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Distribution and movement of 'O' and 'J' stock common minke whales in waters around Japan based on genetic assignment methods

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ABSTRACT

A total of 4,275 western North Pacific common minke whales were examined with a set of 16 microsatellite DNA loci and the program STRUCTURE to assign individuals to either O or J stocks. Samples were available from JARPN/JARPNII (1994–2014; n=2,637), and by-catches (2001–2014; n=1,638), from waters around Japan. Results of the Bayesian clustering analysis confirmed that the whales came from two genetically differentiated stocks, O and J stocks. By using 16 loci, more than 90% of the individual whales were assigned to either stock. Almost all of the individuals collected from the Sea of Japan side belonged to the J stock, whereas almost all of the individuals from the offshore North Pacific belonged to the O stock. Intermediate areas contained individuals from both stocks. The southern part of the Pacific side of Japan was mainly occupied by the J stock, which predominated (around 80% in proportion) throughout the year. In the north part of the Pacific side of Japan the proportion of the J stock animals increased in autumn/winter and decreased in spring/summer, and the O stock showed a reverse pattern.

INTRODUCTION

In the western North Pacific at least two biological stocks of common minke whales *Balaenoptera acutorostrata* are known to exist: the Okhotsk Sea-West Pacific (O stock) and the Sea of Japan-Yellow Sea-East China Sea (J stock) (Omura and Sakiura, 1956; Ohsumi, 1977; 1983). The two stocks are differentiated in morphological and reproductive characters (Omura and Sakiura, 1956; Ohsumi, 1977; Kato, 1992), as well in genetics (Wada and Numachi, 1991 for allozymes; Goto and Pastene, 1997 for mtDNA; and Kanda *et al.*, 2009a; b for microsatellites), suggesting their reproductive isolation. The International Whaling Commission (IWC) had proposed some boundaries for these stocks (Donovan, 1991, Figure 1).

Previous genetic studies showed that both stocks mix with each other spatially and temporally in the southern part of the Okhotsk Sea (northern Hokkaido) (Wada, 1991; Pastene *et al.*, 1998). Since then, a substantial number of genetic samples of western North Pacific common minke whale became available, and modern and more powerful genetic markers became available in recent years. The application of such markers to the new samples made finer studies of stock structure of this species possible for this ocean basin (Pastene *et al.*, 2016a; b).

There are several analytical approaches for estimating

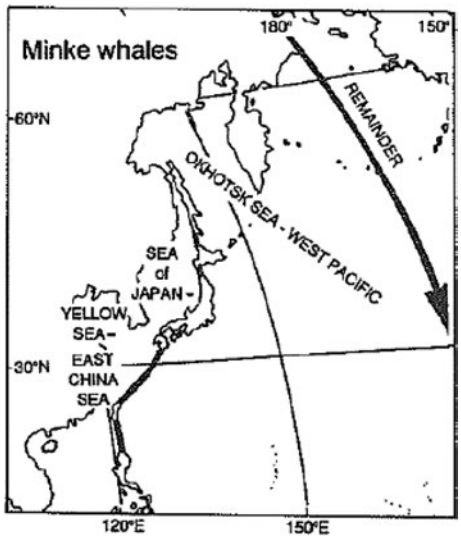

Figure 1. Historical IWC stock boundaries of western North Pacific common minke whale (Donovan, 1991).

the mixing proportion in areas where two or more stocks overlap, based on genetic data. One of those is the frequency-based approach (e.g. Pastene *et al.*, 1998). Under this method the allele or haplotype frequency distribution for regions where only a single stock is considered to be present is determined first ('pure stock' frequencies). Then the proportion of each stock is estimated in overlap

areas by Maximum Likelihood Methods. The other is the individual assignment-based approach, which does not need 'pure stock' assumptions and is based on minimizing departures from Hardy-Weinberg equilibrium (e.g. Pritchard *et al.*, 2000).

The objective of this study was to gain further understanding of the spatial and temporal distribution of the O and J stocks around Japan by using an individual assignment-based approach on the large number of genetic samples of this species collected in waters around Japan.

MATERIALS AND METHODS

Sample collections

To describe the distribution and movement of stocks a total of six Areas were defined for waters around Japan: Areas A=southern part of the Okhotsk Sea; Ba=coastal in the Pacific side off Hokkaido; Bb=coastal in the northern part of the Pacific side of Japan; C=offshore Area in the Pacific side; D=coastal in the southern part of the Pacific side of Japan; and E=coastal Area in the Sea of Japan side (Figure 2). Sample sizes used in this study are shown in Table 1, by Area.

