Temporal Variation in Mitochondrial DNA Haplotypes Composition in Minke Whales from Antarctic Area IV

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ABSTRACT

Variation in mtDNA haplotypes composition in minke whales from Antarctic management Area IV, between and within summer seasons, is examined. With this purpose, we analyzed 306 and 165 whales sampled under special permit during the Antarctic Japanese researches of the 1989/90 and 1991/92 summer seasons, respectively. Fifty-seven mtDNA haplotypes were discriminated in Area IV on the basis of the combination of patterns of six restriction enzymes of which 17 (29.8% of the total) were represented by a single specimen. In order to study the temporal variation in genetic composition between and within summer seasons, we defined six area/time/year groups for Area IV, which were compared for their genetic composition: WE-1989, EE-1989, WL-1989, WE-1991, EE-1991 and WL-1991. WE groups consisted of whales sampled during an early period (early December-middle January) in the western side of Area IV (70°E-100°E); EE groups was composed of whales caught during the early period in the eastern side (100°E-130°E) and WL groups consisted of whales taken during a late period (end of January-middle February) in the western side. Whales from the early and late groups in the western side were distributed in offshore and inshore (including Prydz Bay) waters, respectively. Patterns of mtDNA diversity in the area/time groups were very similar in the 1989/90 and 1991/92 summer seasons. Furthermore mtDNA haplotype frequency distribution between sums for 1989/90 and 1990/91 seasons showed no significant differences. If samples of the two seasons were combined haplotypes compositions were significantly different between the three area/time groups. This indicates that more than one stock migrate to Area IV and the composition changes longitudinally and with progress of time in a feeding season. Our study emphasize the importance of considering the temporal factor in describing the population structure of Southern Hemisphere minke whale.

INTRODUCTION

There have been several attempts, using a variety of methods to define minke whale stocks in the Southern Hemisphere. Studies conducted with this purpose have involved different approaches such as distribution of catches and sightings, morphometrics, marking, ecological and biochemistry. These studies have been summarized by Horwood (1990) and Best (1990). Although phenotypic variation in meristic and non-meristic features have been suggested between Antarctic regions (Doroshenko, 1979; Bushuev and Ivashin, 1986), the objective of identify stocks has not been reached and the several approaches used have failed to reveal any isolated populations of minke whale in the Antarctic.

Recent genetic studies conducted with the purpose of identifying stocks of minke whale in the Southern Hemisphere, have been concentrated mainly in the analysis of samples from the Antarctic management Areas IV and V (Wada, Kobayashi and Numachi, 1991; Hoelzel and Dover, 1991; Amos and Dover, 1991; van Pijlen, Amos and Burke, 1992; Pastene *et al.*, 1992). Most of these works have failed in demonstrating significant genetic differences between minke whales in these areas. Pastene *et al.* (1992) in a more extensive and intensive mitochondrial DNA (mtDNA) survey, found a non-random distribution of mtDNA haplotypes in these areas suggesting the occurrence of different stocks in Areas IV and V but they failed to identify where stock boundaries might be drawn. It was recommended by the Scientific Committee of IWC (IWC, 1992), that biopsy sampling be carried out on breeding grounds, to allow further investigation of the status of Southern Hemisphere minke whale stocks. This recommendation is based on the perception that the breeding stocks in lower latitudes may be more isolated geographically each from another than they are in higher latitudes. If genetic markers are identified independently for each breeding stocks in lower latitudes, then they can be identified in the Antarctic feeding grounds and used to determine the intermingling of stocks.

Recoveries in the Brazil whaling ground of two whales that had been marked some 54° of longitude apart in the Antarctic suggest that whales from different breeding grounds may intermingle in the Antarctic (Best, 1990). It seems that the best way to investigate the stock structure of the southern minke whale is by analyzing samples from low latitudes.

There is very little information on the occurrence of breeding grounds of minke whales in the Southern Hemisphere. The only reliable information arise from Discovery marks recovery analysis (Buckland and Duff, 1989). Two whales marked in the Antarctic were recovered from Brazilian coast. This is the only direct evidence to show that whales caught in low latitudes spend at least some time in the Antarctic (Horwood, 1990).

In this study we examined the temporal changes in mtDNA diversity and haplotype composition in Antarctic Area IV, within and between summer seasons, using samples obtained during the Japanese researches of the 1989/90 and 1991/92 summer seasons.

