Analyses of genetic and non-genetic data do not support the hypothesis of an intermediate stock in sub-area 7

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ABSTRACT

This paper presents evidence of the heterogeneities in distribution pattern by the distance from coast line, body length, seasonal distribution, fluke color pattern, and flipper color pattern between the individuals assigned as the J and O stocks collected from SA7W (140-147°E). In addition, these samples showed evidence of the deviation from the expected Hardy-Weinberg genotypic proportion at the microsatellite loci with homozygous excess. These results deny the hypothesis of the single stock with intermediate genetic characteristics, but support the hypothesis of the mixing of the J and O stocks in SA7W.

INTRODUCTION

Scientific Committee (e.g., IWC, 2003) recommended that the J stock individuals should be excluded from the analyses of the North Pacific minke whales. In order to accomplish the recommendation, Kanda *et al.* (2009a) utilized the Bayesian clustering method to identify stocks among the samples of minke whales from western North Pacific. They identified that minke whales in the samples appeared to come from two genetically different stocks, and approximately 90% of the samples were assigned to one or the other with the rest of the samples was unassigned most likely due to the statistical reason. Geographic distribution of these assigned individuals indicated that these two genetically different groups were the J and O stocks. Using this stock identification, both microsatellite (Kanda *et al.*, 2009b) and mitochondrial DNA (Park *et al.*, 2010) analyses demonstrated that the genetic heterogeneity found in SA7W was due to the mixing of the two stocks. After removal of the individuals assigned to the J stock the genetic heterogeneity between the coastal and offshore samples was disappeared.

Baker and Wade (in press a) proposed a hypothesis of six stocks for western North Pacific common minke whales with one of which is a coastal stock in SA7 (Ow stock) in addition to the J and offshore Oe stocks, while Pastene *et al.* (in press) proposed four hypotheses, all of which indicated the mixing of the J stock and O stock in SA7W. One of the issues to be solved prior to the completion of *Implementation Review* of western North Pacific common minke whales is the stock structure of minke whales in SA7W: whether the whales are the member of the single stock or are the members of the two stocks mixing at the area. The Working Group on the *Pre-Implementation Assessment* of western North Pacific minke whales thus agreed that trying to resolve this issue should be a top priority, using both genetic an non-genetic data (IWC, in press).

The purpose of this document is to show other lines of evidence for the mixing of the two stocks in SA7W, that are observed from the analyses of minke whales taken from the area during the JARPN and JARPNII surveys as well as bycaught from set net along the Pacific coast of Japan.

MATERIALS AND METHODS

A stock can be defined as a group of individuals sharing a common gene pool maintained by random mating. Within the single stock, therefore, samples from large and small body size, from different seasons, and from different areas should show the same genetic characteristics. The single stock hypothesis is rejected when available genetic and non-genetic data show heterogeneities within the samples from SA7W.

Samples of minke whales used were those collected form SA7W (140-147°E) during JARPN/JARPNII offshore surveys from 1994 to 2007 (N=414) and JARPNII coastal surveys (Sanriku and Kushiro) from 2002 to 2007 (N=480). Minke whales that were bycaught on set net fishery conducted along the Japanese coast from 2001 to 2007 were also used (bycatches, N=212). Details of these samples can be found in Kanda *et al.* (2009a) and references therein.

The Bayesian clustering approach was conducted with the genetic data at the 16 microsatellite loci obtained from the total samples of minke whales around Japan (N=2542) using the STRUCTURE version 2.0 (Pritchard *et al.*, 2000) to determine the most likely number of genetically distinct stocks present in our samples and to assign each of the individuals to the inferred stocks (Kanda *et al.*, 2009a). Membership probability of over 90 % was used as a criterion for individual assignment. Again, details of the analysis can be found in Kanda et al. (2009a).

RESULTS

Stock identity

In Kanda *et al.* (2009a), the individuals with the membership probability of over 90% were assigned to one or the other genetic groups. Even with such a high criterion, only about 10% in the individuals from SA7W were unassigned (Table 1). This criterion was arbitrary decided. When the criterion was lower to 75%, 96.2% of individuals were assigned as the pure individuals, and only 42 out of the 1105 individuals were unassigned. Most, if not all, individuals were therefore separated to the two distinct stocks. Principal coordinate analysis in Kanda *et al.* (2010) showed that the unassigned individuals occupied in-between space of the individuals assigned to the J and O stocks. It was concluded that the incomplete assignment of some of the individuals was due to low power. The observed number of the unassigned individuals is too small to think that our samples (N=1105) consisted of randomly collected individuals obtained from an independent single stock occupying SA7W.

