

Further RFLP analysis of mitochondrial DNA in Antarctic minke whales from Areas V and VI

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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 V and VI was conducted using samples of minke whales taken during the 1996/97 JARPA survey. Samples were divided following the same criteria used in the previous analyses in Areas IV and V. They were divided into three longitudinal sectors: Area V Western (130°E-160°E), Area V Eastern (160°E-170°W) and Area VI Western (170°W-140°W) and two time periods (Early and Late). A total of 361 minke whale samples were examined in four area/time groups as follow: VWL, n= 98; VEE, n=67; VEL, n= 105 and VIWE, n= 91. For comparison, the group VIWE of the 1985/86 commercial whaling season was incorporated into the analysis (n= 134). Of the 153 haplotypes of a previous analysis, a total of 60 haplotypes was identified in the 1996/97 samples. Quantification of the mtDNA differentiation among area/time groups was carried out using the Analysis of Molecular Variance (AMOVA). The AMOVA for the four groups of the 1996/97 survey combined showed a no significant PHist value. The combined sample showed significant differences with a sample of the 'western stock' (Area IVWE) but not with a sample of the 'core stock'. The same results were found when each of the 1996/97 area/time groups was compared with those 'standard' samples. No significant differences were found between the two groups VIWE. Consistently, pairwise comparisons showed each of these groups in Area VIW differing significantly from the sample from the 'western stock' but not from that of the 'core stock'. For the four area/time groups of Area V and the single area/time group in Area VI, no significant differences were found between survey years. This result is different from that found in Area III and IV, where evidences of yearly variation have been found.

INTRODUCTION

Based on a large-scale mtDNA survey in minke whales from Antarctic Areas IV and V (Pastene *et al.*, 1993; 1996a), a new interpretation on stock identity in the ordinary form has been possible.

The survey has revealed considerable mtDNA heterogeneity, but little geographic concordance with these putative management areas. Based on the PHI statistics (PHIst) of the Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992), Pastene *et al.* (1996a) proposed the occurrence of a 'core stock' distributed in Area V and eastern part of Area IV in most of the years. Also it was proposed that a group of whales distributes in the western part of Area IV early in the survey season, could belong to a neighboring, genetically differentiated stock (a 'western stock'). A summary of their analysis is presented in Table 1.

These hypotheses suggest 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 a temporal component in the distribution of these two stocks in the western part of Area IV, was suggested (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. For example, samples from adjacent Area VI could be compared with a representative sample of the 'western stock' (as represented by group IVWE in Table 1) and 'core stock' (as represented by a sample from the other groups in Table 1). Pastene *et al.* (1996b) used samples of minke whales taken by commercial operations in the western part of Area VI in the early period of the survey season (December) (VIWE) and compared the mtDNA composition of this area/time/year group with those of group IVWE (Table 1) and a sample from the rest of the groups in Table 1. They found that the sample from group VIWE was significantly different from the 'western stock' sample but no significant differences were found when this group was compared with the 'core stock' sample.

With the 1996/97 JARPA survey operating in sectors VW, VE and VIW, a new set of samples of minke whales became available for different studies, included the mtDNA analysis. Compositions of mtDNA in temporal groups from these sectors are compared with representative samples from the 'western' and 'core' stocks.

MATERIALS AND METHODS

Samples

Samples of the ordinary form minke whale from Areas V and VI were available from the 1996/97 JARPA survey. Samples were grouped into four groups: Area V Western Late (group VWL, n=98), Area V Eastern Early (group VEE, n=67), Area V Eastern Late (group VEL, n=105) and Area VI Western Early (group VIWE, n=91). '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. The geographic distribution of these area/time groups is shown in Fig. 1a,b (data from Nishiwaki *et al.*, 1997). For comparison, we used a sample set from group VIWE (n=134) of the commercial whaling operation of 1985/86 in the western part of Area VI (Pastene *et al.* 1996b) (Fig. 1c).

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 differentiation of haplotypes was quantified using the Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992) as implemented in the computer program *Arlequin* (Schneider *et al.*, 1997). The statistic of primary interest is the PHst, defined as the correlation of random haplotypes within a region or sub-population relative to that of the entire population or species. The significance of the observed variance values was tested using a modification of a matrix permutation procedure available in the computer program *Arlequin*. All tests of statistical significance were based on 10,000 random permutation of the original data sets. The level of significance obtained by this procedure is referred in this paper as the P-value.

