Further Analysis on the Spatial and Temporal Heterogeneity in Mitochondrial DNA Haplotype Distribution in Minke Whales from Antarctic Areas IV and V

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ABSTRACT

Restriction fragment length polymorphisms of mitochondrial DNAs in the ordinary form minke whale were identified and used to examine population structure in Antarctic Areas IV and V. We examined 1,257 minke whales sampled under special permit on the feeding grounds in five survey seasons, two in Area IV and three in Area V. Mitochondrial DNAs extracted from liver tissues were digested with six kinds of six-base polymorphic restriction enzymes. A total of 123 mtDNA haplotypes were discriminated on the basis of the combination of digestion patterns of these enzymes. Spatial and temporal factors were considered for the grouping and analysis of the samples. Haplotypes frequencies were employed to determine genetic relationship between groups of samples. Our results suggest that: a)there is not a significant difference in mtDNA haplotype distribution of the sexes, b)in both Areas IV and V there is not a significant difference in mtDNA haplotype composition between survey seasons in a determined longitudinal sector, c) after pooling samples from different survey seasons, we found a marked spatial and temporal heterogeneity in mtDNA haplotype frequencies distribution in Areas IV and V. The use of this heterogeneity in determining stock structure in Areas IV and V was examined. A group of whales distributed in the western part of Area IV in December (group A) was significantly different in haplotypes composition from all the other spatial and temporal groups analyzed in Areas IV and V. The possibility that this group belong to a relatively discrete breeding stock is discussed.

INTRODUCTION

Recently, several genetic studies have been published on the population structure of the ordinary form minke whale in the Southern Hemisphere, most of them focused particularly in the

Antarctic Areas IV and V (see Donovan, 1991 for a review of IWC stock boundaries): Wada and Numachi (1991) conducted a isozyme survey in the Antarctic that included samples from these management areas. Wada *et al.* (1991) analyzed restriction fragment length polymorphism (RFLP) patterns of the whole mitochondrial DNA (mtDNA) genome and Hoelzel and Dover (1991) analyzed the D-loop region of this molecule. Amos and Dover (1991) studied repeated or satellite DNA sequences. Pastene *et al.* (1993a) conducted a large-scale mtDNA survey in Areas IV and V using minke whale samples from the Japanese Whale Research Programme Under Special Permit in the Antarctic (JARPA).

Most of the studies mentioned above have failed to demonstrate significant genetic differences between groups of minke whales distributed in these areas. Amos and Dover (1991), however, found significant differences between the two management areas in the frequencies of a particular cloned satellite DNA. Several hypothesis were established by these authors to explain such differences. Pastene *et al.* (1993a) divided Areas IV and V into three sectors of 40° longitude for analyzing the mtDNA haplotype frequencies of 318 minke whales. They found significant differences in haplotype frequencies between samples from the western and eastern sectors but the central sector was similar to both the western and eastern sectors. Thus, no stock boundaries could be established from such analysis.

These results are consistent with the hypothesis that more than one stock are involved in these management areas during the feeding season but the inability to determine boundaries suggest some degree of mixing between them. With this regard, it should be pointed out that 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). Under this scenario, the attainment and analysis of biopsy samples from the breeding grounds can be a better approach to estimate stock identity in the southern minke whale than the approach that involves the analysis of samples from the feeding grounds. Unfortunately little is known about the occurrence and location of the breeding grounds of this species.

Pastene *et al.* (1993b) incorporated the temporal factor into the genetic analysis of samples from the feeding grounds of Area IV. They found that the mtDNA haplotype composition of whales sampled in December in the western part of Area IV was significant different from that of whales sampled in the same part during February-March. The use of temporal and spatial heterogeneity in mtDNA haplotype distribution in determining stock structure in Areas IV and V is analyzed.

MATERIALS AND METHODS

Samples

Minke whales used in this study were caught during the JARPA surveys in their summer feeding grounds of Areas IV and V. Whales

were sampled using a random design described by Kato *et al.* (1989). Samples from Area IV were caught in two survey seasons, 1989/90 and 1991/92 (Fujise *et al.*, 1990,1993a) while those from Area V in three seasons, 1988/89, 1990/91 and 1992/93 (Kato *et al.*, 1990; Kasamatsu *et al.*, 1993; Fujise *et al.*, 1993b). The number of samples used in our mtDNA survey are shown in Table 1, by sex and survey season.

Tissue samples
Mitochondrial DNA was isolated from liver tissue that had been frozen at -20°C for 1-4 years, using the procedure described by Pastene *et al.* (1993a).

RFPL analysis
Crude mtDNA (15*ul*) was digested with 2 or 3 units of the following six kinds of six-base sequence recognition endonucleases: *Acc*I, *Ban*I, *Eco*RV, *Hinc*II, *Hpa*I and *Ssp*I. These enzymes were chosen because they were known to be polymorphic in the Antarctic minke whale. Digestion and electrophoresis procedures were those used by Pastene *et al.* (1993a). Distinctive restriction fragment patterns produced by 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. Thus, the composite haplotype for each individual consisted of a string of six letters.

Sample analysis
Samples from Area IV taken in the 1989/90 and 1991/92 survey seasons were grouped in a similar manner as in a previous study (Pastene *et al.* 1993b). Area IV was divided into a western (70°-100°E) and eastern (100°-130°E) sectors. In each of these sectors samples were grouped into an 'early' (December-15 January) and 'late' (16 January-March) groups. Thus, a total of eight area/time/year groups were defined in this area. The period of sampling, and the sample size by sex of each area/time/year group is shown in Table 2. Fig. 1 shows the geographical position of these groups.

In a similar manner as in Area IV, Area V was divided into a western (130°-160°E) and eastern (160°E-160°W) sectors and two temporal categories were established in each of these sectors. As only the eastern sector was surveyed in the 1988/89 survey season, thus, a total of ten area/time/year groups were defined in Area V. Sampling information on these groups are shown in Table 3 and their geographical position in Fig. 2.

Firstly, we analyzed the mtDNA haplotype frequencies distribution between sexes in each of the area/time/year groups. If not significant differences were found, then we pooled samples from both sexes within a particular area/time/year group.

Following this, we compared the mtDNA haplotype composition between different survey seasons in four longitudinal sectors of Areas IV and V. This was conducted separately for 'early' and 'late' sample groups. If not significant differences were found,

then we pooled samples from different survey seasons into a single area/time group.

Following this we investigated the temporal variation in genetic composition in each of the four longitudinal sectors examined. Finally, the genetic differentiation between these longitudinal sectors in Areas IV and V, was investigated.

Data analysis
Mitochondrial DNA frequencies were employed to determine genetic relationship between the samples of the designed area/time groups. Genetic relationships were quantified using the chi-square statistic for heterogeneity of mtDNA haplotype frequencies (Roff and Bentzen, 1989). This Monte Carlo approach, which estimate the significance of the chi-square test computed from the raw data, was useful because several of the haplotypes were represented by a single specimen. In each trial 2,000 randomizations of the original data sets were made.

RESULTS

Mitochondrial DNA fragment patterns
The mtDNA fragment patterns produced by each restriction enzyme used in this study is shown in Table 4. The number of fragment patterns produced by endonucleases *Acc*I, *Ban*I, *Eco*RV, *Hinc*II, *Hpa*I and *Ssp*I were 27, 7, 5, 13, 7 and 28, respectively. The molecular size of each fragment estimated with reference to the mobilities of the standard marker lamda-EcoT14I digest, and the total length in the sum of each fragment, are also shown in Table 4.

