Preliminary RFLP analysis of mitochondrial DNA in the Antarctic minke whale from Areas III and VI

Luis A. Pastene[1], Hirohisa Kishino[2] and Mutsuo Goto[1]

[1]Ecology Section
The Institute of Cetacean Research
4-18 Toyomi-cho, Chuo-ku,
Tokyo 104, Japan

[2]Department of Social and International Relations
The University of Tokyo
8-1 Komaba 3-chome, Meguro-ku,
Tokyo 153, Japan

ABSTRACT

A restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) in the ordinary form minke whale from Antarctic Areas IV and V is extended for analyzing samples from adjacent Areas III and VI. This analysis was made in order to corroborate a new interpretation on stock identity that suggests that at least two stocks distributes in Areas IV and V: a 'core' stock in Area V, eastern part of Area IV and in the western part of Area IV in a late period of the feeding season and a 'western' stock including whales distributed in the western part of Area IV in an early period. A total of 154 and 134 minke whales were analyzed by the Southern hybridization method in the eastern part of Area III and western part of Area VI, respectively. In order to make data comparable to those obtained in Areas IV and V, the same restriction enzymes employed for examining DNAs in a previous analysis, were used: *Acc*I, *Ban*I, *Eco*RV, *Hin*cII, *Hpa*I and *Ssp*I. Quantification of the mtDNA differentiation among whales from the eastern part of Area III and western part of Area VI with representative samples of the 'western' and 'core' stocks, was carried out using the Analysis of Molecular Variance (AMOVA). Minke whales from Area III east were similar to those from the 'western' stock but differed significantly from whales from the 'core' stock. On the other hand, whales from Area VI west differed significantly from whales of the 'western' stock but not with those of the 'core' stock. These results are considered as preliminary due to the fact that samples from Areas III and VI are from past commercial whaling operations, which targeted whales distributed around the pack-ice. A preliminary simulation study indicated that, under the resolution level of the RFLP analysis in Areas IV and V, a sample size of 150-200 is necessary to detect significant genetic differences among putative stocks of minke whale in the Antarctic.

INTRODUCTION

Derived from a large-scale mtDNA survey in minke whales from Antarctic Areas IV and V (Pastene *et al.*, 1993; 1994; 1996), a new interpretation on stock identity in the ordinary form has been possible.

The survey has revealed considerable mtDNA heterogeneity, but little geographic concordance with these putative management areas. Based on the PHI statistics (PHIst) of the Analysis of Molecular Variance (AMOVA) model (Excoffier *et al.*, 1992), Pastene *et al.* (1996) proposed the occurrence of a 'core' stock distributed in Area V and eastern part of Area IV in most of the years. Also it was proposed that a group of whales distributes in the western part of Area IV early in the feeding season could belong to a neighboring, genetically differentiated stock (a 'western' stock).

These hypotheses suggest that the stock structure of the Antarctic minke whale could be more complex than it was thought initially and it could be determined not only by geographical factors but also by temporal factors. For example it was suggested a temporal component in the distribution of these two stocks in the western part of Area IV (Pastene *et al.*, 1996).

In order to check these hypotheses we have begun an analysis of samples from adjacent Areas III and VI. Samples from the eastern part of Area III were analyzed in order to check the hypothesis that whales distributed in the western part of Area IV during an early period of the feeding season could belong to a 'western' stock probably originated in that neighbor area. On the other hand, we have conducted a preliminary analysis of minke whales from the western part of Area VI in order to investigate the possible longitudinal extension (to the east) of the 'core' stock.

HYPOTHESES ON STOCK IDENTITY IN AREAS IV AND V

The hypotheses on stock identity used in this study are based in the results of a large-scale mtDNA survey conducted on minke whales from Antarctic Areas IV and V, which were sampled randomly during the Japanese Whale Research Program Under Special Permit in the Antarctic (JARPA) surveys between 1987/88 and 1994/95 (Pastene *et al.*, 1996).

Mitochondrial DNA's from minke whales from these areas were analyzed with six polymorphic restriction enzymes. A total of 137 unique mtDNA haplotypes were discriminated on the basis of the combination of the restriction patterns of these enzymes. Haplotypes frequencies and the sequence divergence among these 137 haplotypes were used to analyze genetic relationship among whales grouped under longitudinal and temporal criteria, using the AMOVA model.

Samples from Areas IV and V were divided into four longitudinal sectors and two temporal groups, 'early' and 'late', in each of these sectors. Thus, a total of eight area/time groups were examined. A significant source of mtDNA heterogeneity was attributable to a group of minke whales sampled in the western part of Area IV early in the feeding season. By using the samples combined for eight years, no other significant source of heterogeneity was found (Pastene *et al.*, 1996).

The analyses conducted by Pastene *et al.* (1996) permitted the formulation of the following hypotheses (see Fig. 1):

a) A 'core' stock is distributed in Area V and eastern part of Area IV in most of the years.

b) A different 'western' stock distributes in the western part of Area IV during an early period

of the feeding season. Probably the main body of this 'western' stock originates in adjacent Area III.

c) The 'core' stock invades the western part of Area IV during a late period of the feeding season.

Thus, in the western part of Area IV a temporal component in the distribution of these two stocks was suggested. Another explanation given for this temporal component is that the two stocks mix each other in the western part of Area IV in such a way that the 'western' stock is predominant in the early period and the 'core' stock is predominant in the late period.

Pastene *et al.* (1996) also conducted a preliminary analysis for investigating whether the intra-seasonal pattern of mtDNA variation is the same in every austral summer season. They presented preliminary evidences for yearly variation in the western part of Area IV in a late period of the feeding season and in a small sample (n= 12, 1991/92 season) from the eastern part of Area IV in the early period. This finding is being further investigated, but these preliminary evidences suggest at least two scenarios: a possible 'dynamic' boundary line between stocks or a mixing of stocks in Area IV.

MATERIALS AND METHODS

Samples from Area III

Samples of minke whale from the eastern part of Area III (Figs. 2 and 3) were available from the National Research Institute of Far Seas Fisheries, Fishery Agency, Government of Japan. These samples were from past commercial whaling operations. These minke whales were caught in a short period of time, between 6 and 19 December 1978 (corresponding then to the 'early' period category). A total of 154 samples (including muscle, heart and testis) were analyzed in this study. These tissues had been stored at -20°C for a period of 17 years before their use for genetic analysis.

