Examination of mitochondrial DNA heterogeneity in minke whale from Area IV considering temporal, longitudinal and latitudinal factors

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ABSTRACT

In this study we examined the mtDNA heterogeneity in minke whales from Area IV considering geographical (longitudinal and distance from the ice-edge) and temporal factors (early and late period of the survey season). A total of 563 minke whales from two JARPA survey in that Area (1989/90 and 1991/92), were used. samples were divided into two sectors (IVW and IVE) and two periods, early (December-mid January) and late (mid January-March) following a previous study. In addition we further divided the samples into 'offshore' and 'ice-edge' by a line on 60 n.miles from the ice-edge. The homogeneity tests were conducted using the Analysis of Molecular Variance (AMOVA). The only significant source of mtDNA heterogeneity was attributed to the whales distributed in the offshore group of the western part of Area IV in the early period, which is one component of the 'western stock'. However, the ice-edge group of Area IV western early, which is another component of the 'western stock', was not significantly different from the other groups. It is suggested that the main body of the 'western stock' was composed of whales distributed in offshore waters rather those distributed around the ice-edge.

INTRODUCTION

Several studies on stocks identity were conducted during the IWC/SC comprehensive assessment of the minke whale (IWC, 1991). The most intensive and extensive genetic study used allozyme for examining a total of 11,414 whales (Wada and Numachi, 1991). However, the allozyme approach used to examine such large amount of samples was unable to discriminate any stock in the Antarctic. Also another more sensitive molecular approaches used for investigating stock identity in Areas IV and V (e.g. Hoelzel and Dover, 1991; van Pijlen *et al.*, 1991) failed to find any degree of significant genetic heterogeneity in these Areas. All these studies used samples obtained during commercial whaling operations in the Antarctic, which took whales mainly around the ice-edge. For a review of the studies on stock identity in the Antarctic minke whale see Pastene and Goto (1997).

The Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) began in 1989/90 after two feasibility studies in Areas IV and V. During the JARPA surveys, minke whales have been taken randomly from more wider geographical range within Areas IV and V than the past commercial operations. Making use of JARPA samples collected in these management Areas between 1987/88 and 1994/95, a large scale mtDNA analysis involving a total of 2,124 whales was conducted (Pastene *et al.* 1996). Such analysis revealed considerable genetic heterogeneity when the samples were grouped taking into considerations both longitudinal and temporal factors. The results of this study suggested that the stock structure in the Antarctic minke whale could be more complex than though initially and it could involve not only a simple longitudinal division but also a temporal component. Such complex structure could be expected in a abundant population as is the case of the Southern Hemisphere minke whale.

Pastene *et al.* (1996) found that the only source of mtDNA heterogeneity was attributed to whales of the west part of Area IV (70-130E) sampled in the 1989/90 and 1991/92 JARPA surveys, migrating into that sector in the early period of the survey (December). They tentatively suggested that these whales could belong to a different stock (a 'western stock') than the rest of the whales in Areas IV and V ('core stock').

In addition to the longitudinal and temporal factors considered by Pastene *et al.* (1996), we have considered important to investigate the effect of other geographical factors on the stock structure in the minke whale, such as the latitudinal and distances from the ice-edge.

The results of such analysis could be useful for a better understanding of the stock structure in the Antarctic minke whale. It could be also useful to evaluate the utility of past commercial samples for stock identity studies. On this last point, it should be noted that Taylor (1997) proposed an investigation of the utility of existing samples from commercial whaling to address stock identity issues for Antarctic minke whales. The author suggested a computer simulation to sample the JARPA samples in area/time frequencies equivalent to the biased commercial samples, and then repeating the genetic statistics test.

The simulation method proposed by Taylor (1997) considered to include factors such as geographical (longitudinal), temporal and biological (sex and maturity). However, she does not consider the latitudinal factor or the distances from the ice-edge. If the latter factors play an important role in the stock structure of Antarctic minke whale, the utility of past commercial samples could be limited since all the commercial samples were obtained around the pack-ice in any period of the whaling season.

Here we present the results of a preliminary mtDNA analysis that incorporated the latitudinal factor and the distances between sampling position to the ice-edge. Because the only consistent source of mtDNA heterogeneity was found in the group Area IVW early of the 1989/90 and 1991/92 JARPA surveys, we used this group for our analysis.