MtDNA genome size
The mean mtDNA genome size, obtained by averaging the sums of the fragment size estimates, was 16,606±657 (SD).

Mitochondrial DNA haplotypes
Restriction enzyme digestions of mtDNA from the total sample revealed a total of 123 mtDNA haplotypes. The composite patterns of these haplotypes are shown in Table 5.

Mitochondrial DNA haplotype frequencies of the sexes
Neither the area/time/year groups analyzed in Area IV (Table 6) nor the area/time/year groups examined in Area V (Table 7) showed a significant bias in the frequencies of mtDNA haplotypes between the sexes.

Mitochondrial DNA haplotype frequency distribution between survey seasons
Table 8 shows the results of statistical comparisons between the mtDNA haplotype frequencies distribution of area/time groups of the 1989/90 sampling season and that of area/time groups of the 1991/92 sampling season. There is no significant differences in mtDNA haplotype frequencies distribution between two surveys in Area IV. This result is obtained either when the area/time groups or the total samples between two surveys are compared.

Table 9 shows the results of the same analysis in Area V. As in Area IV, there is no significant differences in mtDNA haplotype composition between surveys in Area V. It should be noted, however, that the probability obtained in the comparison made between the three 'late' groups of the eastern part of Area V (160°E-160°W) was particularly low.

Temporal variation in haplotype composition in longitudinal sectors

Table 10 shows the results of chi-square test for heterogeneity in mtDNA haplotype frequencies between 'early' and 'late' sample groups in four longitudinal sectors in Areas IV and V. Comparisons in the western part of Area IV (70°-100°E) and in the eastern part of Area V (160°E-160°W) showed significant differences in haplotypes composition. 'Early' and 'late' sample groups in the eastern part of Area IV (100°-130°E) and in the western part of Area V (130°-160°E) were similar in haplotype composition.

Temporal and spatial heterogeneity in mtDNA haplotype frequencies distribution in Areas IV and V

Because the haplotype compositions of 'early' and 'late' sample groups of the western part of Area IV and eastern part of Area V were different, we treated these groups independently in the rest of the analysis. Fig. 3 shows the geographical position of six groups examined for longitudinal variation in haplotype composition, and Table 11 shows the distribution of 123 mtDNA minke whale haplotypes in these six groups. We have called these groups as A, B, C, D, E and F, respectively. According to this table haplotypes '1' though '5' and haplotypes '8', '10' and '14' were the predominant haplotypes (66.3%). It should be noted here that the frequency of some of these dominant haplotypes were notably different in the group A. For example the frequency of the dominant haplotype '1' was 23.8% in this group while in the other groups was over 30%. The same trend was observed in the frequencies of haplotype '2'. Haplotype '8' was higher in group A than in the rest of the groups. On the other hand, the frequency of haplotype '3' was higher in group F and the frequency of haplotype '5' was higher in group E than in the rest of the groups examined. No particular differences in the frequencies of dominant haplotypes was observed in groups B, C and D.

Table 12 shows the results of a chi-square test for heterogeneity in mtDNA haplotype frequencies in the six groups examined. The frequencies of the group A is significantly different (at the 5% level) from those of all the other groups except that of the group E (7.1%). On the other hand the group F differs in mtDNA haplotype composition with all the other groups except with that of group B. Group B is similar in mtDNA haplotype composition with all the other groups except with group A. The groups C and D are similar in haplotype composition to groups E and B but differ with groups A and F.

DISCUSSION

In this study six polymorphic restriction enzymes were used to digest mtDNA from 1,257 samples of minke whales distributed in Antarctic Areas IV and V, discriminating a total of 123 mtDNA haplotypes. We analyzed the temporal and spatial heterogeneity of these haplotypes to study stock structure in these areas. Levels of mtDNA variability found are very high, as might be expected of a population considered abundant in these areas.

Grouping of samples

As it was pointed out in the introduction of this study, little is known about the occurrence and location of the breeding grounds in the Southern Hemisphere minke whale. Only one breeding ground has been identified (in Brazil) based on Discovery mark recovery analysis (Horwood, 1990, pp. 39-40). Without a previous knowledge on the location of the breeding grounds in the Indian Ocean and western part of the Pacific Ocean, and on the pattern of migration toward the feeding grounds of these breeding stocks, it is difficult to define what longitudinal sectors in the feeding grounds of Areas IV and V should be compare for their genetic composition. For this reason, the longitudinal sectors in the feeding ground analyzed in our study were arbitrarily designed, dividing Areas IV and V into four sectors.

In each longitudinal sector, we divided the samples into 'early' and 'late' sample groups. The 'early' group was composed by individuals sampled from early December to 15 January, while the 'late' group was composed by minke whales sampled from 16 January to March. The criteria used in making such temporal division was based in the findings of Pastene *et al.* (1993b). These authors found that whales sampled in the 'early' period (as defined above) in the western part of Area IV differed significantly from whales sampled in the 'late' period in the same sector, in their mtDNA haplotype composition. It should be noted here that the date of sampling within the 'early' category was not exactly the same between sectors. For example the 'early' category in the western part of Area IV involved samples taken in December only, while the 'early' category in the eastern part involved samples taken in the first half of January.

MtDNA haplotype distribution of the sex

Our analysis began examining the mtDNA haplotype composition of the sexes. None of the area/time groups in the five sampling seasons examined showed a significant differences in mtDNA haplotype frequencies distribution of the sexes. This result is similar to that found by Baker *et al.* (1990) who found no significant differences in the haplotype frequencies of the sexes in the North Hemisphere humpback whale. They examined samples from both feeding and breeding grounds. In a separate analysis (not published here), we also conducted comparisons in the mtDNA haplotype composition of the sexes after grouping samples of different survey seasons (i.e. we conducted such comparisons within each of the groups showed in Table 10). None of the eight test conducted showed significant difference in the haplotype composition of the

sexes.

Inter-surveys comparison in mtDNA composition

Pastene *et al.* (1993a) used samples of the JARPA in order to analyze three contiguous sectors defined in Areas IV and V. In their analysis, they combined in a same sector, samples obtained in more than one survey season. Their assumptions were that the patterns of seasonal movement are the same for a given breeding population in different years and that lateral movement on feeding grounds and patterns of mixing are similar between years. We examined further this in order to conduct genetic comparisons between four longitudinal sectors using samples combined of two or three surveys. None of our inter-surveys comparisons showed a significant differences in haplotype frequencies distribution either when area/time groups or the total samples of different survey seasons were compared. This result is consistent with the hypothesis that the same stocks with similar patterns of lateral movement and mixing are involved in Areas IV and V in different years. This hypothesis is supported by the finding of Pastene *et al.* (1993b) who found a similar pattern of temporal and spatial mtDNA diversity in Area IV, between two survey seasons.

It should be noted here that because the samples were first grouped by longitudinal sectors, the time covered by the sample is not exactly the same between two survey seasons (see Tables 2 and 3 for Areas IV and V, respectively). Inter-surveys comparisons should be further examined in the future by testing samples grouped in different ways, i.e. by grouping the samples by temporal categories first. Furthermore, these analysis should incorporate information on abundance, effort and sampling rate for each survey in the sectors investigated.

