Further analyses of mtDNA RFLP data in the Antarctic minke whale from Areas III-VI

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ABSTRACT

A restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) in the Antarctic minke whale from Areas IIIE, IV, V and VIW was conducted using samples from the 1987/88-2001/02 JARPA surveys. MtDNA heterogeneity had been previously reported for samples in Areas IIIE and IV and this study further examine these samples by adding new data from the 2001/02 JARPA survey. Samples were grouped considering the same longitudinal/temporal strata used in the previous analyses: three longitudinal sectors and two time periods in each (six strata). A total of 4,982 samples was examined in this study. Comparisons were made among these strata as well between these strata and a sample from the 'core stock' of Areas V and VIW. Quantification of the mtDNA differentiation among strata was carried out using the Analysis of Molecular Variance (AMOVA). Both haplotype (Fst) and sequence (PHIst) statistics were used. The pattern of longitudinal and temporal mtDNA heterogeneity is consistent with that found in previous studies supporting the view of a core stock occupying in Areas V, VIW and part of Area IV (E). A different stock could occupy the western part of Area IV and a temporal component to the distribution of these stocks is postulated. Grouping of samples for the analysis of mtDNA has involved arbitrary longitudinal and temporal strata. To better understand the stock structure of Antarctic minke whale in the feeding ground included a better definition of possible geographical and temporal boundaries, grouping of samples should be made considering alternative longitudinal and temporal definitions. Also the incorporation of other factors such as the distance from the ice-edge, are recommended.

KEYWORDS: ANTARCTIC MINKE WHALE, STOCK IDENTITY, GENETICS

INTRODUCTION

One of the main research objectives of the JARPA (the Japanese Whale Research under Special Permit in the Antarctic) is the estimation of biological parameters required for the stock management. The accuracy of the estimation of parameters such as the natural mortality, however, has faced a challenging issue to solve. In the discussion conducted during the 1994 Committee meeting on the preliminary results of the estimation of this parameter, it was noted that the accuracy in the estimation stem largely from the stock identity questions and seasonal variations in the migration patterns for different age groups (IWC, 1995).

Studies on stock identity in the Antarctic minke whale have been based mainly on mitochondrial DNA (mtDNA) RFLP analysis and results of these studies were reported in Pastene *et al.* (1996a) for samples obtained before the JARPA review meeting. Samples obtained after the JARPA review meeting were examined and reported periodically to the annual meeting of the Committee. Following a recommendation from the Committee, microsatellite analyses were conducted preliminary by Abe *et al.* (1998) and more data are being produced and examined actually.

The previous genetic studies have showed that the stock structure in the Antarctic minke whale could be more complex than it was assumed initially and it could be determined by a combination of factors such as geographical (longitudinal), temporal (between and within surveys) and distance from the ice-edge (Pastene *et al.* 1996a; Goto *et al.*, 1998). Furthermore the analysis of genetic data is complicated due to the small effect size of putative populations as well the possibility that sampling is occurring on a mixed assemblage of stocks. The latter could be elucidated by the analysis of samples from putative breeding grounds but these samples are not available and difficult to get from the logistical point of view.

Notwithstanding, results of previous mtDNA analysis (see Pastene *et al.*, 2001 for an update of the analysis in Areas IIIE and IV and Pastene *et al.*, 2002 for an update of the analysis in Areas V and VIW) have been useful and suggested a) substantial geographical and temporal heterogeneity within Areas IIIE-

IV, with some longitudinal/temporal groups differing significantly from the samples in Area V and VIW, b) little heterogeneity (temporal or geographical in Areas V and VIW).

Here we present the results of a new mtDNA analysis in Areas IIIE, IV, V and VIW using all available samples from 1987/88 to 2001/2002. Based on previous results, confirmed with a new analysis in this paper, we assumed that Areas V and VIW are composed of a single biological stock, which we call 'core stock'. Samples in Areas IIIE and IV, incorporating new samples from 2001/02 JARPA survey, are examined and compared to the sample of the 'core stock'.

