SC/52/RMP7

A note on additional mtDNA analysis on western North Pacific minke whale using JARPN samples

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ABSTRACT

The Workshop to review JARPN recommended several new mtDNA analyses to elucidate whether or not additional stock structure exist in sub-area 9. One of the DNA tasks was the estimation of possible correlation across space and/or time with genetic distances between partitions (e.g. Mantel test) and to assess the effect of different stratification. In this note we present the results of such exercise based on mtDNA sequencing data and JARPN samples in sub-areas 7, 8 and 9 in the eastern side of Japan. Results obtained for different stratification of the data set suggests no significant correlation between geographical distances and genetic distances. The mtDNA heterogeneity found in sub-area 9 in 1995 can be explained by a relatively high frequency of a specific haplotype in the sample of the western part of sub-area 9 in 1995, in comparison with the frequencies of such haplotype to the west and east of that sector. Such differences could be reflecting either i) an additional stock structure in sub-area 9 or ii) sampling bias.

KEYWORDS: NORTH PACIFIC, MINKE WHALE, JARPN, STOCK IDENTITY, MANTEL TEST, MANAGEMENT

INTRODUCTION

In a study on stock identity in the Pacific side of Japan presented to the Workshop to review JARPN (Anonymous, 2000), certain degree of mtDNA heterogeneity in sub-area 9 attributed to samples taken in the western side of this sub-area in 1995 was found (Goto *et al.*, 2000). Such result was discussed and the Workshop agreed that further analyses of these data should be carried out to explore this finding further, using stratification differing from those adopted for the sub-areas (Anonymous, 2000).

Annex I of the Workshop Report lists several tasks to be conducted using mtDNA data: i) to estimate possible correlations across space and/or time with genetic distances between partitions (e.g. Mantel test), ii) stratification of samples based in additional parameters (e.g. oceanographical features, distribution of sighting data) and iii) combination with other factors such as biological parameters, fat content, putative migration routes and prey preference (Anonymous, 2000).

Regarding to ii) above Okamura *et al.* (2000) conducted a generalized additive model (GAM)-based analysis of density index of minke whales in the western North Pacific. In their analysis, the process for estimating the density indices was divided into two parts: estimation of the effective search half-width and estimation of the encounter rate. They found that the best model for the first included the wind speed and sightability as covariates and the best for the encounter process included the season, position, sea temperature and the interaction between season and position. They were unable to find any hiatus in the distribution of minke whales in the western North Pacific. If any hiatus had been found then DNA data could have been contrasted between area on either side of such hiatus. Regarding to tasks iii) no analysis has been made.

This paper reports results related to task i) above. To address this task Mantel tests were conducted on several partitions of the JARPN samples of sub-areas 7, 8 and 9.

DATA AND METHODS

Isolation by distance (ISOLDE)

The relationship between genetic and geographical distances was examined by the analysis of isolation by distance. The natural logarithm (ln) of the geographic distance (in kilometers) was plotted against Nei's genetic distance (Nei, 1987) and Fst (Weir, 1990). To test the degree of correlation between genetic distances and the geographical distances, the mantel test was used (Mantel, 1967). The statistical differences of the observed test statistic was found by comparison with 10,000 random permutations of the geographical distance matrix, with a new test statistic estimated each time. The significance level was defined as the proportion of permutations in which the test statistic was equal to or more extreme than the observed value.

Grouping of samples

First we used the total samples from the JARPN taken in the eastern side of Japan. We defined 11 groups as shown in Fig. 1. As shown in this figure for some years (e.g. 1994 and 1999) only a single group was defined due to the small number of samples in these years. In other years two or three groups were defined. Table 1 shows the date of sampling, latitudinal and longitudinal ranges and number of samples for each of these groups.

In the second approach we concentrated in the analysis within years. By considering the number of samples in the eastern side of Japan, we choose the JARPN surveys of 1995, 1997 and 1998. In each of these surveys we defined five, six and four longitudinal groups, respectively. Tables 2A-C shows the date of sampling, latitudinal and longitudinal ranges and number of samples for each of these groups in each year, respectively. Figs. 2-4 show the localities of each group by year.

RESULTS AND DISCUSSION

Fig. 5 shows the relationship between genetic distances and geographical distances using the eleven groups defined in the eastern side of Japan. Fig. 5A refers to Nei's genetic distance while Fig. 5B refers to Fst/(1-Fst). Mantel tests showed that no significant association exists between genetic distances over the geographical scale studied (P=0.2090 and P=0.3304, respectively).

Because Annex I of the Workshop Report suggested that partitions should be kept relatively narrow in terms of space and time to avoid putting potentially heterogeneous samples within same partition, we conducted the same analyses within year. Figs. 6, 7 and 8 show these relationships for the longitudinal groups defined in 1995, 1997 and 1998, respectively. In none of these years a significant association between genetic distances (Nei's distance and Fst) and geographical distances was observed (for 1995 P=0.2090 and P=0.3304, respectively; for 1997 P=0.1012 and P=0.8331, respectively; for 1998 P=0.7518 and P=0.9611, respectively).

In summary the analysis of ISOLDE do not provide new information on the genetic population structure in the western North Pacific and then such analysis can not explain the degree of genetic heterogeneity found in the previous mtDNA analysis (Goto *et al.*, 2000). To understand the origin of the mtDNA heterogeneity, we investigated the distribution of the mtDNA sequencing haplotypes in longitudinal sectors of the western North Pacific (Table 3). We found that the frequency of a specific haplotype ('9'), is notably different between sub-areas 7+8 and sub-area 9. This frequency is particularly high in the western part of sub-area 9 in 1995 (Table 3).

At this stage we are not sure whether such difference in frequency is suggestive of the occurrence of a different stock in sub-area 9 in 1995 or simply reflect a sampling bias. Given the fact that no significant deviation from the Hardy-Weinberg equilibrium was observed in this sub-area (Abe *et al.*, 2000), probably the second explanation is the correct. However, it is desirable to complete the other Workshop tasks in order to reach a definitive conclusion on the possibility of additional stock structure in sub-area 9.