Offshore samples were from the Japanese Whale Research Program under Special Permit in the western North Pacific (JARPN/JARPNII) surveys from 1994 to 2013 in Areas A, Ba, Bb and C. Common minke whale samples obtained from the coastal JARPNII survey between 2002 and 2014 were also used in this study, Kushiro in Area Ba and Sanriku in Area Bb. Samples from bycaught whales in set net fisheries along the Japanese coast from 2001 to 2014 were also used. The bycatches were from Areas A, Ba, Bb, D and E year-round.

DNA extraction

The IWC guidelines for DNA data quality (IWC, 2009) were followed as much as possible (see Kanda *et al.*, 2014). Genomic DNA was extracted from 0.05 g of skin or muscle tissues using standard proteinase K, phenol-chloroform procedure described by Sambrook *et al.* (1989) or using Gentra Puregene kits (QIAGEN). Extracted DNA was stored in the TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Microsatellites

Microsatellite polymorphisms were analyzed using 16 loci: EV1, EV14, EV21, EV37, EV94, (Valsecchi and Amos, 1996), GT23, GT195, GT211, GT310, GT509, GT575 (Bérubé *et al.*, 2000), GATA28, GATA98, GATA417, TAA31 (Palsbøll *et al.*, 1997), DlrFCB14 (Buchanan *et al.*, 1996). EV1, EV14, EV21 were developed from sperm whale, EV37, EV94, GT23, GT310, GT575, GATA28, GATA98, GATA417, TAA31 from humpback whale, and DlrFCB14 from beluga whale. All GT, EV and DlrFCB primers are

Table 1
Sample size used in this study, by Area.

Area	Number of samples
A	128
Ba	1066
Bb	921
C	942
D	535
E	683
Total	4275

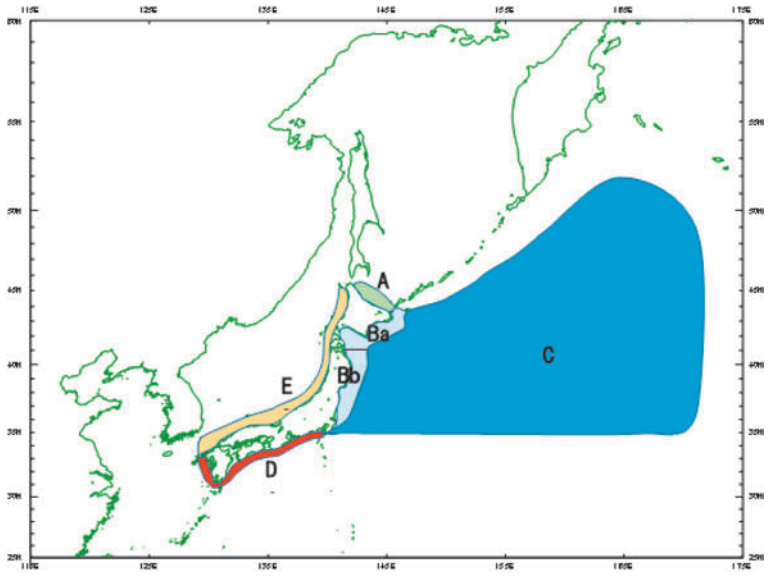

Figure 2. Geographical location of each Area defined for this study.

dinucleotide repeats, TAA31 trinucleotide repeats, and all GATA primers tetranucleotide repeats. Primer sequences and PCR profiles follows those of the original authors with slight modifications.

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), and 2 mM of each dNTP, and 10x reaction buffer containing 20 mM $MgCl_2$ (Takara Shuzo). PCR amplifications followed the manufacturer's instructions for the use of Ex *Taq* DNA polymerase (Takara Shuzo). Amplified products with internal size standard (GENESCAN400HD, Applied Biosystems Japan) were run on a 6% polyacrylamide denaturating gel (Long Ranger™) using a BaseStation TM100 DNA fragment analyzer (Bio-Rad) or were electrophoresed on an Applied Biosystems 3500 Genetic Analyzer. Allele sizes were determined using a 600 LIZ size standard and GeneMapper v. 5.0 (ABI).

Data analysis

The Bayesian clustering approach was implemented with the microsatellite data in the program STRUCTURE version 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 for the analyses of the samples without choosing sample units that did not necessarily correspond to real biological stock boundaries. A conceptual diagram of individual assignment under STRUCTURE is shown in Figure 3.

Posterior probabilities for K were estimated from ten independent runs for each value of K from one to five with only genetic information. These data were calculated based on burn-in period of 10,000 iterations and runs of 100,000 iterations. Individual assignment was then conducted for the most plausible K using the estimated individual proportion of membership probability (90%). The ancestry model 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.

