Preliminary Microsatellite DNA Analysis to Investigate Stock Structure in the Antarctic Minke Whale (*Balaenoptera acutorostrata*)

Hideaki Abe[1], Mutsuo Goto[1], Yoshinao Katsumata[2], Masaki Mizutani[2] and Luis A. Pastene[1]

[1] *Institute of Cetacean Research, 4-18 Toyomi-cho, Chuo-ku, Tokyo 104-0055, Japan*

[2] *Department of Legal Medicine, Nagoya University School of Medicine, 65 Tsurumai-cho, Syowa-ku, Nagoya 466-0065, Japan*

ABSTRACT

A preliminary analysis of stock structure in the Antarctic minke whale was made using five polymorphic microsatellites in a sample of 914 individuals taken by JARPA surveys in IWC management Areas IIIE, IV, V and VIW. Despite these samples were obtained from widely separated locations, little differences were found in allele frequencies. Significant deviations from Hardy-Weinberg equilibrium were detected in the total sample (overall estimation) and Area IIIE using three different statistical approaches, suggesting some degree of stock structure in these Areas. No significant correlation was detected between genetic and geographic distances. Although this microsatellite analysis has a preliminary character and the grouping of samples was not exactly the same as in the mtDNA analyses, there is a possibility that the pattern of nuclear DNA variation could be different from that derived from the maternal-inherited mtDNA analyses. This fact request further investigation.

INTRODUCTION

The minke whale (*Balaenoptera acutorostrata*) has a worldwide distribution and can be regarded as the most abundant baleen whale species. Based primary on distribution data, the Science Committee proposed that Southern Hemisphere minke whale population contained five distinct breeding grounds and should therefore be managed as five separate management units (IWC, 1991). However, until now no firm biological evidences have been presented to support any stock boundary in the Antarctic feeding ground. It is possible that sufficient interchange between adjacent populations exist to counteract the effects of genetic drift.

Several attempts have been made to investigate stock structure in the Antarctic minke whale using various molecular tools (Van Pijlen *et al.*, 1991 ; Hoelzel and Dover, 1991; Wada *et al.*, 1991; Pastene *et al.*, 1993, 1996). These approaches could clarify the genetic divergence between the minke whale of the Southern Hemisphere and those of the

Northern Hemisphere, nevertheless it was quite difficult to detect stock structures within the Antarctic probably due to low genetic variability in this region. Previous studies based on mtDNA RFLP (restriction fragment length polymorphism) analysis showed that more than one stock could occur in Areas IV and V, where the composition of animals from the two putative stocks changed both longitudinally and temporally during feeding season (Pastene *et al.*, 1996). However, information derived from of mtDNA analysis deal with differences in the female component of the population, as mtDNA is transmitted generation to generation across the mother. Information from nuclear genetic markers is required for a more detailed analysis of genetic structure. Indeed, pilot study on nuclear DNA analysis on JARPA minke samples was one of the ten main tasks to address the stock identity problems (IWC, 1997).

Microsatellites are one of the most important nuclear genetic markers and some of them can provide polymorphic information as diploid data in a certain population. This polymorphism can be detected by the PCR amplification (Saiki *et al.*, 1988) using primers complementary to unique flanking sequences. Differences in the sizes of alleles are due to variation in the number of repeats that can be detected by electrophoresis. By now, more than 30 microsatellite loci have been isolated both from mysticeti (Palsbøll *et al.*, 1997) and odontoceti (Buchanan *et al.*, 1996; Richard *et al.*, 1996) genome. In general, microsatellites are conserved in closely related species (Moore *et al.*, 1991) and then we could apply microsatellite loci isolated from humpback whale to population analysis of North Pacific minke whale (Abe *et al.*, 1997).

Here we present a preliminary genetic analysis on the Antarctic minke whale using five microsatellite loci, which were isolated either from humpback or sperm whales.