ACKNOWLEDGMENTS

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Table 1. Latitudinal and longitudinal ranges, date of sampling and sample size of eleven groups used in the test of isolation by distance.

Group	Year	Date	Sub-area	Lat.(N)	Long.(E)	n
I	1994	14July-4Sep.	9	44.19-48.54	158.31-169.30	21
II	1995	26June-14July	9	40.33-42.09	157.12-160.15	39
III	1995	21July-2Aug.	9	43.56-45.58	157.56-161.44	38
IV	1995	7Aug.-15Aug.	9	41.58-47.03	164.03-168.13	21
V	1996	21July-9Aug.	8	41.31-44.44	152.50-156.50	16
VI	1996	26Aug.-5Sep.	7	41.10-43.03	143.14-145.43	30
VII	1997	8May-19June	9	36.42-40.25	158.25-166.45	67
VIII	1997	4July-14July	8	41.36-42.22	152.49-156.24	31
IX	1998	5May-28May	7	38.59-40.32	145.08-149.58	56
X	1998	28May-13June	8	39.19-41.10	150.02-152.22	44
XI	1999	19June-30June	7	40.58-42.48	141.37-145.15	50
Total						413

Table 2. Latitudinal and longitudinal ranges, date of sampling and sample size of groups used in the test of isolation by distance in 1995 (A), 1997 (B) and 1998 (C).

A) 1995 JARPN

Group	Sub-area	Date	Latitude (N)	Longitude (E)	n
1	9	29June-23July	40.34-45.21	157.12-157.59	20
2	9	28June-24July	40.33-40.18	158.01-158.59	24
3	9	26June-27July	40.40-45.24	159.04-159.59	16
4	9	23June-2Aug.	40.49-47.43	160.07-161.44	18
5	9	18June-15Aug.	41.58-47.03	164.03-169.46	22

B) 1997 JARPN

Group	Sub-area	Date	Latitude (N)	Longitude (E)	n
1	8	10July-14July	41.45-42.14	153.43-154.34	19
2	8	6July-12July	41.36-42.19	155.10-156.24	11
3	9	8May-19June	36.42-39.50	158.25-161.52	18
4	9	29May-9June	38.43-39.28	162.00-162.59	20
5	9	11May-11June	38.22-40.25	163.01-163.56	15
6	9	11May-18June	38.40-40.24	164.00-165.19	12

C) 1998 JARPN

Group	Sub-area	Date	Latitude (N)	Longitude (E)	n
1	7	5May-21May	38.59-39.50	145.08-145.50	23
2	7	7May-28May	39.55-40.32	149.02-149.58	25
3	8	28May-13June	39.19-41.10	150.02-150.45	17
4	8	29May-10June	39.47-40.37	151.30-152.22	27

Table 3. Distribution of mtDNA sequencing haplotype by longitudinal sector.

Hap.	<157°E	<=158°E	<=159°E	<=160°E	<=161°E	<=162°E	>162°E	SA7,8,9 Total(A)	(A)-(B)	1995W(B) (157-162°E)
1	7 (0.030)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	2 (0.024)	9 (0.022)	9 (0.026)	0 (0.000)
2	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
3	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
4	1 (0.004)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	1 (0.002)	1 (0.003)	0 (0.000)
5	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)
6	9 (0.039)	1 (0.050)	3 (0.111)	0 (0.000)	0 (0.000)	2 (0.154)	3 (0.036)	18 (0.043)	14 (0.041)	4 (0.051)
7	25 (0.109)	2 (0.100)	3 (0.111)	0 (0.000)	2 (0.087)	1 (0.077)	7 (0.083)	40 (0.096)	35 (0.103)	5 (0.064)
8	7 (0.030)	1 (0.050)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	2 (0.024)	10 (0.024)	9 (0.026)	1 (0.013)
9	20 (0.087)	3 (0.150)	5 (0.185)	3 (0.143)	7 (0.304)	4 (0.308)	8 (0.095)	50 (0.120)	32 (0.094)	18 (0.231)
10	1 (0.004)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	1 (0.002)	1 (0.003)	0 (0.000)
11	10 (0.043)	3 (0.150)	2 (0.074)	1 (0.048)	2 (0.087)	0 (0.000)	2 (0.024)	20 (0.048)	13 (0.038)	7 (0.090)
12	7 (0.030)	0 (0.000)	1 (0.037)	1 (0.048)	0 (0.000)	1 (0.077)	4 (0.048)	14 (0.033)	12 (0.035)	2 (0.026)
13	21 (0.091)	0 (0.000)	1 (0.037)	1 (0.048)	0 (0.000)	0 (0.000)	2 (0.024)	25 (0.060)	24 (0.071)	1 (0.013)
14	12 (0.052)	0 (0.000)	0 (0.000)	3 (0.143)	1 (0.043)	2 (0.154)	5 (0.060)	23 (0.055)	19 (0.056)	4 (0.051)
15	8 (0.035)	0 (0.000)	0 (0.000)	0 (0.000)	1 (0.043)	1 (0.077)	4 (0.048)	14 (0.033)	12 (0.035)	2 (0.026)
16	12 (0.052)	3 (0.150)	0 (0.000)	2 (0.095)	2 (0.087)	0 (0.000)	4 (0.048)	23 (0.055)	19 (0.056)	4 (0.051)
17	11 (0.048)	2 (0.100)	1 (0.037)	0 (0.000)	3 (0.130)	0 (0.000)	10 (0.119)	27 (0.065)	22 (0.065)	5 (0.064)
18	4 (0.017)	1 (0.050)	0 (0.000)	1 (0.048)	1 (0.043)	0 (0.000)	0 (0.000)	7 (0.017)	5 (0.015)	2 (0.026)
19	3 (0.013)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	1 (0.012)	4 (0.010)	4 (0.012)	0 (0.000)
20	3 (0.013)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	0 (0.000)	3 (0.007)	3 (0.009)	0 (0.000)
Total	230	20	27	21	23	13	84	418	340	78

