

Genetic analysis on stock structure in the Antarctic minke whales from the JARPA research area based on mitochondrial DNA and microsatellites

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ABSTRACT

Stock structure in the Antarctic minke whales was investigated using mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP, six restriction enzymes) and microsatellites (six loci), and samples obtained during JARPA surveys from 1987/88 to 2004/05 austral summer seasons in Areas III E, IV, V and VI W. Samples were grouped into the six longitudinal strata used by JARPA surveys: III E (35°-70°E); IV W (70°-100°E) (north and south); IV E (100°-130°E); V W (130°-165°E); V E (165°E-170°W) (north and south) and VI W (170°W-145°W). The total sample sizes used in the mtDNA and microsatellite analyses were 6,256 and 6,260 animals, respectively. The level of genetic diversity was high for both genomes: the nucleon diversity at the mtDNA ranged from 0.856 and 0.891 and the mean expected heterozygosity at the nuclear loci at 0.807 for the total samples. In general results based on both markers were similar, showing substantial spatial genetic heterogeneity among the strata. For both genetic markers whales in the most distant geographic strata (III E, IV and V E, VI W, respectively) were differentiated genetically. Mitochondrial DNA was more sensitive to detect structure in the middle longitudinal strata. Our results are consistent with the hypothesis of at least two stocks distributed in the JARPA research area, possibly related to the proposed breeding areas in the Indian and western South Pacific Oceans, respectively. The pattern of spatial differentiation found provides little support for IWC stock boundaries among III, IV, V and VI. The pattern of genetic differentiation of males and females in the feeding grounds was similar according to the mtDNA analysis, which is consistent with the previous view on whale longitudinal movement based on mark-recapture analysis. The microsatellite heterogeneity tests suggested more structure in females than males. However no significant differences were found when the allele frequencies of males and females were compared in each of the strata. The results of a fine-scale mtDNA analysis suggested a stock division in the sector comprised between 150°-160°E. An area of mixing of stocks around that longitudinal sector can not be discarded. Site fidelity to particular areas of krill concentrations could explain the spatial segregation of stocks of Antarctic minke whales in the feeding grounds.

KEY WORDS: ANTARCTIC, FEEDING GROUNDS, GENETICS, ANTARCTIC MINKE WHALE, EUPHAUSIIDS.

INTRODUCTION

The study on stock structure in the Antarctic minke whale is conducted under the JARPA research objective titled 'elucidation of the stock structure of the Southern Hemisphere minke whales to improve stock management'. Information on stock structure is important for a) the estimation of biological parameters, which should ideally be carried out on the basis of biologically identified stocks and b) the future application of the multi-stock rules of the Revised Management Procedure (RMP) (IWC, 1994).

Hoelzel and Dover (1989) defined three categories of stocks: 'dynamic stock' is the fundamental unit described by a population model or assessment procedure, 'management stock' is the group of whales occurring within a specific geographical boundary which is actively or potentially exploited and 'genetic stock' is a genetically differentiated population within a species. The aim of JARPA is the identification of geographical and temporal boundaries of genetic stocks.

The Antarctic minke whale like all the other balaenopterids (except the Bryde's and pygmy blue whales) are believed to undertake seasonal migrations between feeding grounds south of 60°S in summer and breeding grounds in the tropical or temperate regions in winter. For this species, however,

there is only a single evidence of such linkage, based on mark-recapture data. Two whales that had been marked in the Antarctic were recovered off Brazil (Buckland and Duff, 1989). There are also a few indirect evidences on this linkage based on ecological markers (Nemoto *et al.*, 1980; Ohsumi, 1973).

Only one of the breeding grounds has been identified in Brazil based on Discovery mark recovery analysis (Buckland and Duff, 1989). Of interest for the stock structure studies of JARPA in Antarctic Areas IV and V, however, is the information on location of possible breeding grounds in low latitudes of the Indian and western South Pacific Oceans. Kasamatsu *et al.* (1995) examined Japanese sighting data obtained during 1976 to 1987. Based on spatial distribution in tropical and sub-tropical waters during the latter half of the conception period of the species, they suggested that there were two breeding grounds in the eastern and western South Pacific and two others in the eastern and western Indian Ocean. The breeding grounds that could be related to JARPA research areas in the Antarctic feeding grounds are those in the Indian Ocean and the western South Pacific. Very little is known about the pattern of movement of this species between low latitude breeding grounds and high latitude feeding grounds and we do not know the level of genetic connectivity of whales between these proposed breeding grounds.

In the austral summer 1987/88, JARPA surveys started in IWC Antarctic Areas IV and V, under the assumption that these two Areas are occupied by different genetic stocks. At that time there was little scientific evidence supporting such assumption. Several genetic studies were conducted based on samples obtained during past commercial whaling operations in the Antarctic, mainly in Areas IV and V. Most of these studies failed to detect any substantial genetic heterogeneity (Wada and Numachi, 1991; Hoelzel and Dover, 1991; vanPijlen *et al.*, 1991), or just detected weak differences (Amos and Dover, 1991), between those Areas.

JARPA samples were subsequently used in genetic studies based on mtDNA, which suggested substantial genetic heterogeneity in Areas IV and V and some stock structure hypotheses were proposed, which were not consistent with the current boundaries of Areas IV and V (Pastene *et al.*, 1993; 1996) (see details of previous genetic studies and hypotheses on stock structure in Document SC/D06/J12).

In general the interpretation of genetic results in the Antarctic minke whale has been difficult due to several factors. First and perhaps the most important, is that all the analyses were based exclusively on samples obtained from the feeding grounds, where possible spatial/temporal mixing of stocks could complicate the interpretation of results. Ideally genetic analyses should be based on samples from the breeding grounds where stocks are probably more isolated from each other. Second, past commercial whaling operations were concentrated around the ice-edge therefore samples based in such operations could not be representative enough of the genetic diversity of minke whales (Pastene and Goto, 1999). Third most of the studies that used commercial samples were based on limited sample sizes. Previous genetic studies suggested that the effect size (expected degree of genetic differentiation among stocks) is very small in the Antarctic minke whale (Pastene *et al.*, 1996; Bakke *et al.*, 1996). This factor and the high genetic diversity of this species means that analysis based on small sample sizes are misleading and not very informative.

JARPA surveys were conducted systematically in the feeding grounds of Areas IIIE, IV, V and VIW south of 60°S. Surveys were designed to cover both offshore and ice-edge regions. Therefore JARPA samples are seen as more representative of the genetic diversity than those from past commercial operations. Here a comprehensive genetic study based on all samples collected by JARPA between 1987/88 and 2004/05 is conducted with the aim to test the hypothesis that minke whales in the feeding grounds of Areas IIIE, IV, V and VIW are structured into different genetic stocks.

The analysis is based on the maternal inherited mtDNA and bi-parental inherited microsatellites. Further the analysis is conducted for total samples as well for each sex separately. Such analysis allows for the investigation of possible sex-differentiated pattern of dispersal in the feeding ground. The specific questions to be addressed by our genetic analysis are: Are the Antarctic minke whales distributed in the feeding grounds of Areas IIIE, IV, V and VIW structured into different genetic stocks? If so, what is the geographical and temporal pattern of stock structure? Is such pattern the same for both females and males?

MATERIALS AND METHOD

Samples

Antarctic minke whales sampled by JARPA surveys in Areas IIIE (35°-70°E), IV (70°-130°E), V (130°E-170°W) and VIW (170°-145°W) between 1987/88 and 2004/05, were used in the analysis (Figure 1). All samples were taken randomly along pre-defined track-lines (see Document SC/D06/J2).