MATERIALS AND METHODS

Samples and DNA extraction

Minke whale samples were from the JARPA (Japanese Whale Research Program under Special Permit in the Antarctic) surveys in Area IIIE (1995/96, 1997/98), IV (1989/90) and V, VIW (1996/97) (Table 1). In the isolation by distance analysis, we also used samples taken in North Pacific by the 1996 JARPN (Japanese Whale Research Program under Special Permit in the North Pacific). Total cellular DNA was extracted from several sorts of tissues (muscle, liver, heart) by means of standard proteinase K, phenol-chloroform procedure (Sambrook *et al.*, 1989).

Genotyping of Microsatellite Loci

Nearly 30 primers sets, which were obtained from personal communication (Dr. P. Palsbøll, University of Wales-Bangor, Wales, UK) and previous studies (Valsecchi and Amos, 1996 ; Palsbøll *et al.*, 1997), were tested for PCR amplification on the Antarctic minke whales under several conditions. Five loci turned out to be highly polymorphic and appropriate for population analysis in the Antarctic minke whale. Four of five microsatellite loci contained dinucleotide repeats: GT023 and GT211 (Palsbøll, unpublished) , EV1*Pm* and EV104*Mn* (Valsecchi and Amos, 1996) and the other one comprised of tetranucleotide repeats GATA098 (Palsbøll *et al.*, 1997). It should be noted that some tetramer loci could not be employed in population analysis due to its unstableness (i.e. microvariant alleles; see Puers *et al.*, 1994). Thus, GATA028 and GATA417 loci could not be used in this study even though they had extremely variety of alleles (more than 30 alleles were detected in different length; data not shown). PCR amplification was carried out in 15μl reaction volumes using fluorescently labeled primers. Details of reaction mixture and conditions for PCR amplification were given in our previous paper (Abe *et al.*, 1997).

Statistical analysis
Probability of identity (PI) were calculated for each Area according to the previously described formula (Paetkau *et al.*, 1994). These values were multiplied across loci to give the overall PI. We employed three statistical tests (homozygosity test, likelihood ratio test, exact test) to test departure from Hardy-Weinberg (HW) equilibrium. HW equilibrium was estimated for each locus, and probabilities were combined for each study Area by means of simple equation (Sokal and Rohlf, 1995). The heterogeneity test was conducted using an unbiased estimate of the G-based test, which was performed for all pair of populations for all loci (GENEPOP version 3.1; Raymond and Rousset, 1995).

Analysis of isolation by distance (ISOLDE) was made for minke whales from the four Areas. North Pacific minke whales were used for comparison as outgroup. In order to estimate the genetic differences between the Antarctic minke whale and the North Pacific minke whale, three microsatellites (GT023, GATA098 and EV1*Pm*) were employed as common loci to both hemispheres. The associations between genetic and geographical distances were computed by simple Mantel tests supplied with GENEPOP program. The Mantel spatial correlogram is a useful tool for studying how correlation changes with distance for sample stations that are described by multivariate observations (Manly, 1997).

RESULTS

Genetic diversity and probability of identity
The allelic class frequency distributions of five loci are shown in Table 2. Although some minor private alleles were detected, we could find neither major differences of allele frequency between Areas nor diagnostic private allele. The estimated PI was almost identical among Areas, ranging 2.042×10^{-8} in Area V to 2.838×10^{-8} in Area VI (Table 1). Theoretically, these probabilities are low enough to identify all of individuals in the Antarctic depending on genotype profiles provided in this study.

Tests for Hardy-Weinberg equilibrium
Departures from HW equilibrium were examined by three statistical methods for each Area and microsatellite locus (Table 3). We could find significant deviations in Areas IIIE (for the five loci combined two approaches showed significant departure) and VIW (for the five loci combined one approach showed significant departure). No significant deviations were detected in Area IV and Area V populations at the 5% level. As a whole sample set the three approaches showed significant departure.