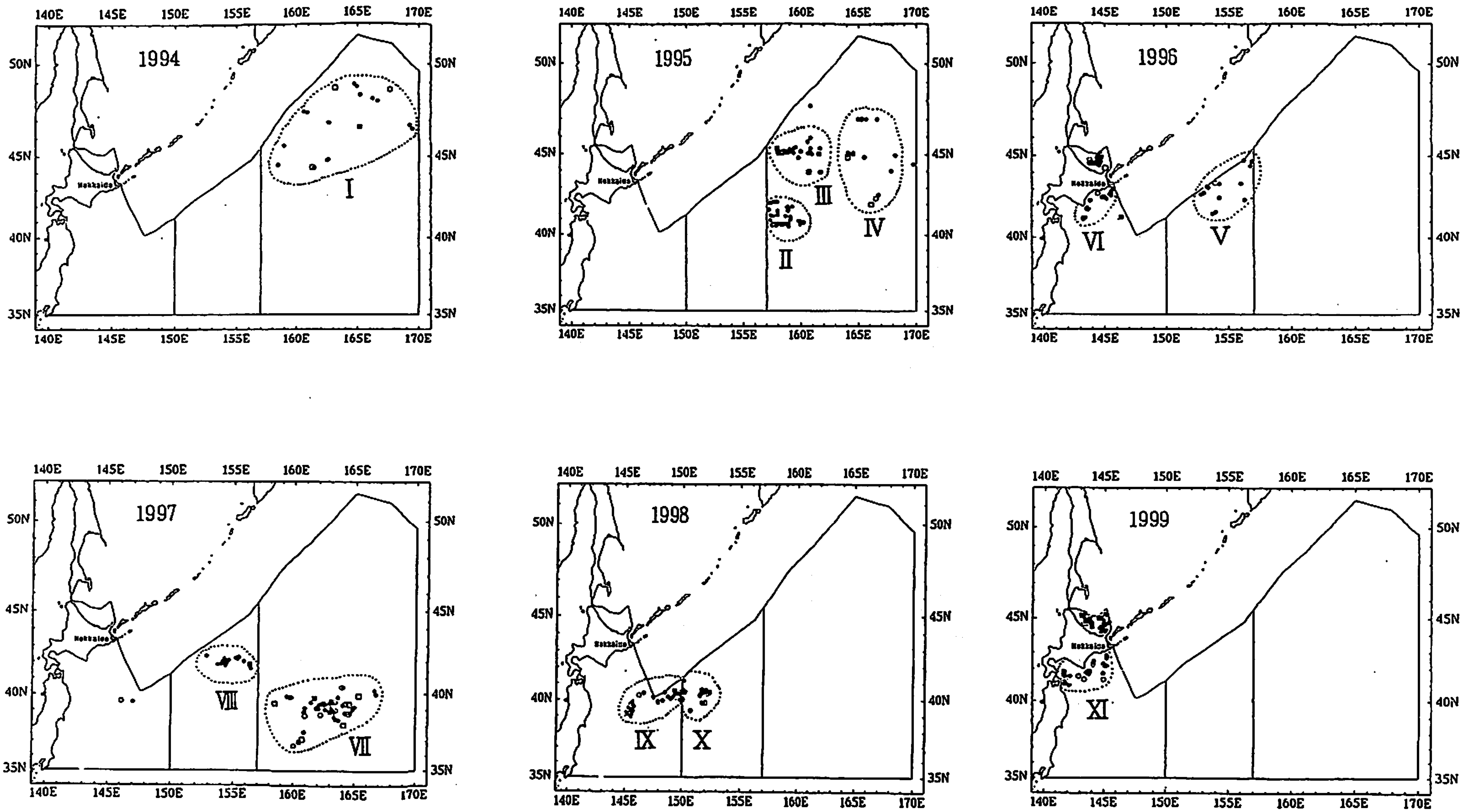


Fig. 1 Geographical distribution of eleven groups used in the test of isolation by distance by year.

