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ABSTRACT

This study examined the genetic population structure of 'O' stock common minke whale in the western North Pacific based on mitochondrial DNA control region sequencing (487bp) and microsatellite DNA (16 loci). Samples used in the tests of homogeneity were obtained during the surveys of the JARPN and JARPNII in sub-areas of the Pacific side of Japan between 1994 and 2014 (n= 2,071 for microsatellite; n= 2,070 for mtDNA). Whales were assigned to the 'O' stock by the analysis of STRUCTURE presented in Pastene *et al*. (2016: SC/F16/JR38). Tests based on both genetic markers and different grouping of the samples showed no evidence of sub-structuring in the 'O' stock common minke whale in the Pacific side of Japan. A simulation exercise showed that the statistical power of the homogeneity test was high. In addition, a Discriminant Analysis of Principal Components (DAPC) based on the total samples used in Pastene *et al*. (2016: SC/F16/JR38) showed clear differentiation between J and O stock whales but no evidence of sub-structuring within the O stock samples. Consequently the results of this study suggested a low plausibility for the hypothesis of sub-division of the O stock common minke whale into OW and OE.

INTRODUCTION

Discussions on stock structure of western North Pacific common minke whale have taken place at the International Whaling Commission Scientific Committee (IWC SC) since 1993, with the latest discussions occurring during the RMP *Implementation Review* (*IR*) in 2013 (IWC, 2014). Samples and data obtained by the JARPN and JARPNII have been essential for the discussions on stock structure at the IWC SC.

A total of 22 sub-areas (Figure 1) and three stock structure hypotheses (Figure 2) were used during the last *IR* of western North Pacific common minke whale (IWC, 2013). The plausibility of the three stock structure hypotheses was discussed at the 2012 IWC SC Annual Meeting. A group of five geneticists summarized their interpretation of the relative support for and against the five hypothesized stocks involved in the different hypotheses (JE, JW, OE, OW and Y) (IWC, 2013 pp135). It should be noted that their evaluation was based on the available genetic information only despite plenty of non-genetic information was available for the *IR*. The result of their evaluation is reproduced in Table 1.

Despite this effort by geneticists it was not possible for the IWC SC to agree on the plausibility of the three stock structure hypotheses. This was in part because the IWC SC has not been able to design an objective and balanced method to evaluate plausibility of stock structure hypotheses. As a consequence, all three main stock structure hypotheses were 'no agreement' and were therefore treated as if they had been assigned 'Medium' plausibility in the trials (IWC, 2013 pp126).

In this study these three hypotheses are further evaluated by conducting hypothesis testing using the total genetic samples available from JARPN and JARPNII till 2014 in the relevant sub-areas shown in Figure 1. This analysis focused only in the investigation of plausibility of O stock sub-structure into OW and OE, given the fact that the genetic evidence for sub-division of the J stocks into JW and JE is low (Table 1).

We examined differences in allele and haplotype frequencies among whales in the sub-areas, particularly we tested whether the frequencies for the whales in the coastal sub-areas (particularly for sub-area 7CN where the OW has been proposed under one of the three hypotheses), differ significantly from whales in offshore areas.

This study also analyzed other pieces of evidences that, according to the five geneticists, provide 'Moderate' support for the hypothesis of the OW (Table 1). For example the results based on PCA (Gaggiotti and Gascuel, 2011) and the suggestion of one or more additional O stocks based on initial one and two locus Wahlund effects (Waples, 2011).

The analyses conducted considered most of the recommendations from the 2009 JARPNII review workshop (IWC, 2010) and from subsequent IWC SC Annual meetings (see Annex 5 of Tamura *et al*., 2016: SC/F16/JR1).

MATERIALS AND METHODS

Samples

For the hypothesis testing, only samples of the 'O' stock common minke whale as assigned by the STRUCTURE analysis (over 90% probability) (see Pastene *et al*., 2016: SC/F16/JR38) were used. Common minke whales samples of the JARPNII offshore component were taken from 2000 to 2014. The JARPN samples from 1994 to 1999 were also used in this study. Table 2 shows the number of individuals used in the present microsatellite and mtDNA analyses, by sub-area and sex, and Figure 3 shows the sighting positions of the collected O stock whales.

The Discriminant Analysis of Principal Components (DAPC) was based on the total samples of J and O stocks (e.g. the same samples used in Pastene *et al*., 2016: SC/F16/JR38).

DNA extraction

The IWC guidelines for DNA data quality (IWC, 2009) were followed as much as possible (see Kanda *et al*., 2014). Skin tissues of minke whales taken during the JARPNII were stored in 95% ethanol until DNA extraction. Genomic DNA was then extracted from 0.05g each of the skin tissues using standard proteinase K, phenol-chloroform procedure described by Sambrook *et al*. (1989). Extracted DNA was stored in the TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Microsatellite analysis

Microsatellite polymorphisms were analyzed using 16 primers: EV1, EV14, EV21, EV37, EV94, (Valsecchi & Amos 1996), GT23, GT195, GT211, GT310, GT509, GT575 (Bérubé *et al*., 2000), GATA28, GATA98, GATA417, TAA31 (Palsbøll *et al*., 1997), and DlrFCB14 (Buchanan *et al*., 1996). EV1, EV14, EV21 were developed from sperm whale, EV37, EV94, GT23, GT310, GT575, GATA28, GATA98, GATA417, TAA31 were from humpback whale, and DlrFCB14 from beluga whale. All GT, EV, and DlrFCB primers were dinucleotide repeat, TAA31 trinucleotide repeat, and all GATA primers tetranucleotide repeat. Most of the primers used here were already tested for amplification on common minke whales by these authors. Primer sequences and PCR profiles follows those of the original authors with slight modifications.

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). 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 an BaseStationTM 100 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 common minke whale DNA of known size that were rerun on each gel.

Data analysis

Level of polymorphism

The number of alleles per locus, expected heterozygosity per locus and inbreeding coefficient per locus were calculated using the software FSTAT 2.9.3 (Goudet, 1995). Statistical tests for deviations from the expected Hardy-Weinberg genotypic proportions were conducted using the software GENEPOP 4.0 (Rousset, 2008).