Grouping of samples

The same geographical sectors used by the JARPA surveys were used in the analysis: IIIE (35°-70°E), IVW (70°-100°E), IVE (100°-130°E), VW (130°-165°E), VE (165°E-170°W) and VIW (170°-145°W) (Figure 1). Because IVW and VE involve a considerable wider latitudinal range, only in these two cases the JARPA's north/south division was considered (Figures 1 and 2).

In each stratum samples were further divided on a monthly basis. This temporal analysis enabled us to check for the possibility of a temporal component in the distribution of stocks. In the 1998/99 survey, the Ross Sea was not accessible and samples in VE were taken only in the northern part. Therefore samples of stratum 'VES' in 1998/99 were included into stratum VEN.

A total of 6,256 minke whale samples (JARPA 1987/88-2004/05) were used for the mtDNA analysis (2,892 females and 3,364 males) and a total of 6,260 (JARPA 1989/90-2003/04) were used for the microsatellite analysis (2,875 females and 3,385 males) (Table 1).

Laboratory procedures

Mitochondrial DNA

Routinely about 300mg of liver were used to extract mtDNA using a mini-prep procedure (Pastene *et al.*, 1993). Crude mtDNA (15ul) was digested with 2 or 3 units of six six-base sequence recognition endonucleases (*AccI*, *BanI*, *EcoRV*, *HincII*, *HpaI* and *SspI*). These enzymes were chosen because they were known to be polymorphic in the Antarctic minke whale. Digestions were carried out under conditions recommended by the manufacturer (Toyobo) and were completed within three hours at 37°C. Digestion was stopped by adding 5ul of dye mixture containing 0.1% bromophenol blue, 50% sucrose, 7M urea and 10mM EDTA. Three-hundred milligrams of frozen liver generated enough mtDNA for about 20 digestions.

Restriction fragments were separated by submarine electrophoresis in 1% agarose gels (SeaKem LE), using a TPE buffer system (Sambrook *et al.*, 1989). After electrophoresis, the gels were stained with ethidium bromide (1mg/500ml) for 15min and were photographed using Polaroid 667 film under 312nm UV irradiation. Sizes of the fragments were obtained in relation to those of the lambda *EcoT14I* marker (Takara Shuzo Co.). 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. Then, the composite haplotype for each individual comprises a string of six letters.

Microsatellites

Genomic DNA was extracted from 0.05g of each skin tissue sample using the protocol described by Sambrook *et al.* (1989). Extracted DNAs were then stored in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0). Microsatellite polymorphisms were analyzed using six sets of primers: EV1, EV104 (Valsecchi & Amos 1996), GT023, GT211, GT4195 (Bérubé *et al.*, 2000), and DlrFCB14, (Buchanan *et al.*, 1996). None of the primers was designed specifically from Antarctic minke whales. All primers were dinucleotide repeats. Although primer sequences follow those of the original authors, an annealing temperature of each of the loci was optimized for Antarctic minke whales used in this study.

Polymerase chain reaction (PCR) amplifications were performed in 15ul reaction mixtures containing 10 to 100ng of DNA, 5pmol of each primer, 0.625 U of *Ex Taq* DNA polymerase (Takara Shuzo), 2mM of each dNTP, and 10 X reaction buffer containing 20mM MgCl₂ (Takara Shuzo). Amplified products with internal size standard (GENESCAN400HD, Applied Biosystems Japan) were run on a 6% polyacrylamide denaturing gel (Long Ranger) using BaseStation100 DNA fragment analyzer (BIO-RAD). Although alleles were visualized using Cartographer software specifically designed for the BaseStation, allelic sizes were determined manually in relation to the internal size standard and Antarctic minke whale DNA of known size that were rerun on each gel.

Statistical analysis

Mitochondrial DNA

The degree of sequence divergence between two mtDNA haplotypes was calculated based on the proportion of DNA fragment shared (formula 5.55 in Nei, 1987). The degree of mtDNA diversity of minke whales in the strata examined was estimated using the nucleon diversity (Nei and Tajima, 1981).

Haplotype frequencies were employed to determine genetic relationships between the samples of the designed strata. Genetic relationships were first quantified using the chi-square statistics for heterogeneity of mtDNA haplotype frequencies (Roff and Bentzen, 1989). This Monte Carlo approach estimates the significance of the chi-square test computed from the raw data. In each trial, 10,000 randomizations of the original data sets were made. The level of significance obtained by this method is referred in this paper as the p-value. A p-value smaller than 0.05 was used as a criterion to reject the null hypothesis of panmixia.

In general analyses followed a stepwise fashion. First, each stratum was tested for monthly variation. Next, north and south strata were tested in IVW and VE. Finally pairwise comparisons were conducted among the longitudinal strata. Analyses were conducted for male and female separately as well for both sample combined. Sequential Bonferroni corrections were made in case of multiple tests.

Samples from several years were pooled in each stratum. This required the assumptions that the pattern of seasonal movement are the same for a given breeding stock in different years and that lateral movement on feeding ground and pattern of mixing are similar between years. In some particular situations analyses by year was conducted to investigate source of genetic heterogeneity.

Additionally, the quantification of the geographical differentiation of mtDNA was carried out using the analysis of molecular variance (AMOVA) of Excoffier *et al.* (1992). The AMOVA program (version 1.55) calculates variance components from a distance matrix and the PHI statistic (PHIst) reflecting the correlation of haplotype diversity at different levels of hierarchical subdivision. The significance of the variance components and PHIst were tested using a random permutation procedure available in the program. For the trials, 10,000 randomizations of the original data sets were made. The level of significance obtained by this procedure is referred in this paper as the p-value. A hierarchical analysis was carried out to estimate differentiation among longitudinal strata, between latitudinal sectors in strata and within strata.

Microsatellite

The GENEPop program (Raymond and Rousset, 1995) was used for calculation of allele frequencies at the six microsatellite loci analyzed and tests for the deviations from expected Hardy-Weinberg genotypic proportions at each of the loci. The number of alleles per locus, allelic richness and heterozygosity were computed using FSTAT 2.9.3 (Goudet, 1995). A conventional hypothesis testing procedure was performed using a heterogeneity test in allele frequencies. The probability test (or Fisher's exact test) with Markov chain method implemented in the GENEPop was used for the heterogeneity tests among minke whales in the designed strata. Statistical significance of the heterogeneity tests was determined using the chi-square value obtained from summing the negative logarithm of p-values over the six microsatellite loci (Sokal and Rohlf, 1995). Sequential Bonferroni corrections were made in case of multiple tests. For the analysis the same stepwise fashion used in the mtDNA, was followed.

RESULTS

Mitochondrial DNA

Mitochondrial DNA fragment patterns

The total number of fragment patterns produced by restriction enzymes *AccI*, *BanI*, *EcoRV*, *HincII*, *HpaI* and *SspI* in the total sample of 6,256 whales were 28, 7, 6, 13, 7 and 31, respectively.

Mitochondrial DNA genome size

The mean mtDNA genome size in the Antarctic minke whale, obtained by averaging the sums of the fragment sizes for all the enzymes was 16,597bp (SD: 668bp).

Mitochondrial DNA haplotypes

Restriction enzyme digestion of mtDNA from the total sample revealed a total of 157 unique mtDNA haplotypes.