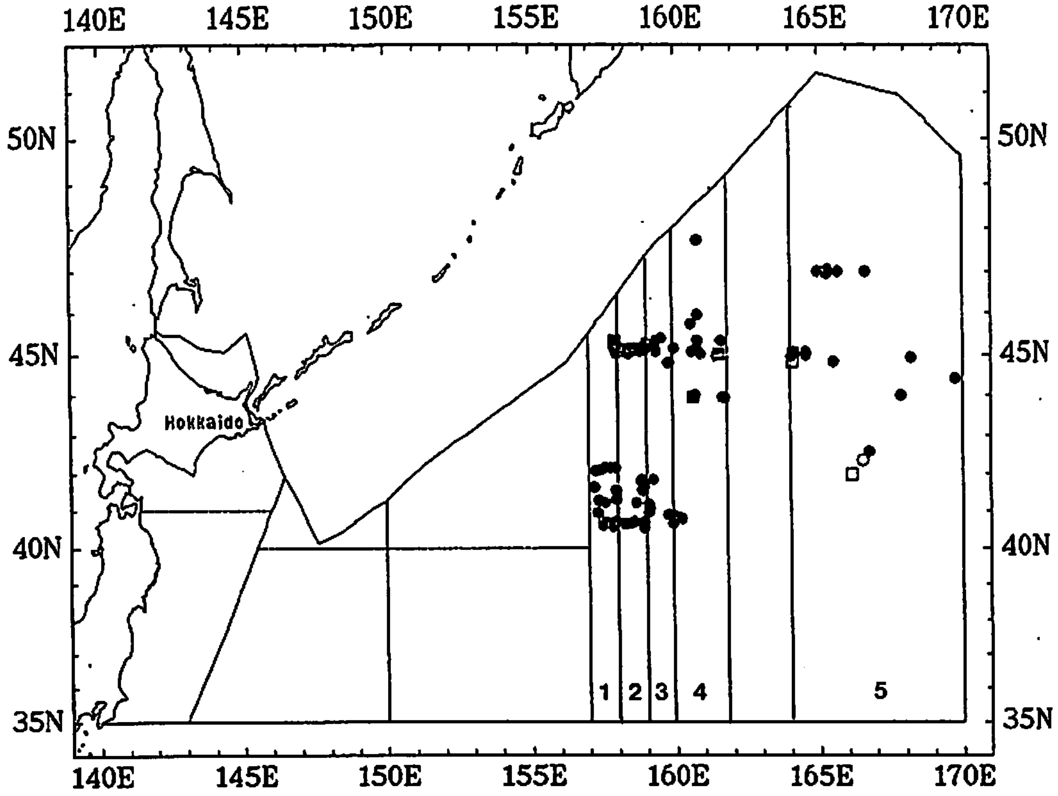


Fig. 2 Geographical distribution of five longitudinally groups in the 1995 JARPN survey used in the test of isolation by distance.

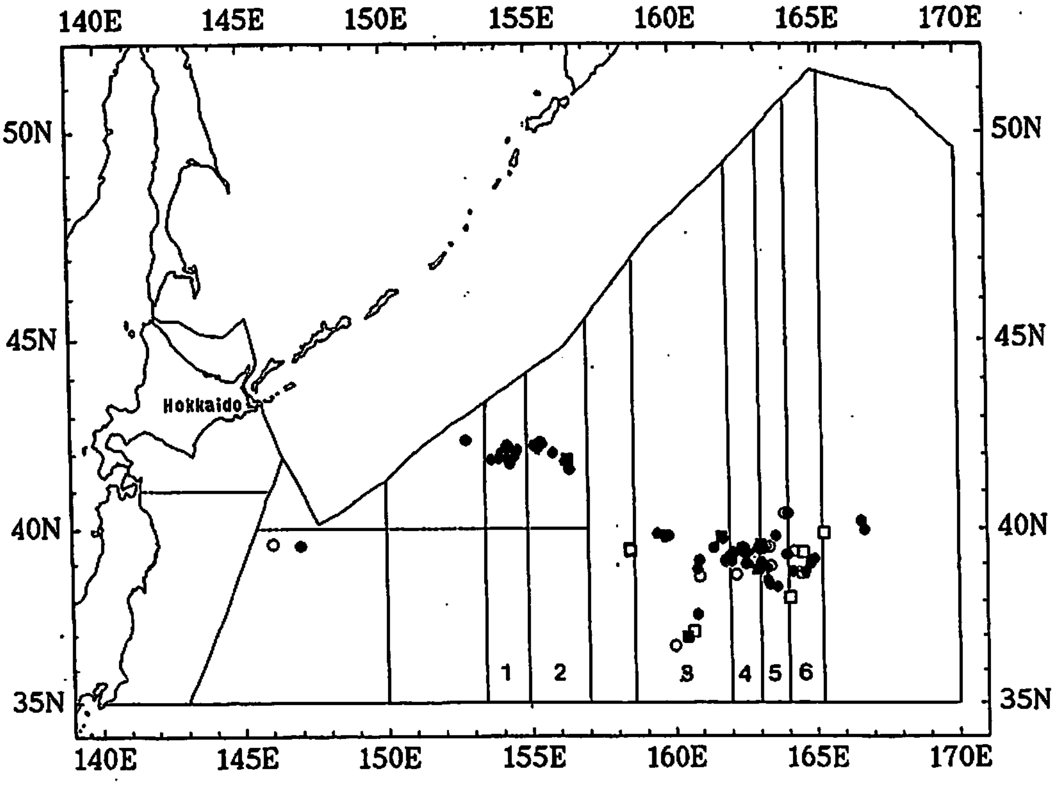


Fig. 3 Geographical distribution of six longitudinally groups in the 1997 JARPN survey used in the test of isolation by distance.