Homogeneity test

In order to detect genetic differences within the 'O' stock sample of common minke whale, conventional hypothesis testing procedure was conducted using heterogeneity test in frequencies of the microsatellite alleles among sub-areas. Null hypothesis to be tested was if the samples came from a genetically same group of common minke whales. If statistically significant allele frequency differences exist, then it could indicate these samples came from genetically different stocks of common minke whales. Probability test (or Fisher's exact test) implemented in GENEPOP 4.0 (Rousset, 2008) was used to conduct the heterogeneity test. Statistical significance was determined using the chi-square value obtained from summing the negative logarithm of *p*-values over the 16 microsatellite loci (Sokal and Rohlf, 1995). The False Discovery Rate (FDR) approach (Benjamini and Yekutieli, 2001) was used for adjustment of *p*-value in case of multiple comparisons. F_{ST} was calculated using FSTAT 2.9.3 (Goudet, 1995).

Assessment of the statistical power

In order to assess the statistical power for the homogeneity test (e.g., Waples and Gaggiotti, 2006), genotypic data were generated using the computer software EASYPOP (Balloux, 2001), and heterogeneity tests were conducted with these generated data. Two stocks (OW and OE) were assumed which consists of diploid individuals with constant sizes and equal sex ratio with random mating. Ratios of effective population size to census population size of 1/3 and 1/4, were assumed (Roman and Palumbi, 2003). The effective population sizes were thus set as 1/3 and 1/4 of the census population sizes. A census population size of 20,000, was used. For each generation, the simulation produces genotype data for 16 independent microsatellite loci for each individual. The number of the loci simulated and maximum number of the allelic states (27) was set based on the observed data. The bidirectional migration model was assumed with an equal migration rate (m). Migration rates ranged from 0.01 to 0.5. A range of F_{ST} between the two assumed stocks was obtained, assuming island model. Mutation rate of $5x10^{-4}$ was chosen to represent microsatellite loci. A total of 100 replicates were made for each simulation parameter set. A total of 5,000 generations for each replicate before collecting data, was run. In the final generation of each replicate, sample of 140 individuals were taken from each population for genetic analysis. The sample size of 140 in this study was approximately equals to the sum of the samples size from SA7 WR and SA7E where the OE stock was assumed, which is considered conservative given that larger sample size was actually used for OW. Homogeneity tests were conducted for the generated data set using pairwise tests of differentiation option in the FSTAT2.9.3 (Goudet, 1995). In this option, for each pair of samples, multi-loci genotypes are randomized between the two samples. The overall loci G-statistic is given and statistical significance was decided with a table wide level of significance at 5%.

Discriminant Analysis of Principal Component

The Discriminant Analysis of Principal Components (DAPC) was used to identify and describe clusters of genetically related individuals (Jombart, *et al*., 2010). DAPC relies on data transformation using PCA as a prior step to DA which ensures that variables submitted to DA are perfectly uncorrelated, and that their number is less than of analysed individuals. Along with the assignment of individuals to clusters, DAPC provides a visual assessment of between-population genetic structures, permitting to infer complex patterns such as hierarchical clustering or clines (Jombart, *et. al*., 2010). Following these authors, the K-means clustering of principal components was used to identify groups of individuals. K-means relies on the same model as DA to partition genetic variation into a between-group and a within-group component, and attempts to find groups that minimize the latter (Jombart, *et al*., 2010). K-means was run with different numbers of clusters, each of which gives rise to a statistical model and an associated likelihood.

DAPC was used to a two data sets:

i) Only individuals belonging to the J and O stocks with a probability larger than 90% according to the criterion used in Pastene *et al*. (2016: SC/F16/JR38).
ii) Only individuals belonging to the O stock with a probability larger than 90% according to the criterion used in Pastene *et al*. (2016: SC/F16/JR38).

Mitochondrial DNA
Sequencing analysis of the 487bp control region of mtDNA was conducted using the primers light-strand MT4 (Árnason *et al*., 1993) and heavy-strand P2 (5'-GAAGAGGGATCCCTGCCAAGCGG-*3'*; Hori *et al*., unpublished). 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 377™ or ABI3100 Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacture. For each sample both strands were sequenced.

Data analysis

Level of polymorphism
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).

Homogeneity test
Conventional hypothesis testing procedure was conducted using heterogeneity test in mtDNA haplotype frequencies among the samples. The randomized chi-square Test of Independence (Roff and Bentzen, 1989) and the conventional F_{ST} were 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 FDR approach was used for adjustment of *p*-value in case of multiple comparisons. F_{ST} for mtDNA was calculated based on the analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992).

RESULTS

Microsatellites
Level of polymorphism
All 16 loci analyzed were polymorphic (Table 3). The total number of alleles per locus ranged from two at the EV21 to 27 at the EV1 with an average of 11.9. Expected heterozygosity at each of the loci ranged from 0.33 at EV21 to 0.88 at GT23 and GT211 with an average of 0.70. No significant deviation from the expected Hardy-Weinberg genotypic proportions was observed. The magnitude of F_{IS} was close or varied around zero as expected if the samples were collected from the same stock.

Homogeneity test
Table 4 shows the results of the heterogeneity tests for different grouping of the samples. No significant differences were found for males, females and sex combined in the three stepwise tests conducted. For males and for a single locus (GATA28) a small *p*-value was found in the comparison 7CS-7CN / 7WR, which remained significant after the FDR correction. Given the number of tests carried out and the fact that the test for mtDNA showed no significant differences in this comparison, no biological meaning is assigned to this result.

Assessment of statistical power for the tests of homogeneity
Table 5 shows the input parameters used and the results of simulation analysis to assess the statistical power for the tests homogeneity. The simulation attempted to test the statistical power for very small genetic divergence between two samples. For instance, estimated F_{ST} values were all smaller than 0.01.

For the homogeneity tests the input parameters for simulating OW and OE were all the same. High statistical power was detected with m=0.01 and 0.02.

DAPC
Figures 4 and 5 show the results of PCA and DAPC analyses, respectively, for the case all samples of J and O stocks were used. Figure 5 was constructed without 'training data', which means that no previous information on J and O stock was used. The approach discriminated clearly between the both stocks (see the STRUCTURE-like plot in Figure 5). Figures 6 and 7 show the results of PCA and DAPC analyses, respectively, for the case O stock samples only were used. Samples from different sources and geographical origin were widely distributed among clusters in Figure 6. When forced to K=2, samples from different sources and geographical origin were equally distributed in the two groups, and the STRUCTURE-like figure did not discriminated between these two groups (Figure 7).

mtDNA
Level of polymorphisms
Table 6 shows the values for the mtDNA diversity. Both indices are relatively high.