First we examined the PH1st and its probability for the four groups of the 1996/97 survey combined. Subsequently, both the total sample and each of the area/time groups of the 1996/97 survey were compared with 'standard' samples of the 'western' and 'core' stocks as defined in Pastene *et al.* (1996a; 1996b). In the comparison we also included the group VIWE of the 1985/86 commercial whaling operation in Area VI.

The sample of the 'western stock' was defined by the group of whales from the western part of Area IV sampled in the early period of the survey season (including samples from the 1989/90 and 1991/92 JARPA surveys, n= 160, group IVWE in Table 1). The sample of the 'core stock' was from longitudes 100°E and 160°E (including samples from the 1987/88-1993/94 JARPA surveys, n= 889) (Fig. 2).

Samples from the western part of Area IV in the late period were not considered in the sample of the 'core stock' because of the preliminary evidences of yearly variation in this group (Pastene *et al.*, 1996a). For the same reason, a small sample of the 1991/92 season (n= 12) from the eastern part of Area IV in the early period was also excluded from the sample representative of the 'core stock' (see Discussion section in Pastene *et al.*, 1996a).

The eastward extension of the 'core stock' sample was limited to 160° E despite the fact that whales from this 'stock' did not differ significantly from whales from the eastern part of Area V (160°E-170°W). This was done because the sample size of group V east early is small (n= 63) and then not significant differences with this group not necessarily means genetic homogeneity (Pastene *et al.*, 1996a).

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. Of them 60 were identified in the 1996/97 JARPA sample in Areas V and VI. A total of 31 were observed in group VWL (n=98), 29 in group VEE (n=67), 28 in group VEL (n=105) and 33 in group VIWE (n=91). The four area/time groups (Table 2) shared the main haplotypes 1 through 5.

The results of the AMOVA indicate that no significant differences were found between male and female samples in each of the four area/time groups. The comparative analysis among these groups was thus conducted for both sexes combined.

Statistical analysis

Samples from the 1996/97 JARPA survey

The statistical analysis by AMOVA began comparing all the groups of the 1996/97 survey. A negative value (-0.30%) of the total variance was due to among group's variation; 100.30% was due to within group's variation. The PH1st value was -0.00295 (P= 0.85070+/-0.00353). All the pairwise comparisons among 1996/97 groups had a negative PH1st value with probabilities over 0.45000.

Comparison between two yearly groups in Area VIW early

Both groups of VIWE (commercial 1985/86 and JARPA 1996/97) did not differ significantly in mtDNA composition (PH1st=0.00396; P=0.14169).

Comparison with 'standard' samples of the 'western' and 'core' stocks

Table 3 shows the results of the comparisons by AMOVA between each of the area/time groups of the 1996/97 JARPA survey and 'standard' samples from the 'western' and 'core' stocks. The results of the comparisons with the 1996/97 samples pooled as well with the commercial samples of 1985/86 (VIWE 85/86), are also shown.

All the single groups as well the 1996/97 samples pooled consistently differ from the 'western stock' sample. When they are compared with the 'core stock' sample the PH1st values are negative (except for the case of the 1985/86 sample in Area VI), all of them with high P values (Table 3).

DISCUSSION

In this report we have examined the pattern of mtDNA variation in minke whales from Area V and western part of Area VI sampled during the 1996/97 JARPA survey. The early sample from Area VI western was taken in December same as the commercial sample from this sector examined in Pastene *et al.* (1996b). Thus a comparison between years for a same sector and period in that Area was possible. A similar scenario was possible for Area V, given the fact that JARPA surveys have operated in this Area in previous years.

In the previous analysis (Pastene *et al.*, 1996b), we found that group VIWE differed significantly from the 'western stock' sample but no significant differences were found when this group was compared with that of the 'core stock'. That result, which it was in order with the geographical location of these putative stocks (group VIWE is closer geographically to the 'core stock' than to the 'western stock'), suggested that the 'core stock' could be distributed in the western part of Area VI. Although the JARPA survey in 1996/97 in Area VI covered the same sector and period as the previous commercial operation, the JARPA samples come from a wider latitudinal area and whales were taken around the ice-edge and in offshore areas (the commercial samples of the 1985/86 season in Area VIW were taken mainly around the pack-ice, Shigemune, personal communication). Thus we were interested to check the previous result with the analysis of more representative samples from Area VIW obtained randomly by JARPA.

Pastene *et al.* (1996b) carried out a preliminary power estimation for their mtDNA analysis on minke whales from Areas IV and V. They calculate that at least 150-200 samples are necessary to detect significant differences between putative stocks in the Antarctic minke whale. It should be noted that the sample sizes for the groups VIWE in the present study were 134 and 91, respectively, smaller figures than those suggested by the power analysis.