Samples from Area VI

Samples from the western part of Area VI (Figs. 2 and 4) were available from the Department of Environment Conservation of the Ehime University. Tissue samples used were from minke whales caught during past commercial whaling operations, between 3 December 1985 and 11 January 1986 (corresponding then to the 'early' period category). A total of 134 samples (including liver and skin) were analyzed from this group.

Samples from Areas IV and V

Samples from Areas IV and V were from minke whales sampled during the JARPA surveys in these areas. Samples from the 1987/88 through 1993/94 austral summer seasons were used in this study (see Pastene *et al.*, 1996).

Biochemical method for examining samples from Area III and VI

Samples from Areas IV and V obtained during the JARPA surveys had been already analyzed by a quick and inexpensive miniprep procedure described by Pastene *et al.* (1993). Under this procedure, crude mtDNA was extracted from 0.3g of frozen liver and then it was directly used for digestion with six-base recognition restriction enzymes. That procedure was useful for JARPA liver samples stored at -20°C for a period up to 7 years.

In the case of samples from Area III and VI, it was not possible the application of the miniprep procedure because in some cases liver tissues were not available. On the other hand, long period of storage combined with non-severe storing conditions had degraded notably the DNA from these samples. An alternative approach, sensitive enough to detect small amount of remaining DNA on one hand and producing analogous data to the miniprep procedure on the other hand, had to be used for examining minke whales from Areas III and VI. We choose the Southern hybridization method for examining minke whales from those areas.

DNA was extracted from muscle, heart, testis or skin tissues. Total DNA (mtDNA+nuclear DNA) was extracted from 0.05g of frozen tissue by the standard technique (Sambrook *et al.*, 1989). In order to make data comparable with those obtained for Areas IV and V, total DNA was digested with the same six restriction enzymes used in the previous analyses in those areas: *Acc*I, *Ban*I, *Eco*RV, *Hinc*II, *Hpa*I and *Ssp*I. Restriction fragments were separated by submarine electrophoresis in 1.2% agarose gels (Takara LO3) using a TPE buffer system and transferred to nylon membranes (Hybond N) by Southern blotting. The hybridization was carried out with a probe of either the Antarctic minke whale or the North Pacific Risso's dolphin mtDNA, labeled with digoxigenin-dUTP with the random primed DNA labeling procedure (Boehringer Mannheim Laboratory). Detection was carried out by enzyme immunoassay as described by Boehringer Mannheim Laboratory.

RFLP analysis

Distinctive restriction fragment patterns produced by each enzyme were assigned letters and individuals were assigned haplotypes consisting of a list of the letters designating the fragment profiles produced by each restriction enzymes. Thus, the composite haplotype for each individual comprises a string of six letters.

Individuals from Areas IV and V had already been characterized for their haplotypes in a previous study (Pastene *et al.*, 1996).

Statistical analysis

We used the AMOVA model for quantifying the temporal and geographical differentiation of mtDNA. The AMOVA program (version 1.55) calculates variance components from a distance matrix and the PHIst reflecting the correlation of haplotypic 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 each trial, 2,000 randomization's of the original data sets were made. The level of significance obtained by this procedure is referred in this paper as the P-value.

Samples from Areas III and VI (as described above) were statistically compared with representative samples of the 'western' and 'core' stocks as defined in Pastene *et al.* (1996).

The sample of the 'western' stock was defined by the group of whales from the western part of Area IV sampled in the early period of the feeding season (including samples from the 1989/90 and 1991/92 JARPA surveys, n= 160). The sample of the 'core' stock was from longitudes 100°E and 160°E (including samples from the 1987/88-1993/94 JARPA surveys, n= 889).

Samples from the western part of Area IV in the late period were not considered here because the preliminary evidences of yearly variation in this group (Pastene *et al.*, 1996). For the same reason, a small sample of the 1991/92 season (n= 12) from the eastern part of Area IV in the early period, was also excluded from the sample representative of the 'core' stock. (see Discussion section in Pastene *et al.*, 1996). We still examining and interpreting the results of yearly variation in these area/time groups, derived from our previous study. In a future analysis, samples from Areas III and VI should be also compared with samples from these groups ordered by years.

The eastward extension of the 'core' stock was limited to 160° E despite the fact that whales from this 'stock' did not differ significantly from whales from the eastern part of Area V (160°E-170°W). This was done because the sample size of group V east early is small (n= 63) and then not significant differences with this group not necessarily means genetic homogeneity.

RESULTS

Restriction fragment patterns

Figure 5 shows examples of restriction fragment patterns produced by six restriction enzymes in minke whale samples from Area III, obtained by the Southern hybridization method. DNA in the samples shown had been isolated from testis tissues. Two JARPA samples were also added for comparison. Restriction fragment patterns produced by these six restriction enzymes using the Southern hybridization methods are analogous to those produced by the miniprep procedure in Areas IV and V in all the cases. Similar results were found when tissues such as muscle, heart and skin were used.

Mitochondrial DNA haplotypes

Pastene *et al.* (1996) discriminated a total of 137 unique mtDNA haplotypes in 2,124 minke whales from Areas IV and V. The analysis involving samples from Areas III and VI discriminated 16 new haplotypes (haplotypes '138' through '153'), eight specific for Area III, six specific for Area VI and two common to both areas.

Table 1 shows the haplotype frequencies in both the 'western' and 'core' stocks samples as well as those in the samples from Areas III eastern early and VI western early. In all these groups haplotype '1' was the predominant haplotype.

Statistical comparison

Tables 2A and 2B shows the results of the comparisons between the sample from Area III east early with both the samples of the 'western' and 'core' stocks. The overall PHIst value was 0.007, which was significant (P= 0.0020) (Table 2A). Table 2B shows the results of the pairwise comparisons. The sample from Area III east early differed significantly (at the 5% significance level) from the sample of the 'core' stock but not from the sample of the 'western' stock.