Table 1. Number of individuals collected from SA7W that were assigned and unassigned to the J or O stock with the membership probability of over 90% or 75%.

	Criterion	
	90%	75%
J stock	196	216
O stock	790	847
Unassigned	119	42

Hardy-Weinberg expected genotypic proportion

Table 2 shows the result of the tests for deviation from the Hardy-Weinberg expected genotypic proportion at each of the 16 microsatellite loci in the sample of all common minke whales collected from SA7W (i.e., both bycatches and JARPN/JARPNII samples (N=1105)). Four of the 16 loci showed the significant deviation from he Hardy-Weinberg expected genotypic proportion. All of these significant results were due to the homozygote excess, supporting the mixture of two stocks.

Table 2. Results of the tests for deviation from the Hardy-Weinberg expected genotypic proportion at 16 microsatellite loci

Locus	p-value
EV37	0.8778
EV1	0.1290
GT310	0.0000*
GATA28	0.0401
GT575	0.0495
EV94	0.6931
GT23	0.0157
GT509	0.0000*
GATA98	0.4467
GATA417	0.0461
GT211	0.0298
EV21	0.1517
DlrFB14	0.0389
EV14	0.0003*
GT195	0.0000*
TAA31	0.0474
All loci	Highly significant

* Significant after correction for the multiple tests.

Baker and Wade (in press b) argued against our interpretation of the mixing of the two stocks from the results of the HW tests by citing Wada (1991). Contrast to our results, Wada (1991) found no deviation from the HW expected genotypic proportion at the Adh-1 locus in the sample from the small-type coastal whaling from SA7W. This contrast occurred most likely because the past commercial whaling sample might have contained the J stock individuals less than the current one did. The area of the coastal whaling at that time was wider than that of the current JARPNII Sanriku survey (e.g., Kanda *et al.*, in press), and chance to obtain the O stock is higher in the offshore area than in the coastal one as shown below. Indeed, mixing proportion of the J stock in the sub-sample of the past commercial whaling (N=141) was estimated as 0.022, compared to 0.179 (N=444) in the current JARPN/JARPNII samples, based on the genetic variation at the control region of mtDNA (M. Goto, unpublished). Furthermore, Wada (1991) relied only on the result from one locus, so that no detection of the HW deviation could have been due to the low statistical power. Genetic diversity at allozymes is low. Therefore, no evidence of the deviation from the HW expected genotypic proportion at Adh-1 locus in the commercial whaling sample from SA7W is not definitive reason to deny the mixing of the stocks at the area.

Distance from the coastal line
Proportion of the minke whales by the distance from the Japanese coastal line of North Pacific side in SA7W was different between those assigned as the J and O stocks (Fig. 1). The proportion of the O stock whales increased toward offshore area. Such clinal distribution supports the mixing of the two stocks.

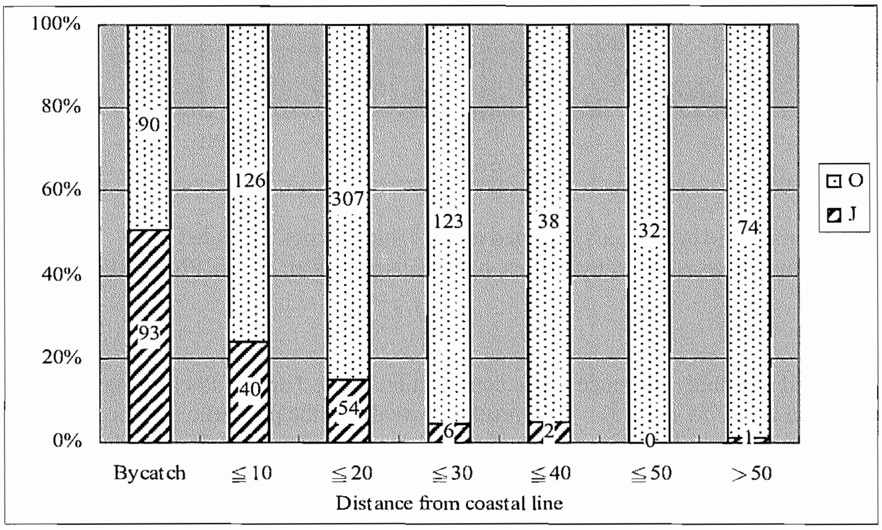

Fig. 1. Proportion of the minke whales assigned to the J and O stock collected from SA7W by the distance from the Japanese coastal line.