The comparative analysis with the 'standard' samples was thus conducted for the two VIWE yearly groups combined (n=225). It should be noted here that both set of samples for group VIWE were obtained during two different operations as explained above, and the validity to pool samples from commercial and JARPA request further consideration. In this exercise we did not find significant differences between group VIWE and the sample of the 'core stock'. The group VIWE combined, however, was significant different from the 'western stock'.

The fact that we were unable to find significant mtDNA differences between the 'core stock' and samples from Area VIWE could be indicating that either:

- 1) There is no stocks segregation between Areas IVE/ V ('core stock') and VIWE or

- 2) Significant differences between the 'core stock' and a hypothesized 'eastern stock' in Area VIW were not detected because the effect size between them is smaller than that between the former and the 'western stock' or
- 3) Stocks in the Antarctic could be better discriminated if the latitudinal component (in addition to the longitudinal and temporal components) is taken into consideration.
- 4) There is marked yearly variation so that in some years Area VIW is occupied in the early period by the 'core stock' and in other years by an hypothesised 'eastern stock'.

Regarding to 1), it should be noted that the mtDNA analysis in the feeding ground using JARPA sample has revealed just a single consistent source of heterogeneity. That is attributed to whales from Area IVW early of the 1989/90 and 1991/92 surveys. The mtDNA studies in a more 'tractable' species, the humpback whale, showed that the only source of mtDNA heterogeneity in the feeding ground was also attributed to Area IV and no significant differences were found among Areas V, VI and I (Pastene *et al.*, 1997). Separation of stocks, however, was more successful when samples from migratory corridors in lower latitudes were used (Pastene and Baker, 1997). Whether additional structure occur in Area VI could be confirmed with the combined analysis of samples of minke whales from both feeding grounds and lower latitudes areas.

Regarding to 2), the estimation of the sample size necessary to detect significant differences between putative stocks in the Antarctic (Pastene *et al.*, 1996b) was made under the assumption that the degree of genetic differentiation among genetic stocks (effect size) in the Antarctic is similar to that between the standard samples of the 'western' and 'core' stocks. If the effect size between a hypothesized 'eastern' stock in Area VIW and the standard sample of the 'core stock' is smaller, then a larger sample size (larger than 150-200) should be analysed to detect significant differences.

Regarding to 3), we have not tested yet for latitudinal component (including distances of whales from the ice-edge) in stock distribution. Future analyses in minke whales from Areas III, IV, V and VI should incorporate the latitudinal component in addition to longitudinal and temporal factors (but see Goto *et al.*, this meeting, for a preliminary analysis of this kind in Area IV).

Regarding to 4), evidences of mtDNA heterogeneity between surveys have been showed for Area III and IV (see below). Such evidences have been detected in Area IIIIE, IVWE and IVWL, but especially in the latter group. Such evidences could be indicative of yearly variation in the pattern of distribution of stocks in the feeding ground. A similar situation could occur in Area VIW.

Yearly variation

Pastene *et al.* (1996a) conducted a preliminary examination of the yearly variation in each of the area/time groups examined. In Table 4 we shows an updated version of such analysis, including Areas IIIIE and VIWE. In general larger PHist values are observed for Areas IIIIE and IVW. In Area IIIIE two early groups are different at the 10 % level and four late groups in Area IVW showed a PHist value, which was significant at the 5% level. Furthermore, although the total PHist value for the early groups of Area IVW was not significant, a pairwise comparison showed a PHist with a P value below 5%. In contrast, groups in Area IVE, VW, VE

and VIW showed very low PHist values, some of them negative, and larger probabilities. Future analyses of yearly variation should include a more detailed examination of the exact geographical position of the samples.

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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: Distribution of mtDNA haplotypes in representative samples of the 'western' and 'core' stocks and in samples from Area VI western early 1985/86 (VIWE 85/86), Area V western late 1996/97 (VWL), Area V eastern early 1996/97 (VEE 96/97), Area V eastern late 1996/97 (VEL 96/97) and Area VI western early 1996/97 (VIWE 96/97). In parenthesis are the sample sizes.

