

## Stock structure of the western North Pacific minke whales based on the allozyme analyses.

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### ABSTRACT

To answer the question whether or not there exists a genetically distinct minke whale stock from the O stock in the North Pacific, allozymes at *Adh-1*, *Gpi* and *6Pgd* were examined on 497 minke whales taken in the JARPN surveys during 1994-1999 by starch gel electrophoresis. The analysis resulted in that there is no evidence for the presence of the W stock in sub-areas 7, 8 and 9, and that there is a considerable mixing of J and O stock whales (male>female) in sub-area 11 in July-August. A point estimate for the proportion of the J stock whales there was calculated as 33% using *Adh-1* data.

### INTRODUCTION

Stocks of the Japanese coastal minke whales were first examined by Omura and Sakiura (1956) followed by Ohsumi (1983). They discriminated two stocks; one distributing in the waters west of Honshu (currently known as the Sea of Japan-Yellow Sea-East China Sea stock or the J stock) and the other distributing in the waters east of Honshu (currently known as the Okhotsk Sea-west Pacific stock or the O stock).

Previous allozyme studies demonstrated genetic isolation between J and O stocks (Wada, 1984; Wada and Numachi, 1991) and partial mixing of the two stocks in sub-area 11 in April (Wada, 1991). This was further examined by Punt *et al.* (1995), Butterworth *et al.* (1996) and Pastene *et al.* (1998). At the RPM Working Group on North Pacific minke whales at the 45th IWC Scientific Committee, a complicated sub-stock scenario was propounded, which needs further information on stock structure. Present paper gives a preliminary result of the allozyme analysis using data from JARPN surveys conducted by Japan under special permit.

### MATERIAL AND METHOD

A small block of liver was collected from 497 (total catch minus one) minke whales taken in the JARPN surveys conducted in sub-areas 7, 8, 9 and 11 of the North Pacific. The three loci *Adh-1*, *Gpi* and *6Pgd*, were examined for genetic variations by starch gel

electrophoresis in the Aminopropyldiethanolamine-citrate buffer, pH 7.0. Gene frequency data from the past Japanese and Korean commercial whalings were extracted from Wada (1991). The deviation of the observed genotype frequency was tested by goodness-of-fit  $G$ -test, and the significance of allele frequency difference among sub-areas was tested by contingency  $G$ -test (Sokal and Rohlf, 1969).

## RESULTS AND DISCUSSION

No variation was found at  $6Pgd$  locus among all samples. At  $Gpi$  locus, 28 whales showed a heterozygous genotype,  $ab$ , and 469 whales, homozygous  $aa$ . The frequency of  $Gpi^b$  was 0.972 for the total sample, ranging from 0.956 in sub-area 11 to 0.978 in sub-area 7. At  $Adh-1$  locus, four alleles and eight genotypes,  $dd$ ,  $dg$ ,  $dh$ ,  $di$ ,  $gg$ ,  $gh$ ,  $hh$ , and  $hi$ , were recognized. The most common allele was  $Adh-1^h$ . The numbers of whales of the five minor genotypes were  $dg=7$ ,  $di=1$ ,  $gg=1$ ,  $gh=6$ , and  $hi=1$ . These 16 whales were excluded from the present analysis.

The observed genotype frequencies at  $Adh-1$  in each sub-area and in total sample showed good fit to the Hardy-Weinberg expectations, and the deviations were all statistically insignificant (Table 1). However, the  $Adh-1^h$  frequency in sub-area 11 showed a considerably low value of 0.480. This is about 0.2 smaller than the value for the O stock (0.681) which was extracted from the past Japanese commercial whaling data in sub-area 7. Spatial heterogeneity of the allele frequency is highly significant for the total sample ( $G_H=23.87$ ,  $df=3$ ,  $p<0.0001$ ), but it turns to be insignificant when sub-area 11 is excluded ( $G_H=2.29$ ,  $df=2$ ,  $p<0.5$ ). Furthermore, the difference in sub-area 11 between the observed genotype frequencies and the expectations calculated using the O stock allele frequencies ( $Adh-1^h=0.681$ ) was highly significant ( $G_D=26.36$ ,  $df=2$ ,  $p<0.00001$ ). The null hypothesis that all samples in sub-area 11 belong to the O stock can be rejected.

To examine further, 76 whales taken in sub-area 11 were tested by month (July and August) and by sex. The difference between the observed genotype frequencies and the expectations calculated using the O stock allele frequencies was greater in July than in August and was greater in male than in female (Table 2). As in the past commercial whaling samples, mixing of J stock whales (mostly by males) in sub-area 11 was clearly shown in the present JARPN samples though the season and sex are different. Although the  $G_D$  value for female was insignificant, the value of  $Adh-1^h=0.533$  suggests that not very small number of J stock females also migrate into sub-area 11 in July-August.