Tables 3A and 3B shows the results of the comparisons between the sample from Area VI with the 'western' and 'core' stocks. The overall PHIst value was 0.007, which was significant (P= 0.0040) (Table 3A). Table 3B shows the results of the pairwise comparisons. Sample from Area VI western early differed significantly (at the 5% significance level) from the

sample of the 'western' stock but not with that of the 'core' stock.

DISCUSSION

A new interpretation of the stock identity in the ordinary form minke whale from Antarctic Areas IV and V was proposed by Pastene *et al.* (1996). Such interpretation suggests that at least two stocks distributes in Areas IV and V. A 'core' stock is present in Area V, eastern part of Area IV and western part of Area IV in a late period of the feeding season. A groups of whales in the latter sector, sampled in an early period of the feeding season, could contain individuals from a neighboring, genetically differentiated 'western' stock, probably originated in Area III (Pastene *et al.*, 1996). In order to examine these hypotheses, we extended our mtDNA analysis for examining samples of minke whale from the eastern sector of adjacent Area III.

On the other hand, we have conducted a preliminary analysis to investigate the possible extension (to the east) of the proposed 'core' stock. This analysis was made by examining some samples of the minke whale available from the western part of Area VI.

In the comparison involving Area III, we found that this sample was significantly different with the 'core' stock but not with the 'western' stock. On the other hand, the sample from Area VI showed significant differences with the 'western' stock but not with the 'core' stock. These preliminary results suggest that the main body of the 'western' stock could be distributed in the eastern part of Area III and that the 'core' stock could extend its distribution into the western part of Area VI.

These results support the interpretation on stock identity given by Pastene *et al.* (1996). However, they should be considered as preliminary and with caution. Sampling procedures in Areas IV and V (by JARPA) are notably different from those of the commercial operations in Areas III and VI. Minke whales examined from Areas IV and V were sampled at random and the sampling had covered both, offshore areas and areas around the pack ice. In contrast, all the minke whales from the eastern part of Area III and western part of Area VI were caught not at random and all they were concentrated around the pack ice.

In the case of minke whales from the eastern sector of Area III, we have examined samples from the early group (December) only. It will be necessary the examination of samples from the late period of the feeding season in that sector. Furthermore, as it can be observed from Fig. 2, the early group of the eastern part of Area III has a more southerly distribution with regard the early group of whales of the western part of Area IV sampled by JARPA. This reflect the nature of the whaling operations, which targeted whales concentrated around the pack-ice. The northern component of the early group should be examined in future. In addition, in the case of Area III east, whales were taken in a short period of time, from 6 to 19 December. Thus the sample examined from Area III is not representative of all the whales distributed in that sector during the feeding season. With this regard, it should be noted that the PHIst value between 'western' and 'core' stocks is 0.0107 (both stocks involve samples randomly taken by JARPA). From Table 2A we can see that the comparison between the sample from Area III east and the 'core' stock present a lower PHIst value (0.0039), although it was significant (P= 0.0440).

With regard samples from the western part of Area VI, it should be noted that these samples were taken during a larger time period within the feeding season than in the case of Area III. However, all these samples were distributed around the pack-ice.

Unlike the case of the western part of Area IV, where using sample from JARPA we have found considerable mtDNA heterogeneity, in the eastern part of Area V we have been unable to demonstrated such heterogeneity (Pastene *et al.*, 1996). However, as these authors discussed, sample size for the early group in that sector is low and no conclusion can be reached yet. If a different stock is distributed in that sector in the early period (like in the case of Area IV west), we should expect that the main body of such stock is distributed in the western sector of Area VI. Our analysis of samples from such sector indicated no significant differences with a representative sample of the 'core' stock. This preliminary result can be interpreted in two ways. First, there is no stock segregation between the 'core' stock and whales from the western part of Area VI, or second, our RFLP analysis has low power for detecting segregation.

Concern has been expressed for situations where stock structure decisions may be based on negative data (no significant value difference among sampled populations) (Dizon and Perrin, 1995). No significant value differences could mean either that there is no population segregation within the sample or that there was inadequate power of the analysis to detect segregation (IWC, 1996). Low power can be the result of small sample size, low resolution due to the small portion of the genome examined, or low effect size (the expected amount of genetic differentiation between populations) (IWC, 1996). If the effect size is high, then the number of samples required in a genetic analysis should be small. Larger number of samples will be necessary if the effect size is low.

In the case of the Southern Hemisphere ordinary form minke whale it seems that the effect size is low as demonstrated by the low PHIst values found in Areas IV and V. Then the expected sample size necessary to detect genetic differences between putative populations could be large. Thus fails to detect differentiation between the 'core' stock and the sample from Area VI, could reveal low power due to small sample size.

Under the assumptions that a third stock present in the western part of Area VI presents a degree of genetic differentiation similar to that found between the 'western' and 'core' stocks in Areas IV and V, we have conducted a simulation study to estimate the sample size necessary to detect such differences. This simulation study is based in the degree of resolution of our RFLP in Areas IV and V.

The sample size in the 'core' stock is 889 and that in the 'western' stock is 160. In our procedure we kept constant the sample size for the latter stock, but 500 different sets of haplotypes frequencies distributions were constructed randomly from the 'core' stock, for sample size of n=50, n=100, n=125, n=150 and n=200. Using AMOVA, each simulated frequency distribution was then compared with that of the 'western' stock. Then, a total of 2,500 statistical comparisons were made.

Fig. 6 shows that the number of cases yielding significant differences increase between n=50 and n=150, for all the cases. After n=150, the curves stabilize between 40 and 50% of the cases for level of significance of 1%, between 70 and 80% of the cases for level of significance

of 5% and between 80 and 90% of the cases for level of significance of 10%.

We concluded that, under the resolution level of our mtDNA RFLP analysis in the Antarctic minke whale, a sample size of 150-200 individuals is necessary to detect significant genetic differences between putative stocks. In the case of the Area III, a sample size of n=154 detected significant differences between Area III east early and the 'core' stock. On the other hand in Area VI west a sample size of n= 134 detected significant differences with the 'western' stock but not with the 'core' stock. If the degree of genetic differentiation between a hypothetical third stock occurring in Area VI west and the 'core' stock are similar or smaller than that between the 'western' and 'core' stocks, then the actual sample size in Area VI could have failed to detect significant genetic differences.

