

Further spatial and temporal analysis of mitochondrial DNA variation in minke whales
from Areas III and IV with considerations on the pack-ice distribution

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ABSTRACT

A restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) in the ordinary form minke whale from Antarctic Areas III and IV was conducted using samples of minke whales taken during the 1995/96 and 1997/98 JARPA surveys. Samples were divided following the same criteria used in the previous analyses. Ten area/time/year groups were defined in Area III and IV: Area III eastern early, Area III eastern late, Area IV western early, Area IV western late and Area IV eastern late, in both 1995/96 and 1997/98 surveys. Of the 153 haplotypes described in a previous analysis, a total of 80 haplotypes was identified in 812 whales sampled in these surveys. Quantification of the mtDNA differentiation among area/time groups was carried out using the Analysis of Molecular Variance (AMOVA). The pattern of mtDNA variation in the two surveys in Areas III and IV was similar. A marginal significant P value was obtained in the comparison among area/time groups for the two surveys pooled. In general the total sample and the individual area/time groups were similar to the 'core' sample of Areas IV and V and different from the 'western' sample (Area IV western early 1989/90, 1991/92) examined previously. Thus the pattern of variation for Area IV western early in 1989/90, 1991/92 was different from that observed in 1995/96, 1997/98. We established two hypotheses consistent with these results. A more detailed examination of the offshore component of group IVWE (to which is attributed mtDNA heterogeneity) of several surveys was conducted, considering the distance from the ice-edge as a new factor. Results of such examination are consistent with the hypothesis of substantial yearly variation in the pattern of mtDNA variation in that area/time group. In addition, a preliminary mtDNA analysis considering different categories of school sizes is conducted.

INTRODUCTION

Pastene *et al* (1996a) conducted an extensive mitochondrial DNA (mtDNA) survey on 2,124 minke whales sampled by the JARPA (Japanese Whale Research Program under Special Permit in the Antarctic) in Areas IV and V between 1987/88 and 1994/95.

The survey revealed considerable mtDNA heterogeneity but little geographic concordance with these putative management Areas. The study showed significant heterogeneity in a single group of whales: those distributed in the western part of Area IV early in the austral summer season ('western' sample). A summary of their analysis is presented in Table 1.

On the basis of their results they hypothesized that the stock structure of the Antarctic minke whale could be more complex than it was thought initially and it could be determined not only by geographic factors (longitudinal) but also by temporal factors. For example it was suggested that a temporal component in the distribution of stocks could occur in the western part of Area IV (Pastene *et al.*, 1996a).

The pattern of mtDNA differentiation shown in Table 1 can be used as a baseline on which future comparisons in mtDNA composition can be based. It should be noted that the Group IVWE in Table 1 ('western' sample) involved whales from two JARPA surveys in Area IV: 1989/90 and 1991/92. After the study by Pastene *et al.* (1996a) two new JARPA surveys have been conducted in Area IV, in 1995/96 and 1997/98. These surveys have also surveyed the eastern part of Area III.

We presented here the results of a mtDNA analysis conducted on minke whale samples from these two new JARPA surveys and compare their mtDNA composition to that of minke whales examined previously in Area IV. We interpreted and discussed the results in the context of other factor (i.e. distance from the ice-edge), which possibly is affecting the pattern of mtDNA variation in the Antarctic feeding ground.

MATERIALS AND METHODS

Samples

Samples of the ordinary form minke whale from Areas IIIE and IV were available from the 1995/96 and 1997/98 JARPA surveys. Samples from each survey were grouped into ten area/time/year groups: Area III eastern early (group IIIEE, n=67 and 86 respectively), Area III eastern late (group IIIEL, n=38 and 13, respectively), Area IV western early (group IVWE, n=131 and 72 respectively), Area IV western late (group IVWL, n=99 and 118, respectively) and Area IV eastern late (group IVEL, n=62 and 126, respectively) (Table 2). 'Early' refers to whales sampled in December and first half of January. 'Late' refers to whales sampled in the second half of January, February and March.