Heterogeneity test
The G-based exact test showed significant differences in two pairwise comparisons: Area III *vs* Area V and Area IV *vs* Area V (Table 4).

Isolation by distance
The relationship between genetic and geographical distances in the Antarctic minke whale is shown in Figs. 1a and 1b. These figures shows pairwise multilocus values of Fst / (1-Fst), plotted against straight-line log-distance between the sighting localities of two samples. Antarctic minke whales are distinguishable from North Pacific minke whales (Fig1a). Moreover, the magnitude of genetic difference within the Antarctic populations was estimated to be a hundred times lower than that existing between both hemispheres. Within Antarctic regions, Mantel tests showed that no significant association exists between genetic distance over the geographical scale studied (10^4 permutations, $P>0.05$).

DISCUSSION

Previously some nuclear DNA-based analyses were conducted on the Antarctic minke whale (Amos and Dover, 1991, Van Pijlen *et al.*, 1991). These studies, however, were restricted to Areas IV and V and were based on a small number of samples. No evidence for genetic heterogeneity was found by these authors. Our preliminary study is the first to use a nuclear DNA marker (microsatellite) to investigate a larger sample set from four IWC Antarctic Areas.

Heterozygosity values in the Antarctic minke whale were higher than those observed in the North Pacific minke whale in our previous study (Abe *et al.*, 1998). Allele frequencies of the five loci used were very similar among Areas. One of the main factor influencing the stock structure is the nature and level of gene flow. Mutation rates of most nuclear loci are so low that new mutations have not had sufficient time to appear and became fixed. In this study, we could not observe difference in allelic distributions between the Antarctic Areas, and the range of genetic distance estimated using isolation by distance was quite narrow ($Fst<0.02$). Generally speaking, if two groups have split too recently to diverge by genetic drift and new mutations, their allele distributions will be very similar, and an F statistic with a value smaller than 0.05 indicates little genetic differentiation (Hartl and Clark, 1997). These are evidences that gene flow could have a strong influence in homogenizing genetic composition in the Antarctic minke whale across biological boundaries that surely existed for a certain period.

Although the gene flow event could have prevented the Antarctic minke whales from gathering diagnostic characters specific for each putative stock, statistical tests for heterogeneity could partially reveal hierarchical structure in the Antarctic. Assuming that all samples used in this study were derived from a panmictic unit, significant deviations from HW equilibrium were found in the whole sample (Table 3) even though no departure from HW proportions were detected in each Area. This is the typical case of Wahlund effect which is well characterized by heterozygote deficiency suggesting the existence of more than one stock.

A previous study based on mtDNA (Pastene *et al.*, 1996) suggested the occurrence of more than one stock in the western part of Area IV and a temporal component to their distribution. In this study, however, we could not detect any deviation from HW equilibrium within area IV. It should be noted, however, that both data set and the grouping are not exactly the same between the two studies. The sample detecting mtDNA heterogeneity in Area IVW early in Pastene *et al.* (1996) was comprised of minke whales from two surveys, 1989/90 and 1991/92 while that our data set for Area IV only included samples from the 1989/90 survey.

Although our microsatelite analysis has a preliminary character, and samples and grouping were not exactly the same as in the mtDNA survey, there is the possibility that the pattern of nuclear DNA variation in the Antarctic could be different from that found in the mtDNA analysis. If this become to be the case, we can cite similar cases in which results from nuclear DNA analysis are different from those derived from mtDNA analysis. For example, previous works on humpback whales showed that even though mtDNA markers demonstrated a significant division between Alaska/Hawaii and the California/Mexico stocks, analysis of the DNA fingerprint and actin sequence data failed to find any differences between them (Palumbi and Baker, 1994). A similar situation has been found in the analysis of Antarctic humpback whales using mtDNA control region sequences and microsatellite (data not shown). Two factors might be concerned with