Finally it should be noted from Tables 2A and 3A, a tiny contribution of molecular variance among groups compared with the variance within group, although significant heterogeneity was detected. This could indicate either an overlap in the distribution of stocks in the areas examined or that genetic isolation among them is not complete. Analysis of samples of minke whales from low latitudes, where we expect a larger degree of isolation among stocks, could assist the interpretation of genetic variation found in the Antarctic. The analysis of such samples has been recommended by the IWC Scientific Committee (IWC, 1996).

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REFERENCES

Dizon, A.E. and Perrin, W.F. 1995. Report of a Workshop on Analysis of Genetic Data to Address Problems of Stock Identity as Related to Management. Paper SC/47/Rep3 presented to the IWC Scientific Committee, May 1995 (unpublished). 66pp

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479-91.

International Whaling Commission. 1996. Report of the Scientific Committee. *Rep. int. Whal. Commn* 46 (in press).

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., Goto, M., Fujise, Y. and Numachi, K. 1994. Further analysis on the spatial and temporal heterogeneity in mitochondrial DNA haplotype distribution in minke whales from Antarctic Areas IV and V. Paper SC/46/SH13 presented to the IWC Scientific Committee, May 1994 (unpublished). 25pp.

Pastene, L.A., Goto, M., Itoh, S. and Numachi, K. 1996. Spatial and temporal patterns of mitochondrial DNA variation in minke whales from Antarctic Areas IV and V. *Rep. int. Whal. Commn* 46 (in press).

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular cloning: A laboratory manual.* 2nd Ed., Cold Spring Harbor Laboratory, NY.

Table 1: Distribution of mtDNA haplotypes in representative samples of the 'western' and 'core' stocks and in samples from Area III eastern early (Area IIIEE) and from Area VI western early (Area VIWE). In parenthesis is the sample size.

Hap. ID	'Core' stock (889)	'Western' stock (160)	Area IIIEE (154)	Area VIWE (134)
1	296	38	49	44
2	84	8	15	7
3	48	8	5	3
4	70	12	13	7
5	33	5	4	3
6	10	7	0	1
7	18	5	4	5
8	25	11	6	2
9	5	3	1	0
10	22	8	7	12
11	12	2	1	4
12	16	1	5	0
13	3	3	2	0
14	28	3	0	4
15	6	2	0	2
16	2	0	1	0
17	1	0	0	0
18	2	0	0	1
19	13	3	2	2
20	6	0	0	2
21	4	1	4	0
22	7	3	1	1
23	14	0	2	1
24	4	0	0	3
25	0	2	0	0
27	1	1	2	1
28	2	2	0	2
29	2	0	2	0
30	8	3	1	2
31	3	1	1	1
32	2	0	0	0
34	6	0	0	0
36	1	0	0	1
39	4	0	0	2
40	1	0	0	0
41	0	1	0	0
42	2	1	3	0
43	1	0	0	1
44	2	1	0	0
45	1	0	0	0

Table1: cont.

46	1	1	0	0
47	19	4	0	5
48	3	0	0	0
49	1	1	0	0
50	1	1	0	0
51	6	2	0	1
52	3	1	0	0
53	1	2	0	0
54	4	2	1	0
55	5	1	3	0
56	2	1	0	0
57	0	1	0	0
58	1	2	0	0
59	2	0	0	0
60	1	1	0	0
61	4	0	0	1
62	7	1	1	0
63	1	0	0	0
65	0	1	0	0
66	1	0	0	0
67	0	1	0	0
68	4	0	0	1
69	1	0	0	0
70	3	0	0	0
73	1	0	0	0
74	1	0	0	0
75	3	0	0	0
76	0	0	1	0
77	1	0	0	0
78	1	0	0	0
79	4	0	0	0
80	1	0	0	0
82	2	0	1	0
84	1	0	1	0
85	1	0	0	0
86	1	0	0	0
87	1	0	0	0
89	0	0	0	1
92	1	0	0	1
93	1	0	0	0
95	1	0	1	0
96	1	0	0	0
97	1	0	0	0
98	1	0	0	0
102	1	0	0	0
104	2	0	0	1

Table 1: cont.

105	2	0	0	0
106	1	0	0	0
107	1	0	0	0
108	1	0	0	0
110	1	0	0	0
111	1	0	0	0
114	0	1	0	0
115	0	1	0	0
116	2	0	0	0
117	0	0	2	0
118	1	0	0	0
121	2	0	0	0
122	1	0	0	0
123	3	0	0	0
124	1	0	0	0
125	1	0	0	0
126	1	0	0	0
128	1	0	0	0
130	1	0	0	0
131	1	0	1	1
132	1	0	0	0
133	1	0	0	0
134	1	0	0	0
135	1	0	0	0
136	0	0	1	0
138	0	0	1	0
139	0	0	1	0
140	0	0	1	0
141	0	0	1	0
142	0	0	1	0
143	0	0	1	0
144	0	0	1	0
145	0	0	1	1
146	0	0	1	1
147	0	0	1	0
148	0	0	0	1
149	0	0	0	1
150	0	0	0	1
151	0	0	0	1
152	0	0	0	1
153	0	0	0	1

Table 2A: Results of the nested analysis of molecular variance applied to three groups of minke whales: a sample from Area III eastern early, 'western' and 'core' stocks. V(A) and V(B) are the molecular variances among and within groups, respectively.

V(A)	V(B)	PHIst	P
0.67%	99.33%	0.007	0.0020

Table 2B: Haplotypic correlation (PHIst below diagonal) and their probabilities (P above the diagonal) among a sample from Area III eastern early (Area IIIEE, n= 154), the 'western stock' (n= 160) and the 'core' stock (n= 889).

	Area IIIEE	'Western' stock	'Core' stock
Area IIIEE		0.6222	0.0440
'Western stock'	-0.0015		0.0020
'Core' stock	0.0039	0.0107	

Table 3A: Results of the nested analysis of molecular variance applied to three groups of minke whales: a sample from Area VI western early, 'western' and 'core' stocks. V(A) and V(B) are the molecular variances among and within groups, respectively.

V(A)	V(B)	PHIst	P
0.66%	99.34%	0.007	0.004

Table 3B: Haplotypic correlation (PHIst below diagonal) and their probabilities (P above the diagonal) among a sample from Area VI western early (Area VIWE, n= 134), the 'western' stock (n= 160) and the 'core' stock (n= 889).

