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Further mtDNA analysis on North Pacific minke whales including JARPN and JARPN II samples from 1994 to 2001

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ABSTRACT

In this paper we present the results of further mtDNA analysis based on the total JARPN and JARPN II samples from 1994 to 2001. Hypothesis testing is based on the North Pacific minke whale sub-areas and three different statistics: χ^2, Hst and Kst*. We also examined alternative grouping of samples in response to suggestions from a member of the North Pacific Minke Whale RMP IST Steering Group. A genetic criterion was used to identify the animals belonging to the J-Stock. These animals were excluded from the analysis. The source of some degree of mtDNA heterogeneity found is attributed to whales sampled in the western part of sub-area 9 in 1995, 2000 and 2001. While the sample size in 1995 is relatively large (n=78), the sample size in 2000 (n=16) and 2001 (n=29) are small. Thus no definitive conclusion can be obtained for these two years. If samples from sub-area 9W in 1995, 2000 and 2001 are excluded from the analysis, no mtDNA heterogeneity is found in sub-areas 7, 8, 9 and 11 for the different groupings used. These results support the one-stock scenario in sub-areas 7, 8, 9 and 11 but the possibility that a different stock occurs sporadically in sub-area 9W, cannot be discarded.

INTRODUCTION

Previous genetic analyses based on JARPN samples and hypothesis testing were summarized by Pastene *et al.* (2000). The most recent genetic analysis was based on mtDNA and microsatellite analyses and used samples from the 2000 JARPN II survey, in addition to those from the JARPN surveys (Goto *et al.*, 2001). The results of these genetic analyses provided no firm evidence for a multi-stock scenario in sub-areas 7, 8 and 9. The mtDNA analysis presented in Goto *et al.* (2001) provided some evidence of mtDNA heterogeneity due to samples from sub-area 9W in two years, 1995 and 2000.

In this paper we presented a new mtDNA analysis based on the total samples available from JARPN and JARPN II in sub-areas 7, 8, 9 and 11 from 1994 to 2001. We used a genetic criteria to identify the J Stock animals present in these sub-areas and exclude them from this analysis following a recommendation of the Scientific Committee.

MATERIALS AND METHODS

Samples and localities

Samples used in this study were taken during the JARPN and JARPN II surveys in sub-areas 7, 8, 9 and 11 from 1994 to 2001. The total number of samples examined in this study (JARPN and JARPN II) is shown in Table 1 by sub-area, month and sex. The geographical localities of minke whales of a total samples is shown in Fig. 1.

Sequencing of the mtDNA control region

The first half of control region of the mitochondrial genome (487bp) was sequenced by the same method used in our previous study (Goto and Pastene, 2000). All the procedures for DNA extraction and amplification of mtDNA control region were the same as in the previous study.

Data analysis

Homogeneity tests were conducted using the randomized χ^2 test (Roff and Bentzen, 1989) and Hudson *et al.* (1992)'s sequence (Kst*) and haplotype (Hst) statistics.

Criteria for definition of 'J' type animals

We tentatively defined 'J' stock animals by the following criteria. Firstly we allocated mtDNA-sequencing haplotypes to three categories as follow:

1) JJ type; haplotypes detected in samples from Sea of Japan side only
2) JO type; haplotypes detected in samples from both Sea of Japan and Pacific side of Japan
3) OO type; haplotypes detected in samples from Pacific side of Japan only

In our analysis we used those samples being characterized by the OO type criteria. Furthermore within this OO type sub-set we excluded those samples defined by two-site criteria (i.e. positions 298 and 463), which are likely to be for the J-stock.

RESULTS

Variability of mtDNA control region sequences of JARPN and JARPN II samples

Table 2 shows mtDNA haplotype frequencies in samples taken by JARPN (1994-1999) and JARPN II (2000 and 2001). Haplotype '9' was predominant in sub-area 9W in the 2000 (37.5%) and 2001 (24.1%) samples. These situations are similar to the case in the 1995 sub-area 9W (23.1%) sample. This haplotype presented a frequency larger than that observed in sub-area 9E in 1995 and those observed in sub-areas 7 and 8.

Homogeneity test

Using three statistical tests, no significant yearly variation was found in sub-areas 7, 8, 9 and 11. In the following analyses we combined samples from different years in these sub-areas. Table 3A shows the results of the homogeneity tests among sub-areas 7, 8 and 9. No significant differences were found between sub-areas 7 and 8 by three statistical tests. On the other hand, the comparison between sub-area 9 and other two sub-areas showed significant differences. These significant differences do not occur when samples from sub-areas 9W in 1995, 2000 and 2001 are excluded from the analysis (Table 3B).