MATERIALS AND METHOD

Samples

Minke whales used in this study were caught in Antarctic Area IV during the Japanese researches conducted during two summer seasons, 1989/90 (Fujise *et al.* 1990) and 1991/92 (Fujise *et al.* 1992). Whales were sampled using a random design described by Kato *et al.* (1989). During the 1989/90 and 1991/92 summer seasons, 326 and 288 ordinal form minke whales were sampled, of which 306 and 165 were used in this study, respectively.

Tissue samples and mtDNA extraction procedure

We isolated mtDNA from liver tissue which had been frozen at -20°C for 1-2 years, using a mini-prep procedure described by Pastene *et al.* (1992).

RFLP analysis

In order to facilitate the analysis of a large number of samples, we used only the most informative enzymes among the 12 six-base sequence recognition endonucleases used in a previous study (Pastene *et al.* 1992). Crude mtDNA (15ul) was digested with 2 or 3 units of the following six enzymes: *Acc*I, *Ban*I, *Eco*RV, *Hinc*II, *Hpa*I and *Ssp*I. Procedures used for digestion and electrophoresis of the samples were the same used by Pastene *et al.* (1992). Profiles for each enzyme were assigned letters. Individuals were assigned haplotypes consisting of a list of the letters designating the fragment profiles produced by each of the six restriction enzymes.

Sampling design and data analysis

Whale samples from the 1989/90 summer season were grouped according the locality and date in which they were caught (Table 1). We divided Area IV arbitrarily into western (70°E-100°E) and eastern (100°E-130°E) sides. Two sampling periods were defined: early (from early December to middle January) and late (from late January to middle February). Samples from the western side taken early (group WE89) were distributed in offshore waters while those taken late in the same side (group WL89) were distributed mainly in inshore waters including Prydz Bay. Whales sampled early in the eastern side (group EE89) were distributed mainly between 61°S and 65°S (Fig. 1). Samples from the eastern side obtained in the late period were not available. The sample size in the three area/time groups from 1989/90 season were 118, 92 and 96, respectively.

Samples from the 1991/92 summer season were grouped under a similar criteria matching location and date of sampling with those samples of the 1989/90 season. In this manner we defined three groups: WE91, WL91 and EE91. Sampling information and geographic distribution of these groups are shown in Table 1 and Fig. 2, respectively. It should be noted here that because the sample was first grouped by longitudinal areas (70-100°E, 100-130°E), the time covered by the sample is not same between the two fishing seasons (Table 1).

Firstly we compared pattern of mtDNA diversity in Area IV, between the 1989/90 and 1991/92 summer seasons. Additionally, the

mtDNA haplotype frequency distribution in groups of samples from the two summer seasons were compared. In a next step, we compared the mtDNA haplotype frequency distribution of the area/time groups within each of the summer seasons.

Haplotypes frequencies distributions between groups of whales were compared using the chi-square statistic for heterogeneity (Roff and Bentzen, 1989). This Monte Carlo approach estimates the significance of chi-square computed from the raw data. In each trial, 2,000 randomizations of the original data sets were made. The degree of sequence divergence between two mtDNA haplotypes was calculated based on the proportion of DNA fragments shared (Nei, 1987). Sequence divergence among haplotypes and haplotypes frequencies in the groups of whales were used to estimate the average number of nucleotide substitutions for a randomly chosen pair of haplotypes in population X (d_X) and the average number of nucleotide substitutions between DNA haplotypes from populations X and Y (d_{XY}). These two parameters were used to estimate mtDNA diversity between groups (d_A) (Nei, 1987).

RESULTS

Mitochondrial DNA fragment patterns

We used the same terminology employed in a previous study (Pastene *et al.*, 1992) for the digestion profiles of the six restriction enzymes. The enzymes *Acc*I, *Ban*I, *Eco*RV, *Hinc*II, *Hpa*I and *Ssp*I showed 12, 5, 3, 6, 3, and 14 restriction fragment patterns, respectively.

Mitochondrial DNA haplotypes

Restriction endonuclease digestion of mtDNA for 471 minke whales from the Antarctic Area IV sampled during two summer seasons, revealed a total of 57 mtDNA haplotypes (Table 2). Of these haplotypes 17 (29.8%) were represented by a single specimen.