RFLP analysis

Crude mtDNA extracted from liver tissues was digested with the same restriction enzymes of the previous study (Pastene *et al.*, 1996a): *AccI*, *BanI*, *EcoRV*, *HincII*, *HpaI* and *SspI*. All the procedures for DNA extraction and DNA digestion were the same as in the previous study.

Statistical analysis

The geographic and temporal differentiation of haplotypes was quantified using the Analysis of Molecular Variance, AMOVA (Excoffier *et al.*, 1992) as implemented in the computer program version 1.55. This program calculates variance components from a distance matrix and the PHist reflecting the correlation of haplotypic diversity at different levels of hierarchical subdivision. The significance of the variance components and PHist were tested using a random permutation procedure available in the program. The level of significance obtained by this procedure is referred in this paper as the P-value. Samples in pairwise comparisons were considered to be significantly heterogeneous when the probability of obtaining the observed, or a more extreme, value of PHist was less than 0.05 in 10,000 simulations.

Statistical analysis was conducted as a stepwise. First we compared females and males in each area/time/year group. If not significant differences were found, females and males were pooled in the subsequent analysis. Second we compared area/time groups between the 1995/96 and 1997/98 surveys. If not significant differences were found, then samples from two surveys were pooled in the subsequent analysis. Finally area/time groups for two surveys combined were compared among them and to the sample of Group IVWE in Table 1 ('western' sample) and to one sample from the rest of the groups in Table 1 ('core' sample). In order to both interpret further the results of the mtDNA analysis and to deal with a JARPA review meeting recommendation, we conducted a preliminary analysis of mtDNA considering the school size as a covariate.

RESULTS

MtDNA haplotypes

The previous study by Pastene *et al.* (1996b), which used six polymorphic restriction enzymes, had discriminated a total of 153 mtDNA haplotypes in the southern ordinary form minke whale from Areas IV and V (JARPA) and Areas III E and VI W (commercial samples). Of them 80 were identified in the 1995/96 and 1997/98 JARPA surveys. The main haplotypes 1 through 5 were shared by the ten area/time/year groups (Table 3).

Homogeneity test

The results of the AMOVA indicates that no significant differences were found between male and female samples in each of the ten area/time/year groups. The subsequent analyses were conducted for both sexes combined.

No significant differences were found between area/time groups of two JARPA surveys. Thus area/time groups of the 1995/96 and 1997/98 surveys were pooled in the subsequent analyses.

Table 4 shows the results of the pairwise comparison by AMOVA among the five area/time groups. The total PH1st value was low (0.003) and marginally significant ($P=0.0652$). Strictly speaking the total sample was not significantly heterogeneous. The low total P value can be explained by certain degree of heterogeneity observed in group IVWL, which presented larger and significant PH1st values in the pairwise comparisons with groups IIIEL and IVWE (Table 4).

Table 5 shows the comparison between the total sample and between the individual area/time groups of the 1995/96 and 1997/98 surveys and the 'western' and 'core' samples of Table 1. The total sample as well most of the individual area/time groups were significantly different from the 'western' sample but similar to the 'core' sample of Table 1. The only exception was group IVWL, which showed a reverse pattern. No trend was observed for Group IVEL although the PH1st value in the comparison with the 'core' sample was smaller (and the P value larger) than in the comparison with the 'western' sample.

DISCUSSION

Spatial and temporal pattern of mtDNA variation

The pattern of mtDNA variation found in samples from the 1995/96 and 1997/98 surveys in Areas III E and IV were similar as no significant differences were observed between individual area/time

groups of the two surveys. While these two surveys were similar in the pattern of genetic variation, both differed markedly from the patterns of mtDNA variation found for the 1989/90 and 1991/92. As it was pointed earlier, in these surveys in Area IV the only source of significant mtDNA heterogeneity was attributable to the group of whales distributed in the western part of Area IV early in the summer season. By contrast, in the surveys of 1995/96 and 1997/98 whales from that particular area/time group did not exhibit any particular degree of heterogeneity. In 1989/90, 1991/92 that particular group was clearly different from the 'core' sample of Areas IV and V (Table 1). In 1995/96, 1997/98 that particular group was similar to the 'core' sample. These results suggest that in recent years the mtDNA composition of the 'core' sample was predominant in Areas IV and IIIIE. It is interesting to note that a sample from 1978 in Area IIIIE showed a mtDNA composition similar to the 'west' sample and different from the 'core' sample (Pastene *et al.* 1996b).