MATERIALS AND METHODS

Grouping of samples

Samples used in this analysis were from the 1989/90 and 1991/92 JARPA surveys in Area IV (Fujise *et al.*, 1990; 1993). Details of the genetic analysis of these samples can be found in Pastene *et al.* (1996).

A total of 563 minke whales (n=307 and 256 from the 1989/90 and 1991/92 surveys, respectively) were used in this study. The samples were divided into several groups in a similar manner as in Pastene *et al.* (1996) with minor modifications. In brief Area IV was divided into two longitudinal sectors, western (W; 70° E-100° E) and eastern (E; 100° E-130° E). Furthermore two time periods were defined in each of these sectors, 'Early' (December-first half of January) and 'Late' (second half of January-March).

In addition to the four area/time groups, latitudinal factor was considered in each group. Each of the groups was divided further into 'offshore' (O) and 'ice-edge' (I) groups by a line of 60 n.miles northwards from the ice-edge. The sample size by longitudinal/temporal/latitudinal groups in 1989/90 and 1991/92 is shown in Table 1. A minor modification was made on the Eastern Early Offshore group of the 1989/90 survey. As the sampling was conducted continuously from January 11th to 17th in the 'Eastern middle zone' (see Fujise *et al.*, 1990), the 'early' period sample here correspond to December-17 January.

Figs. 1 and 2 show the geographical distribution of each latitudinal/longitudinal/temporal groups examined in this study for two JARPA surveys.

Data analysis
Population genetic differentiation of haplotypes were quantified using the Analysis of Molecular Variance procedure (AMOVA; Excoffier *et al.*, 1992) as implemented in the computer program Arlequin (Schneider *et al.*, 1997). The significance of the variance components and PHI-st were tested random permutation procedure available in the program. In each trial, 10,000 randomization of the original data sets were made. The level of significance obtained by this procedure is referred in this paper as P value.

First we compared 'offshore' and 'ice-edge' groups for the combined sample, by survey season. Second we tested for longitudinal and latitudinal factors for the two JARPA surveys combined.
Finally we tested for longitudinal, latitudinal and temporal factors for the two JARPA surveys combined.
Samples from the two surveys were combined in order to increase sample size

RESULTS

Comparison between offshore and ice-edge groups
The results of the AMOVA indicates that no significant differences were found between 'offshore' and 'ice-edge' groups in Area IV in each JARPA survey (Table 2).

Test for longitudinal and latitudinal factors
Table 3 shows the results of the homogeneity test by AMOVA among the four longitudinal/latitudinal groups. The pairwise comparisons among the four groups (Western

Offshore, WO; Western Ice-edge, WI; Eastern Offshore, EO and Eastern Ice-edge, EI) showed no significant differences among them.

Test for longitudinal, latitudinal and temporal factors

Table 4 shows the results of homogeneity test by AMOVA for the seven longitudinal/latitudinal/temporal groups examined. The ELO (Eastern Late Offshore) group was excluded from this analysis because of small sample size (n=1). It can be observed that all the pairwise comparisons involving group WEO shows a higher PHIst values. In three case probabilities are below 1% or 5% level and in two cases below 10% level. All the other pairwise comparisons showed lower PHI-st values and much higher P values.

DISCUSSION

In this study we have conducted a preliminary examination on the possible effect of geographical factors (longitudinal and distance from the ice-edge) and temporal factors (early and late periods in a survey season) on the stock structure in the Antarctic minke whale from Area IV. For conducting such examination we used samples from Area IV taken during the 1989/90 and 1991/92 JARPA surveys. Mitochondrial DNA analysis on these samples had shown significant genetic heterogeneity when the samples were grouped considering longitudinal and temporal factors. Specifically heterogeneity was found in whales from Area IV west sampled during an early period of the survey (Pastene *et al.*, 1996). These authors did not tested for the latitudinal factor including distance from the ice-edge.

The results of our study suggest that geographical factors alone (longitudinal and latitudinal) could be not sufficient to investigate stock identity (Table 3). However, when the temporal factor was included, we detected some degree of heterogeneity in the sample from Area IV western, with the early/offshore group differing from most of the other long./lat./temporal groups in Area IV. This result suggest that the temporal factor is an important factor to be considered for investigating stock identity in the Antarctic minke whale.

The homogeneity test for latitudinal, longitudinal and temporal mtDNA differentiation showed the following two main results regarding the 'western stock' proposed by Pastene *et al.* (1996):

1) Significant differences were observed between whales distributed in the offshore area of the western part of Area IV in the early period (WEO), which is one component of 'western stock', and most of the other groups.