Mitochondrial DNA haplotype distribution of the sexes

Within both 1989/90 and 1991/92 summer seasons, the sex ratio differed in Area IV according the sector and time of sampling (Fujise *et al.*, 1990; Fujise *et al.*, 1992). We compared haplotype frequency between the sexes. In both seasons the mtDNA haplotype frequencies were not significantly different between sexes (P=0.0530 for 1989/90 season and P=0.7915 for 1991/92 season).

Mitochondrial DNA haplotypes distribution of the school sizes

We examined the distribution of mtDNA haplotypes using the total samples in each season between sample taken from schools of one individual and that from two or more individuals. Neither the 1989/90 summer season (P=0.53350) nor the 1991/92 summer season (P=0.3235) showed a significant bias in the mtDNA haplotype frequencies for school size categories.

Mitochondrial DNA diversity

Table 3 shows the mtDNA diversity among and within the area/time groups for the 1989/90 and 1991/92 summer seasons. In both seasons there is as much mtDNA diversity within the groups as between them. An identical within-group pattern of variation of the mtDNA diver-

sity was observed in both seasons: the largest estimate was recorded in the WE groups and the smallest in the WL groups. Comparisons between groups showed also a similar pattern in both summer seasons (Table 3). Largest estimates were recorded between the WE and EE groups and the smallest between the EE and WL groups.

Mitochondrial DNA haplotypes frequency distribution between two summer seasons

Table 2 shows the geographical and temporal distribution of mtDNA haplotypes in minke whales from Area IV in the 1989/90 and 1991/92 summer seasons, respectively. In the 1989/90 season, 52 mtDNA haplotypes were demonstrated in 306 minke whales analyzed. Haplotypes '1' through '5' were the predominant haplotypes (55.6% of the total samples). With regard the 1991/92 season, 34 mtDNA haplotypes were demonstrated in 165 minke whales analyzed in that season. Like in the 1989/90 season, haplotypes '1' through '5' were the predominant haplotypes (61.8% of the total samples). When the sums for the 1989/90 and 1990/91 seasons is considered, frequencies of the predominant haplotypes were very similar in both seasons (Table 2). When both the total samples and the particular area/time groups of two summer seasons are compared, no significant differences in mtDNA haplotype frequency distribution were observed (Table 4).

Mitochondrial DNA haplotype frequency distribution in the 1989/90 summer season

In the 1989/90 summer season, the predominant haplotypes '3', '4' and '5' showed a similar frequency among the three area/time groups (Table 2). Predominant haplotypes '1' and '2', however, exhibited a particular higher frequency in the groups EE89 and WL89 than in the group WE89. The same trend was observed in haplotypes '12', '14' and '19'. The frequency of the relatively common haplotype '6' was notably higher in the WE89 group than in the other two groups. Heterogeneity chi-square decomposition (Table 5) began by estimating the significance of the chi-squares computed from raw data for all three groups. The computed value 125.17 was exceeded only 24 times in 2,000 simulations. It can be concluded that mtDNA haplotypes are not randomly distributed among the three groups. Of the three pairwise comparisons (Table 5), two were found to be significantly different (group WE89 versus group EE89 and group WE89 versus group WL89).

Mitochondrial DNA haplotype frequency distribution in the 1991/92 summer season

With regard the 1991/92 summer season, comparison of the three area/time groups indicated no significant differences in haplotype composition (P= 0.085) (Table 6). Of the three possible pairwise comparisons, however, one was found to be significantly different (group WE91 versus group WL91) (Table 6).

Mitochondrial DNA haplotype frequency distribution for two summer seasons combined

Table 7 shows the results of chi-square tests for heterogeneity among and between area/time groups for samples of the 1989/90 and 1991/92 summer seasons combined. Heterogeneity chi-square decomposition began by estimating the significance of the chi-square

computed from raw data for all three groups. The probability obtained of 0.004 indicates that mtDNA haplotypes are not randomly distributed among these groups. All the three possible pairwise comparisons showed significant differences in mtDNA haplotype composition.

DISCUSSION

The genetic approach has been regarded as one of the most promising approach to be used for identifying stocks. During the last time, several molecular techniques have been developed and applied for examining genetic variation and stock identity in cetacean species. Most of such techniques already have been used on the Southern Hemisphere minke whale. However, beside the mtDNA survey conducted by Pastene *et al.* (1992), none of these studies succeeded in demonstrating significant genetic differences between minke whales in the Antarctic. The latter authors analyzed the mtDNA haplotypes distributions in three broad sectors that involved most of the Areas IV and V. They demonstrated a non-random distribution of mtDNA haplotypes suggesting the possibility that more than one stock might be involved in these areas, but they failed to demonstrate where stocks boundaries might be drawn.