These results are consistent with the following hypotheses:

- i) Significant heterogeneity was not detected in Area IVWE (and Area IIIIE) due to differences in surveys patterns between previous and recent surveys in that sector. For example in the 1989/90, 1991/92 surveys, more samples could have been taken from offshore areas than in recent surveys (1995/96, 1997/98).
- ii) There are yearly changes in the pattern of mtDNA variation in Areas IIIIE and IVW.

In order to find evidence for supporting or rejecting either of these hypotheses, we examined further our samples considering a new factor: the distance between the ice-edge and the sighting position of the whale sampled. We conducted such examination for five surveys in Area IV (1989/90, 1991/92, 1993/94, 1995/96 and 1997/98) and two in Area IIIIE (1995/96 and 1997/98), following the study initiated by Goto *et al.* (1998).

Goto *et al.* (1998) showed that the main source of mtDNA heterogeneity in group IVWE of the 1989/90 and 1991/92 was attributed to the offshore component (whales taken from outside of a line 60n. miles from the ice-edge). Recent analyses conducted on a more fine scale (distance from the ice-edge were defined at 15, 30, 45 and 60 n.miles from the ice-edge) shows that the pattern of differentiation of group IVWE 1989/90 become stronger as distance from the ice-edge increase. Significant differences were observed for the partitioning of 45 and 60n. miles. This pattern was not observed for group IVWE in other JARPA surveys (data not shown). This suggest that the offshore component of the group IVWE is important in order to differentiate genetically this group.

Then if the offshore component in group IVWE was less represented in recent surveys (1995/96, 1997/98) than in the previous survey, then mtDNA heterogeneity could not have been detected in these surveys due to a problem of power of the analysis. In order to check this possibility we investigated the number of samples of the offshore component (45 and 60 n.miles) in four surveys of group IVWE (figures in parenthesis in Table 6). The offshore component is not represented in 1995/96. However, the number of offshore samples in the 1997/98 survey are larger than in 1989/90 and 1991/92. Thus, these data do not provide support for our hypothesis i) above.

It should be noted, however, that differences in distances from the ice-edge do not necessarily mean differences in latitude. Surveys in Area IVWE in 1989/90 and 1991/92 were carried out south

of 55°S while in the 1995/96 and 1997/98 surveys were conducted south of 60°S. We should investigate in the future whether latitude is a factor affecting the pattern of mtDNA variation.

To examine the possibility of yearly variation, we compared the offshore component of group IVWE among different JARPA surveys, for both 45 and 60 n.miles, respectively (Table 6). In each partitioning, no significant differences were found between the 1989/90 and 1991/92 surveys. In each of the partitioning, surveys in 1989/90 and 1991/92 were significantly different from the 1997/98 survey. Thus, these results give support to our hypothesis ii) above.

The only other group showing yearly variation at different level of partitioning was the 'offshore' component of group IVWL. This explain in part certain degree of mtDNA heterogeneity found in group IVWL in the present analysis (Tables 4 and 5) It should be noted that no inter-survey heterogeneity at all was observed for the ice-edge component (in all the partitioning) of groups IVWL and IVEL for which considerable data were available (data not shown). For Area IIIIE just two surveys were available and the sample become very small after additional sub-divisions.