Hap.	CS	WS	VIWE	VWL	VEE	VEL	VIWE
ID	(889)	(160)	85/86 (134)	96/97 (98)	96/97 (67)	96/97 (105)	96/97 (91)
1	296	38	44	34	18	39	26
2	84	8	7	8	4	9	8
3	48	8	3	5	4	6	9
4	70	12	7	10	6	8	9
5	33	5	3	2	4	2	2
6	10	7	1	0	1	1	1
7	18	5	5	1	1	3	0
8	25	11	2	2	0	2	1
9	5	3	0	1	0	0	1
10	22	8	12	2	2	6	0
11	12	2	4	3	0	3	1
12	16	1	0	0	2	1	3
13	3	3	0	0	0	0	0
14	28	3	4	5	2	4	2
15	6	2	2	0	1	0	1
16	2	0	0	1	0	0	0
17	1	0	0	0	0	0	0
18	2	0	1	0	0	3	0
19	13	3	2	1	2	1	1
20	6	0	2	0	0	0	0
21	4	1	0	1	1	0	0
22	7	3	1	1	0	1	0
23	14	0	1	2	2	1	2
24	4	0	3	0	0	0	0
25	0	2	0	0	0	0	0
27	1	1	1	0	0	0	1
28	2	2	2	0	0	1	0
29	2	0	0	0	2	0	1
30	8	3	2	3	1	0	1
31	3	1	1	1	1	0	1
32	2	0	0	0	0	1	1
33	0	0	0	1	1	0	2
34	6	0	0	1	0	1	0
36	1	0	1	0	0	0	0
37	0	0	0	0	1	0	0
39	4	0	2	0	1	1	0
40	1	0	0	0	0	0	0
41	0	1	0	0	0	0	0

Table 2 (cont.)

42	2	1	0	0	0	0	0
43	1	0	1	1	0	0	0
44	2	1	0	0	0	0	0
45	1	0	0	0	0	0	0
46	1	1	0	0	0	0	0
47	19	4	5	0	0	3	4
48	3	0	0	0	0	1	0
49	1	1	0	0	0	0	0
50	1	1	0	0	1	0	0
51	6	2	1	0	2	3	1
52	3	1	0	0	0	0	0
53	1	2	0	0	0	0	0
54	4	2	0	2	0	0	1
55	5	1	0	0	0	0	0
56	2	1	0	0	0	0	0
57	0	1	0	0	0	0	0
58	1	2	0	0	0	0	0
59	2	0	0	0	1	1	0
60	1	1	0	0	1	0	0
61	4	0	1	0	1	0	0
62	7	1	0	1	0	0	0
63	1	0	0	0	1	0	0
64	0	0	0	0	0	0	0
65	0	1	0	0	0	0	0
66	1	0	0	0	0	0	0
67	0	1	0	0	0	0	1
68	4	0	1	0	0	0	1
69	1	0	0	0	0	0	0
70	3	0	0	0	0	0	0
73	1	0	0	0	0	0	0
74	1	0	0	0	0	0	0
75	3	0	0	1	0	0	0
76	0	0	0	0	0	0	0
77	1	0	0	0	0	0	0
78	1	0	0	0	0	0	0
79	4	0	0	0	0	0	0
80	1	0	0	0	0	0	0
82	2	0	0	1	0	0	0
84	1	0	0	0	0	0	0
85	1	0	0	0	0	0	0
86	1	0	0	0	0	1	0
87	1	0	0	0	0	0	0
89	0	0	1	2	1	0	0
90	0	0	0	0	0	0	0

Table 2 (cont.)

91	0	0	0	0	0	0	1
92	1	0	1	1	1	0	0
93	1	0	0	0	0	0	0
95	1	0	0	0	0	1	1
96	1	0	0	0	0	0	0
97	1	0	0	0	0	0	0
98	1	0	0	0	0	0	0
99	0	0	0	0	0	0	0
102	1	0	0	0	0	0	1
104	2	0	1	0	0	0	1
105	2	0	0	1	1	0	0
106	1	0	0	0	0	0	0
107	1	0	0	0	0	0	2
108	1	0	0	0	0	0	0
110	1	0	0	0	0	0	0
111	1	0	0	0	0	0	0
112	0	0	0	0	0	0	1
114	0	1	0	0	0	0	0
115	0	1	0	0	0	0	0
116	2	0	0	0	0	0	0
117	0	0	0	0	0	0	0
118	1	0	0	1	0	0	0
121	2	0	0	1	0	1	0
122	1	0	0	0	0	0	1
123	3	0	0	0	0	0	0
124	1	0	0	0	0	0	0
125	1	0	0	0	0	0	0
126	1	0	0	0	0	0	0
128	1	0	0	0	0	0	0
130	1	0	0	0	0	0	0
131	1	0	1	0	0	0	0
132	1	0	0	0	0	0	0
133	1	0	0	0	0	0	0
134	1	0	0	0	0	0	0
135	1	0	0	0	0	0	0
136	0	0	0	0	0	0	0
138	0	0	0	0	0	0	0
139	0	0	0	0	0	0	0
140	0	0	0	0	0	0	0
141	0	0	0	0	0	0	0
142	0	0	0	0	0	0	0
143	0	0	0	0	0	0	0
144	0	0	0	0	0	0	0
145	0	0	1	0	0	0	0
146	0	0	1	0	0	0	0
147	0	0	0	1	0	0	0
148	0	0	1	0	0	0	0

Table 2: (Cont.)