2) No significant differences were observed between the whales distributed in the ice-edge in the western part of Area IV in the early period (WEI), which is also a component of the 'western stock', and the other groups.

The first result suggests that the main body of the 'western stock' is composed by whales in western part of Area IV in the early period in offshore waters.

The samples in the western early offshore group, which show the genetic differentiation in 1989/90 and 1991/92 survey season, distribute between 58° S and 62° S except for one individual taken at 55° S and between 58° 30' and 62° 30', respectively. Therefore the result are consistent with the hypothesis that a combination of early period and low latitudinal areas could be an important condition to detect stock structure in the Antarctic (Pastene and Goto (this meeting).

The second result suggests that if whales around the ice-edge are considered to represent the past historical commercial samples, then such samples could not be suitable for stock identification study.

In this preliminary analysis we used 60 n.miles as an arbitrary distance separating 'offshore' and 'ice-edge' groups. This figure was based on that used during IDCR cruise in the Antarctic to define northern and southern stratum. Future analyses should consider a set of different distances and to investigate the effect on these different distances on the PHIst and P values.

It should be mentioned that Pastene and Goto (this meeting) suggest certain degree of mtDNA heterogeneity in the late group of Area IV west. This suggest the possibility of mixing of stocks with progress of the survey season. They mentioned that if different stocks migrating into the Antarctic feeding ground mix to each other with progress of the survey season then the mixed assemblage around the ice-edge could make very difficult the discrimination of stocks using such samples. For the late groups further analysis should be carried out.

Finally it should be noted that the power of the statistical analysis decrease when the total samples are divided in several groups. The lack of significant differences in some of our comparisons could reflect decreased power due to small sample size.

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Table 1 : Grouping of the samples. Offshore: samples collected in Northern part of 60 n.miles from ice-edge, Ice-edge: samples collected within 60 miles from ice-edge. WE=Western Early, WLO=Western Late, EEO=Eastern Early, ELI=Eastern Late.

a)89/90 season

	WE	EE	WL
Offshore	12/6-12/29 (43)	1/12-1/17 (27)	1/29-2/13 (47)
Ice-edge	12/10-12/28 (75)	12/31-1/13 (70)	1/21-2/14 (45)

b)91/92 season

	WE	EE	WL	EL
Offshore	12/18-12/28 (22)	1/2-1/8 (12)	2/15-2/25 (76)	3/24 (1)
Ice-edge	12/11-12/15 (20)		1/27-2/14 (71)	1/16-3/11 (54)

Table 2: Statistical analysis between Offshore and Ice-edge by each season. In parenthesis is the sample size.

	Phi-ST	P
89/90 Offshore-Ice-edge (117) (190)	0.00183	0.2275
91/92 Offshore-Ice-edge (111) (145)	0.00006	0.3882

Table 3: Statistical analysis between Offshore and Ice-edge by latitudinal sector. Phi-st: above diagonal, P value: below diagonal. WO=Western Offshore, WI=Western Ice-edge, EO=Eastern Offshore, EI=Eastern Ice-edge.

	WO	WI	EO	EI
WO (n = 188)		0.00003	0.00069	-0.00135
WI (n = 211)	0.3975		-0.00532	0.00097
EO (n = 40)	0.3670	0.7524		0.00001
EI (n = 124)	0.5864	0.2917	0.3999	

Table 4: Statistical analysis between Offshore and Ice-edge by latitudinal and temporal sector. Phi-st: above diagonal, P value: below diagonal. WEO=Western Early Offshore, WEI=Western Early Ice-edge, WLO=Western Late Offshore, WLI=Western Late Ice-edge, EEO=Eastern Early Offshore, EEI=Eastern Early Ice-edge, ELI=Eastern Late Ice-edge.