Body length
Proportions of the individuals assigned as the J and O stock from SA7W differed by body length classes (Fig. 2). The proportion of the O stock increased at the larger classes. Statistical tests indicated that the average body length of the O stock individuals was larger than that of the J stock individuals at all the three sample sources (bycatch, $p<0.01$; coastal, $p<0.05$; offshore, $p<0.01$). Such change is highly unlikely among individuals obtained from a single stock.

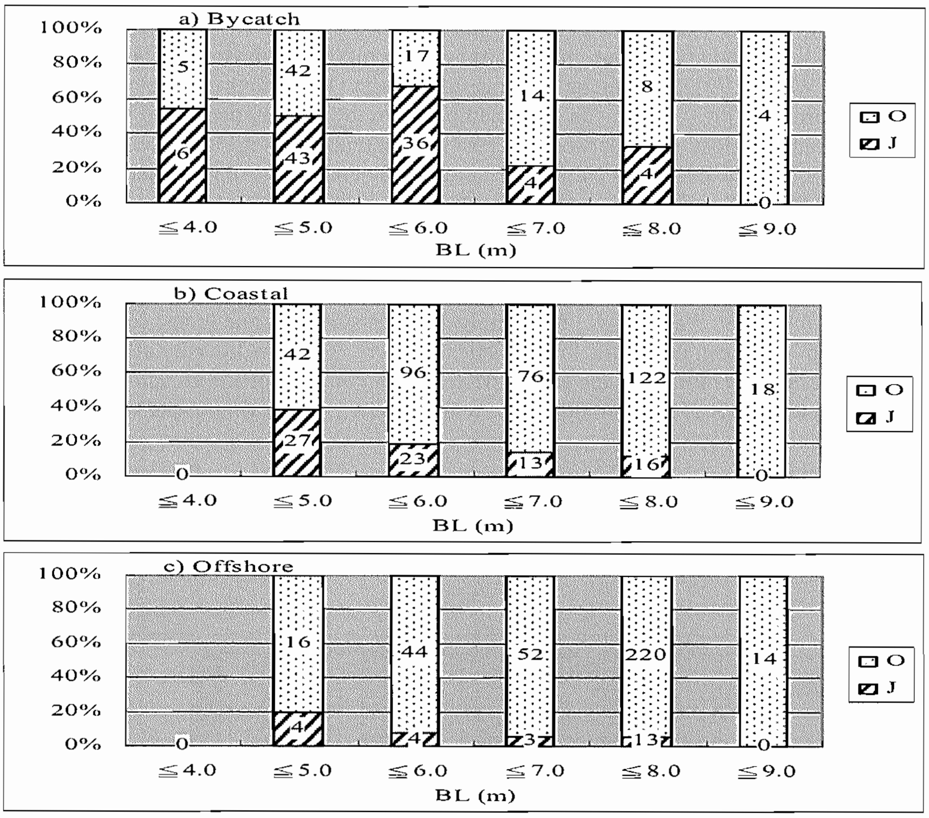

Fig. 2. Proportions of the individuals assigned as the J and O stock collected from SA7W by body length classes. a) bycatches, b) JARPNII coastal, c) JARPN/JARPNII offshore.

Seasonal distribution

Kanda *et al.* (2009a) presented seasonal difference in the composition of the J and O stock along the Japanese coast of the North Pacific side in SA7W (Fig. 3). The result revealed that the proportion of the O stock increased in spring and decreased toward winter season although both the J and O stock individuals stayed year round.