Table 4 shows the results of the homogeneity tests for the comparison between sub-areas 11+7 and 8, sub-areas 11+7 and 9, and sub-areas 11+7+8 and 9. Prior to these analyses, we compared sub-areas 7 and 11. No significant differences were detected (χ^2; P=0.7226, Hst; P=0.7733, Kst*; 0.7491).

There is no significant difference between sub-areas 11+7 and 8. In contrast to that, the comparisons between sub-areas 11+7 and 9 and sub-areas 11+7+8 and 9 shows significant differences, except Kst* statistics (P=0.0842) for the case of sub-areas 11+7 and 9. When the samples from the western part of sub-area 9 in 1995, 2000 and 2001 are excluded from the analysis, no mtDNA heterogeneity is found in sub-areas 7, 8, 9 and 11 for the some groupings used (Table 5).

Table 6 shows the results of comparison between sub-areas 7 + 8W and sub-areas 8E + 9. Sub-area 8 was divided into east and west by 153° E. Significant differences are detected by χ^2 and Hst. When the samples from the western part of sub-area 9 in 1995, 2000 and 2001 are excluded from the analysis, however, no mtDNA heterogeneity is found in this combination (Table 7).

Comparisons between sub-areas 8E and 9 show significant differences by Hst and Kst* and near to significant by χ^2 (P=0.0539)(Table 8).

DISCUSSION

In this paper we presented a new mtDNA analysis based on the total samples available from JARPN and JARPN II in sub-areas 7, 8, 9 and 11. Following a recommendation from the Scientific Committee we excluded the potential J Stock samples from these sub-areas. To identify J-Stock individuals we used a method based on genetic criteria. We feel that most of the J Stock animals had been removed from the research area as we uses an 'extreme' criterion to identify J Stock animals. However we also feel that the results of this genetic method should be contrasted with those based on non-genetic methods (e.g. occurrence of scar, fetal length). Some inconsistencies have been found in the past among results derived from genetic and non-genetic methods. This requires further consideration.

In the 2001 JARPN II survey in sub-area 9W we found a similar scenario to that observed in 1995 and 2000 (Goto *et al.* 2001). The frequency of a particular haplotype '9' in this sample was higher than in the other sub-areas. When the total samples are considered in the statistical analysis, we found some degree of mtDNA heterogeneity due to sub-area 9. However when these samples are excluded, no genetic heterogeneity was observed for the different combinations made.

The number of samples in sub-area 9W in 1995 is relatively high (78). In contrast the sample sizes in sub-area 9W in 2000 and 2001 is smaller, and then no firm conclusion can be obtained from these samples.

In general results from our analysis provide no strong evidence for additional stock structure in sub-areas 7, 8, 9 and 11. Because the heterogeneity observed in sub-area 9W in some years, we cannot discard the possibility of a sporadic occurrence of a different stock in sub-area 9W.

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Table 1. Number of samples collected during the JARPN surveys from 1994 to 1999 and JARPN II in 2000 and 2001, by sub-area, year, month, period and sex.

Sub-area	Year	Month										Total
		Early				Late						
		May		June		July		August		September		
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	
7	1996					1			15	2	13	31
	1997				2							2
	1998	7	49									56
	1999			7	43							50
	2000							1	5	4	14	24
	2001	3	25		22							50
	Sum											213
8	1996						11		5			16
	1997					1	30					31
	1998	1	7	3	33							44
	2001					1	20					21
	Sum											112
9	1994					2	6	1	8		4	21
	1995				14	5	56	4	21			100
	1997	7	20	5	35							67
	2000								16			16
	2001					3	21		5			29
	Sum											233
11	1996							11	19			30
	1999					22	28					50
	Total	18	101	15	149	35	172	17	94	6	31	638

Table 2. MtDNA haplotype frequencies in samples taken by JARPN (1994-1999) and JARPN II (2000-2001).