Mitochondrial DNA diversity

Sequence divergence among haplotypes ranged from 0.00149 to 0.02759. Table 2 shows the estimate of nucleon diversity for minke whales in the primary geographical strata used in this study. The nucleon diversity ranged from 0.8757 to 0.8911.

Geographical distribution of mtDNA haplotypes

A total of 33 haplotypes (21.0% of the total) were represented by a single specimen. Haplotypes '1' to '5' and '10' were the predominant haplotypes (60.1%) found in all strata examined in similar proportions. The number of 'private' haplotypes in strata IIIIE, IVW, IVE, VW, VE and VIW were 3, 9, 4, 10, 8 and 1, respectively. Regardless of their frequencies, 39 haplotypes were common to all strata.

Examination of genetic heterogeneity based on chi-square tests

No significant heterogeneity was detected among months in each of the strata for females, males and both sexes combined, respectively (Table 3). Therefore samples obtained in different months were pooled in each stratum. No significant heterogeneity was found in the comparison between north and south samples in IVW so that both samples were combined for further analyses. In stratum VE some degree of heterogeneity was detected for females ($p=0.0126$) and total samples ($p=0.0111$). An examination of the source of heterogeneity revealed two particular years, 1988/89 and 1992/93. After these samples were excluded, no substantial heterogeneity was detected between north and south samples in this stratum ($p=0.1182$ and $p=0.0825$ for females and total, respectively). The next analysis was conducted by excluding these two years in stratum VE for female and total samples.

Tables 4a and 4b show the results of pairwise statistical comparisons among six longitudinal strata for females and males, respectively. In the case of females the total p-value was 0.0190 while in the case of males was 0.2164. In both cases several p-values below 0.05 were found with some of them being significant after Bonferroni correction. Most of these low p-values occurred in the comparisons between strata in IIIIE, IV and stratum VE. Although some differences occur, the general pattern of variation between females and males are similar.

Table 4c shows the results of the pairwise statistical comparisons among six longitudinal strata for female and male samples combined. The total p-value was significant and several pairwise comparisons resulted in p-values below 0.05. Four of them remained significant after Bonferroni correction (IIIIE-VE, IVW-VE, IVE-VE and IVW-VW). Comparisons between IIIIE, IVW, IVE and VIW resulted in p-values below 0.05 but these were not significant after Bonferroni corrections. This result is probably due to the sample size of VIW, which is the smallest of all strata.

Results of pair-wise comparisons suggest a) no significant differences among IIIIE, IVW and IVE, b) no significant differences between VIW and VE, c) significant differences between IIIIE, IVW, IVE and VE. Stratum VW resulted in significant differences with IVW but not with IVE. A low but not significant p-value (0.0188) was obtained in the comparison between VW and VE. As shown in Table 4c whales in stratum VW seems to be differentiated from both IVW and VE.

To investigate further the pattern of longitudinal variation, two additional fine-scale analyses were conducted. These analyses were conducted for female and male samples combined. Also these two analyses were conducted for all year samples as well total samples minus the two 'variable' years mentioned above. Because the results were similar only the results for the total samples are shown here. The first analysis considered a 'baseline' sample composed of whales from Areas IIIIE+IVW (the most western strata). The haplotype frequencies in this sample were compared with the frequencies in whales distributed to the east, grouped by 10° longitude. Table 5 shows the results of the statistical comparisons. All the comparisons with whales between sector 100°-110°E and 140°-150°E resulted in no significant p-values. Comparisons with whales to the east (from sector 150°-160°E) resulted in significant p-values. The second analysis used two 'baseline' samples: IVW and IVE. The haplotype frequencies of whales in IVW were compared with those of whales to the east grouped by 10° longitude. The haplotype frequencies of whales in IVE were compared with those of whales to the west grouped by 10° longitude. As shown in Table 6a in the comparison based on IVW large p-values were

obtained until sector 140°-150°E. From sector 150°-160°E most of the p-values resulted significant. None comparison made to the west (based on IVE) showed a p-value smaller than 0.05 (Table 6b).

Examination of genetic heterogeneity based on AMOVA

Table 7 shows the results of the nested analysis by AMOVA applied to the eight groups. The analysis was conducted for female and male sampled combined. Only 0.05% of the total molecular variance was accounted for the longitudinal division; a negative value (-0.01%) was accounted for by latitudinal division and >99% by diversity within the groups. None of the p-values were significant although the p-value for PH1st was near-to significant (0.0693). Table 8 shows the results of pairwise comparisons for six longitudinal strata (north and south samples were combined in IVW and VE). In this case the total p-value was significant (0.0383) and several comparisons resulted in p-values below 0.05 again involving comparisons among the most distant strata.

Microsatellites

Genetic diversity

The six microsatellite loci were successfully amplified in Antarctic minke whales and were polymorphic in the samples examined (Table 9). The total number of alleles at the loci in all samples combined ranged from 10 (DirFCB14) to 24 (GT195) with an average of 19.5. Samples from all the strata had the same levels of genetic diversity, as the average number of alleles per locus ranged from 15.8 and 17.7. In addition the average expected heterozygosity per locus was similar among strata ranging from 0.805 and 0.810.

No statistically significant level of deviation from the Hardy-Weinberg genotypic proportion was detected through the strata at each of the six loci (Table 9). The same was true for overall loci in all samples combined (chi-square=16.948, d.f.=12, p=0.152).

Examination of genetic heterogeneity

No significant statistical heterogeneity was detected due to monthly division at the strata (Table 10). Samples taken in different months were pooled in each stratum. Regarding latitudinal division in strata IVW and VE, only the female samples in IVW showed a significant degree of heterogeneity (p= 0.000). An analysis conducted by year at that stratum showed that such heterogeneity was due mainly to samples from 1989/90 and 1991/92. However significant north/south heterogeneity remained even in the case these samples were excluded from the analysis. Therefore the subsequent analysis for females was conducted for strata IVW separated into north and south.

Results of the heterogeneity test for longitudinal variation are shown in Tables 11a, 11b and 11c, for females, males and both samples combined, respectively. In the case of females the total p-value was significant (0.0020) suggesting structure. Pairwise comparisons showed some degree of heterogeneity between IIIE and IVWS. Most of the other significant heterogeneity detected was due to comparisons among the most distant strata. Five comparisons remained significant after Bonferroni correction (IVWN-IVWS, IVWN-VE, IVWN-VIW, IVWS-VE and IVWS-VIW) (Table 11a). In the case of males the total p-value was not significant (0.317) (Table 11b).

In the case of combined samples the total p-value was significant (0.0390) suggesting structure. In pairwise comparisons, p-values smaller than 0.05 were observed among the most distant strata, IIIE, IV and VW, VE, VIW, respectively. Only one comparison remained significant after Bonferroni correction (IVW-VE) (Table 11c).

DISCUSSION

Consistent with the results of previous genetic studies, values of nucleon diversity (mtDNA) and expected heterozygosity (microsatellites) suggested a high degree of genetic diversity in the Antarctic minke whale. The high genetic diversity reflects in part the abundance of this species in the Southern Hemisphere. In fact, some previous studies estimated high N (or Ne) for this species (IWC, 1991).

Our genetic study was conducted in order to respond some research questions outlined earlier, which are addressed below.

Stock structure

Are the Antarctic minke whales distributed in the feeding grounds of Areas IIIE, IV, V and VIW structured into different genetic stocks?