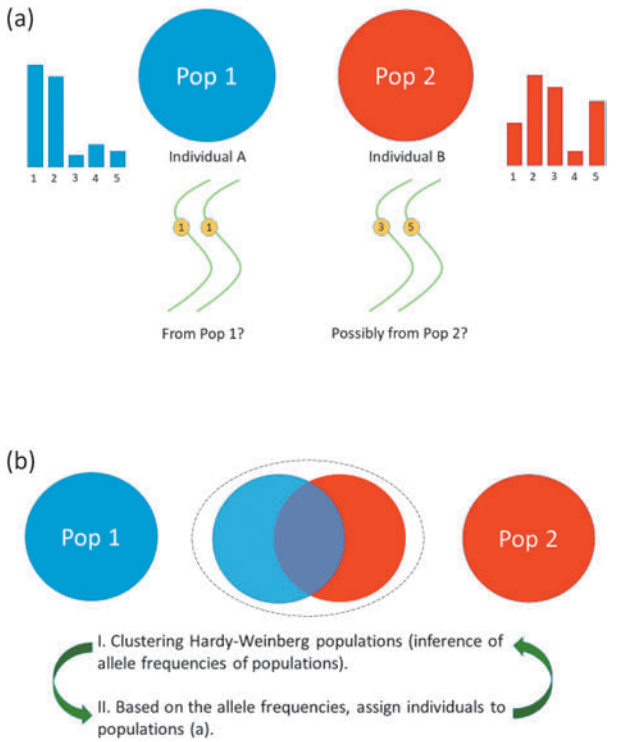

Figure 3. Basic concept of individual assignment: (a) with allele frequency at single loci (bar plot in this figure) in each source population. In this case, it is highly possible that individual A originates from population 1 since it has two alleles that are major in population 1. In contrast, individual B is likely to come from population 2 since it has two alleles that are minor in population 1; (b) with no allele frequency in each source population. In this case, genotypes at multiple loci in each individual enable estimation of allele frequency for the source population and an assignment probability for each individual by repeating the following steps: (I) estimation of allele frequency from tentative clustering in Hardy-Weinberg equilibrium, and (II) individual assignment based on the tentative allele frequency according to the concept of Figure 3a.

RESULTS

Genetic assignment

Bayesian clustering analyses conducted on the total samples (4,275 individuals) without information on their geographic origins presented the highest likelihood probability at K=2 (Table 2). These results confirmed that the samples came from two genetically distinct stocks of common minke whales (O and J stocks). In this study, the individuals with the membership probability of over 90% for either of the two stocks at each of the runs were assigned as pure individuals. All other individuals with the membership probability of less than 90% to the either groups were 'unassigned'.

Spatial distribution of O and J stocks along the Japanese coast

Both the stock-assigned and unassigned individuals were

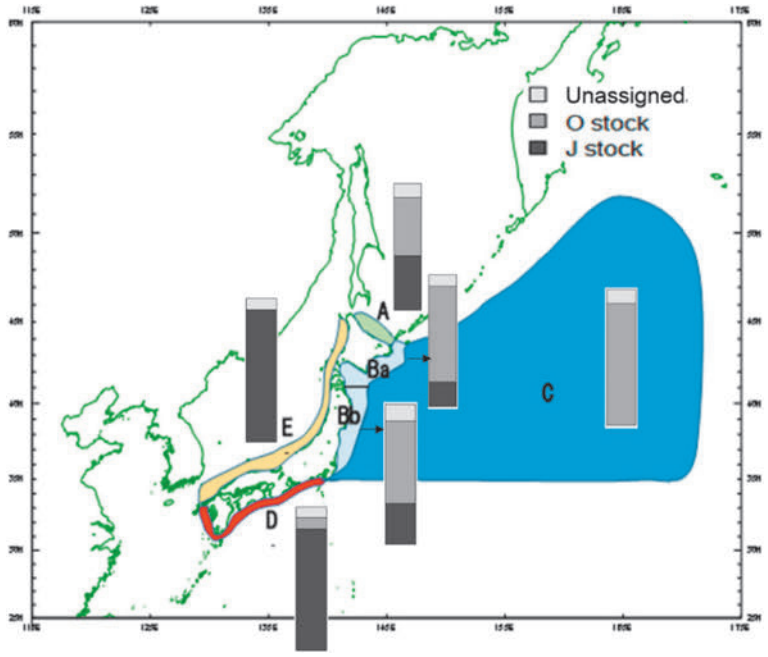

Figure 4. Spatial occurrence of O and J stocks common minke whale in waters around Japan.