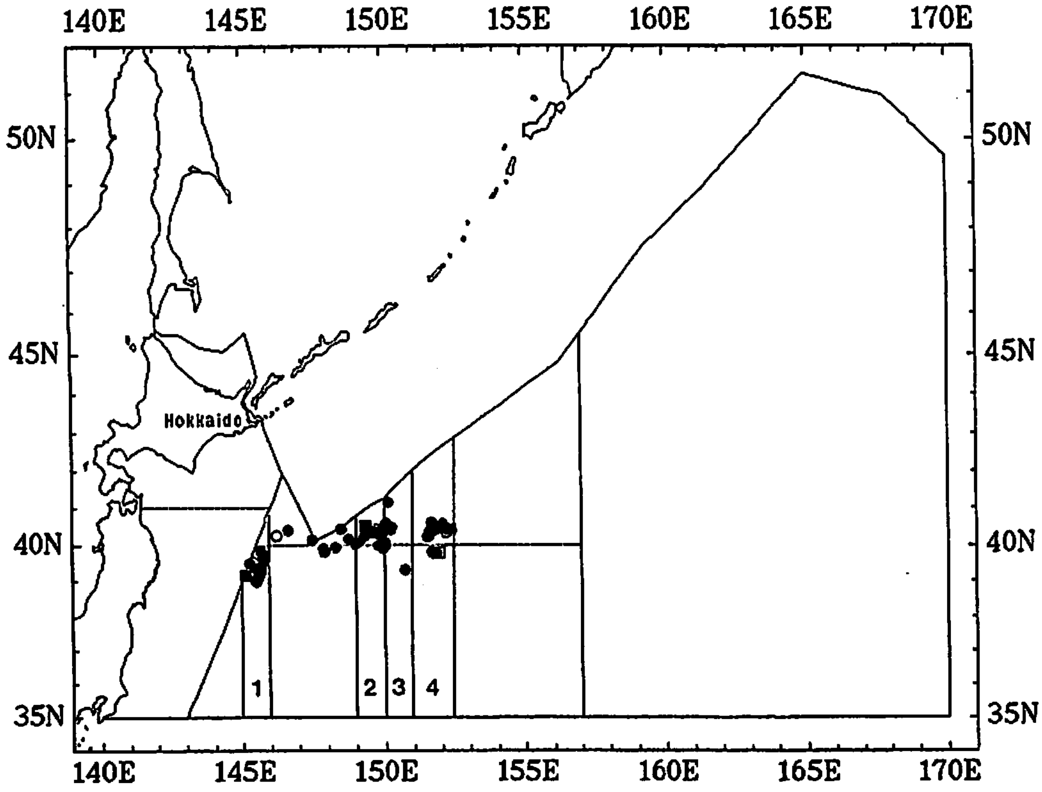
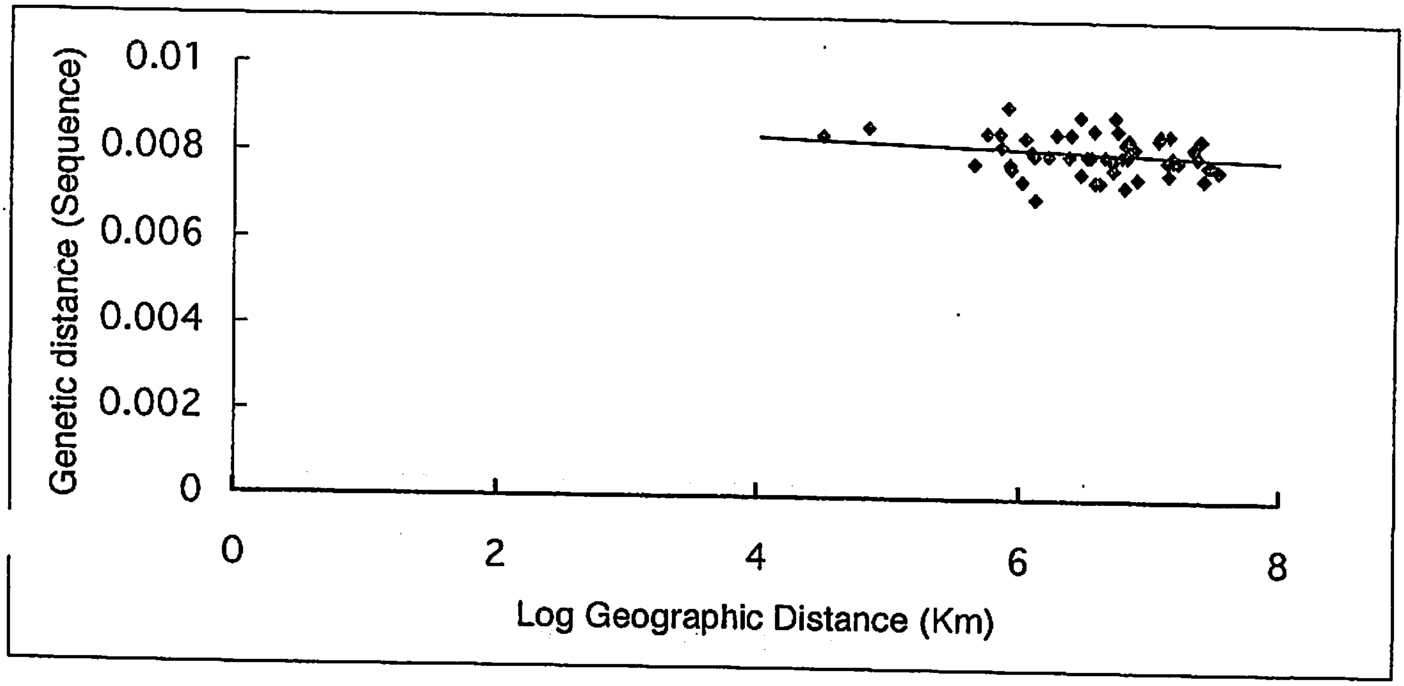


Fig. 4 Geographical distribution of five longitudinally groups in the 1998 JARPN survey used in the test of isolation by distance.

A.