It seems then that our data are more consistent with hypothesis ii) above. The behaviour of the minke whale in the feeding ground could change annually or even within a summer season depending on change in oceanographical conditions governing the dynamics of prey species like the krill. The distribution of Antarctic krill *Euphausia superba*, the main food of the southern minke whale, has been related to bottom topography, sea-ice and hydrographic features (Ichii, 1990). The annual fluctuation of the distribution of krill could affect the distribution of minke whales migrating into the Antarctic during the austral summer. In future, patterns of the mtDNA variation within and between surveys should be examined in the context of the dynamic of the prey species and in the context of the oceanographic features conditioning the distribution of such species.

School size as a new factor in the mtDNA analysis

An additional matter, which need to be addressed is the possible effect of different school sizes on our mtDNA analysis. During the JARPA review meeting a suggestion was made on the statistical analysis of mtDNA data in Areas IV and V. The suggestion read: 'It was recognised that the statistical analysis of the genetic data should consider the inclusion of school size as a covariate because 1) schools of different sizes are not detected with equal probability and 2) of the schools encountered, animals from smaller schools are oversampled relative to animals from larger schools'. We have conducted here a preliminary analysis on this subject.

As sample set we used minke whales from Area IVE sampled between 1989/90 and 1997/98. Samples from Area IVW were excluded from this analysis because the possible occurrence of more than one stock in that sector. Table 7 shows the pairwise comparisons for three categories of school sizes: $n=1$, $n=2$ and $n=or>3$. The total PH1st value for this comparison was 0.003 and presented a marginal significant P value (0.0816). However, none pairwise comparison was significant at the 5% level.

Because minke whales tend to form larger groups near the ice-edge than in offshore areas, we conducted the analysis of school sizes separately for offshore (outside of a line 45 n.miles from the ice-edge) and inshore whales (within of a line 45 n.miles from the ice-edge). The total PH1st value

for the inshore whales was -0.001 , which was not significant ($P=0.5658$). However, the PH1st value for the comparison within the offshore whales was higher (0.027) and significant at the 1% level ($P=0.0004$). Pairwise comparison within this group showed that school sizes of $n=1$ was significantly different from the other categories. This analysis should be considered preliminary and further analyses are being planned. The problem of small sample sizes in some categories, the effect of pooling samples from different years and the consideration of other categories of school sizes should be taken into consideration in these future analyses.

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Table 1: Haplotypic correlation (PHist, below diagonal) and their probabilities (P, above diagonal) among eight area/time groups of minke whales from Areas IV and V. In parenthesis is the sample size. Note that all pairwise comparisons involving group IVWE showed higher PHist values, all of them with P values below 0.01 or 0.05 (After Pastene *et al.*, 1996a).

Area/time group	IVWE (160)	IVWL (383)	IVEE (233)	IVEL (321)	VWE (208)	VWL (264)	VEE (76)	VEL (479)
IVWE	-	0.0345	0.0295	0.0015	0.0060	0.0103	0.0493	0.0040
IVWL	0.0052	-	0.4198	0.3628	0.2104	0.8676	0.5787	0.3058
IVEE	0.0072	-0.0000	-	0.2644	0.1674	0.2489	0.1724	0.3688
IVEL	0.0136	0.0001	0.0007	-	0.6327	0.6892	0.4218	0.5297
VWE	0.0105	0.0010	0.0017	-0.0009	-	0.4733	0.4673	0.7621
VWL	0.0076	-0.0015	0.0009	-0.0009	-0.0004	-	0.8361	0.3403
VEE	0.0087	-0.0015	0.0032	-0.0004	-0.0005	-0.0034	-	0.3093
VEL	0.0083	0.0003	0.0002	-0.0003	-0.0011	0.0003	0.0009	-

Table 2: Summary of minke whale samples from the 1995/96 and 1997/98 JARPA surveys used for the mtDNA analysis.

Survey season	Area IIIIEE		Area IIIIEL		Area IVWE		Area IVWL		Area IVEL	
	M	F	M	F	M	F	M	F	M	F
1995/96	45	22	22	16	100	31	44	55	37	25
1997/98	57	29	12	1	52	20	70	48	74	52

Table 3: Distribution of mtDNA haplotypes in ten area/time/year groups of minke whales of Areas III eastern and IV of the JARPA surveys 1995/96 and 1997/98.