Most of the genetic studies on minke whale in the Antarctic have considered mainly the spatial factor. In this study we have considered both the spatial and temporal factors in the genetic analysis of minke whales sampled on the feeding ground of Area IV. With this purpose we defined and analyzed genetically six area/time/year groups in this area. We expected that if the same stock (or stocks) with similar patterns of distribution and movement is (or are) involved in Area IV each year, then a similar pattern of genetic diversity should be observed in different years for groups of whales sampled from different places of Area IV and during different periods of the feeding season. Our analysis revealed that the patterns of variation of the mtDNA diversity of the area/time groups were very similar in the 1989/90 and 1991/92 summer seasons. Furthermore, comparisons of mtDNA haplotype frequency distribution between total samples and particular area/time groups of these two seasons showed no significant differences.

Our genetic data are consistent with the hyphotesis that the same stock (or stocks) with a similar annual pattern of distribution and movement, might be involved each year in the feeding ground of Area IV. But, is it possible to determine from our genetic data if one or more stocks are involved in Area IV?. If so, is it possible to establish from our data a plausible hyphotesis about its (or their) patterns of movement on breeding grounds?. The overall level of diversity between the groups examined in this study provides no indication of genetic partitioning in the species. MtDNA diversity estimates between the area/time groups, were in general low, althought they were a little higher than that obtained in a previous survey (Wada, Kobayashi and Numachi, 1991) of whales from Areas IV and V.

Under the assumption that specific mtDNA haplotypes might be

related to specific breeding stocks, we searched for answers to the questions established above by examining further the spatial and temporal distribution of mtDNA haplotypes in Area IV. Such analysis was conducted separately for the 1989/90 and 1991/92 seasons and for these two summer seasons combined.

Statistical analysis of the 52 mtDNA haplotypes occurring in Area IV in the 1989/90 season revealed that these haplotypes were not randomly distributed among the three area/time groups analyzed. A significant difference in the mtDNA haplotypes frequency distribution was observed between samples taken early from the western and eastern side of Area IV. The mtDNA haplotypes frequency distribution in whales sampled early in the eastern side and those taken late in the western side were more similar to each other than they were to the samples from the western side taken early. With regard the 1991/92 summer season, the distribution of 34 haplotypes were analyzed in this season. The difference in the number of haplotypes found between the two summer seasons may be related to the difference in sample size anlyzed in each season. Sample size was about 1.9 times smaller in the 1991/92 season than in the 1989/90 season. Of the three pairwise comparisons conducted in the 1991/92 season, only two had results similar to those of the 1989/90 season. Differences in the mtDNA haplotype composition between early samples from the western and eastern sides of Area IV were not evident as they were in the 1989/90 summer season. Pairwise comparison involving the group of whales sampled early in the eastern side in the 1991/92 season should be taken with caution due to the small sample size (N=27) of this group.

Because no significant differences occurred in haplotype composition between the two summer seasons, we compared the haplotype composition of the three area/time groups for both summer seasons combined. The analysis involving the two summer seasons combined showed a greater temporal and spatial heterogeneity in mtDNA haplotype frequency distribution. In this case, haplotype composition was significantly different between the three area/time groups. The apparent similarity in haplotype composition between the EE89 and WL89 groups observed when the 1989/90 season was analyzed separately, was not observed when the samples of two summer season were combined.

On the basis of this marked heterogeneity in haplotype composition, we can suggested that more than one stock occur in Area IV and that composition changes longitudinally and with progress of the feeding season. It should be noted here that mitochondrial DNA diversity was higher in groups of whales sampled in an early stage of the feeding season, and it decreased in groups sampled during a late stage. This might indicate that different stocks occurring in Area IV intermingle with progress of the feeding season. It has been demonstrated that density of minke whales during the feeding season are higher near the ice-edge stratum than in the more northern stratum (Butterworth and Buckland, 1988). It is possible that the congregation of a large number of minke whales in the ice edge in the deep feeding season might contribute to their longitudinal spread.