Temporal variation in mtDNA composition

Because the mtDNA haplotype composition of samples between two surveys showed no significant differences, the following analysis was conducted by pooling samples of two surveys (in Area IV) and three surveys (in Area V). Before conducting inter-longitudinal sectors comparisons, we examined temporal differences in genetic composition in each of the four longitudinal sectors. Our results indicate that significant temporal differences occur in the western part of Area IV and in the eastern part of Area V. The temporal variation found in the eastern part of Area V, however, should be considered as preliminary because the small sample size of the 'early' group of the eastern part of this area. With regard to Area IV our result is similar to that found by Pastene *et al.* (1993b).

Temporal and spatial heterogeneity in mtDNA haplotype composition in Areas IV and V.

A overall picture of the patterns of temporal and spatial heterogeneity in mtDNA haplotype distribution in Areas IV and V was showed in Tables 11 and 12. According to Table 12, the extremes of the overall research areas, groups A and F, respectively are different in haplotype frequencies distribution from most of the other groups. This pattern is more clear in group A than in group

F because the later group showed a composition similar to group B. Group E was lightly different from groups A and B but similar to the central groups C and D. Group B was similar in haplotype composition to all the other groups, except group A and differed lightly from group E.

Although statistical significant differences in haplotype composition were found in several pair-wise comparisons of the six groups, it is noted that these groups were not fully distinguishable by their composite haplotypes. No absolute stock markers were present as shown in Table 11. All the dominant haplotypes (8 of 123 representing 66.3% of the samples analyzed) were shared by all the groups.

There was, however, differences in the frequencies of some dominant haplotypes between Group A and the rest of the groups. It was the case of haplotypes '1', '2' and '8'. Also groups E and F showed differences in the frequencies of dominant haplotypes '5' and '3', respectively, with regard the other groups. Some rare haplotypes were specific for a determined sector. This was seen more frequently in groups in the extremes of the whole research area (groups A, E and F).

Under these considerations it seems that the groups in the extremes of the area, particularly group A, are entities distinguishable genetically, while the groups in the center of the research area, including group B are undistinguishable genetically among them, and in some cases, undistinguishable from groups E or F, as well. Pastene *et al.* (1993a) compared three contiguous longitudinal sectors in Areas IV and V without considerations of temporal variation in each of these sectors. They found that the sectors in the extremes of the research area differed in their haplotype frequencies distribution but the central sector was similar to both extreme sectors. In this study we incorporated the temporal factor and our results indicate that the whales distributed in the western part of Area IV in an early stage of the feeding season are those differing from the whales of the eastern part of Area IV and Area V. The whales distributed in the same western sector in an late stage of the feeding season, on the other hand, was similar to all the other groups of whales of both the eastern part of Area IV and Area V.

Our results are consistent with the hypothesis that Group A represents a discrete breeding stock. Group A was sampled in an early stage of the feeding season (December), thus the degree of mixing with other stocks occurring in the feeding ground could still being low enough to permit genetic identification. The geographical extension in the feeding ground of this hypothesized stock could be investigated with the analysis of samples from the other sectors taken in December. Unfortunately no whales sampled in this month in the eastern part of the Area IV are available and those from the sectors of Area V taken in December are scarce.

On the other hand, genetic distinctness of group F from the

rest of the groups were also clear, although this group was similar to group B. The groups in the central sector (100°-160°E), groups C, D, even group B, are not always discriminated among them, thus, it can be hypothesized that whales distributed in the central sector may represent the occurrence of more than one breeding stock which mix with progress of the feeding season. This require further examination.

Although the recommendation of the Scientific Committee of conducting biopsy sampling in the breeding grounds (IWC, 1992) still a valid suggestion, the analysis of samples from the feeding grounds, on a more strict temporal basis, still provide important information on the population structure of this species.

ACKNOWLEDGEMENTS

We would like to acknowledge the researchers and crew members participating in the research cruises of JARPA of 1988/89, 1989/90, 1990/91, 1991/92 and 1992/93, for sampling design and samples used in this study. Mr. S. Itoh and M. Kawasaki, The Institute of Cetacean Research, assisted in the computer-based analysis.

REFERENCES

Amos, W. and Dover, G.A. 1991. The use of satellite DNA sequences in determining population differentiation in the minke whale. *Rep. int. Whal. Commn* (special issue 13):235-44.

Baker, C.S., Palumbi, S.R., Lambertsen, R.H., Weinrich, M.T., Calambokidis, J. and O'Brien, S.J. 1990. Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. *Nature, Lond.* 344(6263):238-40.

Best, P. 1990. A review of information on stock identity in Southern Hemisphere minke whales. Paper SC/42/SHMi8 presented to the IWC Scientific Committee, 1991 (unpublished). 23pp.

Donovan, G.P. 1991. A review of IWC stock boundaries. *Rep. int. Whal. Commn* (special issue 13):39-68.

Fujise, Y., Yamamura, K., Zenitani, R., Ishikawa, H., Yamamoto, Y., Kimura, K. and Komaba, M. 1990. Cruise report of the research on southern minke whales in 1989/90 under the Japanese proposal to the scientific permit. Paper SC/42/SHMi25 presented to the IWC Scientific Committee, June 1990 (unpublished). 56pp.

Fujise, Y., Ishikawa, H., Saino, S., Nagano, M., Ishii, K., Kawaguchi, S., Tanifuji, S., Kawashima, S. and Miyakoshi, H. 1993a. Cruise report of the 1991/92 Japanese research in Area IV under a special permit for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 43:357-71.

Fujise, Y., Zenitani, R., Saino, S., Ito, S., Kawasaki, M., Matsuoka, K. and Tamura, T. 1993b. Cruise report of the Japanese research authorized under the special permit to take Southern Hemisphere minke whales in 1992/93. Paper SC/45/SHBa12 presented to the IWC Scientific Committee, May 1993 (unpublished). 36 pp.

Hoelzel, A.R. and Dover, G.A. 1991. Mitochondrial D-loop DNA

variation within and between populations of the minke whale (*Balaenoptera acutorostrata*). *Rep. int. Whal. Commn* (special issue 13):171-81.
Horwood, J.W. 1990. *Biology and Exploitation of the Minke Whale*. CRC Press, Boca Raton. 238pp.
International Whaling Commission. 1992. Report of the Sub-committee on Southern Hemisphere Baleen Whales. *Rep. int. Whal. Commn* 43:104-114.
Kasamatsu, F., Yamamoto, Y., Zenitani, R., Ishikawa, H., Ishibashi, T., Sato, H., Takashima, K., and Tanifuji, S. 1993. Report of the 1990/91 southern minke whale research cruise under scientific permit in Area V. *Rep. int. Whal. Commn* 43:505-522.
Kato, H., Hiroyama, H., Fujise, Y. and Ono, K. 1989. Preliminary report of the 1987/88 Japanese feasibility study of the special permit proposal for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 39:235-48.
Kato, H., Fujise, Y., Yoshida, H., Nakagawa, S., Ishida, M. and Tanifuji, S. 1990. Cruise report and preliminary analysis of the 1988/89 Japanese feasibility study of the special permit proposal for Southern Hemisphere minke whales. *Rep. int. Whal. Commn* 40:289-300.
Pastene, L.A., Kobayashi, T., Fujise, Y. and Numachi, K. 1993a. Mitochondrial DNA differentiation in Antarctic minke whales. *Rep. int. Whal. Commn* 43:349-55.
Pastene, L.A., Kobayashi, T., Fujise, Y. and Numachi, K. 1993b. Temporal variation in mitochondrial DNA haplotype composition in minke whales from Antarctic Area IV. Paper SC/45/SHBa13 presented to the IWC Scientific Committee, May 1993 (unpublished). 16pp.
Roff, D.A. and Bentzen, P. 1989. The statistical analysis of mtDNA polymorphisms: chi-square and the problem of small samples. *Mol. Biol. Evol.* 6(5):539-45.
Wada, S. and Numachi, K. 1991. Allozyme analyses of genetic differentiation among the populations and species of the *Balaenoptera*. *Rep. int. Whal. Commn* (special issue 13):125-54
Wada, S., Kobayashi, T. and Numachi, K. 1991. Genetic variability and differentiation of mitochondrial DNA in minke whales. *Rep. int. Whal. Commn* (special issue 13):203-15.