Significant levels of genetic heterogeneity detected for both mtDNA and microsatellites suggest that Antarctic minke whales do not distribute randomly in the research area of Areas IIIE, IV, V and VIW, rather these levels indicate a degree of stock segregation in the feeding grounds. Our genetic analyses therefore, based on two different genetic markers, suggest stock structure of this species in the feeding grounds. The mtDNA heterogeneity found is consistent with previous studies based on the same marker (Pastene *et al.*, 1993; 1996). The present microsatellite analysis was thought more informative than the allozyme analysis conducted in the past (Wada and Numachi, 1991).

What is the geographical and temporal pattern of stock structure?

Our statistical analysis of haplotype and allele frequencies data failed to detect any substantial temporal (monthly) heterogeneity in the research area. It should be noted, however, that when samples are divided by month in each stratum the sample sizes become small. Then the power of this specific analysis is probably low.

The geographic pattern of structure is consistent with at least two-stocks involved in these Areas, which appear to be structured longitudinally. Probably these stocks are related to the breeding areas in the eastern Indian Ocean and western South Pacific suggested by Kasamatsu *et al.* (1995). These authors examined sighting data collected by dedicated Japanese sighting surveys in low latitudes. After consideration of the sighting effort these authors found high encounter rates of Antarctic minke whales in the eastern Indian Ocean (10°-20° S; 100°-110° E) and in the western South Pacific (10°-20° S; 150°-170° W), which were attributed to breeding areas of the Antarctic minke whale (Figure 3). These results are consistent with the significant differences in alleles and haplotype frequencies found between the most distant strata (Areas III, IVW and VE, VIW, respectively); whales in Areas IIIE and IV could be related to breeding grounds in the Indian Ocean and whales in Areas VIW and VE to breeding grounds in the western South Pacific. Assuming this two-stock scenario, the fine-scale analysis using mtDNA demonstrated that it is possible to identify a longitudinal sector where both stocks segregate, 150°-160° E, near the boundary between sectors VW and VE. Therefore the general stock structure archetype for the Antarctic minke whale in the feeding grounds is multiple stocks with spatial (longitudinal) segregation. However given the absence of geographical barriers the possibility of a sector where the two stocks mix can not be discarded. It should be noted that the pattern of stock structure found by our analysis is not consistent with the actual longitudinal boundaries of the IWC Antarctic management Areas.

It is concluded that Antarctic minke whales are genetically structured in the feeding grounds however the genetic divergence between stocks in the Antarctic is very small as revealed by a mtDNA analysis that compared whales from two broad sectors: 70°-165°E and 165°E-145°W. The PHIst from this comparison was 0.001, which was significant ($p=0.0069$).

Some degree of mtDNA latitudinal heterogeneity was observed for females in stratum VE, which were not observed by the microsatellite analysis. On the other hand some degree of microsatellite latitudinal heterogeneity was observed for females in stratum IVW, which were not observed by the mtDNA analysis. The interpretation of such apparent heterogeneity is difficult as results derived from both markers were inconsistent. Further heterogeneity in IVW was due to a single locus (Ev1) only. Although the possibility of additional structure to the west of the research area (e.g. intrusion and mixing with a third stock around Area IIIE) can not be discarded at this stage, such possibility is considered weak (see also Pastene *et al.*, 1996). As mentioned earlier the very high genetic diversity in this species sometimes produces misleading results when the analyses are based on smaller sample sizes. With regard the apparent mtDNA heterogeneity in VE, the alternative explanation is that the pattern of distribution (and mix) of stocks around longitude 165° E change by year.

Our primary analysis was conducted by pooling the samples from several austral summer seasons under several assumptions. Analysis by austral summer seasons have been attempted in the past, however, these have necessarily been based on smaller number of samples. It has been difficult to interpret results of statistical analysis of samples grouped by year. Again given the very high genetic diversity, annual sample size may not be large enough to represent the stock diversity. Notwithstanding when the haplotype frequencies were compared among years in each of the six longitudinal strata examined, no significant differences were found.

Is the pattern of structure the same for females and males?

According to the mtDNA analysis similar pattern of genetic heterogeneity was observed between females and males. This result suggests that the pattern of lateral movement in the feeding grounds is similar between sexes. This is further corroborated by statistical analysis that compared the haplotype frequencies between males and females in each of the strata examined, which showed no significant heterogeneity (data not shown). This is consistent with the results of studies of mark-recapture conducted in the past based on commercial samples, which found no significant differences between sexes in the average longitudinal distance moved (Kato *et al.*, 1993). The results of the heterogeneity test based on microsatellite found more structure in females suggesting more lateral movement of males in the feeding ground. However the statistical analysis comparing allele frequencies between males and females in each of the strata showed no significant heterogeneity, except in the case of stratum IVWN (data not shown). In conclusion our genetic results are more consistent with the view of a similar pattern of lateral movement between sexes in the feeding ground.

Mechanism of segregation of stocks in the feeding ground

As mentioned above the genetic results are consistent with the occurrence of at least two stocks of the Antarctic minke whale in the JARPA research area, which are segregated around the longitudinal sector 150°-160°E. What kind of mechanism is responsible to keep segregated different stocks in the feeding grounds in absence of geographical barriers? The main prey species of the minke whale in the Antarctic is the krill (*Euphausia superba*). Whales migrating south from their respective breeding grounds will not need to conduct extensive longitudinal movements in the feeding grounds if food enough is available in their 'home' longitudinal sector. If the availability of krill is not large enough in their 'home' sector, minke whales will need to move longitudinally in search of other areas of krill concentration. Results of our genetic analysis showed a longitudinal segregation of stocks in the Antarctic suggesting a degree of fidelity of stocks to specific feeding grounds.

Following the above logic, distribution and pattern of movement of different stocks of minke whales in the feeding ground will depend on the distribution of their key prey species, the krill, which in turn depends on bottom topography, sea-ice and hydrographic features (Ichii, 1990). Krill is a circumpolar species, with their main centers of concentration around island groups and along the continental shelf break and slope (Nicol, 2006). Around the Antarctic continent, a series of gyres link the westward-flowing Coastal Current (CC) and the Antarctic Circumpolar Current (ACC) and previous studies have suggested that variation in abundance of krill are linked to the gyral circulation pattern (Nicol, 2006). Figure 4 reproduced from Nicol (2006), shows that historical krill distribution overlaid on maps of known current features suggesting that krill concentrations may be related to surface circulation.

Our genetic results suggest a segregation of stocks around the longitudinal sector 150°-160°E. These longitudes coincide with the aperture of the Ross Sea and from the oceanographic point of view this sector is influenced by the Ross Sea Gyre (Figure 4). Based on these antecedents and the results of our genetic study, it is postulated that Antarctic minke whales originating from breeding grounds in the western South Pacific have some degree of fidelity to the krill concentration associated to the Ross Sea Gyre ('home' sector). On the other hand whales originating in the Indian Ocean have some fidelity to the krill concentration related to the gyre located between 90°-120°E ('home' sector) (Figure 4). Both stocks could expand longitudinally interacting in the area of concentration located between 150°-180°E (Figure 4). Therefore any boundary (or proportion of stocks in areas of mixing) in the Antarctic should be considered as 'soft'.

Consistency with results of independent approaches

In reviewing the results on stock structure derived from JARPA, the Scientific Committee noted that only preliminary conclusions can be drawn at this stage and that more concrete conclusions will be able to be made following the completion of different analyses. It further supported the suggestion that additional analyses using alternative groupings and analytical methods should be conducted (IWC, 2004).