The proportion of the J stock whales in sub-area 11 ( $m_j$ ) is given by

$$0.480 = 0.067 m_j + 0.681 (1-m_j)$$

where, 0.480, 0.067 and 0.681 are the value of  $Adh-1^h$  frequencies for sub-area 11, the J stock and the O stock, respectively. The point estimate of  $m_j$  was calculated as 33%,

which gives the expected number of J stock whales in sub-area 11 as 25 whales. In this case, four whales with *cf* genotype of *6Pgd* and 11 whales with *ab* genotype of *Gpi* are expected to be included in 76 whales taken in sub-area 11. However, since the actually observed numbers of such whales were zero for *6Pgd* and seven for *Gpi*, the value of 33% may be a little overestimated. Wada (1991) showed that a part of J stock whale, mostly pregnant females, migrates into sub-area 11 in April, and that after May O stock whales occupy there. The  $m_j$  value for April have been reported as 64% from *Adh-1* data and 53% from *Gpi* data.

The value of *Adh-1<sup>h</sup>* in sub-area 8 (0.635) was a little smaller than in sub-area 7 (0.685), sub-area 9 (0.699) and the O stock (0.681). However, the null hypothesis that all whales in sub-area 8 belong to the O stock could not be rejected ( $G_D=1.48$ ,  $df=2$ ,  $p<0.5$ ). When the sub-areas 7, 8, and 9 were combined, the observed genotype frequencies showed very good fit both to the Hardy-Weinberg expectations ( $G_D=0.02$ ,  $df=1$ ,  $p>0.90$ ) and to the expectations calculated using the O stock allele frequencies ( $G_D=0.02$ ,  $df=2$ ,  $p<0.98$ ). Therefore, the present allozyme data do not support the presence of any previously unknown stocks in the North Pacific.

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Table 1. Observed genotype frequencies,  $G_D$ -value for deviation from the Hardy-Weinberg expectations ( $G_D$ ), and allele frequency values at *Adh-1* for the JARPN and past commercial whaling samples.

	Sub-areas					O stock*	J stock**
	7	8	9	11	Total		
<i>dd</i>	16 (13.7)	12 (11.9)	14 (16.1)	21 (20.5)	63 (59.4)	56 (61.1)	40 (39.2)
<i>dh</i>	55 (59.6)	41 (41.2)	79 (74.8)	37 (38.0)	212 (219.2)	271 (260.8)	4 (5.6)
<i>hh</i>	67 (64.7)	36 (35.9)	85 (87.1)	18 (17.5)	206 (202.4)	273 (278.1)	1 (0.2)
Total	138	89	178	76	481	600	45
$G_D$	0.80	0.00	0.56	0.05	0.52	0.93	2.14
<i>d</i>	0.315	0.365	0.301	0.520	0.351	0.319	0.933
<i>h</i>	0.685	0.635	0.699	0.480	0.649	0.681	0.067

Figures in parenthesis are the Hardy-Weinberg expectations. The number of degree of freedom for each  $G_D$  value is 1. All tests were insignificant. \*: Data from the Japanese coastal whaling in sub-area 7 (= Region B+C). \*\*: Data from the Korean coastal whaling in sub-area 6.

Table 2. Deviations of the observed genotype frequencies from the expectations calculated using the O stock allele frequencies at *Adh-1*.

	Sub-area	Sub-area	Sub-area 11		Sub-area 11		Sub-area
	8	11	July	August	Male	Female	(7+8+9)
<i>dd</i>	12 (9.1)	21 (7.7)	12 (4.8)	9 (3.0)	14 (4.7)	7 (3.1)	42 (41.2)
<i>dh</i>	41 (38.7)	37 (33.0)	27 (20.4)	10 (12.6)	23 (20.0)	14 (13.0)	175 (176)
<i>hh</i>	36 (41.3)	18 (35.3)	8 (21.8)	10 (13.4)	9 (21.3)	9 (13.9)	188 (187.8)
Total	89	76	47	29	46	30	405
$G_D$	1.48	26.36	21.09	9.30	21.48	5.65	0.02
P	<0.5	<0.00001	<0.0001	<0.01	<0.0001	<0.1	>0.98
<i>d</i>	0.365	0.520	0.543	0.483	0.554	0.467	0.320
<i>h</i>	0.635	0.480	0.457	0.517	0.446	0.533	0.680

The number of degree of freedom is 2 for all tests.