Table 1: Summary of samples of minke whales used for mtDNA analysis.

Season	Area	Female	Male	Total
1988/89	V	46	31	77
1989/90	IV	136	171	307
1990/91	V	151	157	308
1991/92	IV	113	147	260
1992/93	V	144	161	305
Total		590	667	1,257

Table 2: Sampling data of four area/time groups defined in Area IV in the 1989/90 and 1991/92 survey seasons.

Area/time group	Period of sampling	Long. range	Sample size F	M	T
1989/90					
Western Early (WE89/90)	12/6-12/29	70°E-100°E	30	88	118
Western Late (WL89/90)	1/21-2/14	70°E-100°E	50	42	92
Eastern Early (EE89/90)	12/31-1/15	100°E-130°E	49	33	82
Eastern Late (EL89/90)	1/16-1/17	100°E-130°E	7	8	15
1991/92					
Western Early (WE91/92)	12/11-12/28	70°E-100°E	12	30	42
Western Late (WL91/92)	1/27-2/25	70°E-100°E	75	76	151
Eastern Early (EE91/92)	1/2-1/8	100°E-130°E	2	10	12
Eastern Late (EL91/92)	1/16-3/24	100°E-130°E	24	31	55

Table 3: Sampling data of four area/time groups defined in Area V in the 1988/89, 1990/91 and 1992/93 survey seasons.

Area/time groups	Period of sampling	Long. range	Sample size F	M	T
1988/89					
Eastern Early (EE88/89)	1/12-1/14	160°E-160°W	5	5	10
Eastern Late (EL88/89)	1/17-3/27	160°E-160°W	41	26	67
1990/91					
Western Early (WE90/91)	1/13-1/15	130°E-160°E	16	8	24
Western Late (WL90/91)	1/16-3/14	130°E-160°E	48	91	139
Eastern Early (EE90/91)	12/20-1/12	160°E-160°W	7	19	26
Eastern Late (EL90/91)	2/6-2/28	160°E-160°W	80	39	119
1992/93					
Western Early (WE92/93)	12/3-1/15	130°E-160°E	41	42	83
Western Late (WL92/93)	1/20-3/22	130°E-160°E	39	64	103
Eastern Early (EE92/93)	1/2-1/11	160°E-160°W	9	17	26
Eastern Late (EL92/93)	2/4-3/6	160°E-160°W	55	38	93

Table 4: Restriction fragment patterns of minke whale mtDNA produced by six six-base restriction enzymes. Fragment size (Kbp) was estimated with reference to the mobilities of the standard marker lamda-EcoT14I digest.

Enzyme	ID	Fragment Size	Total
*Acc*I	A	4.89,3.43,3.21,2.69,1.27,0.44,0.35	16.28
	B	3.89,3.43,3.21,2.69,1.58,0.88,0.44	16.12
	C	3.89,3.43,3.21,2.69,1.27,0.88,0.44,0.35	15.81
	D	3.89,3.43,3.21,2.69,1.27,0.74,0.44,0.35	16.02
	E	3.89,3.21,2.69,2.60,1.27,0.88,0.77,0.44,0.35	16.10
	F	3.89,3.43,3.21,2.69,1.58,1.31	16.11
	G	3.89,3.49,3.21,2.69,1.27,0.88,0.44,0.35	16.22
	H	3.89,3.49,3.43,2.69,1.27,0.88,0.44,	16.09
	I	3.89,3.65,3.43,2.69,1.27,0.74,0.35	16.02
	J	3.89,3.21,2.69,2.35,1.58,1.02,0.88,0.44	16.06
	K	3.89,3.43,3.21,2.69,1.02,0.88,0.44,0.35	15.91
	L	3.89,3.43,3.21,1,44,1.27,1.27,0.88,0.44,0.35	16.18
	M	4.10,3.89,3.43,2.69,1.06,0.88	16.05
	N	3.89,3.21,2.99,2.69,1.27,0.88,0.44,0.44	15.81
	O	4.10,3.89,3.43,3.21,0.74,0.44,0.35	16.16
	P	4.10,3.89,3.21,2.60,0.88,0.77,0.44,0.35	16.24
	Q	4.10,3.89,3.43,3.21,0.88,0.44,0.35	16.30
	R	3.89,3.65,3.43,2.69,1.27,0.88,0.35	16.16
	S	3.89,3.21,2.69,2.60,1.06,0.88,0.77,0.44,0.35	15.89
	T	3.89,3.43,3.21,2.69,1.58,0.74,0.44,0.35	16.33
	U	3.89,3.21,2.69,2.35,1.58,1.02,0.88,0.44	16.06
	V	3.89,3.43,3.21,1.58,1.44,1.23,0.88,0.44	16.10
	W	3.89,3.43,3.21,1.83,1.06,0.88,0.74,0.44,0.35	15.83
	X	4.89,3.49,3.43,2.69,1.27,0.44	16.21
	Y	3.89,3.43,3.21,2.69,0.88,0.88,0.44,0.44	15.86
	Z	3.89,3.21,2.69,2.60,1.27,0.77,0.77,0.44,0.35	15.99
	A'	3.89,3.21,2.69,2.60,1.27,0.88,0.88,0.44,0.35	16.21
*Ban*I	A	6.87,4.25,2.89,2.05,1.00,0.82	17.88
	B	6.87,2.89,2.42,2.24,1.88,1.00,0.82	18.12
	C	6.87,2.89,2.42,2.05,1.88,1.00,0.82	17.93
	D	5.98,2.29,2.42,2.05,1.88,1.10,1.00,0.82	17.64
	E	6.87,2.42,2.24,2.05,1.88,1.00,0.82	17.28
	F	6.87,4.72,2.42,2.05,1.00,0.82	17.88
	G	6.22,2.89,2.42,2.05,1.88,1.00,0.88	17.34
*Eco*RV	A	16.48,1.49	17.97
	B	9.80,6.75,1.49	18.04
	C	8.17,6.75,1.78,1.49	18.19
	D	9.80,5.51,1.49,1.28	18.08
	E	9.80,4.40,2.42,1.49	18.11
*Hinc*II	A	4.03,3.64,3.25,1.78,1.54,0.89,0.65,0.57,0.44	16.79
	B	4.03,3.64,2.77,1.78,1.54,0.89,0.65,0.57,0.44	16.31
	C	3.64,3.25,2.86,1.78,1.54,0.93,0.89,0.65,0.57,0.44	16.55
	D	5.69,3.64,3.25,1.78,0.89,0.65,0.57,0.44	16.91

Table 4: cont.