The most effective way to address questions on stock identity is to consider results from several techniques, genetics and non-genetics (Donovan, 1991; Pastene *et al.*, 2000; Perrin, 2001; Rugh *et al.*, 2003). In the case of the Antarctic minke whale the application of multi-approaches to investigate stock identity is particularly desirable because previous genetic analysis suggested that the effect size in this species is low. Therefore it is desirable that results obtained using genetics are checked using

independent non-genetic markers. Morphometric and average length of physical maturity analyses were conducted to examine stock structure using JARPA samples (see Documents SC/D06/J10 and SC/D06/J11). In general results of these studies are broadly consistent with those of the genetic analysis that showed the Antarctic minke whales structured into at least two genetic stocks.

Results on mark-recapture analysis conducted during the time of commercial whaling in the areas surveyed by JARPA could assist greatly in the interpretation of results on stock structure found in this study. Kato *et al* (1993) used information from marks both released and recovered within the Antarctic i.e. 110 marks or 101 recaptured individuals. All but one mark were recovered during commercial whaling operations. One mark in his analysis was recovered during a JARPA survey (3 Feb. 1992). Results relevant to our genetic study were: a) patterns of longitudinal movement are very similar between sexes, which is consistent with the genetic results and b) although some individuals moved great distances (over 100° longitude), most whales appear to return to relatively close to the sector in which they were marked (30°-40° longitude apart), which is consistent with a degree of fidelity to some specific feeding grounds proposed by the genetic analysis.

Mark-recapture analysis showed marked movement of whales through longitude 130°E (division between Areas IV and V), which is also consistent with the results of the genetic analysis. With regard boundaries, Kato *et al.* (1993) noted that the absence of mark crossing at 80°E and 160°E suggests the possibility of separate feeding stocks. Following an examination of mark distribution and catching effort they concluded that discontinuity at 80°E could be more suggestive of a boundary between stocks than that at 160°E.

On this regard mark-recapture results are partially consistent with the boundary suggested by genetic analysis. While the boundary at 160°E is consistent with our proposal around that longitude, boundary at 80°E proposed by mark-recapture analysis is not consistent with the results of our study (at least in regard the mtDNA analysis), which suggested that Area III E is similar to Area IV.

It should be noted that two other marks recovered during JARPA surveys were not available for the Kato *et al.* (1993)'s analysis. One of them (elapsed time of 12 years) was marked in the eastern part of Area V and recovered in the western part of this Area (Fujise *et al.*, 1993). The other one (elapsed time of 21 years) was marked in the western part of Area V and recovered in the eastern part of Area IV (Ishikawa *et al.*, 2002).

Further research needs

The following topics are recommended for future studies on stock structure:

- a) Yearly changes in stocks distribution and boundaries (or changes in the proportion of stocks in areas of mixing) in the region 150°E-180° should be investigated in the future in relation to bottom topography, currents and krill distribution.
- b) Western boundary (or areas of mixing) of the stock originating in the eastern Indian Ocean and the eastern boundary (or areas of mixing) of the stock originating in the western Pacific Ocean should be investigated in the future.
- c) To elucidate the possibility of mixing in the feeding ground at a better resolution, samples and genetic analysis of whales from the breeding grounds are necessary. Experiments of satellite tracking would be very useful to examine lateral movement and possible mixing in the feeding grounds as well movement between feeding grounds and lower latitudes wintering grounds.

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Table 1: Number of samples examined by geographical strata, genetic marker and sex.
Areas III E and IV

	III E		IVWN		IVWS		IVW		IV E	
	F	M	F	M	F	M	F	M	F	M
mtDNA	218	311	160	302	462	497	622	799	601	633
Microsatellites*	227	322	164	320	488	534	652	854	526	558

Areas V and VIW

	VW		VEN		VES		VE		VIW	
	F	M	F	M	F	M	F	M	F	M
mtDNA	454	640	205	507	622	168	826	675	170	306
Microsatellites*	488	676	184	491	619	165	803	656	179	319

* Sample size changes among loci. Figures shown are the maximal sample size used among the loci.

Table 2: Nucleon diversity in the Antarctic minke whale, by longitudinal strata. Sample sizes in parenthesis.

Strata (sample size)	Number of haplotypes (private)	Nucleon diversity
III E (529)	65 (3)	0.8898 +/- 0.0103
IVW (1,421)	110 (9)	0.8911 +/- 0.0062
IV E (1,234)	102 (4)	0.8757 +/- 0.0075
VW (1,094)	97 (10)	0.8795 +/- 0.0079
VE (1,502)	104 (8)	0.8775 +/- 0.0068
VIW (476)	71 (1)	0.8822 +/- 0.0114

Table 3: Results of the heterogeneity test for monthly mtDNA variation in each geographic stratum, by sex.

Strata	Sex	Chi-square p-value
III E	F	0.4271
	M	0.5026
	Total	0.2740
IVWN	F	0.3521
	M	0.5791
	Total	0.0720
IVWS	F	0.6142
	M	0.2023
	Total	0.0528
IV E	F	0.1514
	M	0.3043
	Total	0.1926
VW	F	0.9859
	M	0.5060
	Total	0.5929
VEN	F	0.5599
	M	0.7715
	Total	0.5647
VES	F	0.1248
	M	0.9563
	Total	0.3684
VIW	F	0.6062
	M	0.2880
	Total	0.4298

Table 4a: Results of the heterogeneity test for longitudinal mtDNA variation in female samples. The total P-value was 0.0190 suggesting structure. P-values smaller than 0.05 are underlined. Bold show those P-values that remain significant after Bonferroni corrections.

	IIIIE (218)	IVW (622)	IVE (601)	VW (454)	VE (659)	VIW (170)
IIIIE	-	0.4799	0.1149	0.0032	0.0009	0.3639
IVW		-	0.3574	0.0566	<u>0.0046</u>	0.3529
IVE			-	0.3227	0.1272	0.6655
VW				-	<u>0.0204</u>	0.0604
5E					-	0.3176
6W						-

Table 4b: Results of the heterogeneity test for longitudinal mtDNA variation in male samples. The total P-value was 0.2164. P-values smaller than 0.05 are underlined. Bold show those P-values that remain significant after Bonferroni corrections.

	IIIIE (311)	IVW (799)	IVE (633)	VW (640)	VE (675)	VIW (306)
IIIIE	-	0.5843	0.7554	0.3437	0.1619	0.1176
IVW		-	0.8852	0.2104	0.0004	0.0857
IVE			-	0.6661	<u>0.0052</u>	0.1119
VW				-	0.3399	0.7267
5E					-	0.6785
6W						-

Table 4c: Results of the heterogeneity test for longitudinal mtDNA variation in total samples (F+M). The total P-value was 0.0024 suggesting structure. P-values smaller than 0.05 are underlined. Bold show those P-values that remain significant after Bonferroni corrections.

	IIIIE (529)	IVW (1,421)	IVE (1,234)	VW (1,094)	VE (1,283)	VIW (476)
IIIIE	-	0.7812	<u>0.0475</u>	<u>0.0125</u>	0.0002	<u>0.0055</u>
IVW		-	0.9375	0.0024	0.0002	<u>0.0283</u>
IVE			-	0.4537	0.0003	<u>0.0261</u>
VW				-	<u>0.0188</u>	0.3969
VE					-	0.4397
VIW						-

Table 5: Results of the comparison of mtDNA haplotype frequencies between Areas III+IVW (n=1,950) and 10°-sectors in IVE, VW and VE for total samples (female+male). Significant p-values are in bold.