In this study we found that the mtDNA haplotype frequencies were not significantly different between sexes. This analysis should be considered preliminar because it was conducted only for the total sample in each season. A more detailed analysis of the haplotype frequency distribution of the sexes will be conducted in future.

Most of the genetic studies conducted in the Antarctic have been limited to a comparison between Antarctic Areas IV and V. Although these studies have involved the use of powerful techniques, they have considered only the spatial (longitudinal) factor with no significant results. Our study on the variation of haplotype composition in Area IV emphasise the importance of considering the temporal factor in describing the population structure of Southern Hemisphere minke whale.

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Table 1: Sampling data of the area/time groups examined for mtDNA variation in the 1989/90 and 1991/92 summer seasons.

Area/time groups	Period of sampling	Longitudinal range	Latitudinal range	Sample size
1989/90				
WE89	89/12/06-89/12/29	70°E-100°E	55°S-63°30'S	118
EE89	89/12/31-90/01/15	100°E-130°E	61°S-66°S	96
WL89	90/01/21-90/02/14	70°E-100°E	61°S-69°S	92
1991/92				
WE91	91/12/11-91/12/28	70°E-100°E	58°S-64°S	42
EE91	92/01/02-92/01/26	100°E-130°E	61°S-65°30'S	27
WL91	92/01/27-92/02/15	70°E-100°E	63°30'S-69°S	96

Table 2: Geographical and temporal distribution of mtDNA haplotypes in minke whales from Area IV in two summer seasons. Letter sequences from left to right refer to the digestion profiles for the endonucleases: *Acc*I, *Ban*I, *Eco*RV, *Hinc*II, *Hpa*I and *Ssp*I. See Table 1 for explanation of the area/time groups in each season.

Haplotype	1989/90 summer season				1991/92 summer season			
	WE89	EE89	WL89	Total	WE91	EE91	WL91	Total
1 DCCDAA	27	31	35	93	12	10	36	58
2 ECCDAA	4	12	7	23	4	1	8	13
3 DCCDDA	6	3	5	14	2	1	4	7
4 FCCDAB	10	7	10	27	2	2	12	16
5 DCCEAA	4	5	4	13	1	3	4	8
6 DCADAA	7	1	2	10	0	0	2	2
7 DCCDAD	5	1	3	9	0	0	2	2
8 FCCDAF	6	4	0	10	5	2	0	7
9 DACDAD	3	2	0	5	0	1	0	1
10 DCCDAB	6	1	4	11	2	0	6	8
11 BCCDAA	2	1	2	5	0	0	1	1
12 CCCDAA	1	2	3	6	0	1	0	1
13 BCCDAC	3	0	1	4	0	0	0	0
14 ECCEAA	1	2	2	5	2	0	3	5
15 LCCDAA	2	1	0	3	0	1	0	1
16 DDCDAD	1	1	0	2	0	0	0	0
17 ECADAF	0	1	0	1	0	0	0	0
18 ECADAA	2	2	1	5	1	1	2	4
19 BCCDAD	0	2	1	3	0	0	0	0
20 DCADAD	1	1	0	2	0	0	0	0
21 FDCDAD	2	0	2	4	1	1	3	5
22 JCCEAH	0	4	0	4	0	1	1	2