	E	4.03,2.86,2.77,1.72,1.54,0.89,0.65,0.57,0.44	15.47
	F	5.69,3.25,2.38,1.78,1.19,0.89,0.65,0.57,0.44	16.84
	G	4.03,3.54,2.38,1.78,1.54,0.89,0.65,0.57,0.44	15.82
	H	3.64,3.25,2.38,1.78,1.73,1.54,0.89,0.65,0.57,0.44	16.87
	I	4.03,3.64,3.25,1.78,1.54,1.19,0.65,0.57,0.44	17.09
	J	3.64,3.09,2.86,1.78,1.54,0.89,0.65,0.57,0.44	15.46
	K	5.69,3.64,2.86,1.78,0.89,0.65,0.57,0.44	16.52
	L	5.69,3.64,3.25,1.78,1.19,0.65,0.57,0.44	17.21
	M	4.03,3.64,3.25,1.78,1.54,1.40,0.89,0.57,0.44	17.54
HpaI	A	13.60,3.37	16.97
	B	13.60,2.31,1.24	17.15
	C	11.51,3.37,1.63	16.51
	D	6.51,6.22,3.37	16.10
	E	8.03,5.06,3.37	16.46
	F	11.51,3.37,2.31	17.19
	G	11.51,3.37,1.24	16.12
SspI	A	5.74,3.87,3.33,1.78,1.45,0.42	16.59
	B	5.74,3.33,3.33,1.78,1.45,0.55,0.42	16.60
	C	4.40,3.87,3.33,1.78,1.45,1.28,0.42	16.53
	D	4.12,3.87,3.33,1.78,1.60,1.45,0.42	16.57
	E	6.22,3.33,3.33,1.78,1.45,0.42	16.53
	F	4.12,3.33,3.33,1.78,1.60,1.45,0.55,0.42	16.58
	G	5.74,3.87,3.33,1.67,1.45,0.42	16.48
	H	5.74,3.87,3.33,3.33,0.42	16.69
	I	7.10,4.12,3.33,1.60,0.42	16.57
	J	4.40,3.33,2.82,1.78,1.45,1.28,1.03,0.42	16.51
	K	5.74,3.76,3.33,1.78,1.45,0.42	16.48
	L	5.74,3.87,2.69,1.78,1.45,0.60,0.42	16.55
	M	5.74,3.87,3.33,1.88,1.78,0.42	17.02
	N	5.74,3.87,3.76,1.78,1.45,0.42	17.02
	O	5.74,3.33,2.82,1.78,1.45,1.03,0.42	16.57
	P	7.10,5.74,3.33,0.42	16.59
	Q	5.74,3.33,1.94,1.78,1.78,1.45,0.42	16.44
	R	4.12,3.33,1.94,1.78,1.78,1.60,1.45,0.42	16.42
	S	5.74,3.33,3.33,1.78,1.03,0.55,0.42,	16.18
	T	4.12,3.87,3.33,3.33,1.60,0.42	16.67
	U	5.74,5.74,3.33,1.45,0.42	16.68
	V	4.12,3.33,3.33,1.60,1.45,1.28,0.55,0.42	16.08
	W	3.87,3.33,2.82,1.78,1.60,1.45,1.28,0.42	16.55
	X	10.78,3.33,1.78,1.45,0.42	17.76
	Y	5.74,3.87,3.33,1.78,1.03,0.42	16.17
	Z	5.74,3.87,3.76,1.67,1.45,0.42	16.91
	A'	5.74,3.33,3.33,1.67,1.45,0.55,0.42	16.49
	B'	5.30,3.33,3.33,1.67,1.45,0.55,0.42	16.05

Table 5: Composite pattern of 123 minke whale mtDNA haplotypes. Letter sequences from left to right refer to the digestion profiles for the restriction enzymes *Acc*I, *Ban*I, *Eco*RV, *Hin*cII, *Hpa*I and *Ssp*I.

1-	CCBAAA	27-	CCBACD	53-	AABAAG	78-	EDBAAW	104-	CCBIAD
2-	DCBAAA	28-	ECBAAD	54-	ACBIAB	79-	CCEGBX	105-	CCBBAO
3-	CCBACA	29-	ECBAAE	55-	CCBCAA	80-	ICBLAA	106-	QCCAAB
4-	ECBAAB	30-	CABAAA	56-	ECAAAB	81-	ECCAAE	107-	CCBAAY
5-	CCBBAA	31-	CDCAAA	57-	HCBDAD	82-	CCBBAD	108-	YCBAAA
6-	CCAAAA	32-	MCBFEK	58-	PCBAAF	83-	DCBACA	109-	RCBDAH
7-	CCBAAD	33-	ECDAAA	59-	CCBAAN	84-	ECBCAB	110-	CCAAAG
8-	ECBAAF	34-	ICBDAA	60-	OCBAAA	85-	ICBKAA	111-	CCBHFA
9-	CABAAD	35-	GCBAAA	61-	BABAAG	86-	SCBAAB	112-	ICBDAD
10-	CCBAAB	36-	KCBIAI	62-	CCBAAG	87-	CCBCAU	113-	CGCAAA
11-	ACBAAA	37-	LCBAAA	63-	CEBAAA	88-	ECBAAO	114-	CGBAAD
12-	BCBAAA	38-	ABBAAJ	64-	KCBIAP	89-	CABAAU	115-	ECBMAB
13-	ACBAAC	39-	CBBAAD	65-	CCBAAO	90-	DCBEDQ	116-	DGBAAA
14-	DCBBAA	40-	ECBAAG	66-	DCAAAD	91-	TCAAAA	117-	CCBBAZ
15-	HCBAAA	41-	JCBAAA	67-	CCBAAQ	92-	DCBAAY	118-	A'CBAAA'
16-	CDBAAD	42-	ECBAAA	68-	DCAAAR	93-	UCBAAA	119-	ZCBAAB'
17-	DCAAAF	43-	DCBEDA	69-	BCBHFG	94-	CCBBCA	120-	ZCBAAA
18-	RCBDAA	44-	KCCAAA	70-	ECBAAF	95-	CCBAAH	121-	ECBAAA'
19-	DCAAAA	45-	DCBGBA	71-	CCBJGA	96-	VCBAAA	122-	A'CBAAB
20-	ACBAAD	46-	CCCAAB	72-	QCBAAB	97-	WCBIAI	123-	ECBAAB'
21-	CCAAAD	47-	CABAAG	73-	FCBBAT	98-	CCBAAC		
22-	EDBAAD	48-	CCBACH	74-	DCAAAU	99-	ECAGBB		
23-	FCBBAH	49-	CCBBAB	75-	CCBBAU	100-	XBBAAC		
24-	ABBAAC	50-	QCBAAL	76-	ECBAAV	101-	AFBAAA		
25-	FCBBAA	51-	CCBAAM	77-	RCBKAA	102-	CCBHFD		
26-	BCBBAA	52-	NCBBAA	78-	EDBAAW	103-	CBBAAA		

Table 6: Results of a chi-square tests for heterogeneity in mtDNA haplotype frequencies **between sexes in four area/time groups in Area IV in the survey seasons of 1989/90 and 1991/92**. Probability calculated by the Monte Carlo approach of Roff and Bentzen (1989). A total of 2,000 simulations were made in each test.