these discrepancies. One possible explanation is that results of statistical tests may be influenced (changed) due to the small degree of differentiation among stocks product of high level of gene flow between them. Distinguishing between partially isolated stocks connected by ongoing gene flow remain one of the important challenges when doing these type of analyses. Second, the fixation of distinct alleles at nuclear loci almost certainly required more time than did the mtDNA divergence (Avise and Ball, 1990). Therefore, it is reasonable to conclude that we will not have a diagnostic nuclear marker which can provide alleles specific for a certain stock of Antarctic minke whale. For the case of the Antarctic minke whale more research is necessary before conclude that the pattern of mtDNA and nuclear DNA variation is different.

The heterogeneity test suggested certain degree of heterogeneity in Area V. This result should be taken with caution as no significant deviation from the HW equilibrium was observed in Areas IV and V. It is necessary to investigate further the apparent differentiation of minke whales from Area V.

By taking into consideration the results of the HW test in our analysis, we could say that the actual boundary between Areas IV and V at 130°E is artificial and that a 'core' stock could occur in the main body of these Areas. However, to confirm this, further research is necessary to investigate the apparent differentiation of Area V as mentioned earlier.

Finally, our study would arise a problem concerning the number of independent loci that are required for the examination of genetically close related stocks. Previous study that examined this question in relation to the segregation of ancient polymorphisms suggested that in general more than five loci are needed to resolve the species phylogeny accurately (Wu, 1991). It is obvious that this number will be considerably higher if the internal nodes are relatively short as in the case of Antarctic minke whales. In this study, three microsatellite loci were sufficient to detect difference between the Antarctic and the North Pacific minke whales (Fig1a), but five loci were insufficient for revealing unambiguously genetic structure within the Antarctic minke whale. In examining Fig 1a, it should be considered that genetic differences between the Antarctic ordinary form minke whale and North Pacific minke whale are at the level of species differentiation (Wada and Numachi, 1991; Pastene *et al.*, 1994). Some scientists reiterated that an adequate number of loci should be employed in order to have sufficient statistical power to identify potential problems. Thus, it is necessary to isolate novel microsatellite loci which can provide highly polymorphic information of intra-populations.

ACKNOWLEDGMENTS

All samples used in this study were obtained during the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) surveys. Sincere thanks are due to researchers and crew members that participated in those surveys. We are extremely grateful to P.J. Palsbøll (School of Biological Sciences, University of Wales-Bangor, Wales, UK) for providing unpublished data of microsatellite primers.

REFERENCES

Abe, H., Goto, M., Palsbøll, P.J. and Pastene, L.A. 1997. Preliminary microsatellite analyses of western North Pacific minke whales, *Balaenoptera acutorostrata*. Paper SC/49/NP12 presented to the IWC Scientific Committee, October 1997 (unpublished) 12pp.

Abe, H., Goto, M. and Pastene, L.A. 1998. Further microsatellite analyses in the western North Pacific minke whales, *Balaenoptera acutorostrata*. Paper SC/50/RMP8 presented to the IWC Scientific Committee, May 1998 (unpublished) 10pp.

Amos, B. 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.

Avise, J.C. and Ball Jr, R.M. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *Oxford Surveys in Evolutionary Biology*. 7:45-67.

Buchanan, F.C., Friesen, M.K., Littlejohn, R.P. and Clayton, J.W. 1996. Microsatellites from the Beluga whale (*Delphinapterus leucas*). *Mol. Ecol.* 5:258-64.

Hoelzel, A.R. and Dover, G.A. 1991. Mitochondrial D-loop variation within and between populations of the minke whale (*Balaenoptera acutorostrata*). *Rep. int. Whal. Commn.* (special issue 13):171-81.

Hartl, D.L. and Clark, A.G. 1997. Population Substructure. pp. 111-162. *Principles of Population Genetics*. 3rd Edn. Sinauer Associates, Sinauer, Massachusetts. i-xiii+542pp.