149	0	0	1	0	0	0	0
150	0	0	1	0	0	0	0
151	0	0	1	0	0	0	0
152	0	0	1	0	0	0	1
153	0	0	1	0	0	0	0

Table 3: Comparison using the AMOVA between area/time/year groups of minke whales and representative samples of the 'western' and 'core' stocks. Area VIW early 1985= whales caught in December in the western part of Area VI during the 1985/86 whaling season (Pastene *et al.*, 1996b); Area VIW early 1996= whales caught in December in the western part of Area VI during the 1996/97 JARPA survey; Area VW late 1996= whales caught in January-March in the western part of Area V during the 1996/97 JARPA survey; Area VE early 1996= whales caught in December in the eastern part of Area V during the 1996/97 JARPA survey.

	'Western Stock' (n=160)		'Core Stock' (n=889)	P
	PHist	P	PHist	
Area VIW early 1985 (n=134)	0.00708	0.03990	0.00085	0.26087
Area VIW early 1996 (n=91)	0.01218	0.01880	-0.00091	0.54705
Area VIW total 1996 (n=104)*	0.00832	0.04160	-0.00091	0.58084
Area VW late 1996 (n=98)	0.00814	<u>0.05489</u>	-0.00228	0.81712
Area VE early 1996 (n=67)	0.01640	0.01520	-0.00157	0.61034
Area VE late 1996 (n=105)	0.01367	0.01430	-0.00112	0.60924
Total sample 1996 (n=361)	0.01475	0.00090	-0.00048	0.64894

* Include 13 minke whale samples obtained in Area VIW in the 'late' period.

Table 4: Yearly variation examined by AMOVA in area/time groups of minke whales from Areas III, IV, V and VI. V(A) and V(B) are the molecular variances among and within years (austral summer seasons), respectively.

Area/time group	V(A)	V(B)	PHist	P
IIIEE (2)*	0.83%	99.17%	0.00827	<u>0.07250</u>
IVWE (3)**	0.35%	99.65%	0.00352	0.16700
IVWL (4)	0.47%	99.53%	0.00466	0.05070
IVVE (2)	0.01%	99.99%	0.00005	0.40930
IVEL (3)	-0.54%	100.54%	-0.00541	0.93670
VWE (2)	0.10%	99.90%	0.00104	0.33160
VWL (3)	-0.39%	100.39%	-0.00386	0.94670
VEL (5)	0.08%	99.92%	0.00084	0.29590
VIWE (2)*	0.39%	99.61%	0.00396	0.14169

* One of the groups is from commercial whaling operation (historical samples)

** Although the total PHist value was not significant, the pairwise comparison between 1989/90 and 1995/96 showed a significant PHist value (PHist=0.00870; P=0.03930).

