bayesian clustering method conducted in this paper then failed to detect evidence of the existence of multiple stocks in the North Pacific. Although it was based on three polymorphic allozyme loci, Wada and Numachi (1991) already reported the lack of temporal and spatial genetic heterogeneity in samples of the North Pacific sei whales (632) collected from past commercial whaling in 1974 and 1975. Heterogeneous distributions of sei whales observed from the catch history and marking data therefore could simply reflect favored feeding sites and do not necessary indicate existence of multiple stocks. In fact, it is known that oceanographic environment (e.g., limit of Kuroshio extension, flow pattern of Alaska gyre, etc.) and oceanic geography (Emperor Seamount Chain) influence distributions and variety of prey species around the 174°W and 155°W (e.g., Kawamura, 1982; Bakun, 2006; Gregr and Bodtker, 2007; Murase *et al*., 2009), limiting east-west movement of sei whales in the feeding ground.

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REFERENCES

Árnason, Ú., Gullberg, A., and Widegten, B. 1993. Cetacean mitochondrial DNA control region: Sequences of all extant baleen whales and two sperm whale species. *Mol. Biol. Evol.* 10:960-970.

Bakun, A. 2006. *Wasp-waist* populations and marine ecosystem dynamics: navigating the "*predator pit*" topographies. *Progr. Oceanogr*. 68:271-288.

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo-di-Sciara, G., Sears, R., Sigurjónsson, J., Urban-Ramirez, J., and Palsbøll, P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus, 1758); analysis of mitochondrial and nuclear loci. *Mol. Ecol.* 7:585-599.

Bérubé, M., Jørgensen, H., Mcewing, R., and Palsbøll, P.J. 2000. Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeanglliae*. *Mol. Ecol.* 9:2181-2183.

Buchanan, F.C., Friesen, M.K., Littlejohn, R.P., and Clayton, J.A. 1996. Microsatellites from beluga whale *Delphinapterus leucas*. *Mol. Ecol.* 5:571-575.

Donovan, G.P. 1991. A review of IWC stock boundaries. *Rep. int. Whal. Commn. (special issue 13)* :39-68.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) 3.5c. University of Washington, Seattle.

Goudet, J. 1995. FSTAT, version 1.2: a computer program to calculate F-statistics. *J. Hered.* 86:485-486.

Gregr, E.J. and Bodtker, K.M. 2007. Adaptive classification of marine ecosystems: identifying biologically meaningful regions in the marine environment. *Deep. Sea. Res.* 54:385-402.

Horwood, J. 1987. *The sei whale: population biology, ecology and management.* London: Croom Helm.

Horwood, J. 2002. Sei whales. In: *Encyclopedia of Marine Mammals*, Perrin, W.F., Würsig, B., and Thewissen, J.G.M. (eds.). San Diego, CA: Academic Press.

Kanda, N., Goto, M., and Pastene, L.A. 2006. Genetic characteristics of western North Pacific sei whales, *Balaenoptera borealis*, as revealed by microsatellites. *Mar. Biotechnol.* 8:86-93.

Kanda, N., Goto, M., Kato, H., McPhee, M.V., and Pastene, L.A. 2007. Population genetic structure of Bryde's whales (*Balaenoptera brydei*) at the inter-oceanic and trans-equatorial levels. *Cons. Genet.* 8:853-864.

Kawamura, A. 1982. Food habits and prey distributions of three rorqual species in the North Pacific ocean. *Sci. Rep. Whales Res. Inst.* 34:59-91.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111-120.

Masaki, Y. 1976. Biological studies on the North Pacific sei whale. *Bull. Far. Seas. Fish. Res. Lab.* 14:1-104.

Masaki, Y. 1977. The separation of stock units of sei whales in the North Pacific. *Rep. int. Whal. Commn. (special issue 1)* :71-79.