International Whaling Commission. 1991. Report of the subcommittee on Southern Hemisphere minke whale. *Rep. int. Whal. Commn.* 41:113-31.

International Whaling Commission. 1997. Report of the scientific comittee. *Rep. int. Whal. Commn.* (in press) 88pp.

Manly, B.F.J. 1997. Distance matrices and spatial data. pp. 172-204. *Randomization, Bootstrap and Monte Carlo Methods in Biology*. 3rd Edn. Chapman & Hall, London. i-xix+399pp.

Moore, S.S., Sargeant, L.L., King, T.J., Mattick, J.S., Georges, M. and Hetzel, D.J.S. 1991. The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics*. 10:654-60.

Paetkau, D. and Strobeck, C. 1994. Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.* 3:489-95.

Palsbøll, P.J., Bérubé, M., Larsen, A.H. and Jørgensen, H. 1997. Primers for the amplification of tri- and tetramer microsatellite loci in cetaceans. *Mol. Ecol.* 6:893-95.

Palumbi, S.R. and Baker, C.S. 1994. Opposing views of humpback whale population structure using mitochondrial and nuclear DNA sequences. *Mol. Biol. Evol.* 11:426-35.

Pastene, L.A., Kobayashi, T., Fujise, Y. and Numachi, K. 1993. Mitochondrial DNA differentiation in Antarctic minke whales. *Rep. int. Whal. Commn.* 43:349-55.

Pastene, L.A., Fujise, Y. and Numachi, K. 1994. Differentiation of mitochondrial DNA between ordinary and dwarf forms of southern minke whale. *Rep. int. Whal. Commn.* 44:277-81.

Pastene, L.A., Goto, M., Itoh, S. and Numachi, K. 1996. Spacial and temporal patterns of mitochondrial DNA variaton in minke whales from Antarctic Areas IV and V. *Rep. int. Whal. Commn.* 46:305-14.

Puers, C., Hammond, H.A., Caskey, C.T., Lins, A.M., Sprecher, C.J., Brinkmann, B. and Schumm, J.W. 1994. Allelic ladder characterization of the short tandem repeat polymorphism located in the 5í flanking region to the human coagulation factor XIII A subunit gene. *Genomics.* 23(1):260-4.

Raymond, M. and Rousset, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity.* 86: 248-9.

Richard, K.R., Whitehead, H. and Wright, J.M. 1996. Polymorphic microsatellites from sperm whales and their use in the genetic identification of individuals from naturally sloughed piece of skin. *Mol.Ecol.* 5:313-5.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullius, K.B. and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science.* 239:487-91.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular Cloning. A Laboratory Manual.* 2nd Edn. Cold Spring Harbour Laboratory, Cold Spring Harbor, New York. i-xxiii+434pp.

Sokal, R.R. and Rohlf, F.J. 1995. *Biometry: the Principles and Practice of Statistics in Biological Research.* W.H.Freeman and Company, New York. i-xix+887pp.

Valsecchi, E. and Amos, W. 1996. Microsatellite markers for the study of cetacean populations. *Mol.Ecol.* 5:151-6.

Van Pijlen, I.A., Amos, B. and Dover, G.A. 1991. Multilocus DNA fingerprinting applied to population studies of the minke whale *Balaenoptera acutorostrata. Rep. int. Whal. Commn.* (special issue 13):245-54.

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.

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.

Wu, C.I. 1991. Inferences of species phylogeny in relation to segregation of ancient polymorphisms. *Genetics.* 127:429-35.

Table 1. Summary of minke whale samples, proportion of male, heterozygosity and total expected probability of identity (PI).