	WEO	WEI	WLO	WLI	EEO	EEI	ELI
WEO (n = 65)		0.00598	0.02431	0.01406	0.01608	0.01760	0.01135
WEI (n = 95)	0.1500		0.00588	0.00306	-0.01018	0.00230	-0.00023
WLO (n = 123)	0.0064	0.0963		0.00262	0.00162	-0.00206	-0.00609
WLI (n = 116)	0.0400	0.2053	0.2112		0.00078	0.00296	-0.00346
EEO (n = 39)	0.0884	0.9342	0.3363	0.3706		0.00063	-0.00247
EEI (n = 70)	0.0407	0.2737	0.5858	0.2441	0.3771		-0.00423
ELI (n = 54)	0.0985	0.4135	0.8674	0.6428	0.4963	0.6400	

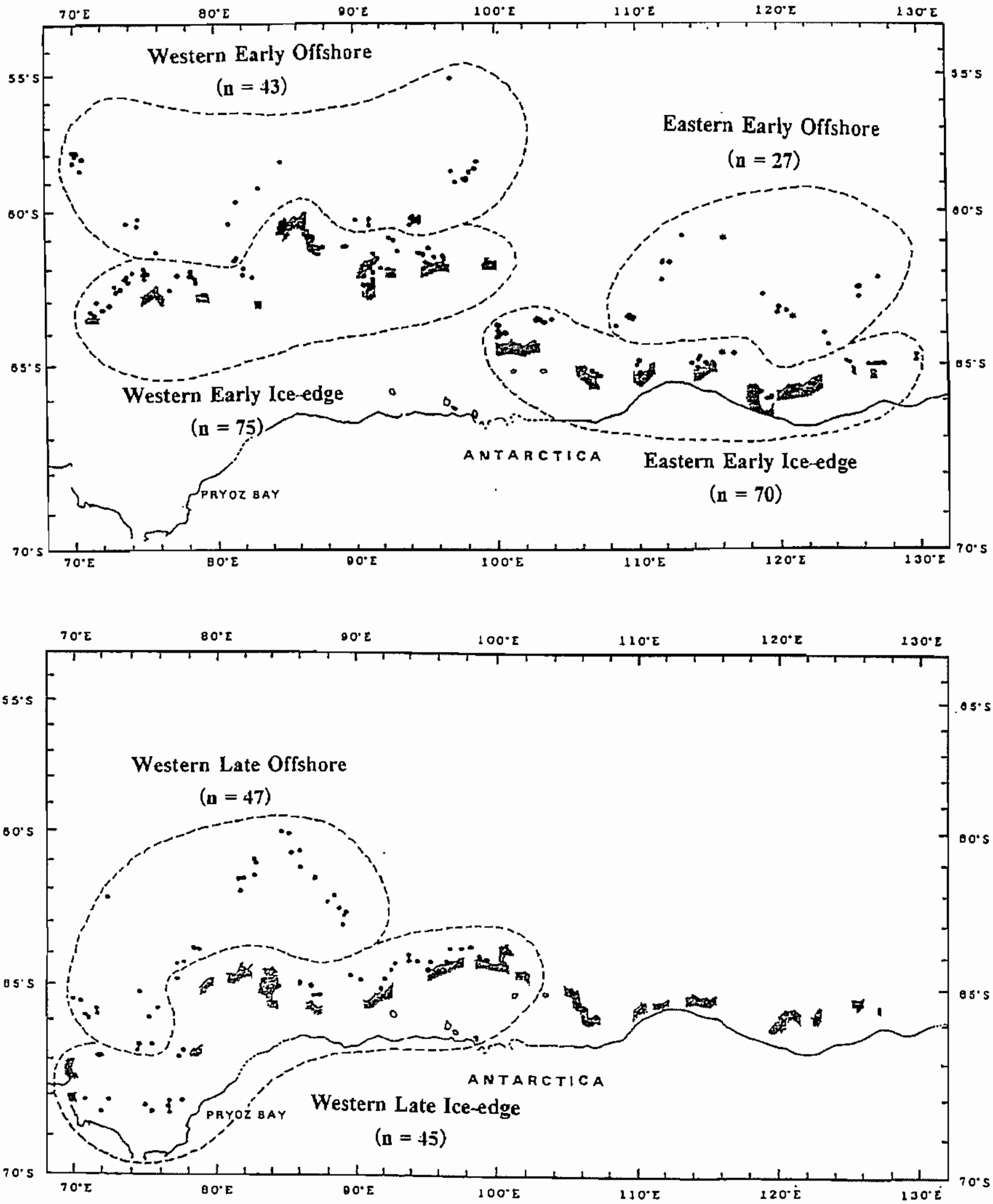

Fig. 1: Geographical distribution of the six lat./long./time groups of ordinary minke whales examined for mtDNA variation in the 1989/90 JARPA survey in Area IV. Upper: 'Early' in the season, Lower: 'Late' in the season. Sample size in each group is in parenthesis. This figure is made based on Fig. 4 in Fujise *et al.* (1990)