Murase, H., Hakamada, T., Kiwada, H., Inagake, D., Okazaki, M., Tojo, N. and Matsuoka, K. 2009. Preliminary results of prediction of sei whale (*Balaenoptera borealis*) distribution and abundance in

the whole North Pacific basin. Appendix 2 10pp. *In*: Hakamada, T. Re-assessment of the effect on the stocks of JARPN II catches. Paper SC/J09/JR36 presented to the JARPN II review workshop, Tokyo, January 2009 (unpublished).
Nei, M. 1987. *Molecular Evolutionary Genetics.* New York: Columbia University Press.
Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Compu. Appl. Biosci.* 12**:**357-358.
Palsbøll, P.J., Bérubé, M., Larsen, A.H., and Jørgensen, H. 1997. Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. *Mol. Ecol.* 6:893-895.
Pastene, L.A., Goto, M., Kanda, N., Zerbini, A.N., Kerem, D., Watanabe, K., Bessho, Y., Hasegawa, M., Nielsen, R., Larsen, F., Palsboll, P.J. 2007. Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata*. *Mol. Ecol.* 16: 1481-1500.
Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
Rice, D.W. 1998. *Marine mammals of the world: systematics and distribution.* Lawrence, KS: Society for Marine Mammalogy.
Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.
Roff, D.A. and Bentzen, P. 1989. The statistical analysis of mtDNA polymorphisms: χ^2 and the problem of small samples. *Mol. Biol. Evol.* 6:539-545
Rousset, F. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molec. Ecol. Resources* 8:103-106.
Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular cloning: A laboratory manual. 2nd Ed*., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
Tamura, T., Matsuoka, K., and Fujise, Y. 2009. Methodology and survey procedure under the JARPN II - offshore component- with special emphasis on whale sampling procedures. Paper SC/J09/JR4 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 16pp.
Valsecchi, E., and Amos, W. 1996. Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* 5:151-156.
Wada, S., Oishi, M., and Yamada, T. 2003. A newly discovered species of living baleen whale. *Nature* 426:278-281.
Wada, S., and Numachi, K. 1991. Allozyme analyses of genetic differentiation among the populations and species of the *Balaenoptera*. *Rep. int. Whal. Commn. (special issue 13)* :125-154.

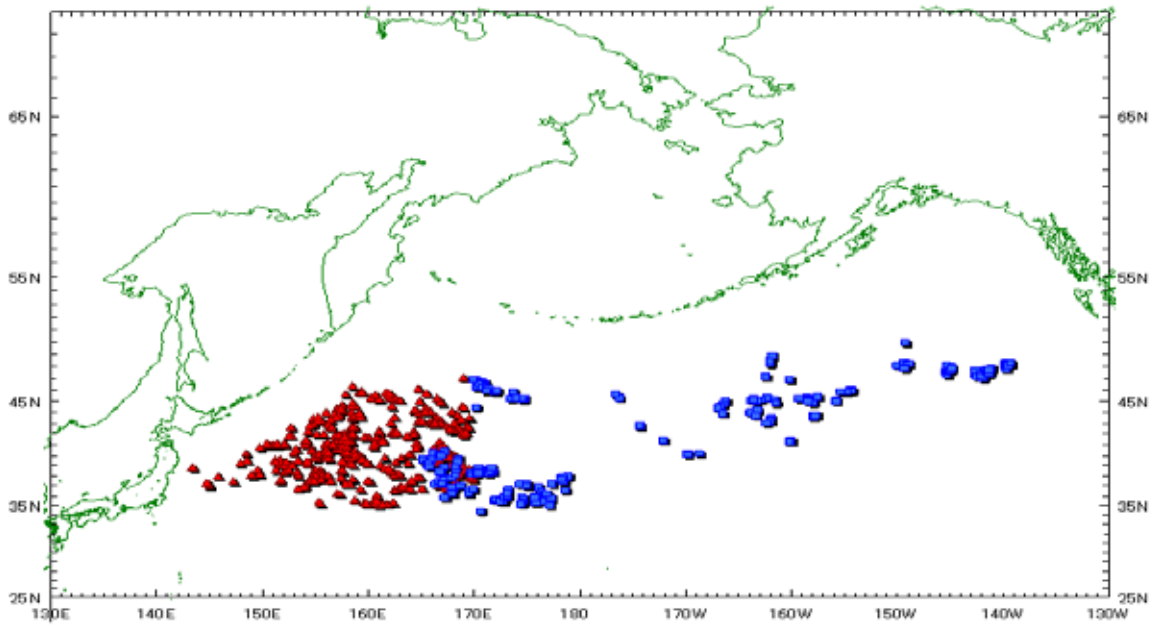

Fig. 1. Sampling locations of sei whales in the North Pacific.
Triangle: JARPNII, Square: commercial whaling

Fig. 2 The neighbor-joining tree of mtDNA haplotype of North Pacific sei whales.
Closed circles indicate nodes with bootstrap values above 50% in 1,000 simulations. An end code of each of the haprotypes represents: S = shared haplotypes between JARPNII and commercial whaling samples, J = unique haplotypes in the JARPNII samples, C = unique haplotypes in commercial whaling samples.