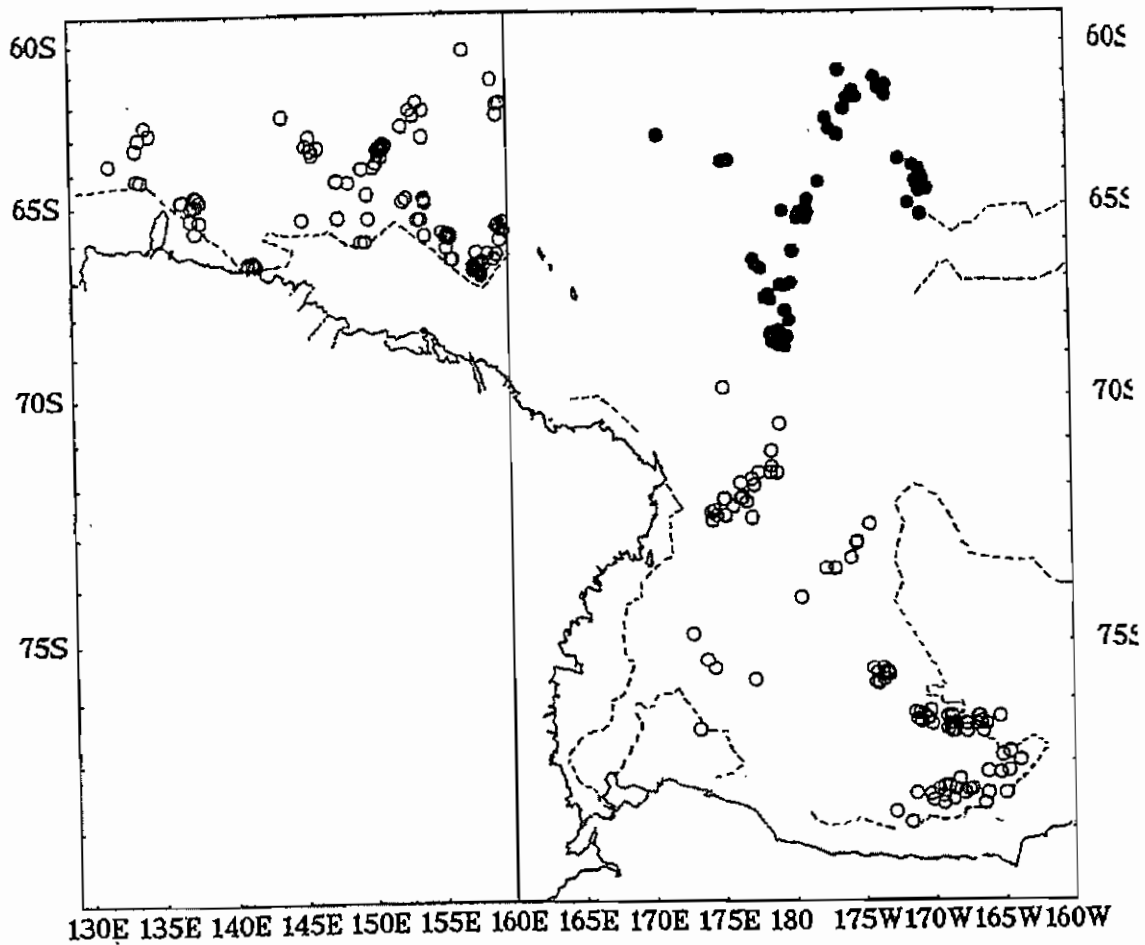


Figure 1a: Geographic distribution of minke whales examined in the mtDNA analysis in Area V (1996/97 JARPA). Those whales distributed in the west part of Area V belong to group VW late (VWL). Those distributed in the eastern part of Area V belong to group VE early (closed circle, VEE) and group VE late (open circle, VEL). Dotted lines indicate position of the ice-edge.