Sectors	Chi-square p-value
(IVE) 100-110E (394)	0.9114
(IVE) 110-120E (443)	0.2859
(IVE) 120-130E (397)	0.2344
(VW) 130-140E (278)	0.2687
(VW) 140-150E (309)	0.1228
(VW) 150-160E (418)	0.0001
(VW-E) 160-170E (243)	0.0044
(VE) 170E-180 (599)	0.0253
(VE) 180-170W (605)	0.0001
(VIW) 170W-145W (620)	0.0024

Table 6a: Results of the comparison of mtDNA haplotype frequencies between Area IVW (n=1,421) and 10°-sectors in IVE, VW and VE for total samples (female+male). Significant p-values are in bold.

Sectors	Chi-square p-value
(IVE) 100-110E (394)	0.9903
(IVE) 110-120E (443)	0.7616
(IVE) 120-130E (397)	0.6005
(VW) 130-140E (278)	0.5040
(VW) 140-150E (309)	0.2115
(VW) 150-160E (418)	0.0009
(VW-E) 160-170E (243)	0.0366
(VE) 170E-180 (599)	0.0920
(VE) 180-170W (605)	0.0007
(VIW) 170W-145W (620)	0.0167

Table 6b: Results of the comparison of mtDNA haplotype frequencies between Area IVE (n=1,234) and 10°-sectors in IVW and IIIE, for total samples (female+male).

Sectors	Chi-square p-value
(IVW) 90-100E (440)	0.4355
(IVW) 80-90E (401)	0.8874
(IVW) 70-80E (580)	0.6682
(III E) 60-70E (224)	0.0529
(III E) 50-60E (150)	0.4166
(III E) 35-50E (155)	0.2036

Table 7: Results of the nested analysis of molecular variance of Antarctic minke whale mtDNA haplotypes in Areas III, IV, V and VIW. The p-value is the probability of a more extreme variance component or PHIst than that observed, in comparison to a null distribution of these values on 10,000 random permutations of the data matrix. PHIct and the among longitudinal sectors variance component involves the permutation of whole longitudinal/latitudinal groups among longitudinal sectors; the PHIsc and the among latitudinal groups within longitudinal sectors involves the random permutation of individuals among longitudinal/latitudinal groups within longitudinal sectors; the PHIst and the within longitudinal/latitudinal groups components involves the random permutation of individuals among the eight longitudinal/latitudinal groups.

	df	% total variance	PHI	P
Among long. Sectors	5	0.05	CT: 0.000	0.2750
Among latitudinal	2	-0.01	SC: -0.000	0.4777
Within groups	6,248	99.96	ST: 0.000	0.0693

Table 8: Haplotypic correlation (PH1st, below diagonal) and their probabilities (P, above diagonal) among six (north/south combined in IVW and VE) longitudinal groups of Antarctic minke whales from Areas IIIE, IV, V and VIW. In parenthesis is the sample size. Underline indicates those haplotypic correlations presenting a p-value below 0.05. The total p-value was 0.0383 suggesting structure.

	III (529)	IVW (1,421)	IVE (1,234)	VW (1,094)	VE (1,502)	VIW (476)
III	-	0.3791	0.1993	0.0523	<u>0.0323</u>	<u>0.0500</u>
IVW	0.0001	-	0.9241	0.0723	<u>0.0065</u>	<u>0.0328</u>
IVE	0.0005	-0.0004	-	0.4112	0.0727	0.1243
VW	0.0013	0.0006	-0.0000	-	0.8278	0.7884
VE	0.0014	0.0013	0.0006	-0.0003	-	0.9895
VIW	0.0020	0.0016	0.0008	-0.0006	-0.0009	-

Table 9: Number of alleles (#A), allelic richness (AR), expected heterozygosity (He) and test result for expected Hardy-Weinberg genotypic proportion (HW) at six microsatellite loci analyzed in the Antarctic minke whale.

	IIIIE	IVWN	IVWS	IVE	VW	VEN	VES	VIW	Total
EV1									
#A	15	16	14	17	18	17	16	18	22
AR	14.7	16.0	12.4	14.3	15.3	15.8	14.5	17.8	14.8
He	0.817	0.821	0.820	0.817	0.821	0.829	0.821	0.821	0.821
HW	0.749	0.390	0.199	0.774	0.269	0.396	0.047	0.330	
EV104									
#A	17	16	17	17	16	17	16	16	17
AR	17.0	16.0	16.5	16.7	16.0	16.7	16.0	16.0	16.4
He	0.907	0.905	0.903	0.908	0.906	0.907	0.908	0.908	0.906
HW	0.284	0.782	0.258	0.810	0.919	0.061	0.867	0.973	
GT211									
#A	19	18	22	21	22	20	21	19	23
AR	18.9	18.0	19.7	19.1	19.4	19.3	20.3	19.0	19.4
He	0.891	0.891	0.896	0.888	0.893	0.893	0.892	0.894	0.892
HW	0.069	0.046	0.294	0.533	0.833	0.602	0.482	0.470	
DlrFCB14									
#A	9	8	8	8	9	8	8	8	10
AR	8.6	8.0	7.4	7.5	8.0	7.4	7.1	7.9	7.6
He	0.480	0.476	0.446	0.461	0.456	0.478	0.453	0.450	0.463
HW	0.921	0.090	0.591	0.989	0.220	0.362	0.154	0.037	
GT195									
#A	19	19	21	23	20	17	20	19	24
AR	18.9	19.0	19.7	20.7	18.3	16.8	18.8	18.9	19.2
He	0.870	0.860	0.872	0.864	0.867	0.864	0.866	0.862	0.866
HW	0.185	0.665	0.643	0.302	0.721	0.551	0.569	0.830	
GT23									
#A	19	18	21	19	21	19	18	19	22
AR	18.8	18.0	19.3	18.6	19.5	18.8	17.8	18.9	19.0
He	0.895	0.888	0.892	0.892	0.896	0.888	0.896	0.892	0.892
HW	0.110	0.389	0.458	0.741	0.114	0.881	0.103	0.547	
All loci									
#A	16.3	15.8	17.2	17.5	17.7	16.3	16.5	16.5	19.7
AR	16.2	15.8	15.8	16.1	16.1	15.8	15.7	16.4	16.1
He	0.810	0.807	0.805	0.805	0.807	0.810	0.806	0.805	0.807
HW	0.173	0.188	0.455	0.950	0.512	0.451	0.139	0.447	

Table 10a: Results of the heterogeneity test for monthly female microsatellite variation in each geographic stratum.

Sector	Microsatellite locus						
	EV1	EV104	GT211	DlrFCB14	GT195	GT23	All loci
III E	0.227	0.549	0.434	0.207	0.485	0.670	0.509
IVWN	0.281	0.255	0.854	0.589	0.873	0.960	0.858
IVWS	0.596	0.199	0.707	0.139	0.954	0.500	0.582
IV E	0.383	0.924	0.651	0.155	0.255	0.680	0.601
VW	0.924	0.619	0.328	0.054	0.291	0.583	0.387
VEN	0.005	0.906	0.228	0.569	0.931	0.406	0.151
VES	0.841	0.113	0.596	0.615	0.554	0.438	0.656
VIW	0.057	0.441	0.899	0.501	0.520	0.706	0.533

Table 10b: Results of the heterogeneity test for monthly male microsatellite variation in each geographic stratum.