	JARPN+JARPN II 1994-2001			JARPN 1995		JARPN II	
						2000	2001
Hap.	SA7	SA8	SA9	West	East	SA9	SA9
1	9	1	2	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	3	1	1	0	0	0	1
5	0	0	0	0	0	0	0
6	12	3	10	4	0	1	0
7	20	12	20	5	4	3	2
8	7	1	3	1	2	0	0
9	22	10	43	18	0	6	7
Freq.	0.103	0.089	0.185	0.231	0.000	0.375	0.241
10	1	0	0	0	0	0	0
11	8	5	12	7	1	0	2
12	6	5	7	2	2	0	0
13	15	9	4	1	1	0	0
14	8	7	13	4	1	0	2
15	6	4	7	2	0	0	1
16	16	4	12	4	1	0	1
17	9	6	19	5	3	2	1
18	2	2	3	2	0	0	0
19	2	1	1	0	0	0	0
20	4	0	1	0	0	0	1
21	3	1	3	1	0	0	0
22	1	0	0	0	0	0	0
23	2	0	1	0	1	0	0
24	1	0	0	0	0	0	0
25	1	2	4	2	0	0	0
26	1	1	0	0	0	0	0
27	1	0	1	0	0	0	0
28	2	0	0	0	0	0	0
29	5	4	6	0	0	0	1
30	9	1	10	1	2	1	0
31	2	1	3	2	0	0	1
32	1	0	0	0	0	0	0
33	2	0	0	0	0	0	0
34	3	4	4	1	1	1	0
35	2	2	0	0	0	0	0
36	4	5	3	1	0	0	2
37	3	0	2	1	0	0	0
38	2	1	1	1	0	0	0
39	1	2	5	3	0	0	0
40	0	1	0	0	0	0	0
41	0	1	0	0	0	0	0
42	0	1	0	0	0	0	0
43	1	3	1	0	0	0	1
44	0	1	0	0	0	0	0
45	1	2	1	0	0	0	1
46	1	1	0	0	0	0	0
47	0	1	0	0	0	0	0
48	0	1	1	1	0	0	0
49	0	1	1	0	0	0	0
50	0	1	2	0	0	1	0
51	0	1	1	0	0	0	0
52	0	0	6	2	1	0	1
53	3	0	4	3	0	0	0
54	0	0	1	1	0	0	0
55	0	0	1	1	0	0	0
56	0	0	1	1	0	0	0
57	0	0	1	1	0	0	0
58	0	0	1	0	1	0	0
59	0	0	3	0	1	0	1
60	0	0	3	0	0	0	1
61	0	0	1	0	0	0	0
62	1	0	0	0	0	0	0
64	1	0	0	0	0	0	0
66	3	0	0	0	0	0	0
75	0	2	0	0	0	0	0
81	2	0	0	0	0	0	0
87	1	0	0	0	0	0	0
88	0	0	1	0	0	1	0
89	1	0	0	0	0	0	0
90	1	0	0	0	0	0	0
91	1	0	0	0	0	0	0
92	0	0	1	0	0	0	1
93	0	0	1	0	0	0	1
Total	213	112	233	78	22	16	29

Table 3A. Comparison among sub-areas 7, 8 and 9 by three statistical tests (χ^2; upper, Hst; middle, Kst*; lower). Figures shown are P values.

		SA7 n=173	SA8 n=96
		0.1542	
SA8	n=96	0.5020	
		0.4233	
		0.0149	0.0500
SA9	n=192	0.0156	0.0177
		0.0570	0.0042

Table 3B. Comparison among sub-areas 7, 8 and 9 by three statistical tests (χ^2; upper, Hst; middle, Kst*; lower). Samples from west side of sub-area 9 in 1995, 2000 and 2001 were excluded from these analyses. Figures shown are P values.

		SA7 n=173	SA8 n=96
		0.1573	
SA8	n=96	0.5003	
		0.4320	
		0.1631	0.1812
SA9	n=92	0.6918	0.3646
		0.6310	0.1487

Table 4. Results of comparison by three statistical tests (χ^2, Hst and Kst*) between sub-areas 11+7 and 8, sub-area 11+7 and 9 and sub-areas 11+7+8 and 9. Figures shown are P values.

			χ^2	Hst	Kst*
SA11+7 N=224	vs	SA8 n=96	0.1588	0.572	0.3275
SA11+7 N=224	vs	SA9 n=192	0.0255	0.0256	0.0842
SA11+7+8 N=320	vs	SA9 n=192	0.0162	0.0049	0.0083

Table 5. Results of comparison by three statistical tests (χ^2, Hst and Kst*) between sub-areas 11+7 and 8, sub-area 11+7 and 9 and sub-areas 11+7+8 and 9. Samples from west side of sub-area 9 in 1995, 2000 and 2001 were excluded from these analyses. Figures shown are P values.

			χ^2	Hst	Kst*
SA11+7 n=224	vs	SA8 n=96	0.1588	0.572	0.3275
SA11+7 n=224	vs	SA9 n=92	0.1977	0.7272	0.7763
SA11+7+8 n=320	vs	SA9 n=92	0.1826	0.5889	0.4948

Table 6. Results of comparison by three statistical tests (χ^2, Hst and Kst*) between sub-areas 7 + 8W and sub-areas 8E + 9. Sub-area 8 was divided into east and west by 153° E. Figures shown are P values.