	Area VIWE	'Western' stock	'Core' stock
Area VIWE		0.0350	0.2129
'Western' stock	0.0083		0.0000
'Core' stock	0.0011	0.0107	

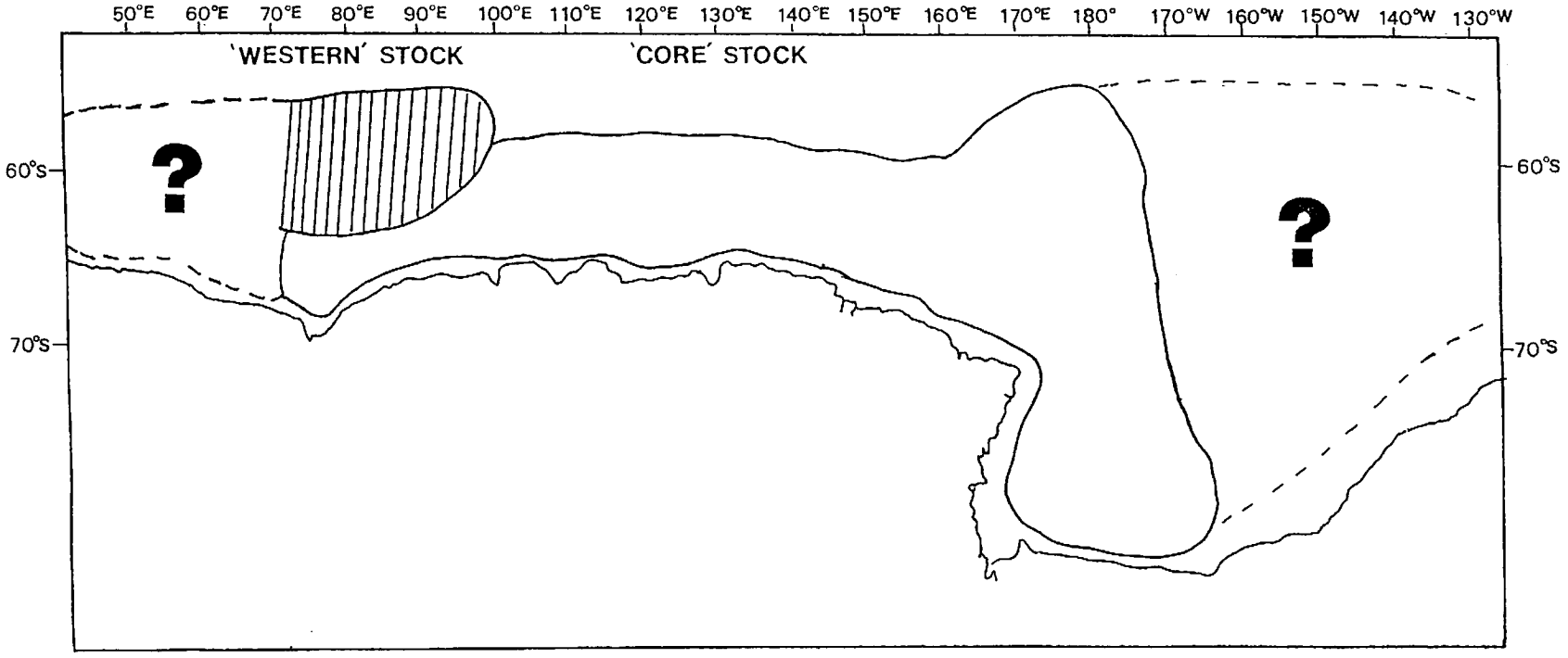

Fig. 1: Schematic representation of the hypothesis on stock identity in the Antarctic ordinary form minke whale from Areas IV and V. The hypothesis is derived from the results of an extensive mtDNA RFLP analysis conducted using minke whales from Areas IV and V, sampled during JARPA surveys. The hypothesis suggests that at least two stocks distributes in Areas IV and V. A 'core' stock distributes in Area V and eastern part of Area IV and a 'western' stock distributes in the western part of Area IV during an early period of the feeding season. The 'core' stock distributes in the western part of Area IV late in the season. Thus, a temporal component in the distribution of these stocks is suggested for the western part of Area IV. The temporal component could indicate either: 1) in the western part of Area IV the 'western' stock is present in the early period and the 'core' stock is present in the late period when the 'western' stock leave that sector or 2) two socks mix each other in the western part of Area IV in such a way that the 'western' stock is predominant in the early period and the 'core' stock is predominant in the late period. Preliminary evidence of yearly variation in this pattern of intraseasonal variation was given and discussed by Pastene *et al.* (1996).