Area Group	Sample Size			Probability	SE
	Female	Male	Total		
1989/90					
WE89/90	30	88	118	0.6265	0.0108
WL89/90	50	42	92	0.2860	0.0101
EE89/90	49	33	82	0.0990	0.0067
EL89/90	7	8	15	---	---
Total Area IV 89/90	136	171	307	0.0960	0.0066
1991/92					
WE91/92	12	30	42	---	---
WL91/92	75	76	151	0.4385	0.0111
EE91/92	2	10	12	---	---
EL91/92	24	31	55	0.3465	0.0106
Total Area IV 91/92	113	147	260	0.5735	0.0111

Table 7: Results of a chi-square tests for heterogeneity in mtDNA haplotype frequencies **between sexes in area/time groups in Area V** in the survey seasons 1988/89, 1990/91 and 1992/93.

Area Group	Sample size			Probability	SE
	Female	Male	T		
1988/89					
EE88/89	5	5	10	----	----
EL88/89	41	26	67	0.2165	0.0092
Total Area V 88/89	46	31	77	0.3525	0.0107
1990/91					
WE90/91	16	8	24	----	----
WL90/91	48	91	139	0.4780	0.0112
EE90/91	7	19	26	----	----
EL90/91	80	39	119	0.1805	0.0086
Total Area V 90/91	151	157	308	0.3375	0.0106
1992/93					
WE92/93	41	42	83	0.9630	0.0042
WL92/93	39	64	103	0.1015	0.0068
EE92/93	9	17	26	----	----
EL92/93	55	38	93	0.8285	0.0084
Total Area V 92/93	144	161	305	0.7160	0.0101

Table 8: Results of chi-square test for heterogeneity in mtDNA haplotype frequencies **between area/time groups of two surveys seasons in Area IV.** In parenthesis is the sample size used in each test.

Comparisons	Probability	SE
WE89/90 (118) v/s WE91/92 (42)	0.2905	0.0102
WL89/90 (92) v/s WL91/92 (151)	0.6110	0.0109
EE89/90 (82) v/s EE91/92 (12)	0.5940	0.0110
EL89/90 (15) v/s EL91/92 (55)	0.7010	0.0102
Total 1989/90 (307) v/s Total 1991/92 (260)	0.4965	0.0112

Table 9 : Results of chi-square test for heterogeneity in mtDNA haplotype frequencies **between area/time groups of three surveys seasons in Area V.** In parenthesis is the sample size used in the tests.

Comparisons	Probability	SE
WE90/91 (24) v/s WE92/93 (83)	----	----
WL90/91 (139) v/s WL92/93 (103)	0.4725	0.0111
EE90/91 (26) v/s EE92/93 (26)	0.9835	0.0056
EL90/91 (119) v/s EL92/93 (93) v/s EL88/89 (67)	0.0645	0.0055
Total 1990/91 (308) v/s Total 1992/93 (305) v/s Total88/89 (77)	0.2190	0.0925

Table 10: Results of a chi-square test for **temporal** heterogeneity in mtDNA haplotype frequencies in four longitudinal sectors of Area IV and V. In parenthesis is the sample size used in each test.

Comparisons	Probability	SE
70°-100°E Early (160) v/s Late (243)	0.0220	0.0033
100°-130°E Early (94) v/s Late (70)	0.6725	0.0105
130°-160°E Early (107) v/s Late (242)	0.4895	0.0112
160°E-160°W Early (62) v/s Late (279)	0.0220	0.0033

Table 11: Geographical and temporal distribution of 123 minke whale mtDNA haplotypes in Areas IV and V. See Table 12 for statistical comparison between groups.

	70-100°E		100-130°E	130-160°E	160°E-160°W		Total
Haplotype	Early	Late	Total	Total	Early	Late	
	A	B	C	D	E	F	
1	38	83	58	114	19	89	401
2	8	23	16	27	4	22	100
3	8	10	5	19	3	23	68
4	12	25	11	32	5	20	105
5	5	11	8	10	5	6	45
6	7	4	1	6	0	5	23
7	5	5	2	5	1	5	23
8	11	3	8	9	2	4	37
9	3	3	3	1	0	1	11
10	8	13	2	8	1	12	44
11	2	3	1	5	0	3	14
12	1	4	5	2	1	5	18
13	3	1	0	1	0	0	5
14	3	6	2	12	1	9	33
15	2	0	2	2	0	1	7
16	0	0	1	1	0	0	2
17	0	0	1	0	0	0	1
18	0	1	0	2	0	0	3
19	3	3	5	2	0	4	17
20	0	2	2	3	1	2	10
21	1	0	1	3	0	2	7
22	3	5	2	2	0	2	14
23	0	2	5	5	1	0	13
24	0	2	0	2	0	2	6
25	2	0	0	0	0	0	2
26	0	1	0	0	0	0	1
27	1	2	0	0	0	2	5
28	2	0	1	0	0	0	3
29	0	0	1	1	0	5	7
30	3	2	0	5	1	2	13
31	1	1	0	1	1	1	5
32	0	0	0	2	0	2	4
33	0	0	0	0	0	1	1
34	0	0	0	4	1	0	5
35	0	0	0	0	1	0	1
36	0	0	0	1	0	2	3
37	0	0	0	0	0	1	1
38	0	0	0	0	1	0	1
39	0	1	3	1	0	1	6
40	0	0	0	1	0	0	1
41	0	0	1	0	0	0	1
42	1	1	1	0	0	3	6
43	0	0	0	1	0	0	1
44	1	0	1	1	0	2	5
45	0	0	1	0	0	2	3
46	1	0	1	0	0	0	2
47	5	4	3	7	1	2	22
48	0	0	1	0	0	0	1
49	1	0	1	0	0	0	2
50	1	1	0	0	0	0	2
51	2	1	0	4	0	4	11
52	1	0	0	0	0	0	1
53	2	0	0	0	0	0	2
54	2	0	0	2	0	0	4
55	1	0	0	3	1	0	5
56	1	0	0	0	0	1	2
57	1	0	0	0	0	0	1
58	2	0	0	0	0	0	2
59	0	2	1	1	0	1	5
60	1	1	0	0	0	1	3

Table 11: Cont.