Homogeneity test
Table 7 shows the results of the tests. For the three stepwise tests conducted no significant heterogeneity was found for males, females and sexes combined.

DISCUSSION

As noted earlier, the main objective of the present genetic analyses was the evaluation of the plausibility of additional structure in the O stock common minke whale, e.g. the plausibility of an OW stock in coastal areas of Japan. For doing this, the total samples obtained by JARPN and JARPNII till 2014 were analyzed with two genetic markers, which are commonly used in most of the genetic studies on stock structure presented and discussed at the IWC SC meetings. It is believed that the combined use of mtDNA control region sequences and microsatellite DNA at 16 loci on large sample sizes is a strong tool to investigate genetic differences in weakly differentiated stocks.

Firstly the present study addressed all 'simple issues' recommended by the 2009 JARPNII review workshop. For example the workshop recommended description of procedures to ensure data quality. In response to this recommendation and other one from the JARPAII review workshop, a document was prepared and presented to the IWC SC in 2014 (Kanda *et al*., 2014). The IWC SC welcomed this document and agreed that it responded appropriately to the recommendation (IWC, 2015). Another example is the 2009 workshop recommendation to use the False Discovery Rate approach instead of the Bonferroni correction for the adjustment of *p*-values in cases of multiple comparisons. This approach was used in the present study.

More extensive and long-term recommendations from the 2009 JARPNII review workshop were responded in this document as well as in other stock structure documents presented previously or to this workshop. See more details on the responses to previous recommendations in Annex 5 of Tamura *et al*. (2016: SC/F16/JR01).

On the genetic information providing 'moderate' evidence for the case of OW (Table 1), three were considered the most relevant: hypothesis testing, PCA and one and two locus Wahlund effects.

Hypothesis testing
Table 1 shows that one of the evidence for the case of OW was 'significant F_{ST} differentiation comparing non-purged samples'. Results of tests based on non-purged samples are very difficult to interpret. This is the reason of why the IWC SC had recommended genetic analyses separately for J and O stocks, and

specifically recommended the use of alternative methods for exclusion of J stock animals in the analyses focused on the O stock (IWC, 2003). In fact several genetic studies have conducted analyses separately for the O stock based on the output of the work of Kanda *et al*. (2009a; b; 2010), e.g. hypothesis testing (Kanda *et al*., 2009c) and PCA (Gaggiotti and Gascuel, 2011) based on purged samples.

During IWC SC discussions the circularity for the use of purged samples for hypothesis testing analyses of the O stock was argued, because this process would exclude individuals that are genetically intermediate to the mean O and J genotypes (presumably in the unknown samples) (IWC, 2010). It was also noted in those discussions that the problem largely would disappear if all or virtually all assignments can be made with a high degree of certainly (IWC, 2010).

As shown by Pastene *et al*. (2016: SC/F16/JR38), the 'unknown' samples are not whales genetically intermediate to the O and J stocks but rather they are the results of the low power due to the number of microsatellite loci. Eventually all or virtually all individuals would be assigned to either J or O stock if a larger number of loci are used. Furthermore the unknown individuals are widely distributed in the western North Pacific, both temporally and geographically. Therefore this provides a strong rational for the use of J-purged samples in further analyses on O stock substructure using alternative analytical approaches.

As expected, both genetic markers provided no evidence of significant heterogeneity suggesting that the common minke whales used in the analyses belong to a single stock. The F_{ST} values were very small and none was significantly different from zero. Following recommendations from the 2009 JARPNII review workshop an assessment of the power of the homogeneity test was conducted (Kanda *et al*., 2009d, this study), and the power was concluded to be high.

PCA and DAPC

In providing 'moderate' support for OW, Table 1 stated that 'PCA results using J-purged O stock sample provided support for an additional stock in OW compared to OE'. This conclusion in Table 1 was based on the PCA work by Gaggiotti and Gascuel (2011). These authors concluded that 'these results suggest that minke whales in sub-area 7-9 exhibit a hierarchical structure that comprises two main genetic clusters corresponding to the putative J and O stocks identified by STRUCTURE analyses and further substructuring within the O-stock. This substructuring, however, is not correlated with body length or geographic position so for the moment it has no clear biological explanation'. It should be noted that the authors did not speculate on whether or not such 'substructuring' was related to possible additional stock structure in common minke whales.

It should be also noted that Gaggiotti and Gascuel (2011) noted that there was a good concordance between results obtained with STRUCTURE and PCA methods and suggested that the substructuring of the O-stock would be revealed by a STRUCTURE analysis focused only on this stock. Kanda *et al*. (2010) conducted STRUCTURE analyses based only on O stock samples but they did not find any evidence of substructuring.

Based on this brief summary it is concluded that the previous PCA evidences in support of an OW stock cannot be considered 'moderate' as indicated in Table 1, but very weak.

The DAPC approach used in this study is a new methodological approach, which retain all assets of DA without being burdened by its limitations. It relies on data transformation using PCA as a prior step to DA, which ensure that variables submitted to DA are perfectly uncorrelated, and that their number is less than that of analysed individuals (Jombart *et al*., 2010). This approach used on the large microsatellite DNA data set of common minke whale provided no evidence of substructuring of O stock in sub-areas 7, 8 and 9.

One and two locus Wahlund effects

In providing 'moderate' support for OW, Table 1 noted the 'suggestion of one or more additional stocks based on initial one and two locus Wahlund effects'. This suggestion was originally provided by Waples (2011): when only two distinct stocks exist in a given area, the largest departure from equilibrium (departure from F_{IS}) or higher linkage disequilibrium should be seen at the loci or pairs of loci that show the largest or strong allele frequency differences between the two distinct stocks (describe as θ). Because

the observed relationship of these genetic indices in the samples from SA 7, 2 and 11 were weaker than the expected relationship estimates from artificial mixtures of only J and O individuals, Waples (2011) suggested that samples might have contained individuals from more than two stocks.

It should be noted that Waples (2011) recognized the novel character of the approach used and that 'more evaluations are needed to determine how robust the results are'. Apart from this recognition, Kanda and Hatanaka (2012) carried out a simulation exercise in response to Waples (2011)'s analyses and concluded that the weaker observed relationship of the indices could be due to the small sample size rather than the mixture of individuals from more than two stocks.

Based on this brief summary it is concluded that the Waples (2011)'s evidence in support of an OW stock cannot be considered 'moderate' as indicated in Table 1, but very weak.