Area	Year	Period	Total (n)	Male	Mean obz. Hz.	Mean exp. Hz.	PI ($x10^{-8}$)
III	95/96 JARPA	26.Nov. - 22.Dec.	69	0.652	0.8783	0.8602	2.633 (Area III total)
III	97/98 JARPA	7.Dec. - 31.Dec.	94	0.649	0.8206	0.8686	-
IV	89/90 JARPA	6.Dec. - 14.Feb.	325*	0.564	0.8711	0.8674	2.377
V	96/97 JARPA	5.Jan. - 12.Mar	330	0.4	0.8706	0.8690	2.042
VI	96/97 JARPA	30.Nov. - 3.Jan.	96	0.680	0.8631	0.8684	2.838

Abbrev . : obz.=observed, exp.=expected, Hz.=Heterozygosity

* except dwarf minke whales

Table 2. Microsatellite loci and allele frequencies in the Antarctic minke whales.

GT211	Area III	Area IV	Area V	Area VI
85	0.037	0.062	0.044	0.057
87	0.046	0.034	0.033	0.031
88	0.009	-	-	-
89	-	0.005	-	0.005
90	-	-	0.002	-
91	-	0.005	-	-
92	0.003	-	0.006	-
94	-	-	0.002	0.005
98	0.006	-	-	-
100	0.021	0.011	0.015	0.010
102	0.046	0.045	0.049	0.057
104	0.067	0.069	0.094	0.052
106	0.227	0.183	0.160	0.182
108	0.150	0.155	0.154	0.130
110	0.117	0.129	0.112	0.109
112	0.101	0.108	0.129	0.109
114	0.083	0.092	0.082	0.089
116	0.037	0.043	0.053	0.073
118	0.034	0.031	0.043	0.042
120	0.006	0.015	0.014	0.026
122	0.003	0.008	0.008	0.016
124	0.006	0.003	0.002	0.005
126	-	0.002	-	-
132	-	0.002	-	-

GATA098	Area III	Area IV	Area V	Area VI
92	0.012	-	-	0.005
96	0.037	0.026	0.020	0.005
100	0.071	0.057	0.034	0.042
104	0.185	0.183	0.132	0.182
108	0.262	0.263	0.283	0.193
112	0.074	0.115	0.135	0.125
116	0.191	0.151	0.194	0.219
120	0.127	0.142	0.150	0.172
124	0.028	0.038	0.040	0.031
128	0.012	0.025	0.014	0.026

EV1 *Pm*	Area III	Area IV	Area V	Area VI
117	-	-	0.002	-
121	-	-	0.002	-
123	0.016	0.018	0.018	0.021
125	0.037	0.040	0.026	0.031
127	0.214	0.206	0.220	0.208
129	0.261	0.260	0.238	0.281
131	0.230	0.243	0.223	0.208
133	0.109	0.112	0.133	0.120
135	0.047	0.051	0.061	0.063
137	0.037	0.023	0.024	0.036
139	0.019	0.018	0.011	0.010
141	0.016	0.017	0.011	0.005
143	0.012	0.011	0.029	0.010
145	0.003	-	-	-
147	-	-	0.002	-
149	-	-	-	0.005
155	-	-	0.002	-
159	-	-	0.002	-

EV104 *Mn*	Area III	Area IV	Area V	Area VI
133	0.006	0.002	-	-
135	0.016	0.011	0.008	0.017
137	0.016	0.019	0.022	0.006
139	0.047	0.043	0.052	0.078
141	0.053	0.061	0.028	0.083
143	0.022	0.043	0.058	0.022
145	0.053	0.050	0.060	0.033
147	0.053	0.048	0.048	0.072
149	0.071	0.099	0.087	0.083
151	0.106	0.140	0.122	0.150
153	0.174	0.154	0.123	0.122
155	0.143	0.127	0.168	0.189
157	0.078	0.102	0.092	0.067
159	0.050	0.040	0.065	0.056
161	0.068	0.043	0.037	0.011
163	0.037	0.017	0.010	0.006
165	0.009	-	0.020	0.006