61	0	1	0	3	0	2	6
62	1	1	1	5	0	1	9
63	0	0	1	0	0	0	1
64	0	1	0	0	0	1	2
65	1	1	0	0	0	0	2
66	0	1	1	0	0	0	2
67	1	0	0	0	0	0	1
68	0	2	0	2	0	1	5
69	0	0	1	0	0	0	1
70	0	0	1	2	0	0	3
71	0	0	0	0	1	0	1
72	0	0	0	0	2	0	2
73	0	1	0	1	0	0	2
74	0	1	0	0	0	0	1
75	0	1	0	3	1	0	5
76	0	0	1	0	0	0	1
77	0	0	0	1	0	0	1
78	0	0	0	1	0	0	1
79	0	0	0	4	0	0	4
80	0	0	0	1	0	0	1
81	0	1	0	0	0	0	1
82	0	1	0	0	0	1	2
83	0	1	0	0	0	0	1
84	0	0	0	1	0	0	1
85	0	0	0	0	0	1	1
86	0	0	0	1	0	1	2
87	0	0	1	0	0	1	2
88	0	0	0	0	0	1	1
89	0	0	0	0	0	2	2
90	0	0	0	0	1	1	2
91	0	0	0	0	0	1	1
92	0	0	0	0	0	1	1
93	0	0	0	0	0	1	1
94	0	0	0	0	0	1	1
95	0	0	0	0	0	3	3
96	0	0	0	1	0	0	1
97	0	0	0	1	0	0	1
98	0	0	0	1	0	0	1
99	0	0	0	0	1	0	1
100	0	0	0	0	1	0	1
101	0	0	0	0	1	0	1
102	0	0	0	1	0	0	1
103	0	0	0	0	0	2	2
104	0	0	0	2	0	0	2
105	0	0	0	1	0	0	1
106	0	0	0	1	0	0	1
107	0	0	0	0	0	1	1
108	0	0	0	0	0	2	2
109	0	0	0	0	0	1	1
110	0	0	0	1	0	0	1
111	0	0	0	1	0	0	1
112	0	0	0	0	0	1	1
113	0	0	0	0	0	1	1
114	1	0	0	0	0	0	1
115	1	0	0	0	0	0	1
116	0	1	0	2	0	0	3
117	0	1	0	0	0	1	2
118	0	0	0	1	1	0	2
119	0	1	0	0	0	0	1
120	0	1	0	0	0	0	1
121	0	0	0	1	1	0	2
122	0	0	0	0	1	0	1
123	0	0	0	2	0	0	2
Total	160	243	164	349	62	279	1,257

Table 12: Results of a chi-square test for heterogeneity in mtDNA haplotype frequencies in area/time groups from Areas IV and V. In parenthesis is the number of sample size used in each test. Bold mark indicates significant differences in haplotype frequencies distribution at 5% level.

	70-100°E Early (160) **A**	Late (243) **B**	**100-130°E** Total (164) **C**	**130-160°E** Total (349) **D**	**160°E-160°W** Early (62) **E**	Late (279) **F**
70-100°E						
Early (160) **A**		**0.0220**	**0.0205**	**0.0050**	0.0705	**0.0060**
Late (243) **B**			0.2460	0.4955	0.0695	0.6455
100-130°E						
Total (164) **C**				0.0705	0.1635	**0.0340**
130-160°E						
Total (349) **D**					0.1910	**0.0500**
160°E-160°W						
Early (62) **E**						**0.0220**

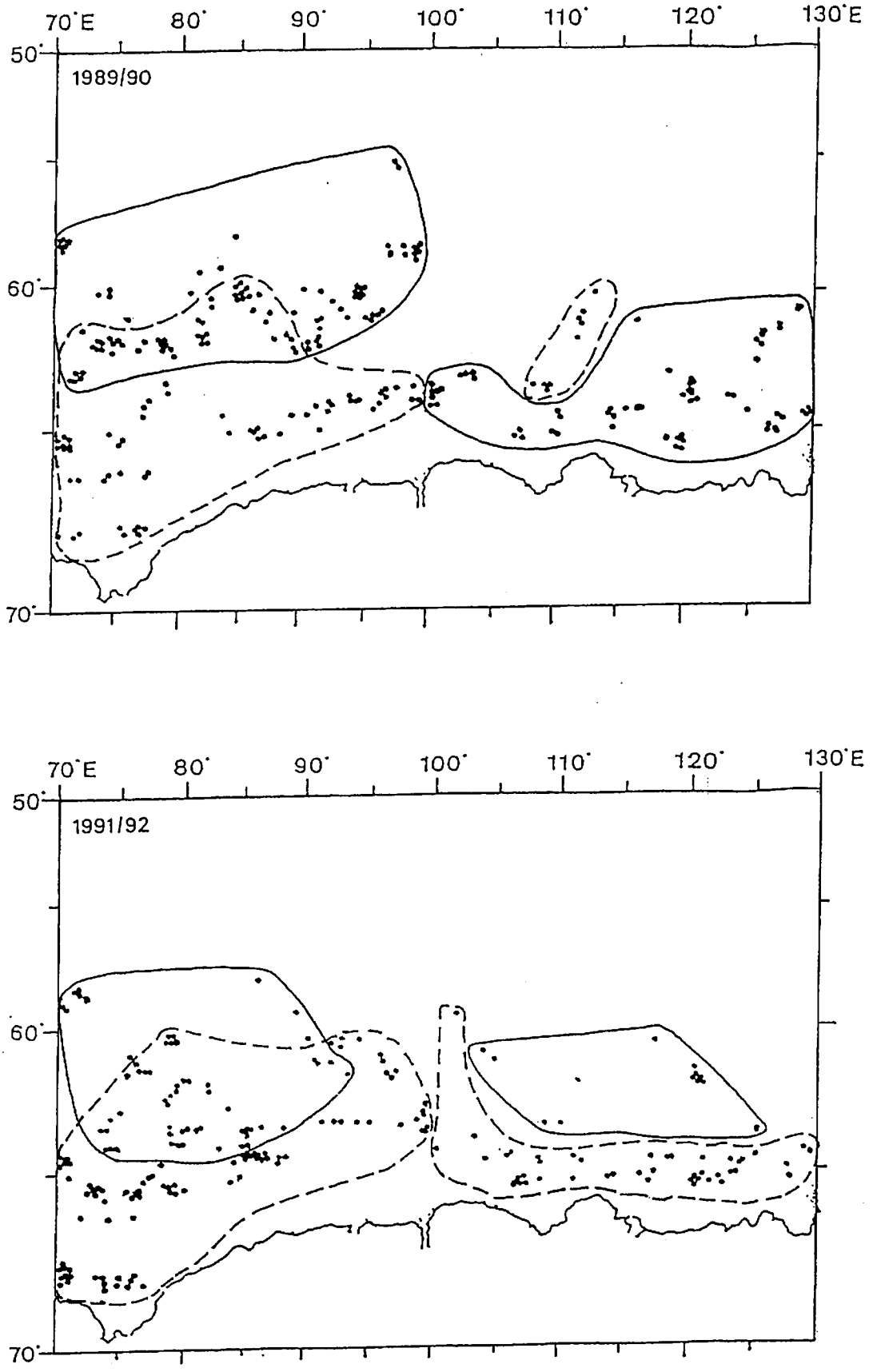

Fig. 1: Geographical distribution of the eight area/time/year groups of minke whale examined for mtDNA variation in Area IV. Upper: 1989/90 survey season, Lower: 1991/92 survey season. Solid line indicate 'early' group, dotted line indicate 'late' group. Sampling data of these groups are show in Table 2.