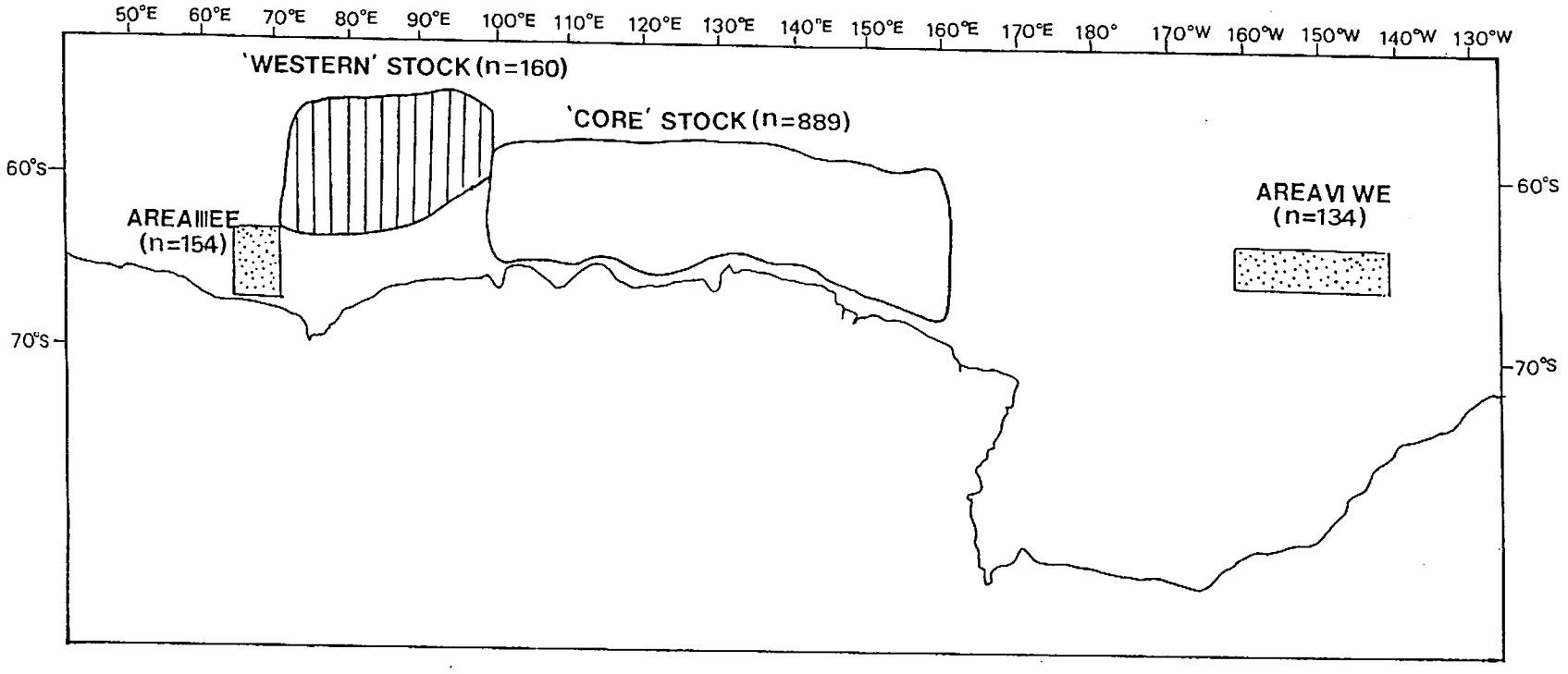

Fig. 2: Geographical distribution of representative samples of the 'western' and 'core' stocks and those from the eastern part of Area III in the early period (Area IIIEE) and western part of Area VI in the early period (Area VIWE). Samples obtained during JARPA surveys from 1987/88 to 1993/94 austral summers were used for 'western' and 'core' stocks. Samples from the western part of Area IV in the late period were not considered within the 'core' stock because preliminary evidences of yearly variation in this group. On the other hand, samples from the eastern part of Area V were not considered in the 'core' stock despite the fact that whales from this stock did not differ significantly from whales from the eastern part of Area V (Pastene *et al.*, 1996). This was done because the sample size of group VEE is small (n= 63) and not significant differences with this group not necessarily means genetic homogeneity.

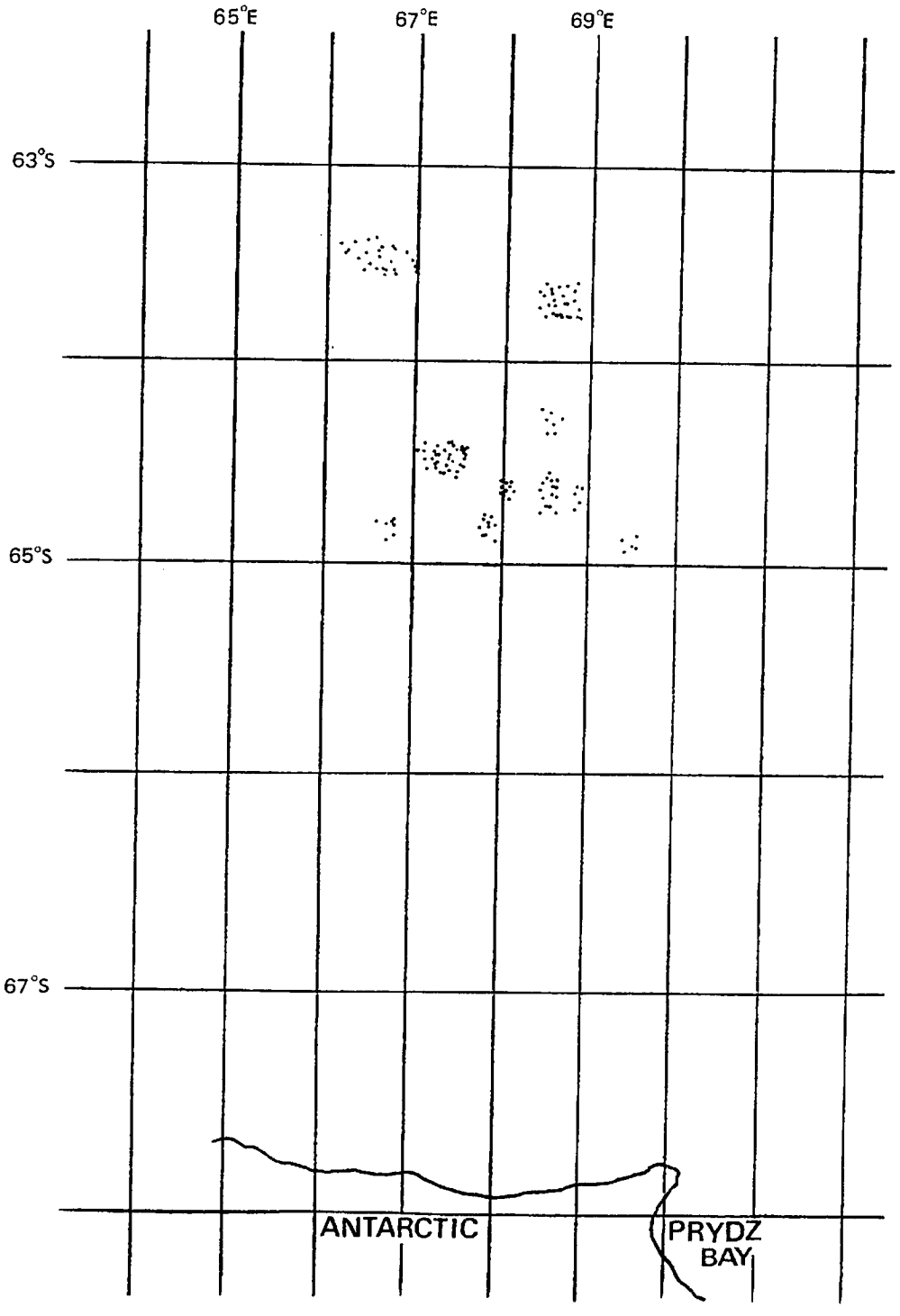

Fig. 3: Geographical distribution of samples from Area III eastern early. Samples are from limited longitudinal and latitudinal ranges. All these samples were taken around the pack ice in a short time period: from 6 to 19 December 1978.