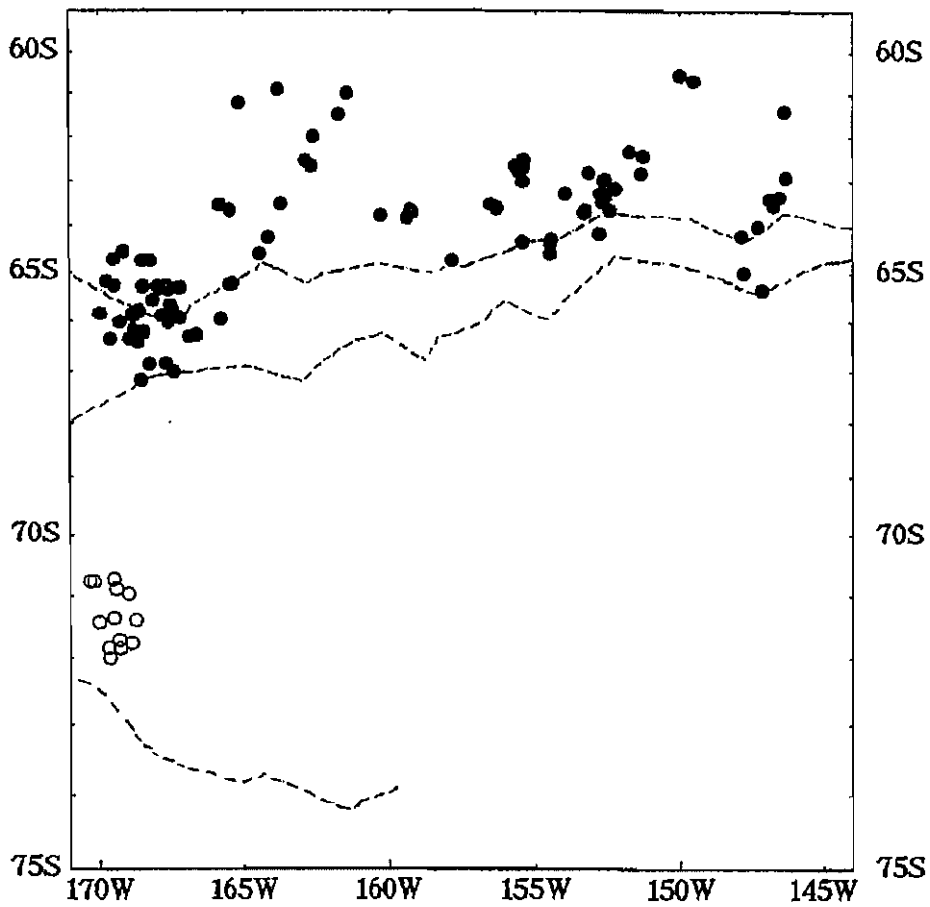


Figure 1b: Geographic distribution of minke whales examined in the mtDNA analysis in Area VI (1996/97 JARPA). These whales belong to group VIW early (closed circle, VIWE) and VIW late (open circle, VIWL). Group VIWL was not used in the pair-wise comparisons because of small sample size. Dotted lines indicate the position of the ice edge.

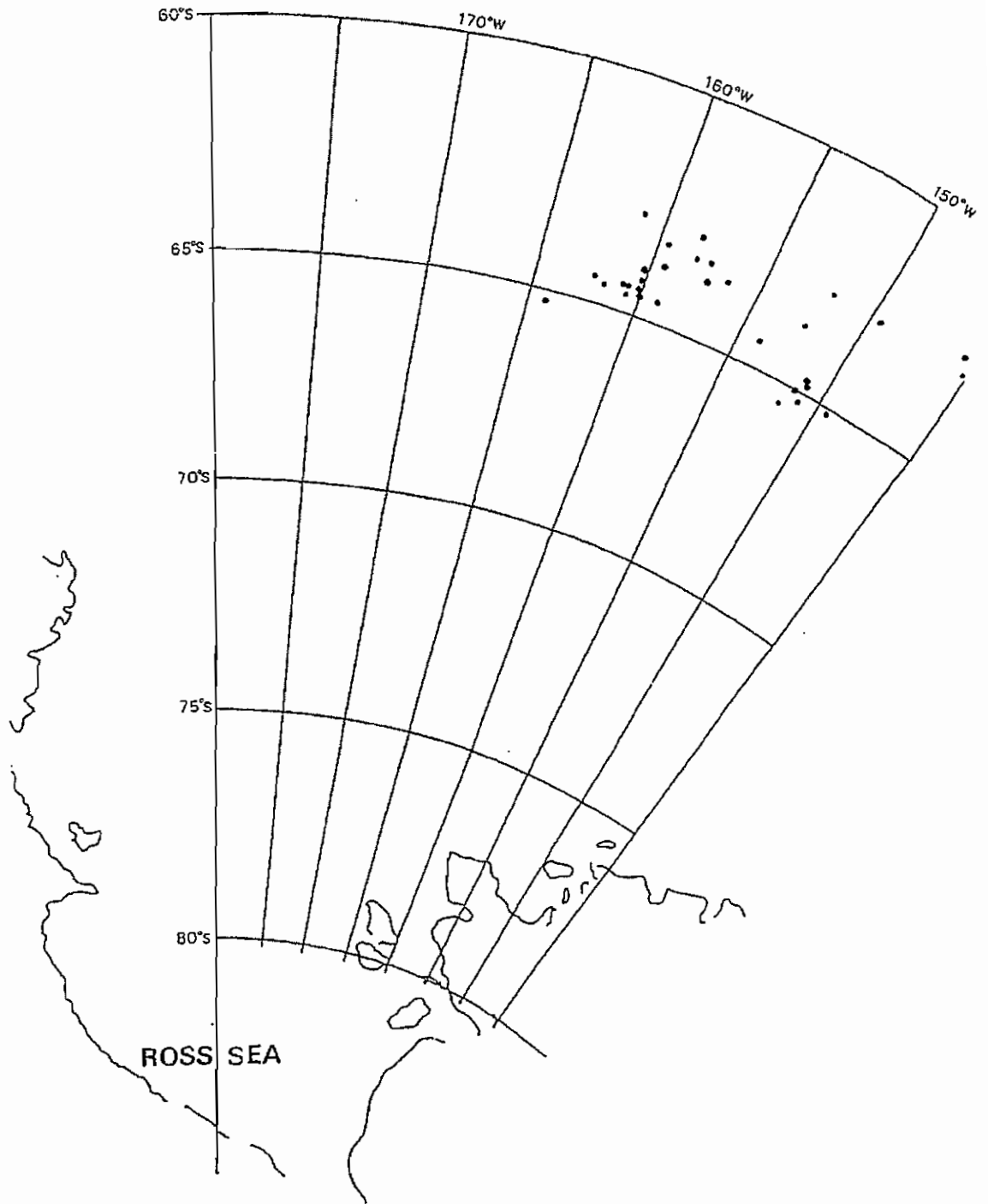


Figure 1c: Geographic distribution of minke whales taken in a commercial operation in the west part of Area VI in December 1985. These whales belong to group VIW early 1985/86. All these whales were caught around the ice-edge (Shigemune, personal communication).