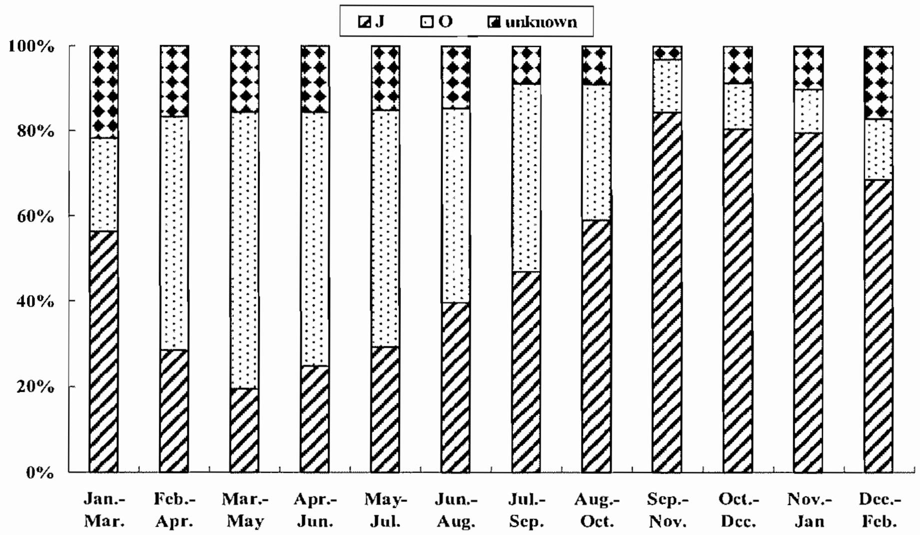

Fig 3. Seasonal change in composition of the bycatches assigned as the J, O, and unknown categories in SA7W (from Kanda *et al.*, 2009a). Each bar was expressed as three months intervals.

Fig. 4 shows the detail look of the bycatches from April to June and from October to December, where clear difference in stock composition was observed from the seasonal analysis shown above. The seasonal difference between the two stocks was caused by the decrease of the O stock whales in winter. Again, this kind of the difference is highly unlikely to happen within a single stock. This seasonal pattern was well consistent with the migration pattern of the two stocks described in Hatanaka and Miyashita (1997) and Hatanaka *et al.* (2010).

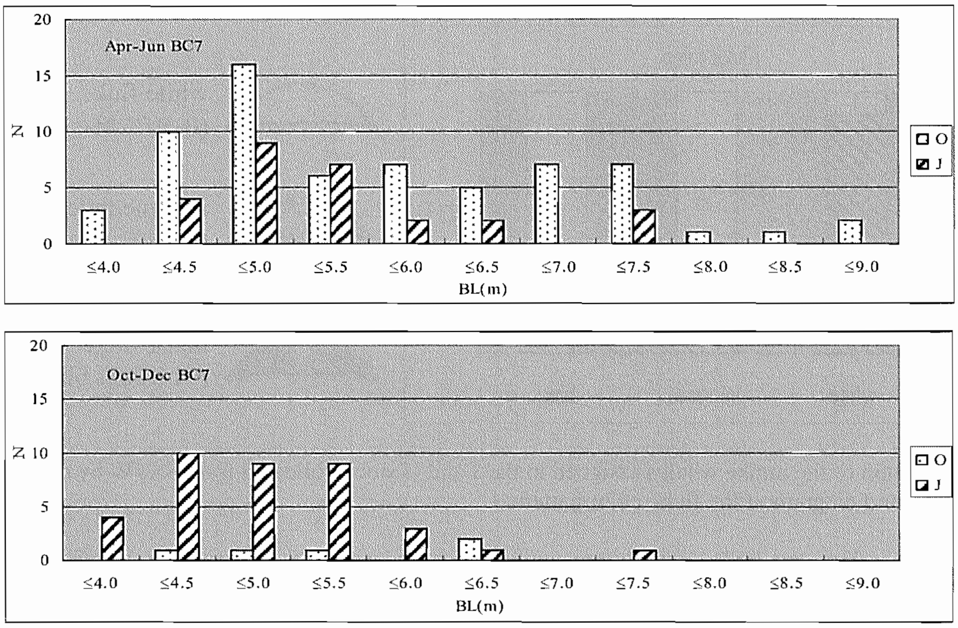

Fig. 4. Frequency of bycatches from SA7W assigned as the J and O stock from April to July (above) and from October to December (below) by body length.

Size of white band of the flipper

Kato *et al.* (1992) first reported that flipper coloration was a valuable trait for the stock identity of North Pacific common minke whales. Nagatsuka (2010) then found that size of the white band of the flipper was different between the individuals assigned as the J and O stocks sampled from 2007 JARPNII coastal and offshore samples. The size of the white band was calculated as the size of the white band over the total size of the flipper (%). In this paper, the average size of the white band was compared between the individuals collected only from SA7W (Fig. 5). The size of white band tended to be larger in the J stock than in O stock ($p<0.001$).