Sample size		P value		
SA7+8W	SA8E+9	χ^2	Hst	Kst*
213	248	0.0270	0.0291	0.2096

Table 7. Results of comparison by three statistical tests (χ^2, Hst and Kst*) between sub-areas 7 + 8W and sub-areas 8E + 9. Sub-area 8 was divided into east and west by 153° E. Samples from west side of sub-area 9 in 1995, 2000 and 2001 were excluded from these analyses. Figures shown are P values.

Sample size		P value		
SA7+8W	SA8E+9	χ^2	Hst	Kst*
213	148	0.1671	0.4138	0.7536

Table 8. Results of comparison by three statistical tests (χ^2, Hst and Kst*) between sub-areas 8E and 9. Sub-area 8 was divided into east and west by 153° E. Figures shown are P values.

Sample size		P value		
SA8E	SA9	χ^2	Hst	Kst*
56	192	0.0539	0.0396	0.0099

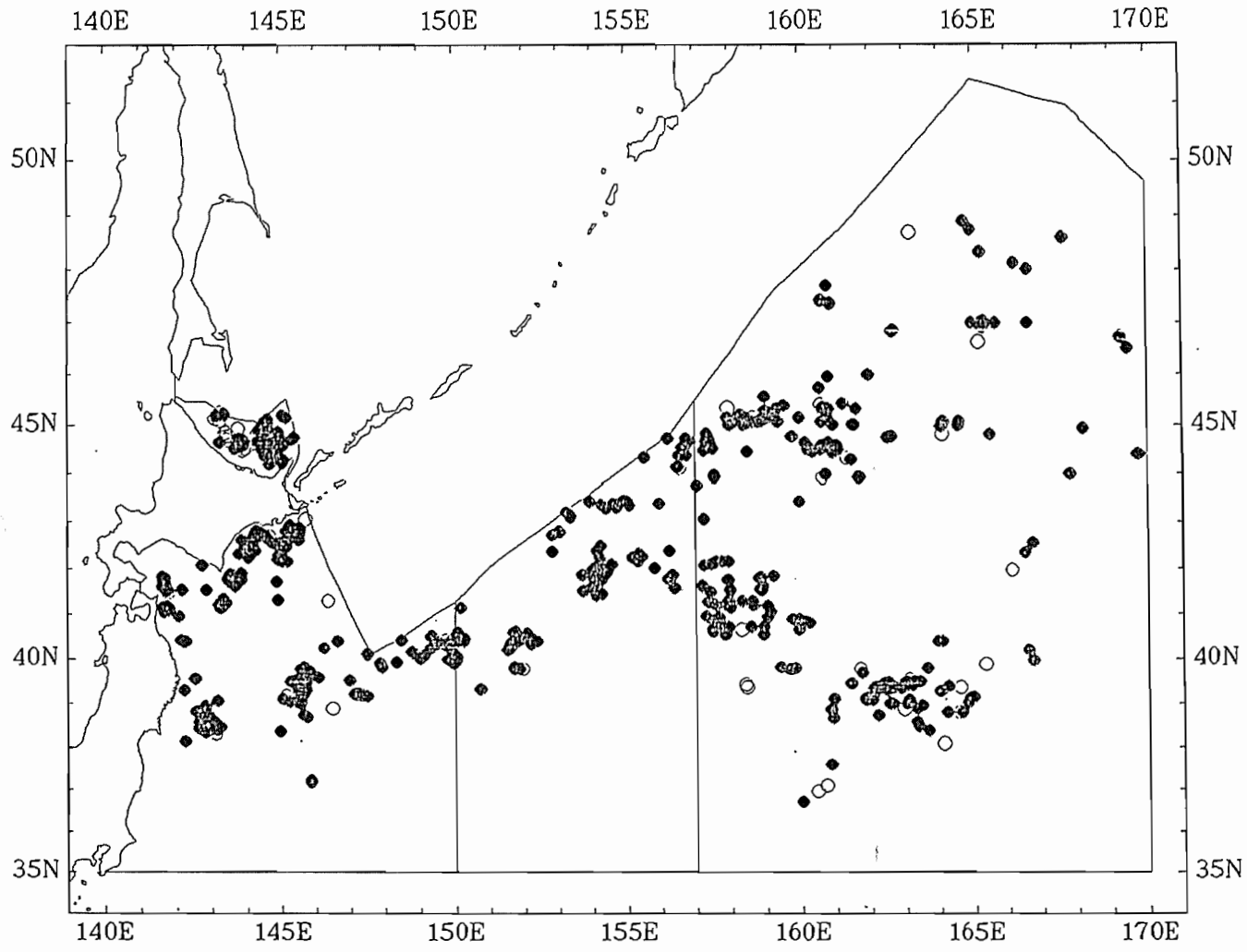

Fig. 1. Geographical positions of North Pacific minke whales sampled in the JARPN and JARPN II surveys from 1994 to 2001. ○: female, ●: male.