In conclusion the main evidences listed in Table 1 as moderate support to the existence of a OW stock are considered weak. The results of the genetic analyses in the present study (hypothesis testing including an assessment of the statistical power and DAPC) provided strong support for a single O stock in sub-areas 7, 8 and 9.

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Table 1. Evaluation of the main components of stock structure hypotheses of common minke whale in the western North Pacific based on genetic data (IWC, 2013).

Stock	Evidence for:	Evidence against:
Y	**Moderate**: Significant microsatellite DNA F_{st} values between SA5 and Sea of Japan samples, and seasonal evidence for mixing based on HW deviation.	**Low**: No significant F_{st} based on mtDNA, but small sample sizes. Microsatellite DNA F_{st} values small.
JW (J)	**High**: Case for a core J stock is strong based on various data.	N/A
JE	**Low**: Significant mtDNA F_{st} comparing 6E and 2 based on non-purged dataset. Differential haplotype frequencies for two most common haplotypes. Weak suggestion of one or more additional stocks based on initial one and two locus Wahlund effects.	**Moderate or High**: F_{st} values were very small. Some mtDNA haplotype data suggest mixing between J and O (e.g. the number of haplotypes per individual is 0.19 in 2BC compared to 0.08 in 6BC and 0.10 in 8 and 9). Possible considerations of temporal aspects of comparisons (with bycatch not representing the same time period as the hunt). PCA found no evidence for differentiation between 2C and 6E. One and two locus Wahlund effect method requires further trials, as evidenced by preliminary simulation data presented in SC/64/NPM9. Fis results for SA2 indicate a mixture (not a new pure stock) and appear consistent with a mixture of just O and J.
OW	**Moderate**: PCA results using J-purged O stock sample provided support for an additional stock in OW compared to OE. Significant F_{st} differentiation comparing non-purged samples. Suggestion of one or more additional stocks based on initial one and two locus Wahlund effects. Haplogroup data suggests different frequencies in 7CN and 7CS regions compared to O-stock (8 and 9).	**Low or Moderate**: PCA work requires simulation analysis to evaluate effects of purging. PCA regression against length was not significant. Small but positive Fis considering all loci together suggests mixing in 7W-K and 7W-S regions. One and two locus Wahlund effect method requires further trials, as evidenced by preliminary simulation data presented in SC/64/NPM9. Haplogroup data (based on two SNPs) not clearly inconsistent with mixing.
OE (O)	**High**: Case for a core O stock is strong based on various data.	N/A

Table 2. Sample size of the 'O' stock whales used in this study, by sub-area and sex. Whales were assigned to the 'O' stock based on the analysis of STRUCTURE (see SC/F16/JR39).

Sub-area	**Microsatellites**			**mtDNA**		
	Males	Females	Total	Males	Females	Total
11	28	20	48	28	20	48
7CN	534	205	739	534	205	739
7CS	219	220	439	219	220	439
7E	41	4	45	41	4	45
7WR	80	9	89	80	9	89
8	207	17	224	206	17	223
9	439	48	487	439	48	487
Total	1548	523	2071	1547	523	2070

Table 3. The number of alleles (A), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), and test result for the expected Hardy-Weinberg genotypic proportions (HW) at 16 microsatellite loci of 'O' stock of western North Pacific minke whale. Bold indicate statistically significant differences after FDR correction.

Microsatellite loci	A	H_E	HW	F_{IS}
EV37	12	0,73	0,773	0,000
EV1	27	0,83	0,587	0,014
GT310	14	0,82	0,899	0,001
GATA28	20	0,84	0,052	0,005
GT575	12	0,82	0,029	-0,011
EV94	8	0,67	0,791	0,002
GT23	15	0,88	0,223	0,007
GT509	20	0,85	0,857	0,005
GATA98	6	0,63	0,490	0,002
GATA417	13	0,76	0,026	0,003
GT211	16	0,88	0,179	0,000
EV21	2	0,33	0,577	-0,013
DlrFB14	5	0,37	0,264	0,010
EV14	6	0,57	0,438	-0,001
GT195	12	0,81	0,040	-0,005
TAA31	3	0,39	0,229	0,010
Overall	11,94	0,70	0,057	0,002

Table 4. Results of the microsatellite DNA heterogeneity test among 'O' stock minke whale from different sub-areas, by sex and total samples. Bold indicate statistically significant differences after FDR correction.

Microsatellite loci	7CS x 7CN					
	P -values			F_{ST}		
	Sex combined	Male	Female	Sex combined	Male	Female
EV37	0,055	0,025	0,869	0,001	0,002	-0,001
EV1	0,924	0,655	0,924	0,000	0,000	-0,001
GT310	0,288	0,759	0,327	0,000	-0,001	0,001
GATA28	0,720	0,473	0,707	0,000	0,000	0,000
GT575	0,572	0,779	0,024	0,000	0,000	0,008
EV94	0,583	0,391	0,864	0,000	-0,001	-0,001
GT23	0,742	0,816	0,363	0,000	-0,001	0,000
GT509	0,650	0,682	0,891	0,000	0,000	-0,001
GATA98	0,530	0,790	0,180	0,000	0,000	0,002
GATA417	0,589	0,690	0,330	0,000	-0,001	0,000
GT211	0,052	0,081	0,637	0,001	0,001	0,000
EV21	0,118	0,458	0,116	0,001	-0,001	0,004
DlrFB14	0,117	0,202	0,520	0,000	-0,001	-0,001
EV14	0,514	0,384	0,451	-0,001	-0,001	0,000
GT195	0,077	0,122	0,363	0,000	0,000	0,000
TAA31	0,213	0,486	0,684	0,001	0,000	-0,002
Overall	0,181	0,447	0,574	0,000	0,000	0,001

Microsatellite loci	7CS-7CN x 7WR					
	P -values			F_{ST}		
	Sex combined	Male	Female	Sex combined	Male	Female
EV37	0,832	0,590	0,343	-0,002	-0,001	-0,006
EV1	0,263	0,490	0,053	0,000	0,001	0,013
GT310	0,412	0,335	0,972	0,000	0,000	-0,018
GATA28	0,020	**0,001**	0,738	-0,001	0,000	-0,005
GT575	0,625	0,828	0,390	-0,001	-0,002	0,008
EV94	0,163	0,186	0,401	0,005	0,005	0,013
GT23	0,855	0,542	0,841	-0,002	-0,001	-0,014
GT509	0,808	0,776	0,736	0,000	0,001	0,005
GATA98	0,877	0,745	0,267	-0,002	-0,002	-0,007
GATA417	0,421	0,784	0,071	-0,002	-0,003	-0,015
GT211	0,923	0,901	0,710	0,000	-0,001	0,002
EV21	0,635	0,474	0,554	-0,002	-0,001	-0,008
DlrFB14	0,978	0,862	0,753	-0,003	-0,003	-0,022
EV14	0,436	0,379	0,920	-0,002	-0,002	-0,014
GT195	0,884	0,678	0,562	-0,001	-0,001	-0,010
TAA31	0,172	0,205	1,000	-0,003	-0,003	-0,029
Overall	0,741	0,438	0,804	-0,001	-0,001	-0,005