Table 1. Collection year, survey period, total sample size (N), and longitudinal subdivision of the individuals in the samples collected from JARPNII and past commercial whaling.

Year	Survey period	N	Longitudinal sample groupings*								
			All			Female			Male		
JARPNII (143°E - 170°E)			-155E	-165E	-170E	-155E	-165E	-170E	-155E	-165E	-170E
2002	July - September	39	2	37	0	1	23	0	1	14	0
2003	May - August	50	14	33	3	9	17	1	5	16	2
2004	June - September	100	0	45	55	0	26	27	0	19	28
2005	May - August	100	21	56	23	12	28	9	9	28	14
2006	June - August	100	29	46	25	14	24	14	15	22	11
2007	May - August	100	20	63	17	9	27	10	11	36	7
			All			Female			Male		
Commercial (165°E - 139°W)			-180E	-150W	-139W	-180E	-150W	-139W	-180E	-150W	-139W
1972	June - August	27	11	16	0	8	8	0	3	8	0
1973	May - August	274***	169	41	64	84**	21**	26	85	20**	38

*Based on sighting positions for the JARPNII samples and collecting position for the commercial whaling samples.
**Sample size in the mtDNA analyses was one individual fewer than microsatellite analysis
***Sample size in the mtDNA analyses was three individuals fewer than microsatellite analysis

Table 2. The number of alleles (A), expected heterozygosity (He), and test result for expected Hardy-Weinberg genotypic proportions (HW) at 17 microsatellite loci, haplotype number, haplotype diversity, and nucleotide diversity (standard error) at mtDNA analyzed in the JARPNII and commercial whaling samples of sei whales. n.s. = not significant, n.a.= not available.

	JARPNII			Commercial		
Microsatellites	A	He	HW	A	He	HW
DlrFCB17	18	0.872	n.s.	20	0.889	n.s.
EV1	15	0.834	n.s.	17	0.835	n.s.
EV14	23	0.856	n.s.	n.a.	n.a.	n.a
EV21	6	0.621	n.s.	6	0.653	n.s.
EV94	6	0.684	n.s.	6	0.673	n.s.
EV104	7	0.724	n.s.	8	0.723	n.s.
GATA28	11	0.810	n.s.	11	0.819	n.s.
GATA53	3	0.480	n.s.	3	0.501	n.s.
GATA98	7	0.733	n.s.	n.a.	n.a.	n.a.
GATA417	11	0.777	n.s.	n.a.	n.a.	n.a.
GGAA520	10	0.797	n.s.	9	0.805	n.s.
GT23	12	0.604	n.s.	11	0.606	n.s.
GT011	4	0.441	n.s.	4	0.449	n.s.
GT211	6	0.312	n.s.	6	0.320	n.s.
GT271	4	0.133	n.s.	3	0.129	n.s.
GT310	4	0.511	n.s.	5	0.471	n.s.
GT575	5	0.592	n.s.	5	0.598	n.s.
average	8.9	0.634		8.1	0.605	
mtDNA						
Haplotype number	81			54		
Haplotype diversity	0.929			0.915		
Nucleotide diversity	0.0078			0.0080		
(standard error)	(0.0001)			(0.0002)		

Table 3. Results (p-values) of the heterogeneity tests among the JARPNII samples collected from different survey years within the same longitudinal areas (left) and among the samples collected from the different longitudinal areas (right). See text for longitudinal subdivision.