Sector	Microsatellite locus						
	EV1	EV104	GT211	DlrFCB14	GT195	GT23	All loci
III E	0.085	0.411	0.566	0.840	0.772	0.287	0.512
IVWN	0.011	0.638	0.558	0.832	0.285	0.520	0.223
IVWS	0.739	0.202	0.984	0.112	0.610	0.964	0.678
IV E	0.613	0.540	0.276	0.874	0.442	0.687	0.827
VW	0.859	0.530	0.257	0.412	0.879	0.946	0.893
VEN	0.560	0.621	0.961	0.703	0.512	0.485	0.931
VES	0.845	0.906	0.801	0.400	0.633	0.111	0.776
VIW	0.415	0.507	0.236	0.092	0.381	0.792	0.356

Table 10c: Results of the heterogeneity test for monthly microsatellite variation in each geographic stratum (total samples)

Sector	Microsatellite locus						
	EV1	EV104	GT211	DlrFCB14	GT195	GT23	All loci
III E	0.102	0.457	0.384	0.489	0.770	0.333	0.430
IVWN	0.359	0.513	0.675	0.535	0.562	0.719	0.842
IVWS	0.627	0.202	0.955	0.011	0.625	0.515	0.212
IV E	0.518	0.985	0.237	0.893	0.376	0.871	0.878
VW	0.954	0.568	0.116	0.176	0.824	0.961	0.662
VEN	0.334	0.486	0.507	0.853	0.732	0.220	0.706
VES	0.740	0.433	0.343	0.692	0.732	0.405	0.817
VIW	0.192	0.423	0.388	0.217	0.538	0.935	0.500

Table 11a: Results of the heterogeneity test for microsatellite variation in females Antarctic minke whales from different longitudinal sectors. The total p-value was 0.002 suggesting structure. In bold are those p-values that remained significant after Bonferroni correction.

Sector	Microsatellite locus						All loci
	EV1	EV104	GT211	DlrFCB14	GT195	GT23	
All sectors	0.013	0.060	0.841	0.004	0.654	0.162	0.002
III E x IVW-N	0.867	0.038	0.385	0.636	0.412	0.404	0.352
III E x IVW-S	0.001	0.343	0.234	0.535	0.066	0.955	0.011
III E x IVE	0.045	0.404	0.210	0.659	0.534	0.964	0.349
III E x VW	0.290	0.692	0.048	0.702	0.364	0.982	0.440
III E x VE	0.283	0.928	0.412	0.171	0.483	0.960	0.658
III E x VIW	0.049	0.225	0.574	0.013	0.186	0.814	0.032
IVW-N x IVW-S	0.001	0.082	0.521	0.022	0.454	0.119	0.001
IVW-N x IVE	0.069	0.019	0.728	0.281	0.908	0.040	0.027
IVW-N x VW	0.299	0.078	0.432	0.184	0.445	0.140	0.112
IVW-N x VE	0.083	0.000	0.887	0.028	0.494	0.084	0.000
IVW-N x VIW	0.055	0.000	0.850	0.005	0.872	0.284	0.000
IVW-S x IVE	0.095	0.993	0.774	0.151	0.572	0.149	0.305
IVW-S x VW	0.234	0.634	0.798	0.333	0.019	0.358	0.171
IVW-S x VE	0.244	0.484	0.662	0.019	0.012	0.002	0.001
IVW-S x VIW	0.137	0.073	0.967	0.000	0.601	0.136	0.002
IVE x VW	0.872	0.217	0.089	0.612	0.927	0.112	0.322
IVE x VE	0.618	0.460	0.882	0.567	0.865	0.209	0.836
IVE x VIW	0.293	0.220	0.985	0.027	0.994	0.521	0.299
VW x VE	0.639	0.334	0.293	0.214	0.996	0.370	0.562
VW x VIW	0.263	0.063	0.797	0.030	0.883	0.670	0.159
VE x VIW	0.190	0.143	0.982	0.002	0.956	0.737	0.061

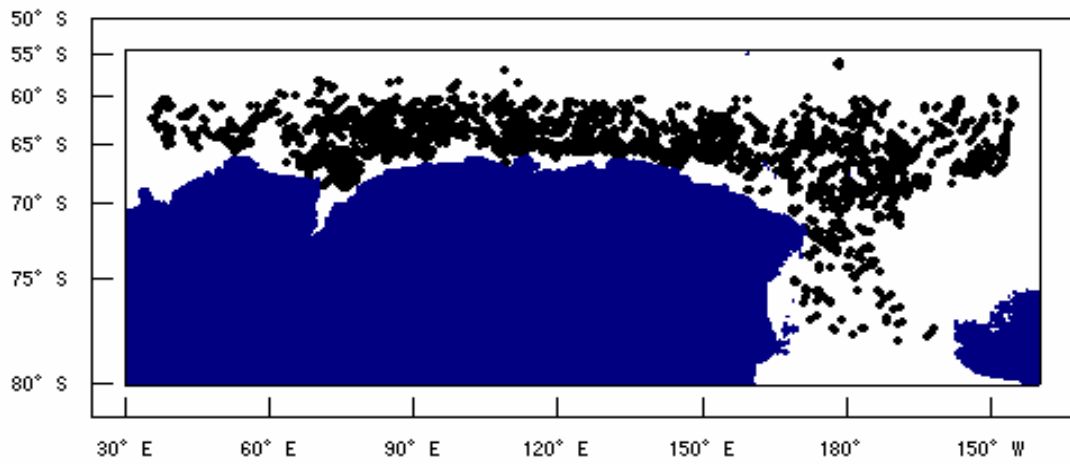
Table 11b: Results of the heterogeneity test for microsatellite variation in male Antarctic minke whales from different longitudinal sectors. The total p-value was 0.317.

Sector	Microsatellite locus						
	EV1	EV104	GT211	DlrFCB14	GT195	GT23	All loci
All sectors	0.522	0.514	0.731	0.285	0.025	0.744	0.317

Table 11c: Results of the heterogeneity test for microsatellite variation in Antarctic minke whales from different longitudinal sectors (female and male samples combined). The total p-value was 0.039 suggesting structure. In bold are those p-values that remained significant after Bonferroni correction.

Sector	Microsatellite locus						
	EV1	EV104	GT211	DlrFCB14	GT195	GT23	All loci
All sectors	0.081	0.192	0.430	0.224	0.067	0.179	0.039
IIIE x IVW	0.048	0.334	0.309	0.406	0.323	0.507	0.190
IIIE X IVE	0.300	0.386	0.309	0.224	0.522	0.535	0.429
IIIE x VW	0.097	0.370	0.145	0.488	0.034	0.664	0.076
IIIE x VE	0.298	0.698	0.396	0.093	0.103	0.432	0.193
IIIE x VIW	0.017	0.137	0.491	0.213	0.096	0.489	0.030
IVW x IVE	0.834	0.652	0.773	0.742	0.944	0.203	0.933
IVW x VW	0.551	0.704	0.550	0.379	0.000	0.308	0.017
IVW x VE	0.169	0.063	0.772	0.008	0.256	0.009	0.002
IVW x VIW	0.080	0.008	0.604	0.253	0.772	0.676	0.072
IVE x VW	0.717	0.696	0.167	0.621	0.008	0.268	0.106
IVE x VE	0.770	0.462	0.288	0.383	0.706	0.433	0.716
IVE x VIW	0.414	0.196	0.691	0.537	0.692	0.472	0.682
VW x VE	0.396	0.386	0.203	0.741	0.179	0.304	0.343
VW x VIW	0.118	0.279	0.620	0.674	0.386	0.950	0.565
VE x VIW	0.273	0.455	0.641	0.254	0.768	0.409	0.606