Microsatellite loci	7CS-7CN-7WR x 7E x 8 x 9					
	P -values			F_{ST}		
	Sex combined	Male	Female	Sex combined	Male	Female
EV37	0,344	0,243	0,462	0,001	0,001	-0,002
EV1	0,931	0,933	0,541	-0,001	-0,001	0,001
GT310	0,216	0,373	0,471	0,001	0,001	-0,002
GATA28	0,485	0,418	0,380	-0,001	0,000	-0,005
GT575	0,817	0,548	0,892	0,000	-0,001	-0,002
EV94	0,568	0,313	0,954	0,000	0,001	-0,007
GT23	0,467	0,330	0,482	0,000	0,001	-0,004
GT509	0,350	0,385	0,347	0,000	-0,001	0,000
GATA98	0,349	0,243	0,576	-0,001	0,000	-0,005
GATA417	0,764	0,440	0,952	0,000	0,000	-0,006
GT211	0,577	0,779	0,459	0,000	-0,001	0,000
EV21	0,456	0,525	0,668	0,000	0,000	-0,004
DlrFB14	0,216	0,163	0,470	0,000	0,001	-0,006
EV14	0,680	0,721	0,790	-0,001	-0,001	0,004
GT195	0,308	0,354	0,303	0,000	0,000	-0,001
TAA31	0,792	0,913	0,026	0,000	-0,001	0,013
Overall	0,847	0,711	0,791	0,000	0,000	-0,002

Table 5. Input parameter sets used for generating simulated data set using EASYPOP to assess statistical power in our samples and results of the homogeneity tests with the simulated data. The following were fixed in all sets other than shown in the table: diploid, random mating, equal sex ratio, subpopulations of constant Ne, mutation rate of 0.0005, and 100 replicates each with 5000 generations.

	n	N	Ne	m	Nem	*Fst*	S	L	A	% rejecting panmixia
N=3Ne	2	19980	6660	0.01	66.6	0.0037	140	16	27	100
	2	19980	6660	0.02	133.2	0.0019	140	16	27	87
	2	19980	6660	0.05	333	0.0008	140	16	27	32
	2	19980	6660	0.1	666	0.0004	140	16	27	16
	2	19980	6660	0.2	1332	0.0002	140	16	27	8
	2	19980	6660	0.5	3330	0.0001	140	16	27	4
N=4Ne	2	20000	5000	0.01	50	0.0050	140	16	27	100
	2	20000	5000	0.02	100	0.0025	140	16	27	93
	2	20000	5000	0.05	250	0.0010	140	16	27	35
	2	20000	5000	0.1	500	0.0005	140	16	27	16
	2	20000	5000	0.2	1000	0.0002	140	16	27	4
	2	20000	5000	0.5	2500	0.0001	140	16	27	5

N=census population size, Ne=effective population size, m=migration rate, Nem=number of migrants per generation, S= number of sample size, L = number of loci analyzed, A=possible number of allelic states.

Table 6. Results of mtDNA diversity for western North Pacific common minke whale.

Number of haplotypes	Nucleotide diversity (SE)	Haplotype diversity
102	0.00784 (0.00009)	0.94763

Table 7. Results of the mtDNA heterogeneity test among 'O' stock minke whale from different sub-areas, by sex and total samples.

7CS × 7CN					
Chi-square *p*-value			F_{ST}		
Sex combined	Male	Female	Sex combined	Male	Female
0.223	0.432	0.707	0.00046	-0.00028	-0.00072

7CS-7CN × 7WR					
Chi-square *p*-value			F_{ST}		
Sex combined	Male	Female	Sex combined	Male	Female
0.238	0.434	0.690	-0.00097	-0.00144	0.02005

7CS-7CN-7WR × 7E × 8 × 9					
Chi-square *p*-value			F_{ST}		
Sex combined	Male	Female	Sex combined	Male	Female
0.066	0.110	0.952	0.00030	0.00035	0.00152

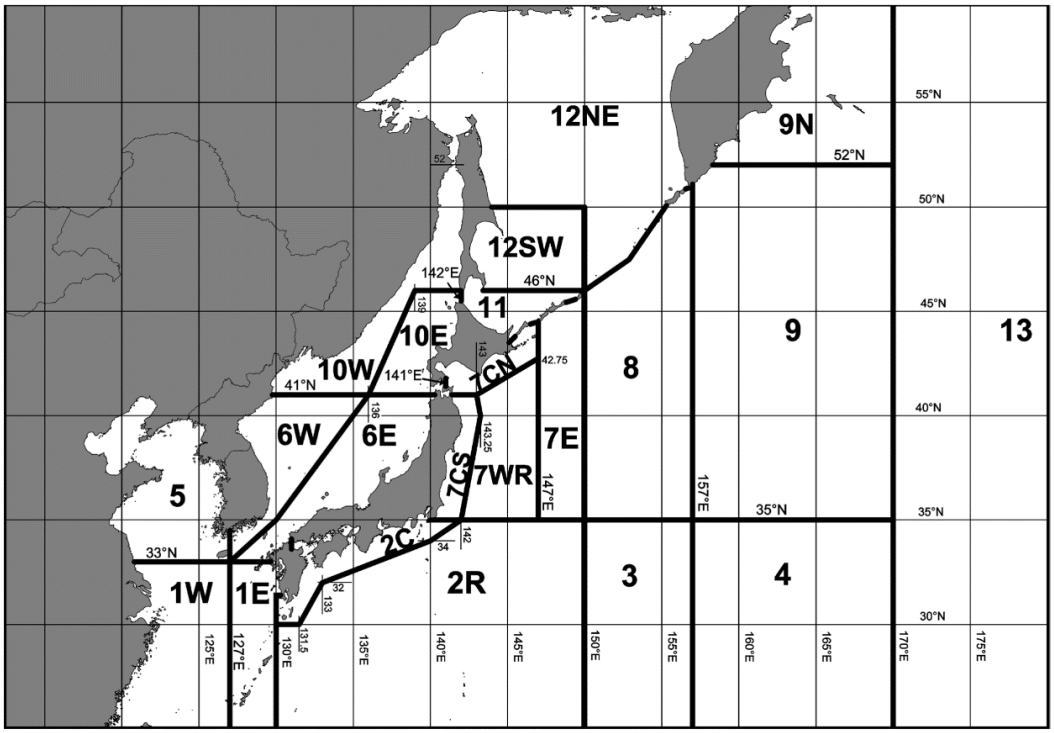


Figure 1. Sub-areas used for the management of common minke whale under the RMP (IWC, 2013).