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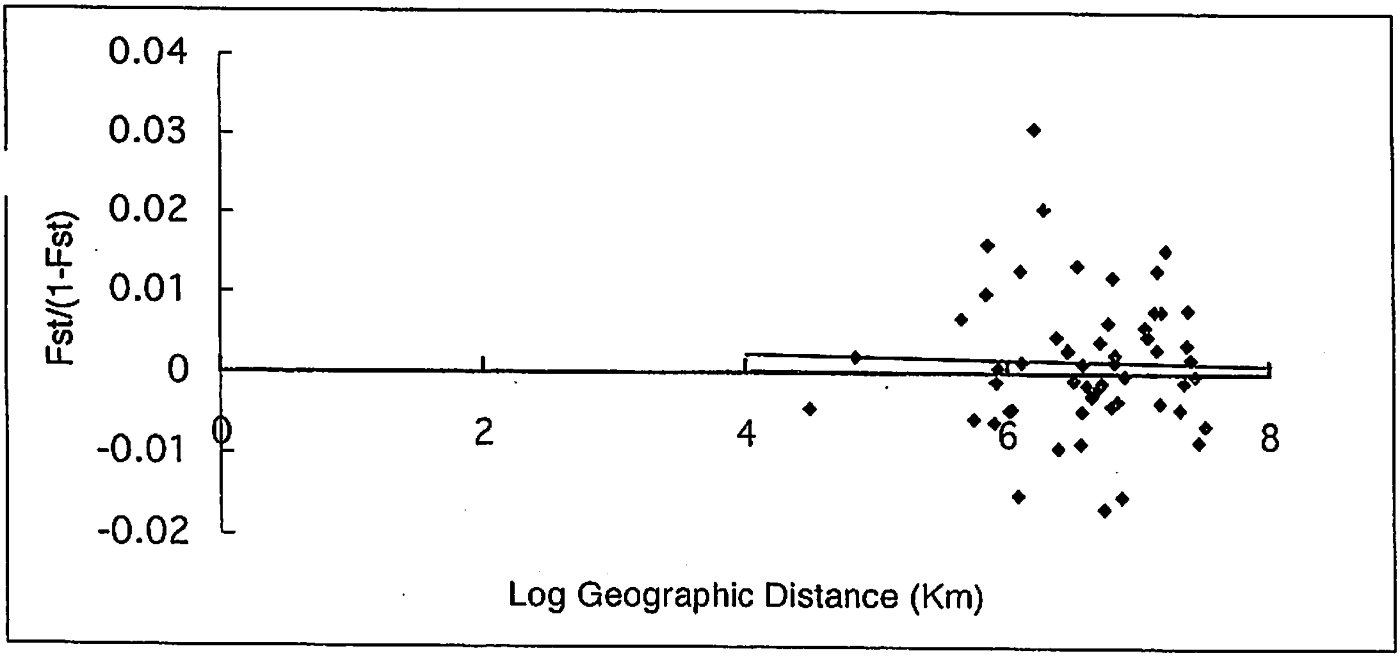
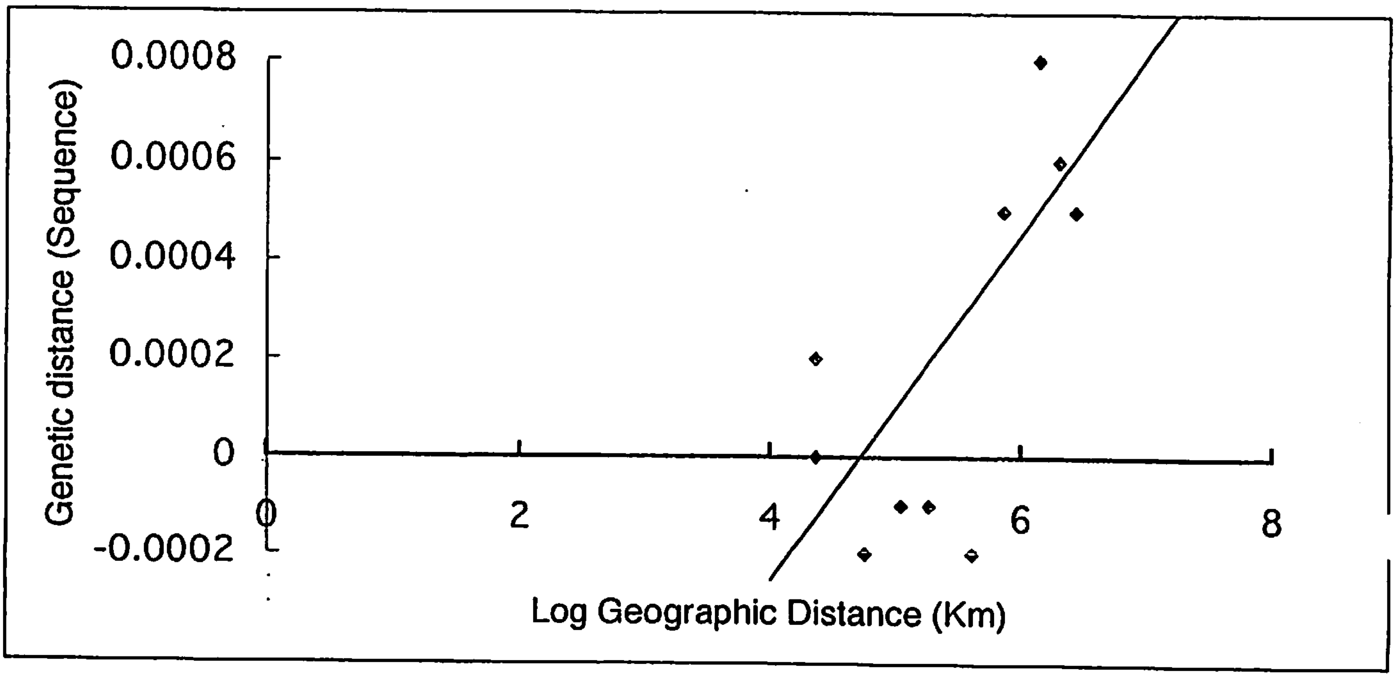


Fig. 5 Plot of the natural logarithm (ln) of the geographical distance against (A) Nei's genetic distance and (B) Fst/(1-Fst) among eleven groups of samples from 1994-1999.

A.