MATERIALS AND METHODS

Samples

Samples of the Antarctic minke whale from Areas IIIE-VIW obtained from the 1987/88-2001/02 JARPA surveys were examined in this study. For each survey, samples were grouped into Area III Eastern Early (group IIIEE, n=342), Area III Eastern Late (group IIIEL, n=78), Area IV Western Early (group IVWE, n=476), Area IV Western Late (group IVWL, n=738), Area IV Eastern Early (group IVEE, n=252), Area IV Eastern Late (group IVEL, n=865), Area V Western Early (group VWE, n=219), Area V Western Late (group VWL, n=703), Area V Eastern Early (group VEE, n=195), Area V Eastern Late-North (group VELNo, n=293), Area V Eastern Late-South (group VELSo, n=555), Area VI Western Early (group VIWE, n=194) and Area VI Western Late (group VIWL, n=72). Divisions between western and eastern sector in Areas IV and V were at 100°E and 165°E, respectively. Division between north and south in group VEL was at 69°S (the south stratum involving the Ross Sea). Thus a total of 4,982 samples was examined in this study. '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 number of samples is shown in Table 1.

RFLP analysis

Crude mtDNA extracted from liver tissues was digested with six polymorphic restriction enzymes, same as in the previous studies (Pastene *et al.*, 1996a): *Acc*I, *Ban*I, *Eco*RV, *Hinc*II, *Hpa*I and SspI. All the procedures for DNA extraction and digestion were the same as in the previous study.

Statistical analysis

The geographical/temporal differentiation of mtDNA was quantified using the Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992) as implemented in the computer program AMOVA ver. 1.55. The statistics of primary interest are the haplotype (Fst) and sequence (PHIst), both of which were used. The significance of the observed variance values was tested using a modification of a matrix permutation procedure available in the computer program. All test of statistical significance were based on 10,000 random permutations of the original data sets. The level of significance obtained using this procedure is referred in this paper as the P value.

Grouping of samples

Samples in Area IIIE and IV were grouped in the same manner as in previous studies (see Table 1). Samples were grouped into three longitudinal sectors (IIIE, IVW and IVE) and two time periods (early and late) resulting in a total of six strata. 'Early' refers to samples collected in December and first half of January; 'late' refers to samples collected between the second half of January and March. First we tested for yearly variation in each of the longitudinal/temporal stratum. If no significant heterogeneity was found, we pooled samples from different years. The pooled longitudinal/temporal strata or longitudinal/temporal/year stratum (in the cases where substantial yearly variation was found), were compared with the sample of the 'core stock'.

RESULTS

mtDNA haplotypes

Characterization of the mtDNA RFLP haplotypes was documented in previous papers (Pastene *et al.*, 1996a;1996b). By using a set of six polymorphic restriction enzymes, a total of 153 haplotypes had been discriminated in the Antarctic minke whale. Several of these had been discriminated in commercial samples from Areas III and VI (see Pastene *et al.*, 1996b). As this study is concentrated on JARPA samples only, the actual number of haplotypes examined here is 134.

Definition of a sample of the 'core stock'

Previous analyses in Area V and VIW had showed no significant geographical or temporal heterogeneity in Areas V and VIW (Pastene *et al.*, 2002). The analysis in these Areas was repeated using data as grouped in Table 1, but excluding data from 1998/99 JARPA survey. As discussed in the previous report that survey had a narrow geographical and temporal covering of the research area with regard the other surveys and it is considered that such samples could not be representative of the genetic diversity (Pastene *et al.*, 2002). For PHIst the results of the new analysis were the following: among longitudinal sectors, PHIct=-0.001 (P=0.9070); among temporal groups within longitudinal sector, PHIsc=0.000 (P=0.9070) and within long//temporal PHIst=-0.001 (P=0.6100). For Fst the results were the following: among longitudinal sectors Fct=0.001 (P=0.3801); among temporal groups within longitudinal sector: Fsc=-0.000 (P=0.5940) and within long//temporal Fst=0.000 (P=0.3010). Therefore we assumed that Areas V and VIW are composed of a single biological stock, the 'core stock', which was used to compare samples in Areas IIIE and IV under different groupings (n=1,869).