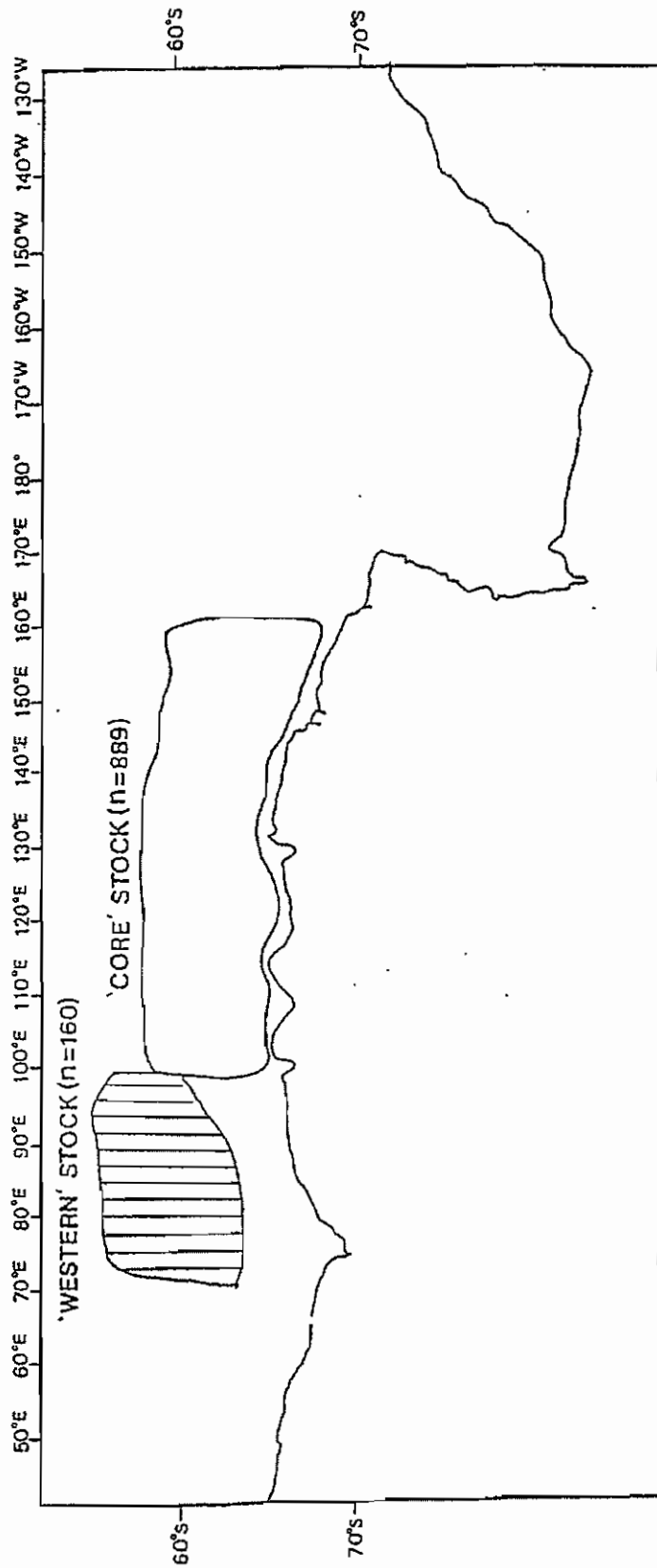


Fig. 2: Geographical distribution of representative samples of the 'western' and 'core' stocks. Samples obtained during JARPA surveys from 1987/88 to 1993/94 austral summers were used for 'western' and 'core' stocks. Samples from the western part of Area IV in the late period were not considered within the 'core' stock because preliminary evidences of yearly variation in this group. On the other hand, samples from the eastern part of Area V were not considered in the 'core' stock despite the fact that whales from this stock did not differ significantly from whales from the eastern part of Area V (Pastene *et al.*, 1996a). This was done because the sample size of group VEE is small ($n=63$) and not significant differences with this group not necessarily means genetic homogeneity.