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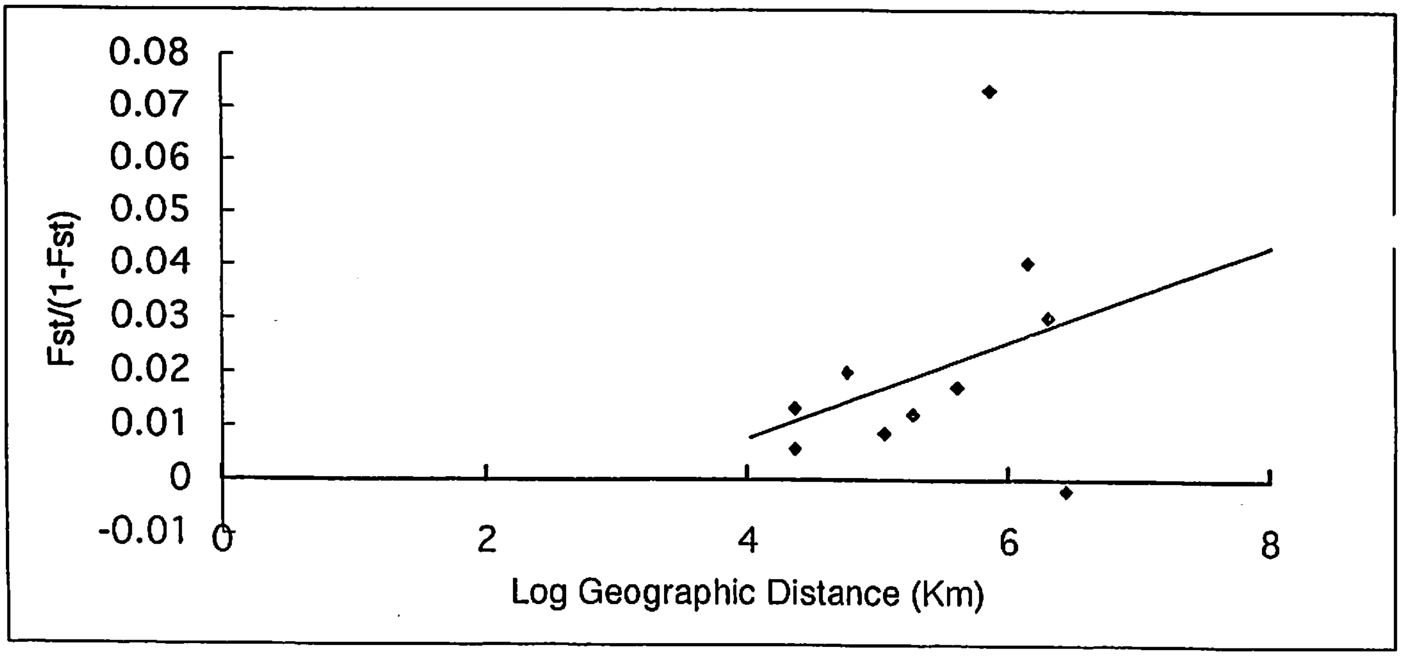
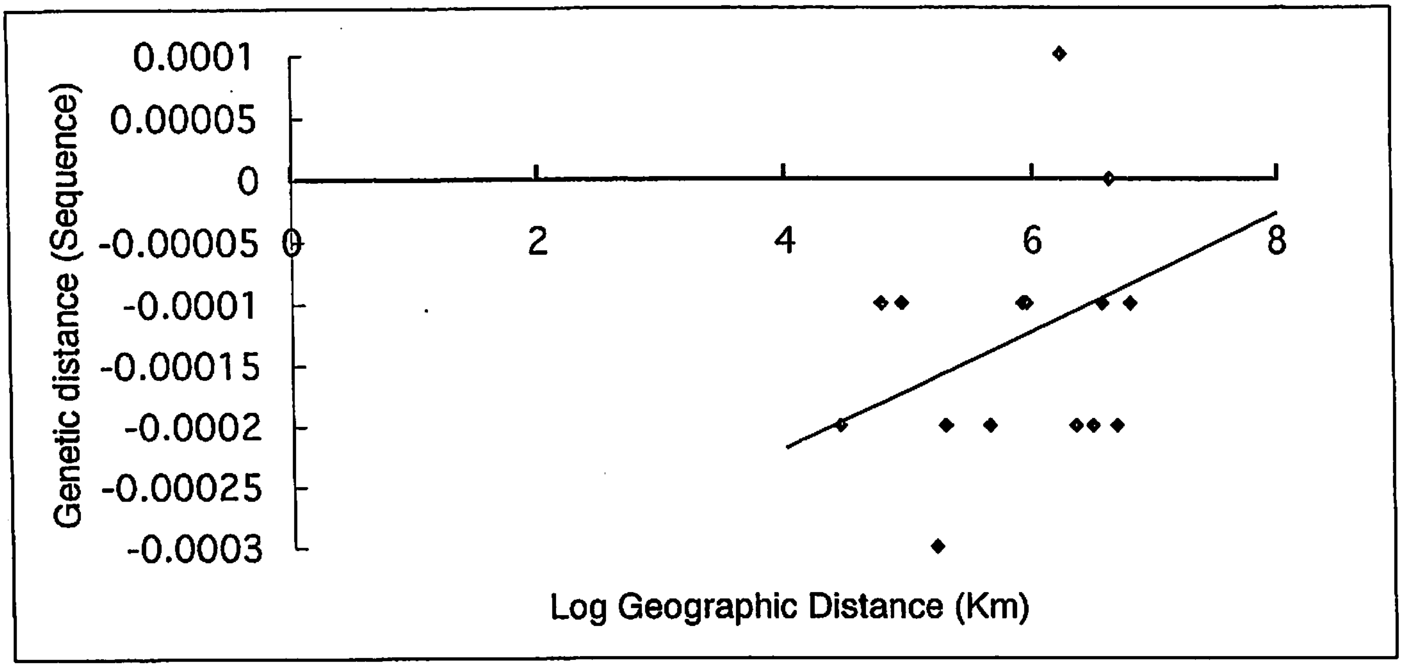


Fig. 6 Plot of the natural logarithm (ln) of the geographical distance against (A) Nei's genetic distance and (B) Fst/(1-Fst) among five groups within 1995.

A.