23 BBCDAC	0	0	2	2	0	0	0	0
24 JCCEAA	2	0	0	2	0	0	0	0
25 CCCEAA	0	0	1	1	0	0	0	0
26 DCCDDD	0	0	2	2	1	0	0	1
27 FCCDAD	2	1	0	3	0	0	0	0
28 DACDAA	1	0	0	1	1	0	2	3
29 DDDDAA	1	0	0	1	0	0	0	0
30 DBCDAD	0	3	0	3	0	0	1	1
31 NCCDAA	0	1	0	1	0	0	0	0
32 FCCDAA	1	1	0	2	0	0	1	1
33 OCDDAA	0	1	0	1	1	0	0	1
34 ECCJCA	0	1	0	1	0	0	0	0
35 DCDDAB	0	1	0	1	1	0	0	1
36 DACDAG	5	1	0	6	0	0	3	3
37 DCCDDH	0	1	0	1	0	0	0	0
38 DCCEAB	1	0	0	1	0	0	0	0
39 SCCDAL	1	0	0	1	0	0	0	0
40 DCCDAM	2	0	1	3	0	0	0	0
41 RCCEAA	1	0	0	1	0	0	0	0
42 BACDAG	1	0	0	1	1	0	0	1
43 FCCLAB	3	0	0	3	0	0	0	0
44 DCCFAA	1	0	0	1	0	0	0	0
45 FCADAB	1	0	0	1	0	0	0	0
46 LCCGAD	1	0	0	1	0	0	0	0
47 TCCDAF	1	0	0	1	1	0	0	1
48 DCCDAN	0	0	1	1	0	1	1	2
49 SCCDAA	0	0	1	1	1	0	0	1
50 CACDAG	0	0	1	1	0	0	0	0
51 DCCDAG	0	0	1	1	1	0	0	1
52 DECDAA	0	1	0	1	0	0	0	0
53 OCCLAP	0	0	0	0	0	0	1	1
54 DCCDAO	0	0	0	0	1	0	1	2
55 ECADAD	0	0	0	0	0	1	0	1
56 DCCDAQ	0	0	0	0	1	0	0	1
57 ECADAR	0	0	0	0	0	0	2	2
Total	118	96	92	306	42	27	96	165

Table 3: Mitochondrial DNA diversity among and within the area/time groups analyzed in Area IV for the 1989/90 and 1991/92 summer seasons. Along diagonal is the within groups sequence divergence. Above the diagonal is the between groups sequence divergence. See Table 1 for explanation of the area/time groups. In parenthesis is the number of samples analyzed.

	1989/90				1991/92		
	WE89 (N=118)	EE89 (N=96)	WL89 (N=92)		WE91 (N=42)	EE91 (N=27)	WL91 (N=96)
WE89	0.00304	0.00280	0.00255	WE91	0.00270	0.00269	0.00239
EE89		0.00257	0.00228	EE91		0.00265	0.00238
WL89			0.00205	WL91			0.00213

Table 4: Results of chi-square tests for heterogeneity between groups of samples from two different summer seasons. Comparison between two summer seasons are made for the total samples obtained in the 1989/90 and 1991/92 seasons and for the particular area/time groups. Probability calculated from a Monte Carlo approach. See Table 1 for explanation of the area/time groups. In parenthesis is the number of samples analyzed.

Groups comparisons	Chi-square raw data	Probability
Total 89/90 (N=306) v/s Total 91/92 (N=165)	50.09	0.7670 (S.E. 0.00945)
WE89 - WE91 (N=118) (N=42)	46.35	0.2245 (S.E. 0.00933)
EE89 - EE91 (N=96) (N=27)	22.04	0.9430 (S.E. 0.00518)
WL89 - WL91 (N=92) (N=96)	27.97	0.6505 (S.E. 0.01066)

Table 5: Results of chi-square tests for heterogeneity among and between area/time groups of minke whale sampled randomly during the 1989/90 summer season. Probability calculated from a Monte Carlo approach. See Table 1 for explanation of the area/time groups. In parenthesis is the number of samples analyzed.

Groups comparisons	Chi-square raw data	Probability
WE89 - EE89 - WL89* (N=118) (N=96) (N=92)	125.17	0.0120 (S.E. 0.00243)
WE89-EE89*	57.94	0.0235 (S.E. 0.00339)
WE89-WL89*	52.20	0.0470 (S.E. 0.00473)
EE89-WL89	45.97	0.1065 (S.E. 0.00690)

* = significant at 5% level

Table 6: Results of chi-square tests for heterogeneity among and between area/time groups of minke whale sampled randomly during the 1991/92 summer season. Probability calculated from a Monte Carlo approach. See Table 1 for explanation of the area/time groups. In parenthesis is the number of samples analyzed.

Groups comparisons	Chi-square raw data	Probability
WE91 - EE91- W191 (N=42) (N=27) (N=96)	78.55	0.0850 (S.E. 0.00624)
WE91-EE91	22.40	0.7710 (S.E. 0.00940)
WE91-WL91*	39.77	0.0420 (S.E. 0.00449)
EE91-WL91	33.35	0.1000 (S.E. 0.00671)

* = significant at 5% level

Table 7: Results of chi-square tests for heterogeneity among and between area/time groups for samples of the 1989/90 and 1991/92 summer seasons combined. In parenthesis is the number of samples analyzed.