Yearly variation in the longitudinal/temporal strata

Results of the statistical analysis of yearly variation in the longitudinal/temporal strata are shown in Table 2, for both Fst and PHIst statistics. For comparison we included both previous results and the results that used new samples from the 2001/02 survey. Previous results had indicated significant yearly differences using PHIst and a near-to-significant difference for Fst. Results of the analysis incorporating new samples from 2001/02 showed low P values but they were not significant.

Comparison with a sample of the 'core stock'

Longitudinal/temporal strata in Table 2 were compared statistically to the sample of the 'core stock'. In consideration of the low P value indicated in Table 2, stratum IVWE was compared to the 'core stock' for both total samples and on a yearly basis.

Table 3 shows the results of the comparisons between strata in Areas III and IV with the 'core stock', for both Fst and PHIst statistics. No significant differences were found in the comparison involving samples from Area III. Results for the late sample in this Area can be explained by the small sample size (78). Both statistics showed significant or near-to-significant differences in the comparison involving strata IVWE and IVWL. In the comparison involving stratum IVEE, Fst showed no significant differences and PHIst showed near-to-significant differences. Both statistics showed no significant differences in the comparison with stratum IVEL. Pairwise comparisons among the strata in Table 3 resulted generally in large P values.

In the comparison of stratum IVWE on a year basis, some comparisons with the 'core stock' resulted in significant differences, other not (Table 3). As in the case of the yearly variation analysis, small sample size precludes the attainment of conclusive results. The stratum IVWE from 1989/90 was the most differentiated from the core stock.

DISCUSION

As in previous analyses we found remarkable mtDNA heterogeneity in our sample from Areas IIIE-VIW. The pattern of mtDNA heterogeneity suggests the occurrence of more than one stock in these Areas. Results were similar to those found in the previous analysis of Area IIIE and IV (Pastene *et al.* 2001). However, yearly variation in stratum IVWE was less evident after the addition of samples from the 2001/02 survey. Although low P values were observed in the analysis of yearly variation, these values were not significant. In the comparison with the 'core stock', samples from western part of Area IV (early and late) showed significant differences; samples from eastern part of Area IV as well samples from Area III showed not significant differences. The latter results make sense for contiguous Area IVE but they are surprising in the case of Area III, which is geographically far from the 'core stock'. Results of the comparison between stratums IVWE (yearly basis) are difficult to interpret due to the small sample size.

Our results are consistent with the hypothesis of more than one stock in Areas IIIE-VIW, with a core stock occurring in Areas V, VIW and eastern part of Area IV. Other stock could be distributed in the western part of Area IV. We can not discard the possibility of these stocks overlapping in part of Area IV, with a temporal component to their distribution. Wada (1984) examined mark-recapture data and concluded that a possible division occurs at 80°E (western part of Area IV). More recently a study showed differences at the trace elements concentrations between samples collected on both sides of this longitude division (Kunito *et al.*, 2002). Our results are somewhat consistent with these independent results. However it is considered that in the case of the Antarctic minke whale hard boundaries scenarios have low plausibility

given the dynamics of oceanographic and prey species conditions. The distribution of Antarctic krill concentrations has been related to bottom topography, sea-ice and hydrographic features (Ichii, 1990). The Antarctic Coastal Current (East Wind Drift) runs along the contours of the continental shelf from Adelie Land and approximately 105°E. Between 105°E and 95°E the current curves to the north and then eastward (Tchernia and Jeannin, 1984). The longitudinal sector where the current curves to the north could change annually within the longitudinal range mentioned above, This could have some effect on annual fluctuation of the distribution of krill concentrations and this, in turn, could affect the distribution of minke whales around the 100°E. The dynamics of the prey species, which is related to oceanographic features in that area, could help explain the findings of our investigation.