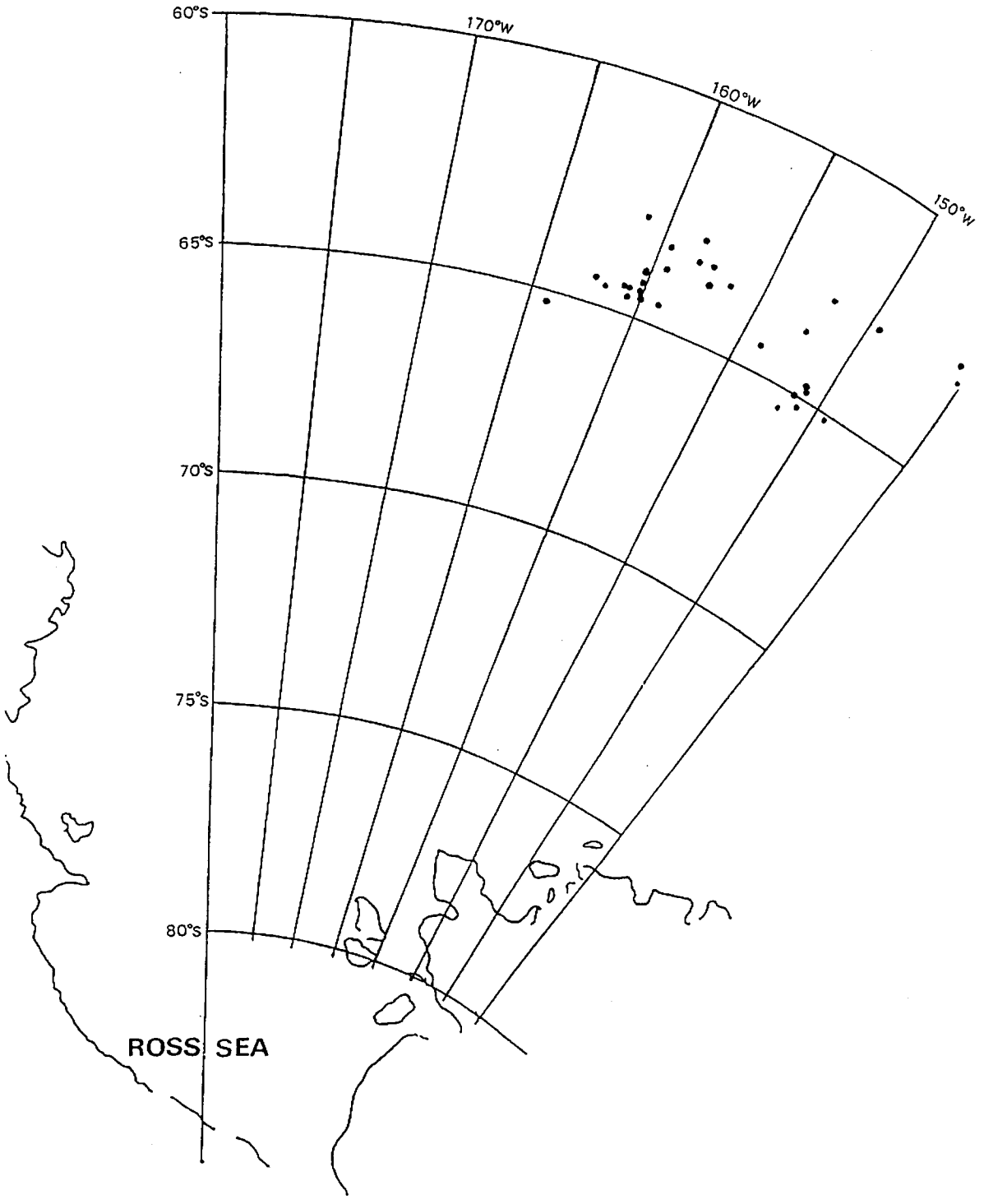

Fig. 4: Geographical distribution of samples from Area VI western early. As in the case of Area III, the samples are from a limited latitudinal and longitudinal range. All the samples were taken around the pack ice between 3 December 1985 and 11 January 1986.