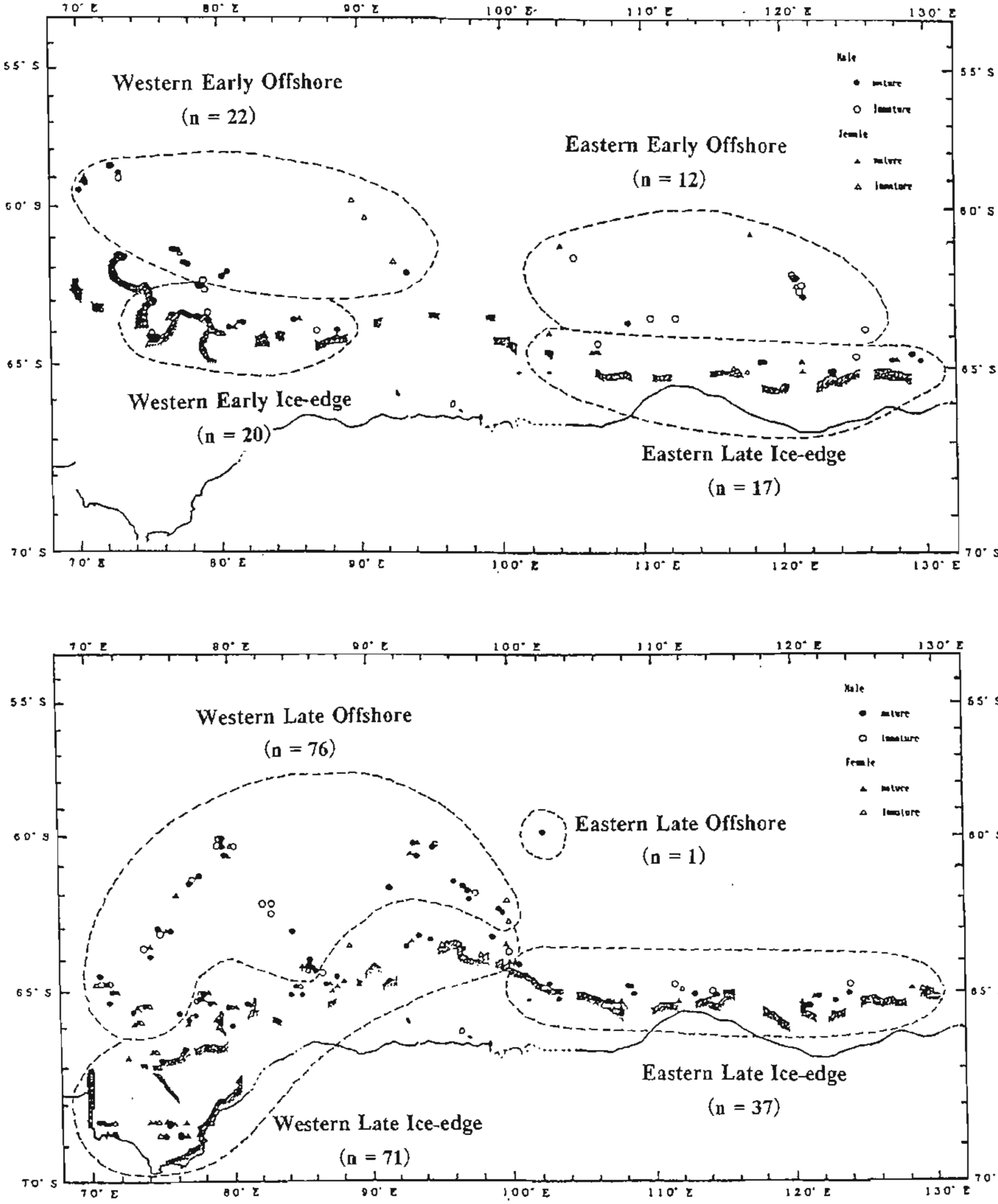

Fig. 2: Geographical distribution of the six lat./long./time groups of ordinary minke whales examined for mtDNA variation in the 1991/92 JARPA survey in Area IV. Upper: 'Early' in the season including a part of samples of Eastern Late Ice-edge group, Lower: 'Late' in the season. Sample size in each group is in parenthesis. This figure is made based on Fig. 6 in Fujise *et al.* (1993)