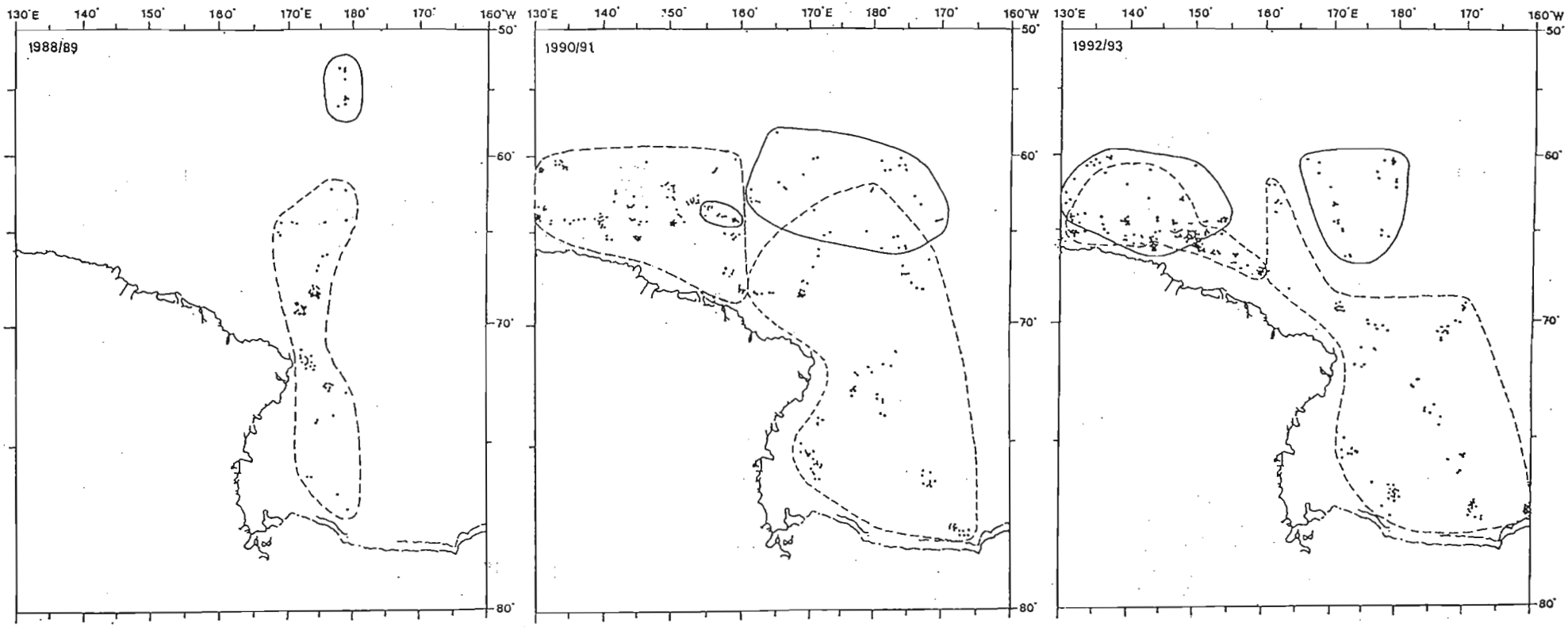

Fig. 2: Geographical distribution of ten area/time/year groups of minke whale examined for mtDNA variation in Area V. Left: 1988/89 survey season; Central: 1990/91 survey season; Right: 1992/93 survey season. Solid line indicate 'early' group, dotted line indicate 'late' group. Sampling data of these groups are showed in Table 3.

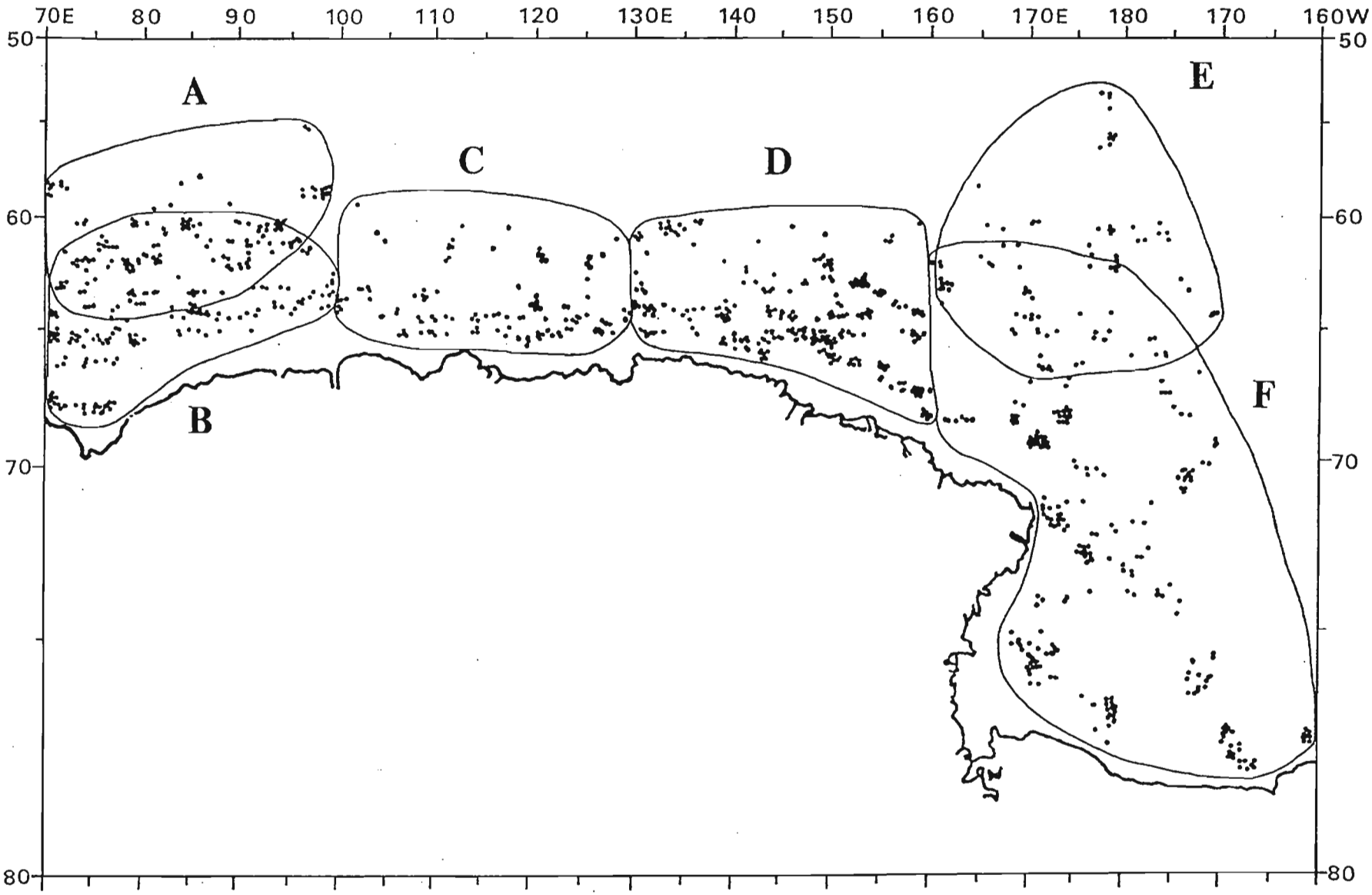

Fig. 3: Geographical distribution of six areal or area/time groups examined for mtDNA variation in Areas IV and V. **A**: 6 Dec-28 Dec, n=160; **B**:21 Jan-25 Feb, n=243; **C**: 6 Dec-24 March, n=164; **D**: 3 Dec-22 March, n=349; **E**: 20 Dec-12 Jan, n=62; **F**: 4 Feb-6 March, n=279. See haplotype frequencies of these groups in Table 11 and the statistical comparisons between them in Table 12.