GT023	Area III	Area IV	Area V	Area VI
88	0.003	0.011	0.005	-
90	0.003	0.006	0.002	0.005
94	0.015	0.012	0.011	0.016
96	0.009	0.006	0.008	0.010
98	0.019	0.028	0.030	0.042
100	0.043	0.028	0.035	0.021
102	0.052	0.039	0.052	0.047
104	0.052	0.051	0.050	0.068
106	0.049	0.076	0.078	0.047
108	0.160	0.146	0.133	0.146
110	0.111	0.110	0.139	0.120
112	0.145	0.136	0.142	0.130
114	0.194	0.194	0.160	0.224
116	0.074	0.094	0.090	0.047
118	0.043	0.037	0.040	0.036
120	0.019	0.006	0.018	0.026
122	0.003	0.012	0.003	0.010
124	0.003	0.006	0.005	0.005
126	-	-	0.002	-
128	-	0.002	-	-

Table 3. Result of three tests for Hardy-Weinberg equilibrium. Probabilities below 5%, 10% are shown as bold and underline respectively, and overall estimation is listed in the right bottom (shaded).

Area III	Likeli. test	Exact test	Homoz. test
EV1 *Pm*	0.4693	0.4683	0.5625
EV104 *Mn*	0.1647	0.1489	0.5726
GATA098	0.1982	0.0640	**0.0106**
GT211	**0.0045**	**0.0033**	0.2259
GT023	0.5982	0.6356	0.9303
prob.	**0.0275**	**0.0102**	0.1522

Area VI	Likeli. test	Exact test	Homoz. test
EV1 *Pm*	**0.0417**	0.1044	0.4138
EV104 *Mn*	0.3900	0.2933	0.6517
GATA098	0.1208	0.1494	0.2648
GT211	0.3140	0.5525	0.3569
GT023	0.1533	0.1248	0.8384
prob.	**0.0466**	0.0962	0.6589

Area IV	Likeli. test	Exact test	Homoz. test
EV1 *Pm*	0.8527	0.9498	0.1016
EV104 *Mn*	0.2931	0.3741	0.9998
GATA098	0.3298	0.2209	0.2474
GT211	0.7786	0.8903	0.1651
GT023	0.7780	0.7938	0.5866
prob.	0.8157	0.8331	0.2826

Area V	Likeli. test	Exact test	Homoz. test
EV1 *Pm*	0.4852	0.6015	0.1369
EV104 *Mn*	0.1683	0.1453	0.0706
GATA098	0.2025	0.2024	0.4064
GT211	0.8480	0.8801	0.0828
GT023	0.4417	0.5507	0.8787
prob.	0.4258	0.4837	0.0908

Overall	Likeli. test	Exact test	Homoz. test
EV1 *Pm*	0.6791	0.8357	**0.0199**
EV104 *Mn*	0.1440	0.1519	0.0841
GATA098	**0.0055**	**0.0014**	**0.0043**
GT211	0.1422	0.2258	0.1255
GT023	**0.0122**	**0.0332**	0.6395
prob.	**0.0020**	**0.0026**	**0.0014**

Note : Overall estimations were done with all the Antarctic samples used in this study (Overall).

Abbrev. : Likeli.=Likelihood ratio, Homoz.=Homozygosity

Table 4. Genetic differentiation between the four minke whale study areas.

Between areas		Microsatellite Loci						Probability	
		GT23	**GATA98**	**EV1 *Pm***	**EV104 *Mn***	**GT211**	-2*Sum(InP)	5% (18.307)	1% (23.209)
Area III	**vs**								
	Area IV	0.6387	**0.0466**	0.9786	0.1149	0.1491	15.2078	NS	NS
	Area V	0.9573	**0.0010**	0.6645	**0.0061**	0.1180	29.2504	**0.00113**	**0.00113**
	Area VI	0.9643	0.0728	0.9700	**0.0352**	0.3461	14.1905	NS	NS
Area IV	**vs**								
	Area V	0.3772	**0.0419**	0.2230	**0.0007**	0.1312	29.9747	**0.00086**	**0.00086**
	Area VI	0.5313	0.1082	0.7231	**0.0339**	0.9079	13.3244	NS	NS
Area V	**vs**								
	Area VI	0.6180	0.0969	0.8793	**0.0210**	0.6750	14.4011	NS	NS