Table 2

Results of the Bayesian clustering method analyzed for overall samples.

K	Log P (k/x)	Variance	Pr (k/x)
1	−210753.0	85.2	~0.0
2	−202085.7	873.1	~1.0
3	−203026.1	3693.0	~0.0
4	−202960.7	4761.0	~0.0
5	−204283.6	8075.9	~0.0

grouped based on the defined Areas (Figure 4). Almost all of the individuals collected from the Sea of Japan side (Area E) belong to the J stock, whereas almost all of the individuals from the offshore North Pacific (Area C) belong to the O stock. Area D (southern part of the Pacific side of Japan) was mainly occupied by the J stock. Areas A (northern Hokkaido) and Ba and Bb (northern part of the Pacific side of Japan) represent areas where both stocks overlap geographically.

Temporal distribution of O and J stocks along the Pacific coast of Japan

Figure 5 shows the temporal distribution of the O and J stocks in Areas Ba, Bb and D in the Pacific side of Japan, expressed as three months moving average. In Area D, the J stock was predominant (around 80% in proportion) throughout the year. In Areas Ba and Bb the proportion of the J stock increased in autumn/winter and decreased in spring/summer. Conversely the proportion of O stock decreased in autumn/winter and increased in spring/summer.

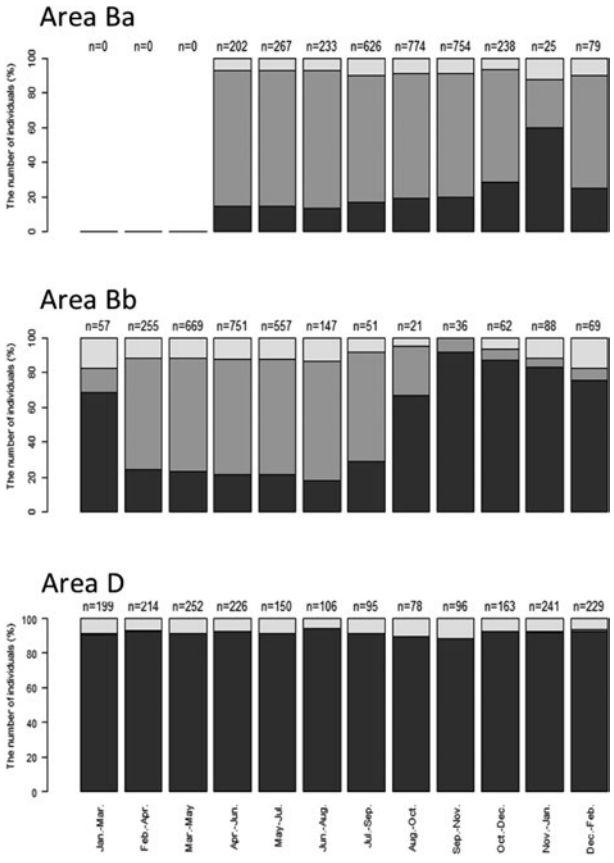

Figure 5. Monthly occurrence of O and J stocks in Areas Ba, Bb and D in the Pacific side of Japan. Each bar is expressed as three months moving average. Sample size is on the top of each bar. The sampling years in Areas Ba and Bb were 1994–2014. In Area D sampling years were 2001–2014.

DISCUSSION

Assignment methods based on genetic data

There are several methods for assigning individuals to source populations. Dawson and Belkhir (2001) developed a maximum likelihood method for assigning the individuals in a sample to source populations, on the basis of their genotypes at co-dominant marker loci. Cornuet *et al.* (1999) used a partially Bayesian exclusion test that uses an exclusion simulation method in the Geneclass software. Mantel *et al.* (2002) showed that the fully Bayesian assessment test of Prichard *et al.* (2000) performed better than the partially Bayesian exclusion test of Cornuet *et al.* (1999). However they recognized that the fully Bayesian method required the assumption that the true population origin was sampled. Given this background the fully Bayesian clustering approach of Pritchard *et al.* (2000), implemented in the STRUCTURE program, was used in this study to assign individuals to either O and J stocks, which are highly differentiated genetically.

Temporal and spatial distribution of O and J stocks

Results of the Bayesian clustering analysis confirmed that the whales came from two genetically differentiated stocks, O and J stocks. By using 16 loci, more than 90% of the individual whales were assigned to either stock. Almost all of the individuals collected from the Sea of Japan side (Area E) belonged to the J stock, whereas almost all of the individuals from the offshore North Pacific (Area C) belonged to the O stock. Intermediate areas (Areas A, Ba and Bb) contained individuals from both stocks. In Area D the J stock was predominant (around 80% in proportion) throughout the year. In Areas Ba and Bb the proportion of the J stock increased in autumn/winter and decreased in spring/summer.