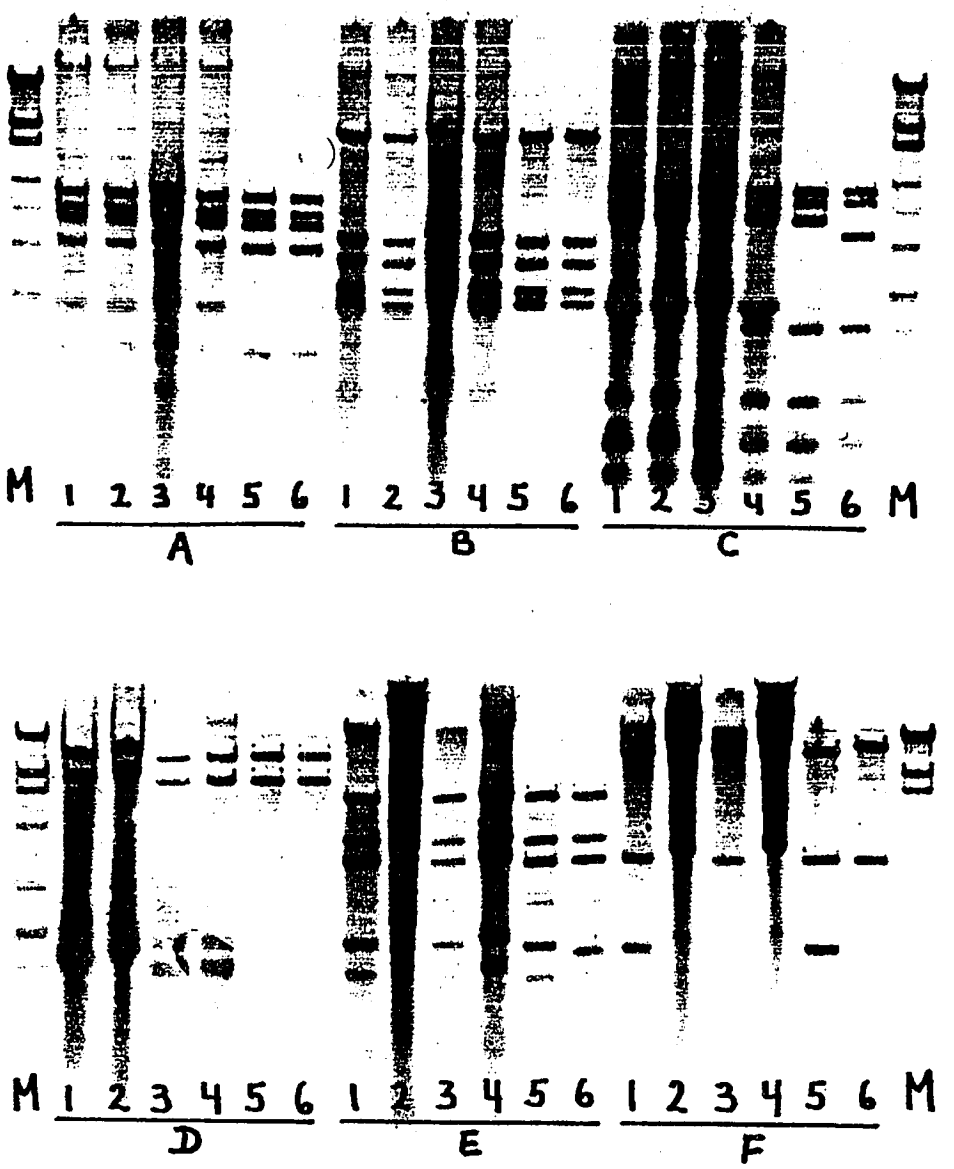

Fig. 5: Restriction fragment patterns of six restriction enzymes used to examine mtDNA by the Southern hybridization method: A= *Acc*I, B= *Ban*I, C= *Hin*cII, D= *Eco*RV, E= *Ssp*I and F= *Hpa*I. M is the lambda DNA marker added to check fragment position and size. Samples 1 through 4 are DNA extracted from testis tissues of minke whales from Area III. Samples 5 and 6 are DNA extracted from liver tissues of minke whales from Area IV sampled by JARPA.

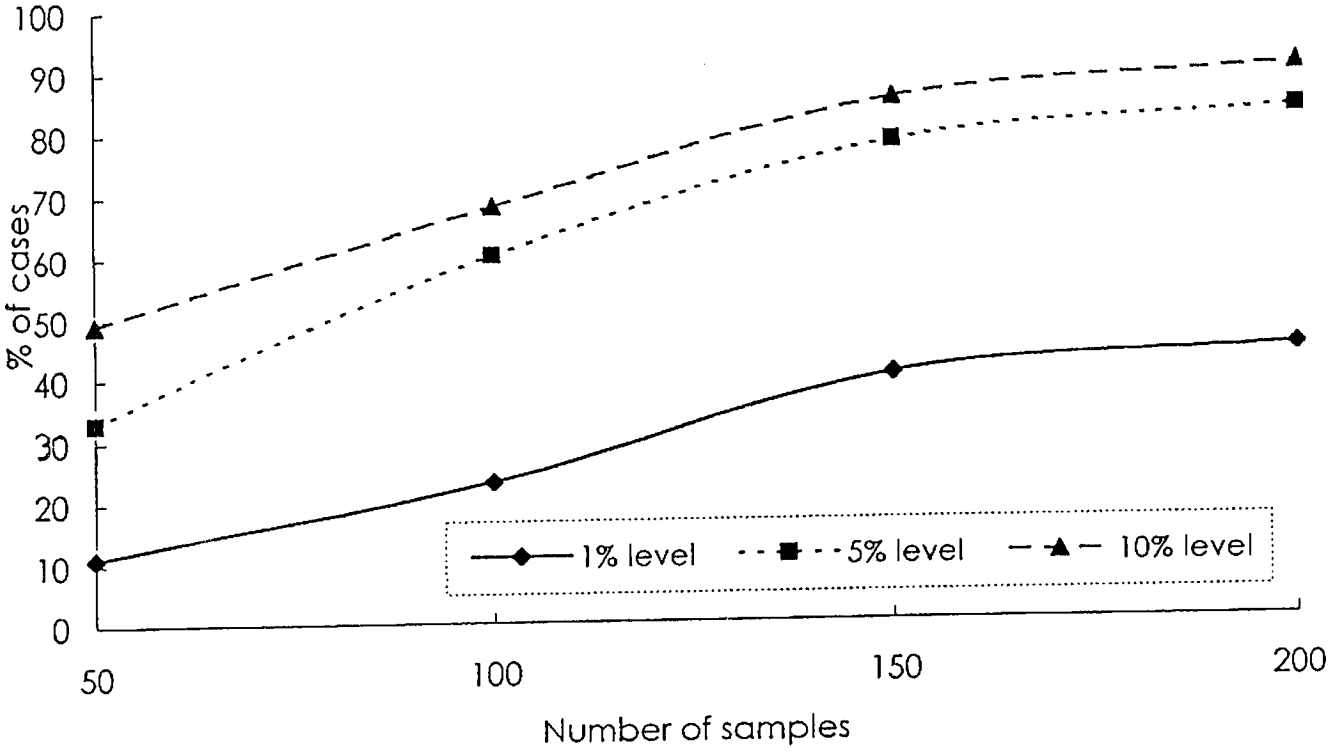

Fig. 6: Relationship between sample size and percentage of cases (in 500 simulated cases) that showed significant mtDNA differences (at the 1, 5 and 10% significance levels) in comparisons between simulated haplotype frequencies obtained from the 'core stock' and the haplotype frequency of the 'western stock'