The geographical and temporal groupings used in our analysis are arbitrary. Further examination of the pattern of mtDNA variability should be examined using new longitudinal lines and more fine time scale. Analysis considering alternative geographical and temporal stratification could explain for example why samples from Area IIIE in this study were not different from the 'core stock' while Area IVW showed significant differences. Additional analysis should also incorporate component such as the distance from the ice-edge. Goto *et al.* (1998) examined samples from Area IV from two JARPA surveys (1989/90 and 1991/92). Apart the longitudinal and temporal division they separated the samples as 'offshore' (more than 45n.miles from the ice-edge) and 'ice-edge' (within 45n.miles from the ice-edge). They found that the 'offshore' samples in Area IVWE were more informative on stock structure than the 'ice-edge' sample. Then apart from longitudinal and temporal considerations information on ice-edge should be taken into account in future groupings. As explained earlier the identification of geographical and temporal boundaries is necessary for the estimation of biological parameters.

Following a recommendation from the JARPA review meeting Abe *et al.* (1999) used a set of five microsatellites to examine a sub-set of the samples used in this study. This study has continued and actually microsatellite data from six JARPA surveys have been obtained and analysis is under way.

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Table 1: Number of Antarctic minke whale samples used in the mtDNA analysis, by JARPA survey and longitudinal/temporal stratum. See text for definition of these groups.

Survey	III EE	III EL	IV WE	IV WL	IV EE	IV EL	V WE	V WL	V EE	V ELNo	V ELSo	VI WE	VI WL	TOT
87/88						227								227
88/89									10	56	39			105
89/90			118	92	80	15								305
90/91							31	149	20	33	77			310
91/92			42	148	14	55								259
92/93							83	110	26		87			306
93/94				145	138	22								305
94/95							105	24	13	65	106			313
95/96	67	38	130	101		62								398
96/97								119	67	21	105	91	13	416
97/98	88	14	73	120		127								422
98/99								169		100	40		59	368
99/00	107		47	50	1	213								418
00/01								132	59	18	101	103		413
01/02	80	26	66	82	19	144								417
TOT	342	78	476	738	252	865	219	703	195	293	555	194	72	4,982

Table 2: Results of the statistical analysis of yearly variation in the longitudinal/temporal groups in Areas IIIE and IV, for two statistics Fst and PHIst. See Table 1 for the surveys and sample sizes used in each test. No test was conducted for group IIIEL because the small sample size in this group. P values below 5% are shown in **bold**. P values below 10% are shown underlined.

	Previous results				Including 2001/02 samples			
	Fst	P	PHIst	P	Fst	P	PHIst	P
III EE	-0.002	0.663	-0.001	0.523	0.004	0.122	0.001	0.314
IV WE	0.004	0.070	0.008	**0.024**	0.003	0.099	0.005	0.0529
IV WL	0.001	0.201	0.001	0.330	0.002	0.143	0.000	0.4188
IV EE	-0.003	0.707	-0.002	0.534	-0.004	0.747	-0.000	0.4342
IV EL	-0.000	0.554	0.001	0.365	-0.001	0.612	0.000	0.4441

Table 3: Results of the statistical comparisons between groups in Areas III and IV and the sample of the 'core stock' (n=1,869). See text for definition of the 'core' sample and Table 1 for longitudinal/temporal groups. Results are shown for both Fst and PHIst statistics. P values below 5% are shown in **bold**. P values below 10% are shown underlined. Sample size in parenthesis.

Groups	Fst	P	PHIst	P
IIIEE Total (342)	0.0008	0.1022	0.0005	0.2008
IIIEL Total (78)	-0.0021	0.7747	-0.0006	0.4949
IVWE Total (476)	0.0016	**0.0159**	0.0012	0.0519
IVWE 1989/90 (118)	0.0059	**0.0138**	0.0110	**0.0012**
IVWE 1991/92 (42)	-0.0005	0.4250	0.0010	0.3435
IVWE 1995/96 (130)	-0.0006	0.5676	-0.0014	0.7525
IVWE 1997/98 (73)	0.0062	**0.0422**	0.0077	**0.0409**
IVWE 199/00 (47)	0.0042	0.1430	0.0036	0.1949
IVWE 2001/02 (66)	0.0025	0.1775	0.0000	0.4075
IVWL (738)	0.0008	**0.0462**	0.0016	**0.0081**
IVEE (252)	-0.0002	0.4568	0.0020	0.0502
IVEL (865)	0.0002	0.1933	0.0005	0.1103