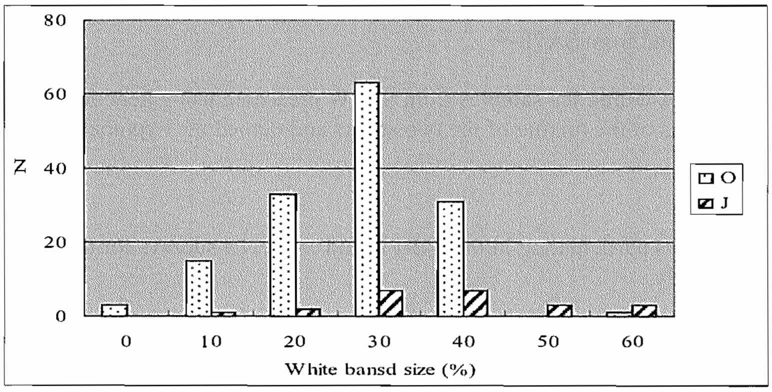

Fig. 5. Size of the white band of the flipper among the minke whales assigned as the J and O stock collected from SA7W.

Fluke color pattern

Nagatuska (2008) showed that minke whales had different black and white patterns on the underside of their flukes, and then separated the sampled whales to three different fluke color types (Fig. 6). Nagatsuka (2010) then found the differences in frequencies of these three types between the individuals assigned as J and O stocks sampled during 2007 JARPNII coastal and offshore surveys. In this paper, the frequencies of the three types were recalculated for the samples collected only from SA7W (Fig. 6). The frequencies were different between the individuals assigned as the J and O stocks (p<0.001).

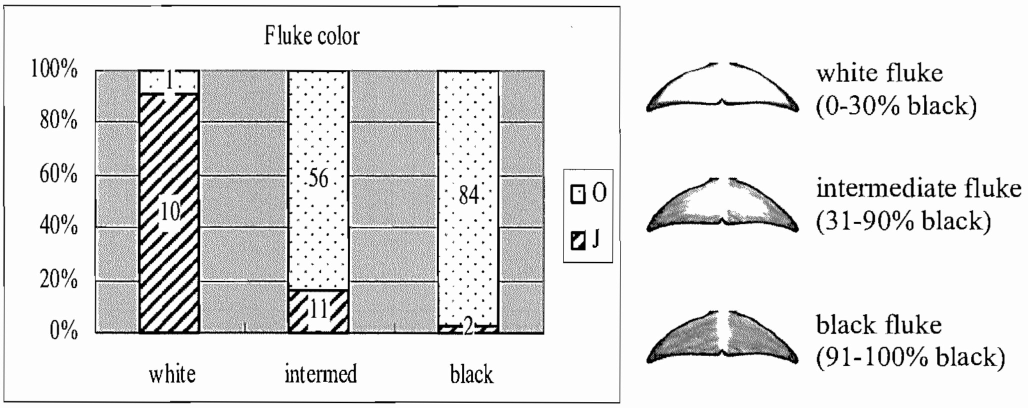

Fig. 6. Proportion of the minke whales assigned to the J and O stock collected from SA7W by the fluke color pattern, and diagram of the fluke color pattern.

The heterogeneities in these two body color patterns within the samples from SA7W support the mixing of the two stocks in the area.

CONCLUSION

A stock can be defined as a group of individuals sharing a common gene pool maintained by random mating. This means that, within a single stock, samples from large and small body size, from different seasons, and from different areas should show the same genetic characteristics. However, our samples from SA7W did not match to the expectation. All of the results presented in this paper showed the heterogeneities within the samples for such cases.

Although they recognized that the available data could have indicated either hypothesis, Wade and Baker (2010) concluded, without presenting any strong supporting evidence, that hypothesis of the single stock with intermediate genetic characteristics was more plausible than that of the two stocks mixing for the stock structure of minke whales in SA7W. Our results denied the possibility that our SA7W samples might have come from a single group of minke whales sharing a common gene pool maintained by random mating and thus did not show any evidence for the single stock hypothesis. It is also important to note that after the removal of the individuals assigned to the J stock, no evidence of the heterogeneities were observed between the individuals collected from SA7W and from SA7E-9.

Non-random distributions of the whales within the samples from SA7W presented using genetic and non-genetic data support the hypothesis of the mixing of the two stocks and denied the hypothesis of the single stock.

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