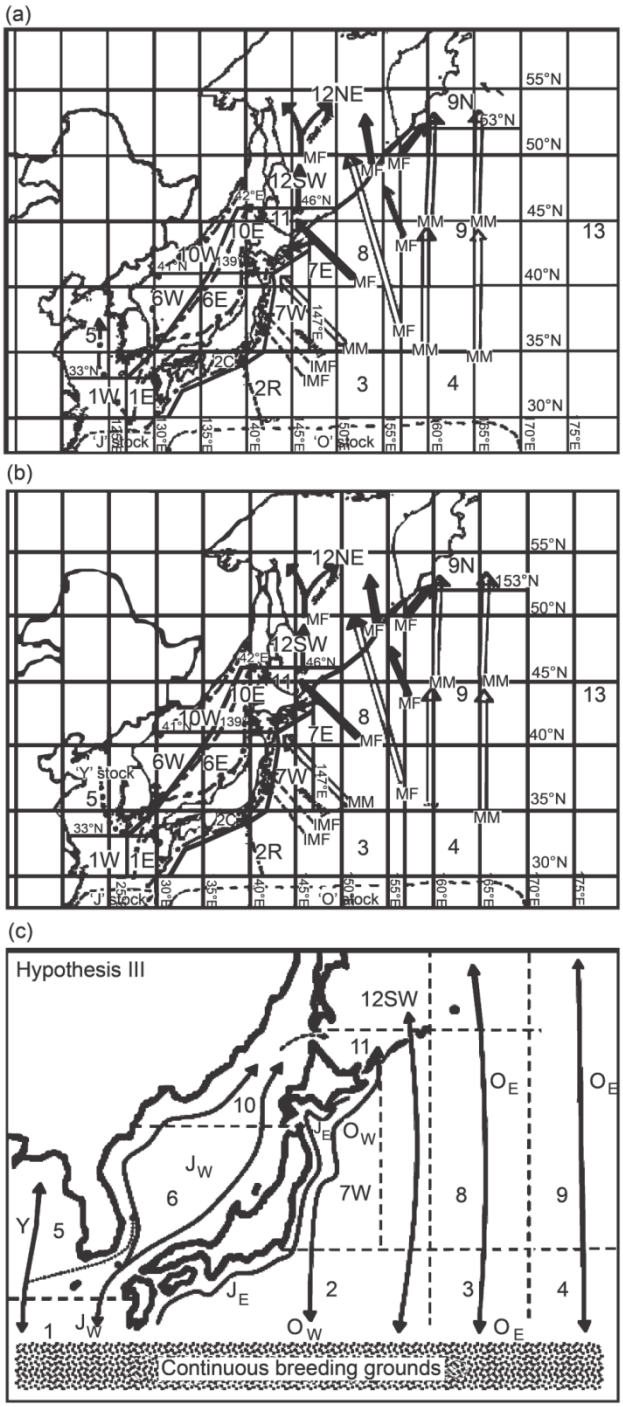


Figure 2. Stock structure hypotheses used for management under the RMP (IWC, 2013).

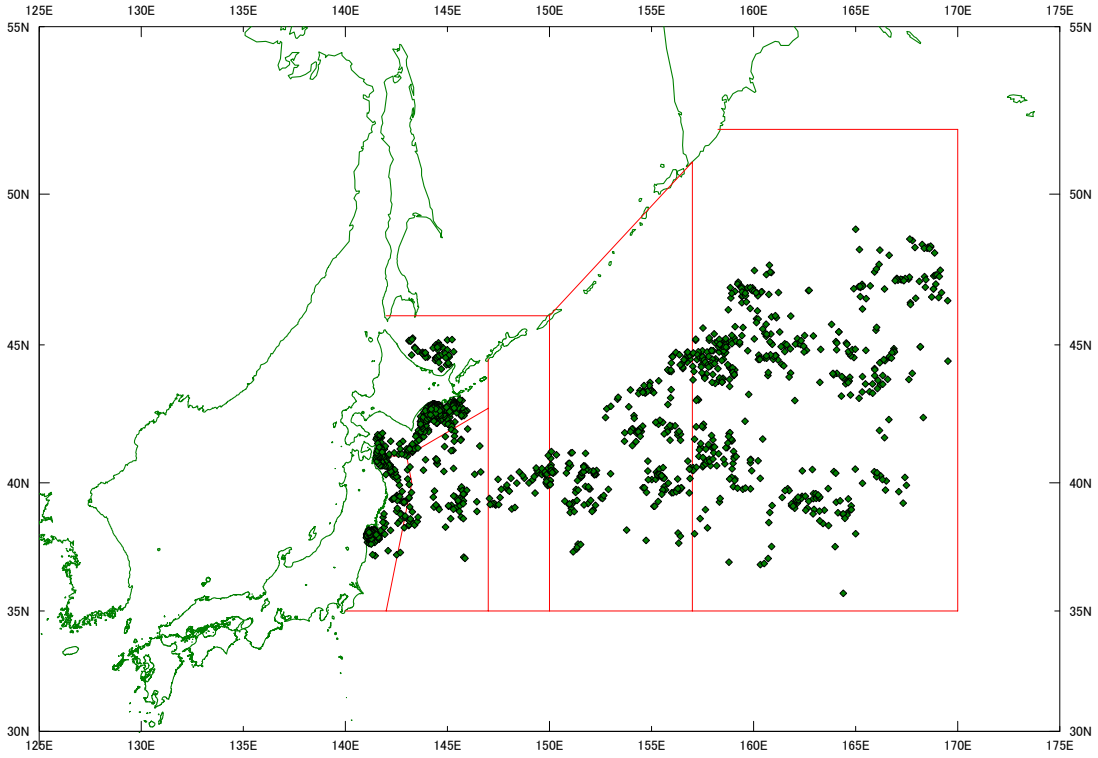


Figure 3. Geographical distribution of 'O' stock common minke whales used in this study.