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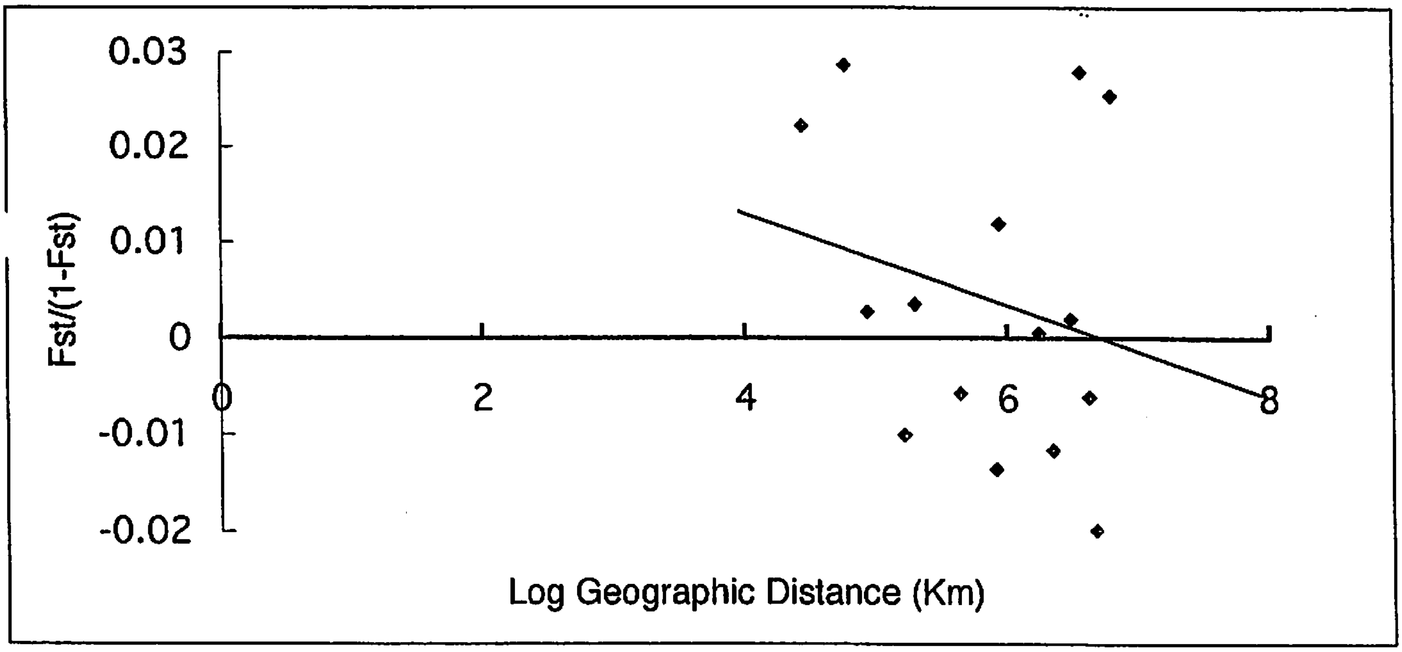

Fig. 7 Plot of the natural logarithm (ln) of the geographical distance against (A) Nei's genetic distance and (B) Fst/(1-Fst) among six groups within 1997.

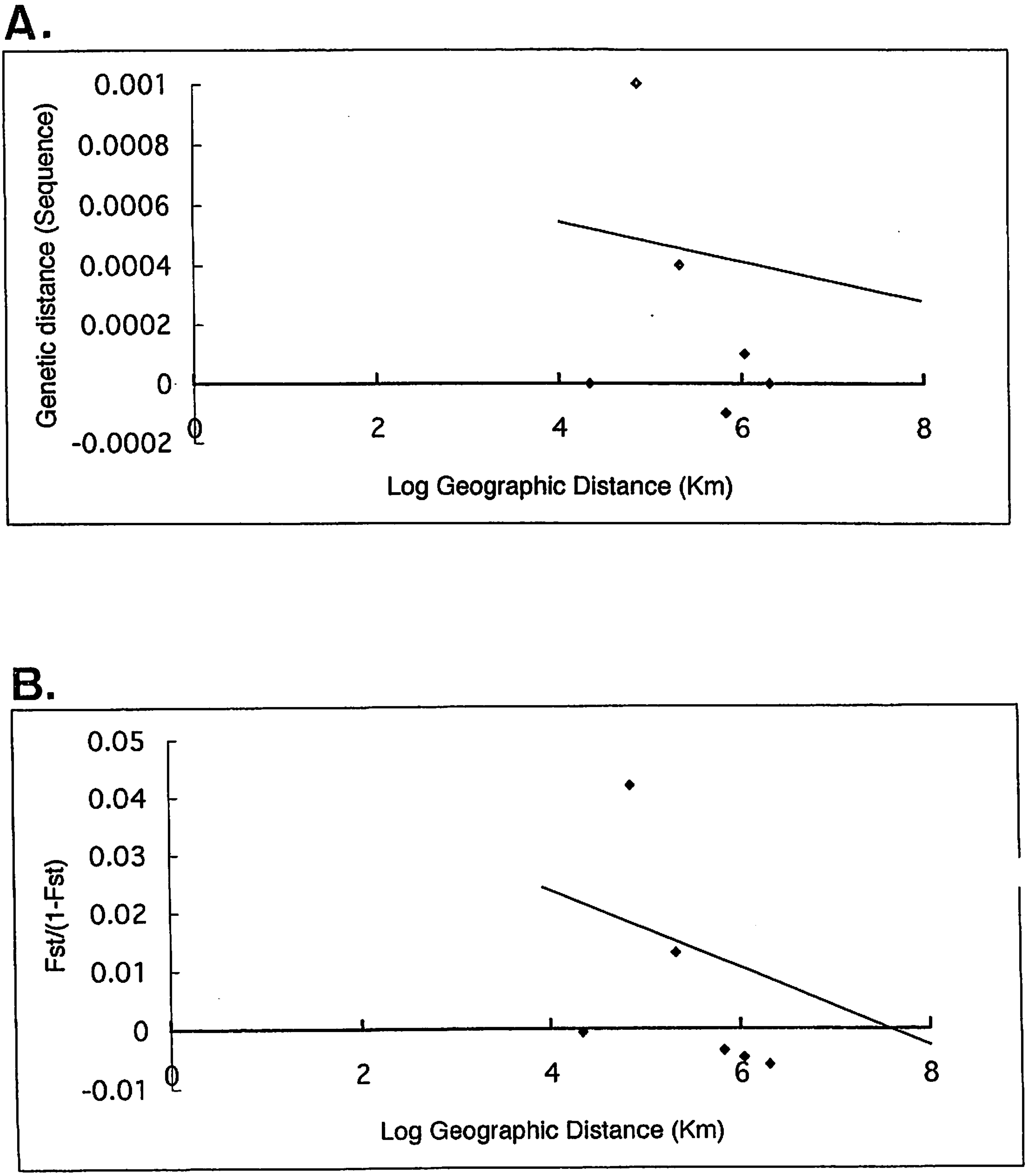

Fig. 8 Plot of the natural logarithm (ln) of the geographical distance against (A) Nei's genetic distance and (B) Fst/(1-Fst) among four groups within 1998.