MALES



FEMALES

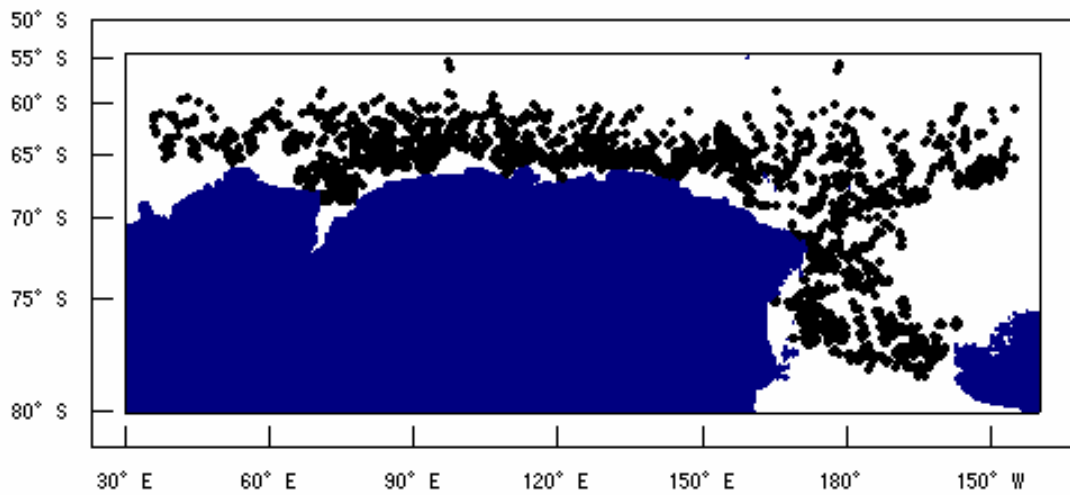


Figure 1: Geographical distribution of Antarctic minke whales sampled by JARPA and examined in this study (upper figures: males; lower figure: females).

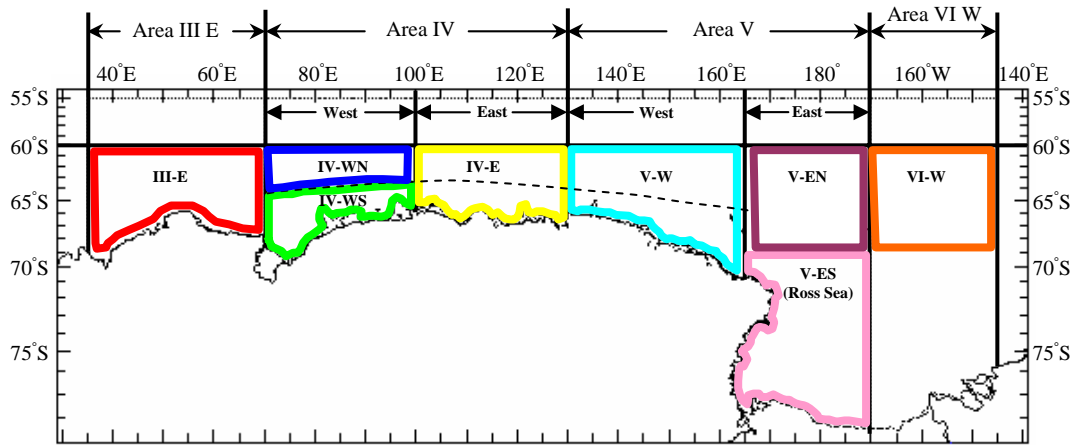


Figure 2: Geographic strata used in the present genetic analyses of stock structure in the Antarctic minke whale.

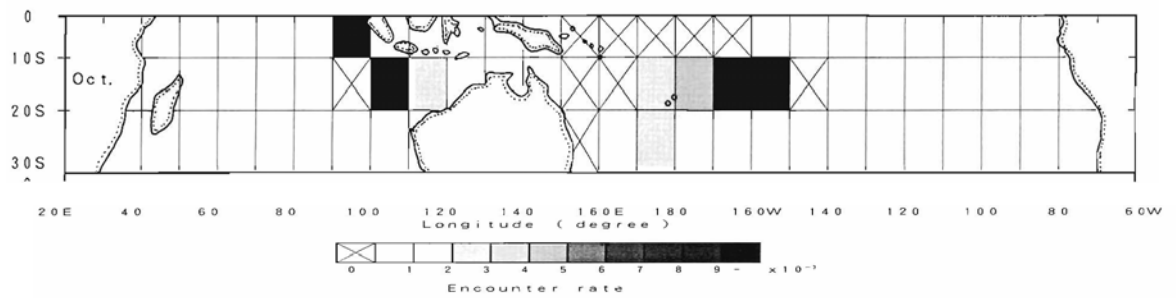


Figure 3: Encounter rates of Antarctic minke whales in 10° squares of latitude and longitude in waters 0° - 30° S in October (from Kasamatsu *et al.*, 1995).

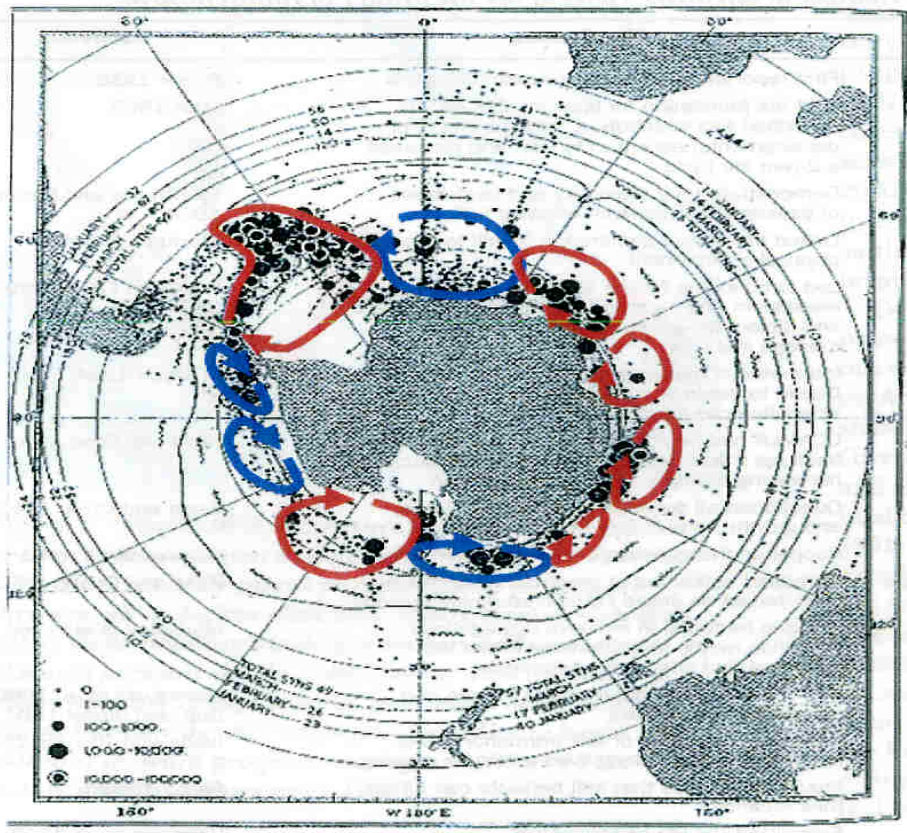


Figure 4: Distribution of krill in summer, overlaid with surface ocean circulation pattern. Red gyres are well documented; blue ones are more speculative (Taken from Nicol, 2006).