	Samples from different years within the same areas									Samples from different areas		
	All			Female			Male					
	-155E	-165E	-170E	-155E	-165E	-170E	-155E	-165E	-170E	All	Female	Male
Microsatellites												
DlrFCB17	0.433	0.230	0.943	0.294	0.252	0.997	0.715	0.794	0.271	0.781	0.234	0.124
EV1	0.491	0.119	0.921	0.657	0.322	0.937	0.781	0.112	0.896	0.585	0.652	0.741
EV14	0.078	0.919	0.807	0.143	0.174	0.669	0.284	0.918	0.297	0.449	0.559	0.543
EV21	0.917	0.199	0.633	0.816	0.036	0.784	0.317	0.372	0.625	0.377	0.284	0.417
EV94	0.845	0.090	0.158	0.377	0.291	0.831	0.125	0.130	0.008	0.499	0.511	0.341
EV104	0.973	0.752	0.689	0.840	0.578	0.364	0.738	0.755	0.243	0.632	0.754	0.610
GATA28	0.070	0.787	0.250	0.029	0.088	0.274	0.266	0.646	0.298	0.074	0.258	0.398
GATA53	0.674	0.171	0.532	0.996	0.177	0.588	0.466	0.023	0.452	0.171	0.366	0.322
GATA98	0.553	0.531	0.013	0.790	0.201	0.456	0.598	0.921	0.019	0.628	0.554	0.597
GATA417	0.431	0.715	0.201	0.777	0.665	0.507	0.359	0.476	0.374	0.870	0.823	0.725
GGAA520	0.068	0.248	0.673	0.740	0.599	0.831	0.018	0.058	0.305	0.506	0.594	0.704
GT23	0.190	0.300	0.052	0.132	0.083	0.193	0.854	0.440	0.044	0.777	0.084	0.979
GT011	0.560	0.173	0.273	0.769	0.633	0.852	0.019	0.238	0.127	0.124	0.020	0.112
GT211	0.505	0.894	0.118	0.329	0.916	0.191	0.890	0.225	0.112	0.318	0.490	0.823
GT271	0.333	0.217	0.825	0.446	0.098	0.737	0.526	0.210	1.000	0.243	0.224	0.906
GT310	0.896	0.340	0.217	0.737	0.215	0.244	0.929	0.133	0.949	0.319	0.813	0.023
GT575	0.374	0.153	0.211	0.697	0.183	0.623	0.045	0.485	0.342	0.192	0.071	0.187
mtDNA	0.226	0.132	0.884	0.206	0.321	0.707	0.776	0.891	0.682	0.085	0.158	0.263

Table 4. Results (p-values) of the heterogeneity tests among the commercial whaling samples from the different longitudinal areas. See text for the longitudinal subdivisions of the samples.

	All	Female	Male
Microsatellites			
DlrFCB17	0.612	0.466	0.759
EV1	0.981	0.552	0.464
EV21	0.723	0.803	0.635
EV94	0.448	0.959	0.099
EV104	0.355	0.212	0.416
GATA28	0.585	0.631	0.613
GATA53	0.882	0.973	0.596
GGAA520	0.682	0.830	0.333
GT23	0.257	0.472	0.266
GT011	0.201	0.565	0.365
GT211	0.792	0.421	0.901
GT271	0.165	0.136	0.349
GT310	0.760	0.679	0.767
GT575	0.425	0.901	0.171
mtDNA	0.862	0.175	0.906

Table 5. Results (p-values) of the heterogeneity tests between the JARPNII and commercial whaling samples collected from the area of between 165°E-180°.

	All	Female	Male
Microsatellites			
DlrFCB17	0.153	0.095	0.069
EV1	0.021	0.027	0.482
EV21	0.616	0.773	0.841
EV94	0.984	0.392	0.305
EV104	0.496	0.539	0.580
GATA28	0.085	0.254	0.293
GATA53	0.230	0.687	0.218
GGAA520	0.350	0.748	0.026
GT23	0.212	0.455	0.200
GT011	0.228	0.163	0.391
GT211	0.297	0.571	0.171
GT271	0.962	0.258	0.533
GT310	0.135	0.344	0.023
GT575	0.097	0.838	0.066
mtDNA	0.276	0.066	0.526

Table 6. Results (p-values) of the heterogeneity tests among the JARPNII and commercial whaling samples collected from the five different longitudinal areas (left) and from west and east of 180° (right). See text for the longitudinal subdivision of the samples.

	5 different areas			East and west of 180°		
	All	Female	Male	All	Female	Male
Microsatellites						
DlrFCB17	0.820	0.399	0.742	0.303	0.130	0.733
EV1	0.954	0.530	0.719	0.323	0.013	0.105
EV21	0.422	0.593	0.291	0.450	0.869	0.127
EV94	0.435	0.972	0.164	0.755	0.824	0.600
EV104	0.507	0.703	0.521	0.237	0.807	0.179
GATA28	0.498	0.707	0.547	0.479	0.971	0.484
GATA53	0.692	0.829	0.643	0.760	0.911	0.413
GGAA520	0.725	0.873	0.703	0.828	0.805	0.705
GT23	0.595	0.085	0.815	0.684	0.494	0.632
GT011	0.198	0.279	0.203	0.113	0.595	0.259
GT211	0.885	0.474	0.986	0.283	0.277	0.503
GT271	0.137	0.148	0.725	0.854	0.953	0.541
GT310	0.584	0.912	0.130	0.117	0.960	0.104
GT575	0.741	0.405	0.401	0.644	0.806	0.535
mtDNA	0.179	0.157	0.540	0.313	0.080	0.802

Table 7. Estimated posterior probability of number of stocks (K) for the pooled samples of sei whales collected from JARPNII and past commercial whaling in the North Pacific computed using STRUCTURE.