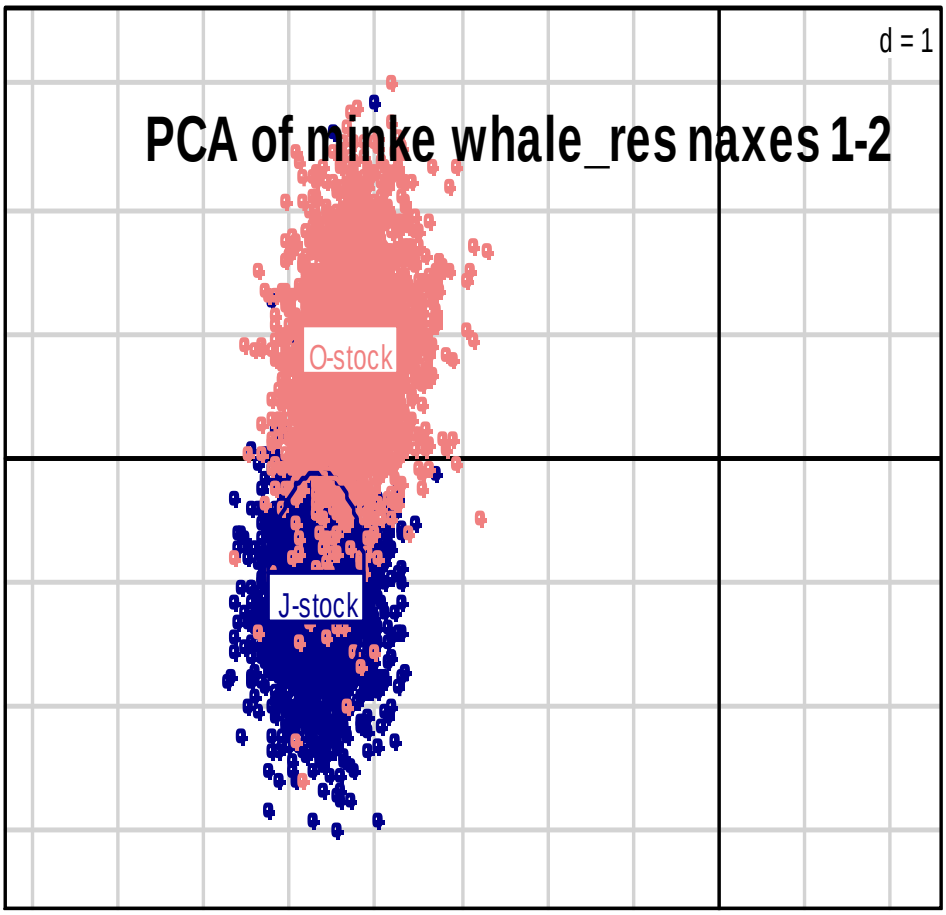
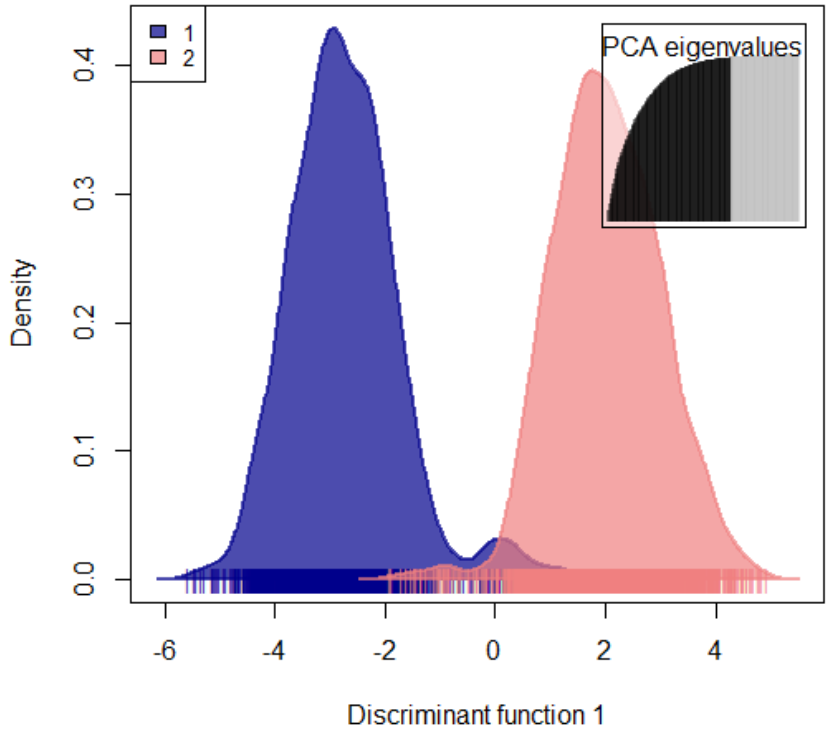


Figure 4. A result of PCA analysis for the common minke whale. The horizontal and vertical axes show the first and second principal components, respectively. The difference colors mean stocks (J and O) assigned by *STRCTURE*, but that sort of information was not used in the analysis itself.



Grp= 2	J-stock	O-stock
1	1,546	102
2	62	2,163

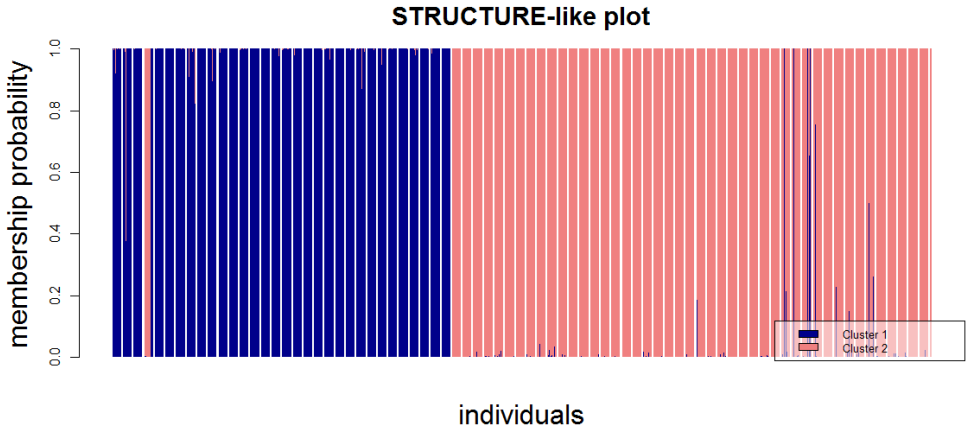


Figure 5. Results of DAPC analyses without any training data. Here training data mean the one with known origins. Error rates were small enough.