Groups comparisons	Chi-square raw data	Probability
WE(89+91)-EE(89+91)-WL(89+91)* (N=160) (N=123) (N=188)	145.74	0.0040(S.E. 0.00141)
WE(89+91)-EE(89+91)*	64.58	0.0315(S.E. 0.00391)
WE(89+91)-WL(89+91)*	69.74	0.0055(S.E. 0.00165)
EE(89+91)-WL(89+91)*	64.92	0.0040(S.E. 0.00141)

* = significant at 5% level

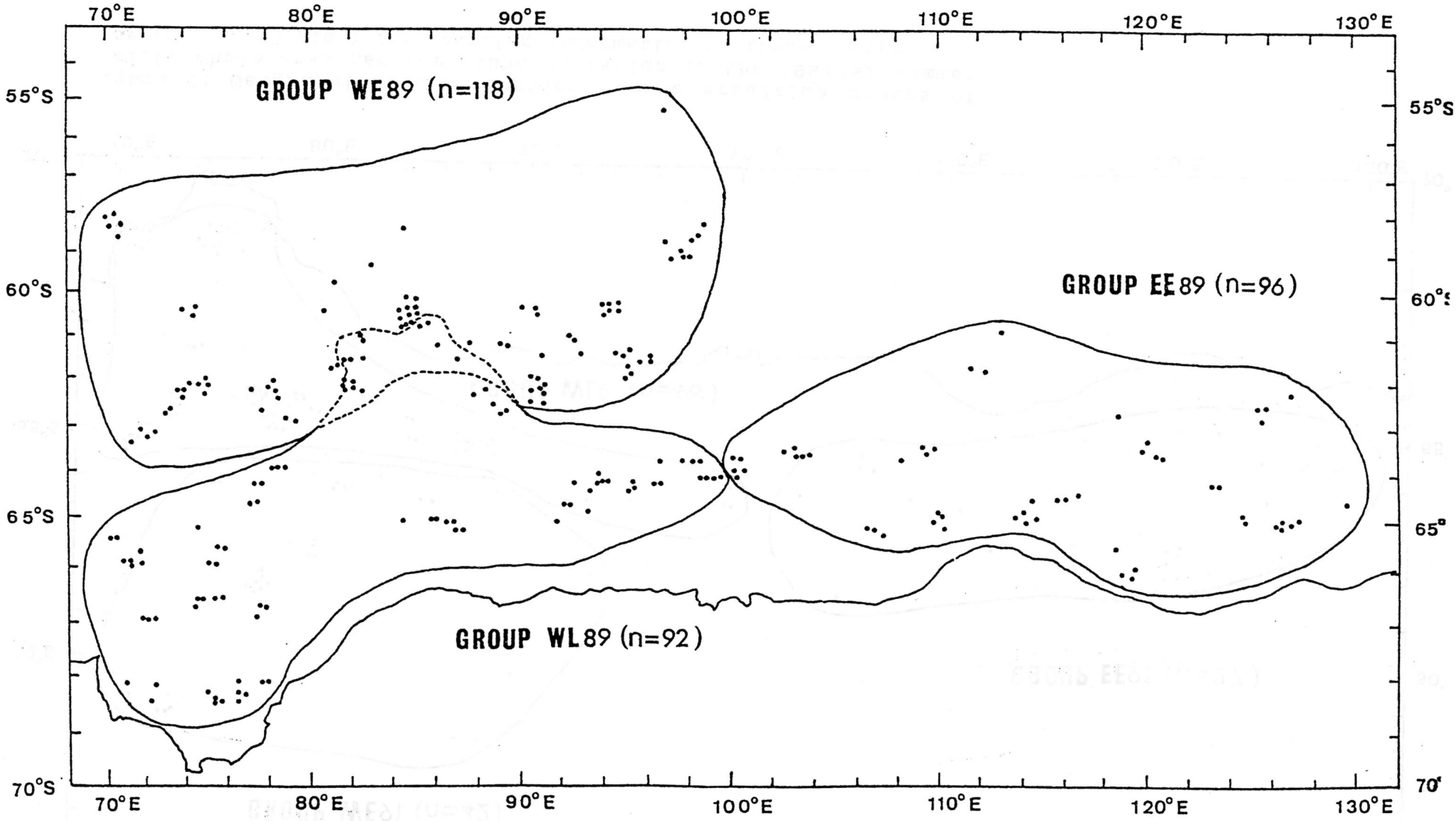

Figure 1: Geographical distribution of the area/time groups of minke whale examined for mtDNA variation in the 1989/90 summer season. See Table 1 for sampling information of these groups. Dotted line indicate an area of spatial overlapping between individuals from groups WE89 and WL89.