Hap.	IIIEE		IIIEL		IVWE		IVWL		IVEL	
	95/96	97/98	95/96	97/98	95/96	97/98	95/96	97/98	95/96	97/98
1	23	23	9	8	38	29	34	25	18	41
2	7	8	3	1	14	10	7	14	8	10
3	4	4	1	0	6	2	2	6	2	5
4	5	5	4	0	7	0	11	11	5	11
5	4	6	3	1	5	1	1	5	2	3
6	1	1	1	0	1	0	2	2	0	3
7	2	3	1	0	3	1	3	1	3	3
8	0	4	0	0	7	1	3	5	3	2
9	0	0	0	0	0	0	1	0	0	1
10	3	4	1	0	4	2	3	4	2	3
11	0	0	1	0	2	2	1	0	0	1
12	2	2	0	0	0	0	0	3	0	2
13	0	0	0	0	0	0	0	1	0	2
14	2	2	2	0	4	1	1	0	1	1
15	0	0	0	0	2	0	0	1	1	0
16	0	0	0	0	0	0	0	1	0	0
18	0	0	0	0	0	0	0	1	0	2
19	1	0	0	0	4	1	2	3	2	3
20	0	0	1	0	1	0	0	4	1	1
21	0	1	0	0	1	2	1	0	2	0
22	0	3	0	0	0	0	1	3	0	1
23	1	1	2	2	5	2	0	2	0	1
24	0	2	0	0	0	1	3	0	1	0
27	1	1	0	0	0	0	0	1	0	0
28	0	0	0	0	0	0	5	1	0	0
29	0	1	1	0	1	1	1	2	0	1
30	1	0	0	0	0	0	0	3	2	0
31	1	1	3	0	2	0	2	1	2	0
32	0	0	0	0	1	1	0	0	1	1
34	0	0	0	1	0	1	0	1	0	1
35	0	0	0	0	0	1	0	0	1	0
36	0	0	0	0	0	0	0	0	0	1
42	0	0	0	0	0	1	0	0	0	1
44	1	1	0	0	1	0	0	1	0	0
46	0	0	0	0	0	0	1	0	1	0
47	1	4	1	0	3	2	2	2	0	4
48	2	1	0	0	0	1	0	1	0	1
51	1	0	0	0	1	1	2	2	0	2
52	0	0	0	0	0	0	0	0	0	1
54	1	0	0	0	3	0	1	0	0	2
55	0	0	0	0	0	0	0	2	0	0
59	0	1	0	0	2	0	1	0	0	0
60	0	1	0	0	2	1	1	0	0	0
61	0	1	1	0	0	0	0	1	0	1
62	0	0	0	0	0	1	3	1	0	1
63	0	1	0	0	0	1	0	0	0	0
64	0	0	0	0	0	0	1	0	0	0
66	0	0	1	0	0	0	0	0	0	0
68	0	0	0	0	0	0	0	1	0	2
69	0	0	0	0	2	0	0	0	0	1
70	0	0	1	0	0	0	0	0	0	0
72	0	0	0	0	0	1	0	0	0	0

Table 3: cont.

73	0	0	0	0	0	1	0	0	0	0
75	1	0	0	0	0	0	0	1	0	1
76	0	1	0	0	2	0	0	0	0	3
79	0	0	0	0	0	0	0	0	1	0
82	0	0	0	0	2	0	1	0	0	0
84	1	0	0	0	0	0	1	0	0	0
87	0	0	0	0	1	0	0	0	0	0
89	0	0	0	0	1	0	0	0	0	0
90	0	0	0	0	1	0	0	0	0	0
95	0	0	0	0	0	1	0	0	0	0
96	0	0	0	0	0	0	0	0	1	0
99	1	1	0	0	0	0	0	0	0	0
103	0	0	0	0	0	1	0	0	0	1
107	0	1	0	0	0	0	0	0	0	0
108	0	0	0	0	0	0	1	0	0	0
116	0	0	0	0	0	0	0	0	0	1
117	0	0	0	0	1	0	0	0	0	0
121	0	0	0	0	0	0	0	1	1	0
123	0	0	0	0	1	0	0	1	0	0
124	0	0	0	0	0	0	0	1	0	0
125	0	0	0	0	0	1	0	0	0	0
129	0	0	0	0	0	0	0	0	0	1
130	0	1	0	0	0	0	0	1	0	0
131	0	0	0	0	0	0	0	0	1	0
141	0	0	0	0	0	0	0	0	0	1
143	0	0	0	0	0	0	0	1	0	0
147	0	0	1	0	0	0	0	0	0	1
152	0	0	0	0	0	0	0	0	0	1
Total	67	86	38	13	131	72	99	118	62	126