The fact that the J stock is distributed in Area D throughout the year suggests that the Kuroshio Current, which is one of the strongest west-boundary currents of the subtropical gyre, is working as the stock boundary between O and J stocks.

It is important to note that the individuals from the JARPN/JARPNII and those from the bycatch samples differ in their body length. Average body length of the JARPN/JARPNII samples including both the offshore and coastal components was 6.67 m (SD=1.13) and that of the all bycatch sample was 4.94 m (SD=0.99). Kato (1992) estimated mean body length at sexual maturity of North Pacific minke whales to be 6.3 m for males and 7.1 m for females, so that the bycatch sample in the present study

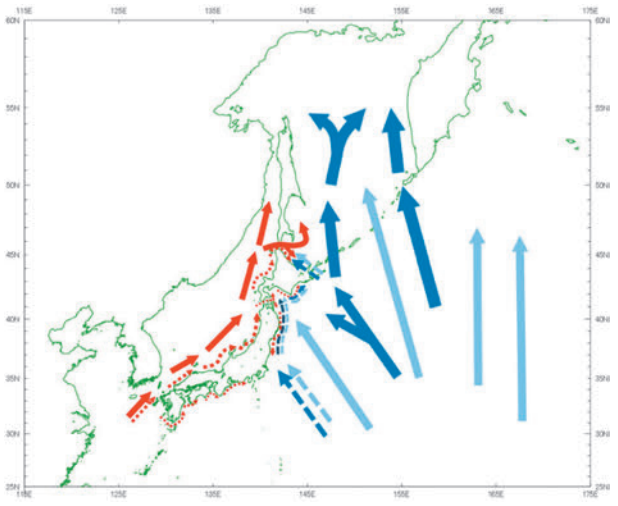

Figure 6. Assumed feeding migration route of O stock and J stock animals (modified after Hatanaka and Miyashita, 1997 and Goto *et al.*, 2010). Dark blue: female of O stock animals, light blue: male of O stock animals, red: J stock animals. Solid line: mature, dotted line: immature.

consisted mostly, if not all, of immature whales. The observed difference in the maturity status between the individuals from the bycatch and JARPN/JARPNII samples, however, could indicate that the patterns of the temporal and spatial distributions illustrated with the bycatches for the Areas Ba, Bb and D in this study may be different to some extent from those of adults. In regard to Area D, common minke whales from the offshore Area are not available. A related concern can be also seen in Area A. The number of J stock individuals in Area A differed between the bycatch and JARPN samples. This difference could be due to the immature/mature, temporal, or both factors, but we were not able to distinguish among these possibilities. Although we substantially increased our understanding of common minke whale distribution in the waters around Japan from this study, our sample set is still missing samples from some areas to depict the whole picture of distribution and movement of the two stocks. Genetic samples from the central and northern Okhotsk Sea are therefore highly desirable.

The pattern described above confirms the migration pattern proposed by Hatanaka and Miyashita (1997) for the O stock and that proposed by Goto *et al.* (2010) for the J stock (see a summary of movements in Figure 6).

Possibility of additional structure with the O and J stocks

STRUCTURE has generated considerable discussion in the IWC Scientific Committee (SC) (e.g. IWC, 2007; 2010). One major concern is the well-documented difficulty that STRUCTURE had in detecting weakly differentiated

populations/stocks, e.g. the approach could not detect additional structure within the O and J stocks.

In this regard one of the issues discussed at the IWC SC was the significance of the unassigned individuals that could not reliably be assigned to either O or J stocks (IWC, 2010). Some IWC SC members have argued that some if not all of the unassigned individuals, might belong to a different stock. Alternatively, these unassigned individuals could be the product of low statistical power of the analysis. Taguchi *et al.* (2017) showed that the proportion of unassigned individuals decreased with an increase of the number of loci used. Therefore the most likely explanation for the unassigned individuals is the low power of the analysis due to a small number of loci used.

On the other hand, Pastene *et al.* (2016b) conducted hypothesis testing to examine the genetic population structure of O stock common minke whales assigned by STRUCTURE, and found no significant heterogeneity in the sample providing support for a single O stock in the western North Pacific. A simulation exercise showed that the statistical power of the test was high.

More recently Tiedemann *et al.* (2017) investigated the spatial distribution of parent/offspring pairs based on microsatellite genotype profiles. Several pairs were found in which the parents were distributed in offshore waters and the offspring in the coastal waters in the Pacific side of Japan, a pattern difficult to reconcile with a multiple O stocks scenario in the western North Pacific.

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