K	Log P (x/k)	s.d.	Probability
1	-28392.7	52.6	~1.0
2	-28801.6	1151.2	~0.0
3	-29868.3	3287.5	~0.0

Appendix 1. The number of alleles (A), allelic richness (RA), and expected heterozygosity (He) at 17 microsatellite loci, haplotype number, haplotype diversity, and nucleotide diversity (standard error) at mtDNA analyzed in the JARPNII samples of sei whales collected from 2002 to 2007.

	2002			2003			2004			2005			2006			2007		
Microsatellites	A	RA	He	A	RA	He	A	RA	He	A	RA	He	A	RA	He	A	RA	He
DlrFCB17	15	15.0	0.868	13	12.2	0.861	18	15.4	0.884	17	14.3	0.863	14	12.6	0.868	15	13.4	0.879
EV1	12	12.0	0.807	14	13.1	0.810	14	12.3	0.834	14	12.3	0.819	14	11.5	0.844	15	13.1	0.858
EV14	14	14.0	0.869	15	13.6	0.855	16	13.4	0.855	16	12.6	0.856	16	13.7	0.855	16	13.0	0.865
EV21	6	6.0	0.638	5	5.0	0.634	6	5.4	0.639	5	4.6	0.605	6	5.4	0.609	6	5.3	0.619
EV94	6	6.0	0.613	6	5.8	0.704	6	5.9	0.689	6	5.9	0.719	6	5.8	0.669	6	5.6	0.673
EV104	5	5.0	0.747	6	5.8	0.731	6	5.4	0.715	7	6.0	0.738	6	5.3	0.721	5	4.9	0.712
GATA28	10	10.0	0.822	8	8.0	0.799	9	8.8	0.829	9	8.2	0.809	9	7.9	0.781	9	8.3	0.821
GATA53	3	3.0	0.351	3	3.0	0.469	3	3.0	0.484	3	3.0	0.464	3	3.0	0.516	3	3.0	0.509
GATA98	6	6.0	0.728	7	6.8	0.763	6	5.8	0.725	6	5.9	0.729	6	5.9	0.715	6	6.0	0.742
GATA417	8	8.0	0.765	9	8.5	0.774	9	7.7	0.781	9	7.8	0.773	8	7.3	0.780	9	7.9	0.788
GGAA52	8	8.0	0.796	7	6.8	0.794	10	8.5	0.806	9	7.5	0.758	8	7.9	0.816	9	8.1	0.805
GT23	6	6.0	0.493	9	8.6	0.561	8	6.2	0.560	11	8.6	0.680	11	8.7	0.603	9	7.8	0.624
GT011	4	4.0	0.471	4	3.8	0.386	4	3.6	0.398	4	3.9	0.461	4	3.8	0.550	3	3.0	0.356
GT211	4	4.0	0.331	4	3.8	0.343	5	4.0	0.297	4	3.6	0.290	6	4.6	0.302	5	4.0	0.341
GT271	3	3.0	0.146	2	2.0	0.040	3	2.6	0.141	3	2.9	0.167	3	2.4	0.105	4	3.3	0.160
GT310	3	3.0	0.388	3	3.0	0.461	3	3.0	0.519	3	3.0	0.487	4	3.4	0.553	4	3.4	0.550
GT575	4	4.0	0.561	4	4.0	0.613	4	4.0	0.594	5	4.4	0.590	5	4.5	0.582	5	4.2	0.591
average	6.9	6.9	0.611	7.0	6.7	0.623	7.6	6.8	0.632	7.7	6.7	0.636	7.6	6.7	0.639	7.6	6.7	0.641
mtDNA																		
Haplotype number		19			20			34			31			35			36	
Haplotype diversity		0.919			0.908			0.941			0.937			0.931			0.918	
Nucleotide diversity		0.0088			0.0076			0.0079			0.0083			0.0076			0.0068	
(standard error)		(0.0006)			(0.0005)			(0.0003)			(0.0003)			(0.0003)			(0.0004)	