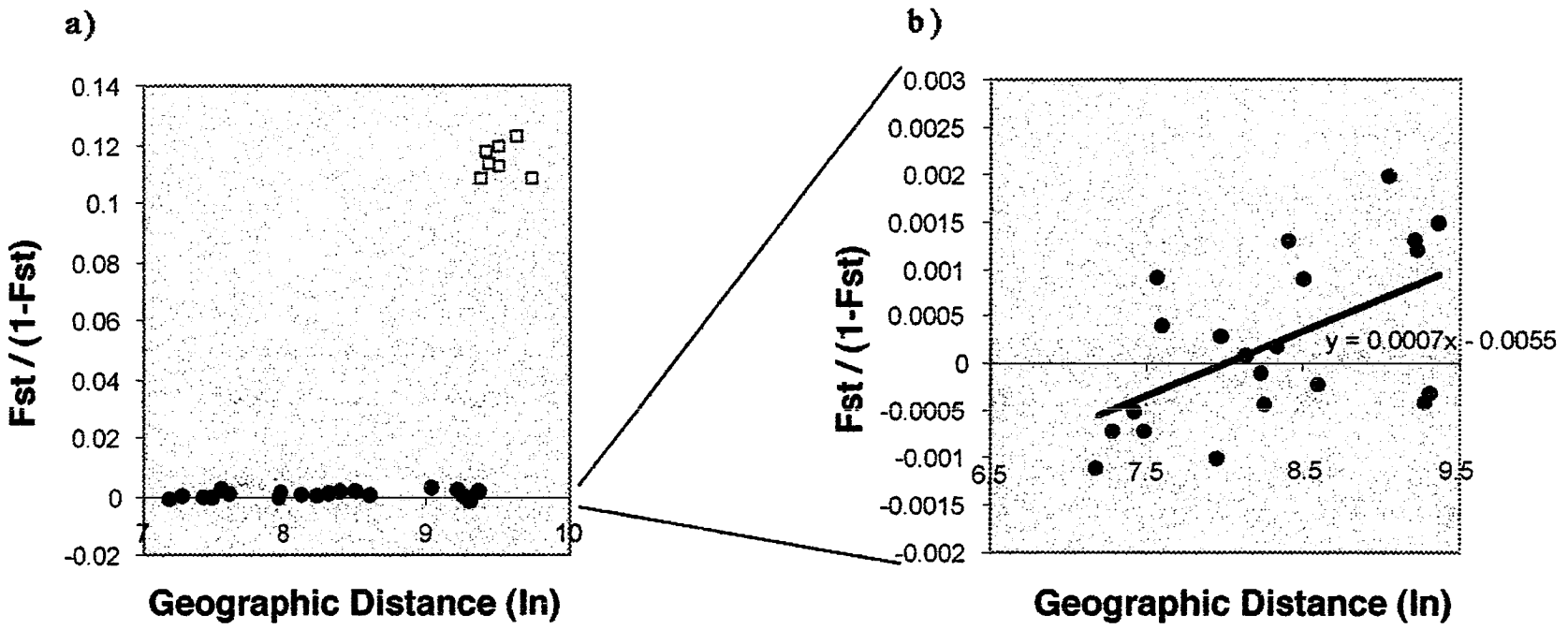

Fig.1 Result of isolation by distance (ISOLDE) analysis. Black circles (●) indicate the comparisons within Antarctic populations ,and open squares (□) denote the comparisons between Antarctic population and North Pacific population.