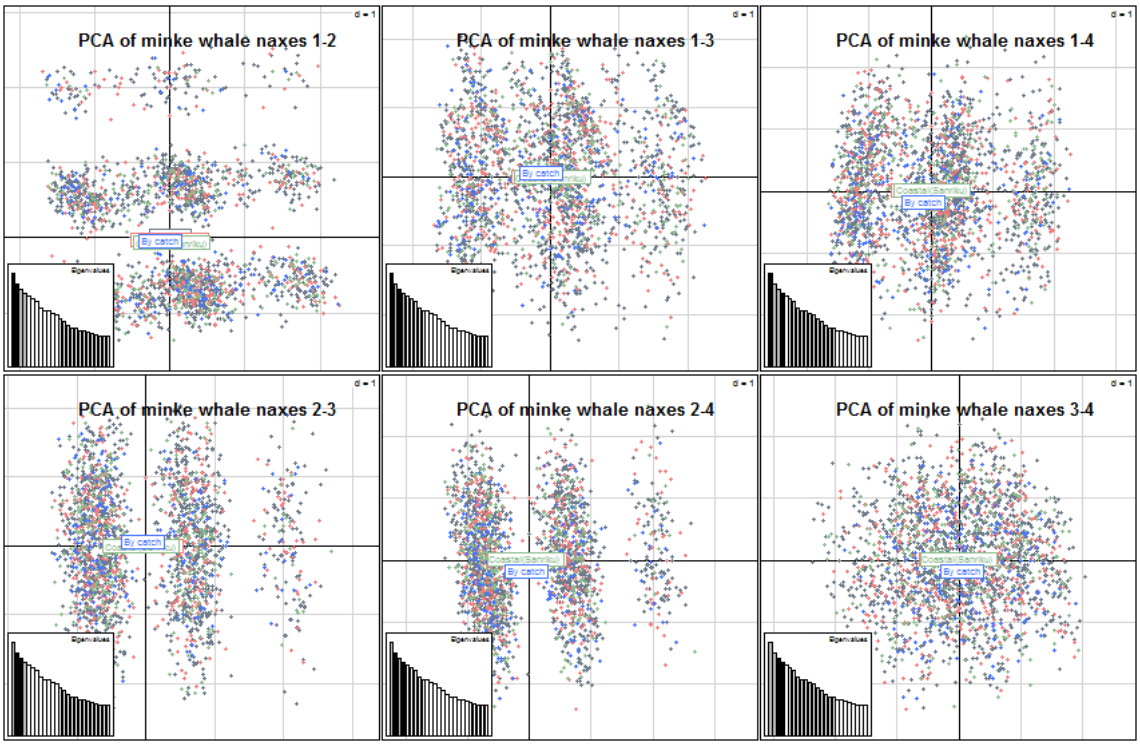
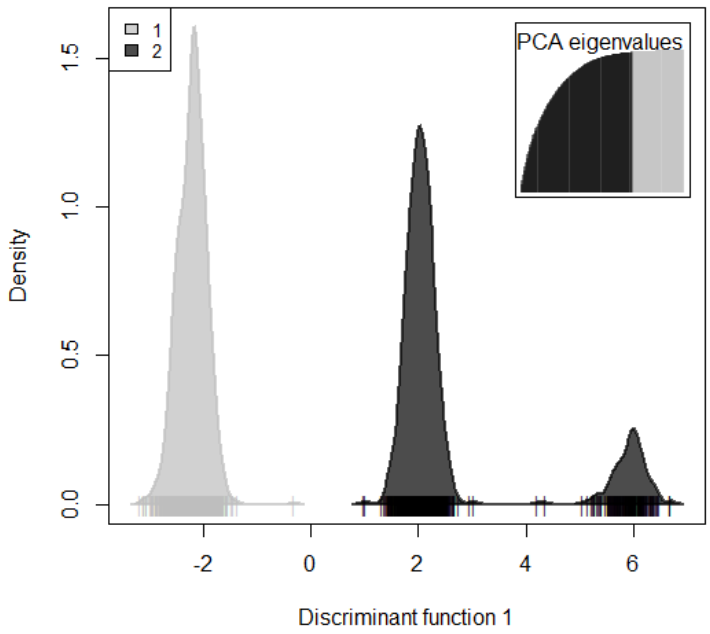


Figure 6. A result of PCA analysis for the common minke whales within O-stock (Ow-Oe, K=2)



Grp= 2	Offshore	Kushiro	Sanriku	Bycatch
1	662	265	198	114
2	599	202	145	80

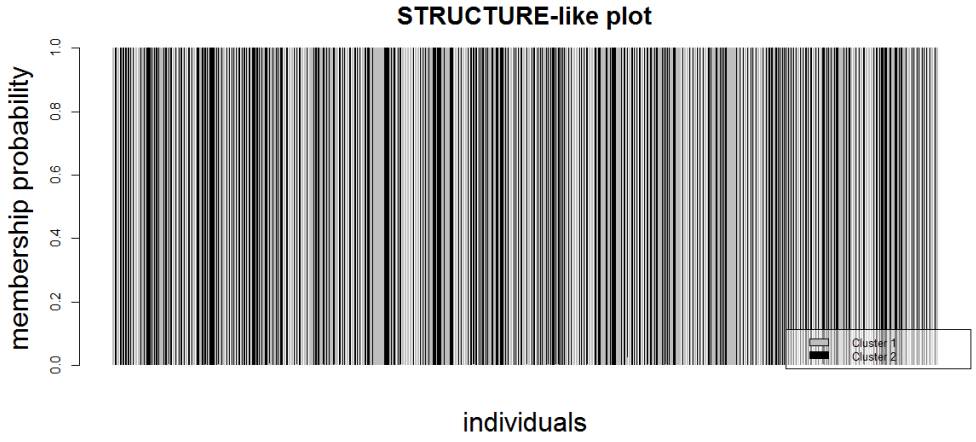


Figure 7. Results of DAPC analysis without any training data. Here training data mean the individuals with known origins. Error rates were small enough.