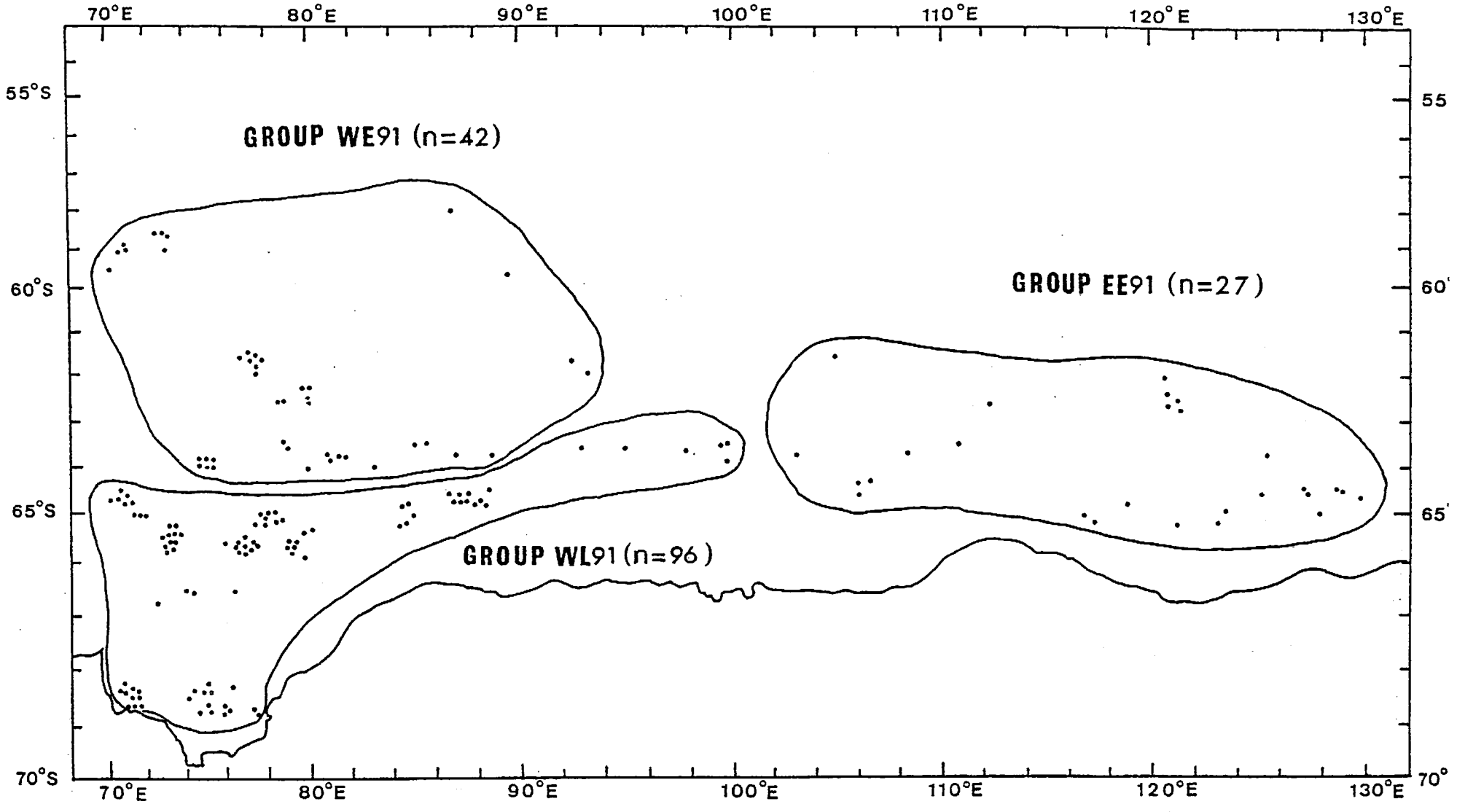

Figure 2: Geographical distribution of the area/time groups of minke whale examined for mtDNA variation in the 1991/92 summer season. See Table 1 for sampling information of these groups.