Table 4: Results of the statistical analysis by AMOVA for pairwise comparisons among area/time groups in Areas III eastern and IV (samples for surveys 1995/96 and 1997/98 combined). The total PHist value was 0.003 (P=0.0652). Figures in parenthesis are the sample sizes. Figures in bold indicate probability below 0.05.

Area/time gr.	Area IIIEE (n=153)	Area IIIEL (n=51)	Area IVWE (n=203)	Area IVWL (n=217)	Area IVEL (n=188)
Area IIIEE		PHist=0.0034 P=0.2349	PHist=-0.0003 P=0.4665	PHist=0.0023 P=0.1658	PHist=-0.0007 P=0.5286
Area IIIEL			PHist=-0.0026 P=0.6150	PHist=0.0174 P=0.0178	PHist=0.0090 P=0.073
Area IVWE				PHist=0.0080 P=0.0095	PHist=0.0004 P=0.3407
Area IVWL					PHist=-0.0012 P=0.6296

Table 5: Comparison by AMOVA between the total sample and individual groups of the 1995/96 and 1997/98 surveys and the 'western' and 'core' samples of Table 1. Figures in parenthesis are the sample sizes. Figures in bold indicate probability below 0.05.

	Core sample (889)	Western sample (160)
Area III+IV 95/96+97/98 (812)	PHist=-0.0002, P=0.6135	PHist=0.0060, P=0.0128
Area IIIEE (153)	PHist=-0.0016, P=0.8339	PHist=0.0063, P=0.0462
Area IIIEL (51)	PHist=0.0048, P=0.1447	PHist=0.0255, P=0.0056
Area IVWE (203)	PHist=-0.0006, P=0.6180	PHist=0.0153, P=0.0011
Area IVWL (217)	PHist=0.0046, P=0.0108	PHist=-0.0020, P=0.7555
Area IVEL (188)	PHist=-0.0005, P=0.5478	PHist=0.0024, P=0.1583

Table 6: Analysis of yearly variation by AMOVA for the 'offshore' (whales sighted outside a line of 45 or 60n. miles from the ice edge) component of the group Area IVWE, by survey year. Figures in parenthesis are sample sizes. Figures in bold indicate probability below 0.05.

a) 45 n. miles from the ice-edge

	1989/90 (n=45)	1991/92 (n=24)	1997/98 (n=61)
1989/90		PHIst=-0.0085 P=0.5092	PHIst=0.0709 P=0.0000
1991/92			PHIst=0.0289 P=0.0320

b) 60 n. miles from the ice-edge

	1989/90 (n=43)	1991/92 (n=20)	1997/98 (n=55)
1989/90		PHIst=-0.0088 P=0.6162	PHIst=0.0693 P=0.0000
1991/92			PHIst=0.0285 P=0.0280

Table 7: Examination of mtDNA heterogeneity considering different categories of school sizes: SCH1=school size of one animal; SCH2=school size of two individuals and SCH3=school size of 3 or more individuals. Figure in parenthesis are sample sizes.

	SCH1 (202)	SCH2 (117)	SCH3 (182)
SCH1		PHIst=0.0056; P=0.0594	PHIst=0.0029; P=0.1249
SCH2			PHIst=0.0005; P=0.3615