Genetic population structure in the western North Pacific minke whale examined by mtDNA control region sequencing analysis

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ABSTRACT

Population structure in the western North Pacific minke whale was examined by mitochondrial DNA (mtDNA) control region sequencing analysis. A total of 396 samples obtained from past commercial whaling in Korea in 1982 (sub-area 6, n=28) and from JARPN surveys between 1994 and 1998 (total=368, sub-area 7: n=89, sub-area 8: n=91, sub-area 9: n=188), was examined. In addition, six minke whale samples from the eastern North Pacific (four from California and two from Alaska) were compared with that from western North Pacific. A total of 61 unique haplotypes were defined by the 32 variable sites detected in a 487bp consensus segment in the first half of mtDNA control region. Homogeneity tests using the haplotype (Hst) and sequence (Kst*) statistics were conducted. As expected, sub-area 6 (Korea) was discriminated from sub-areas 7, 8 and 9 (eastern side of Japan). No significant heterogeneity was found in the comparison among sub-areas 7, 8 and 9. As regard to the six eastern North Pacific samples, each sample presented different haplotype. However, all of those haplotypes were represented in sub-areas 7, 8 and 9. Regarding sub-areas 6, 7, 8 and 9, results of the sequencing analysis were similar to those obtained by a previous RFLP analysis.

INTRODUCTION

Previous mtDNA analyses on the western North Pacific minke whales were based on RFLP analysis of mtDNA control region (Goto and Pastene 1997a; 1997b; 1998). They used samples from both past coastal whaling in Korea and Japan and from the JARPN (Japanese Whale Research Program under Special Permit in the North Pacific). Results derived from these analyses confirmed the genetic differentiation between minke whales from coastal Korean and minke whales from the eastern side of Japan. On the other hand no significant differences were found between coastal and offshore minke whales.

These studies also confirmed the previous view that minke whales from two different stocks mix to each other on a spatial and temporal scale in the Okhotsk coast of Hokkaido (sub-area 11). Pastene *et al.* (1998) using mtDNA control region haplotype data, estimated the proportion of J stock female animals in the April sample in sub-area 11 in 0.4075 (SE=0.0806) and that of J stock male animals in August in 0.3147 (SE=0.1160).

The possibility of additional structure in offshore areas of the western North Pacific still being in dispute at the Scientific Committee. One of the arguments given is that RFLP-

based analyses have low resolution to detect stocks with low levels of genetic differentiation. Considering such argument, Goto and Pastene (1998) presented the results of a preliminary sequencing analysis to investigate further the stock structure in the western North Pacific minke whale. It is known that a sequencing analysis has higher resolution power than RFLP analyses. That preliminary sequencing analysis used a limited number of samples from sub-areas 9 and 7 and used as outgroup the Korean samples, which were previously known to be from a different stock.

We present here the results of a sequencing analysis that used all the samples taken in JARPN surveys between 1994 and 1998, except those from sub-area 11. The Korean samples are used as outgroup. In addition we used six minke whale samples from the eastern North Pacific.

MATERIALS AND METHODS

Samples and localities

Minke whales used in this study were sampled during the JARPN surveys between 1994-1998. As out group we used Korean minke whales caught in the past by Korean coastal whaling operations in September and October 1982. In addition, six minke whale samples from the eastern North Pacific (four from California and two from Alaska) were incorporated into the analysis. Five regions were defined using the geographical position of the samples taken (Fig. 1): Korea (sub-area 6 defined by the Working Group on North Pacific Minke Whale Management Trials, including one individual from sub-area 5), Pacific coast of northern Japan (sub-area 7), two offshore areas (sub-area 8 and 9) and eastern North Pacific (ENP group). A total of 402 samples (n=28 in sub-area 6, n=89 in sub-area 7, n=91 in sub-area 8, n=188 in sub-area 9 and n=6 in ENP) was examined. The total number of JARPN samples examined in this study by sub-area, month and sex are shown in Table 1. The thirty samples from sub-area 11 collected in 1996 JARPN survey in August were excluded from this study, because J and O stock animals temporary mix within this sub-area in August (Pastene *et al*, 1998). The six minke whale samples from the eastern North Pacific were collected by biopsy sampling and from stranded animals and were available from the Southwest Fisheries Science Center. For these six samples, the PCR products amplified by MT4 (Arnason *et al.*, 1993) and Dlp5R (Baker *et al.*, 1997) were electrophoresed on agarose gel and target fragments were excised. The excised gels were sent from La Jolla to ICR in Japan. The purification of the excised fragment and sequencing were followed by procedures mentioned below.

Tissue used, DNA extraction and amplification of the mtDNA control region

Using established protocols (Sambrook *et al.*, 1989), genomic DNA (mtDNA + nuclear DNA) was isolated from liver, muscle or skin tissue. The first half of control region of the mitochondrial genome was amplified by using the polymerase chain reaction (PCR) (Hoelzel, 1992). In order to amplify the approximately 550 bp minke whale mtDNA including control region, primers light-strand MT4 (*5'*-CCTCCCTAAgACTCAAggA-Ag-*3'*) and heavy-strand Dlp 5R (*5'*-CCATCgAgATgTCTTATTTAAggggAAC-*3'*) were used. PCR products were purified by MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing were performed with the same primers, using AmpliTaq FS

Sequencing Kit (Perkin-Elmer, Inc). The cycle sequencing products were purified by AutoSeq G-50 spin Columns (Pharmacia Biotech) and then sequenced on an ABI 377 Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacture. For each sample both strands were sequenced. Sequences were aligned using Sequence Navigator (Applied Biosystems, Inc).

Data analysis

The degree of mtDNA diversity within each geographical sub-area was estimated using the index of nucleotide diversity (Nei, 1987 pp. 256). The Kimura's two parameters method (Kimura, 1980) was used for estimating genetic distances between two sequences. The net genetic distances between sub-areas were estimated from equation 10.21 of Nei (1987). Homogeneity test between and within sub-areas were conducted using the Hudson *et al.* (1992)'s sequence (Kst*) and haplotype (Hst) statistics. The degree if divergence was inferred as being larger than zero, if an equal or more extreme value of Kst*/Hst was observed in less than 5% of 10,000 Monte Carlo simulations.

RESULTS

Variability of mtDNA control region sequences

A 487 base pairs of mtDNA control region (the 5'-end) was analyzed for the total samples of 402 individuals. A total of 32 polymorphic site defined 61 haplotypes (Table 2). Except for one in/del site, all substitutions were transitions.

Nucleotide and nucleon diversity

Table 3 shows the nucleotide diversity for each of the four sub-areas examined. Nucleotide diversity were 0.00457+/-0.00090, 0.00879+/-0.00048, 0.00832+/-0.00050 and 0.00727+/-0.00028 for sub-areas 6, 7, 8 and 9, respectively. Estimated nucleon diversity was 0.5529 for sub-area 6, 0.9632 for sub-area 7, 0.9602 for sub-area 8 and 0.9435 for sub-area 9. The nucleotide and nucleon diversity for the samples of the eastern North Pacific (ENP, n = 6) were 0.00493+/-0.00104 and 1.0000, respectively.

Geographical distribution of haplotypes

In the 28 individuals from sub-area 6, five haplotypes were detected (Table 2). The predominant haplotype '1' was shared with other three sub-areas and one rare haplotype '4' was shared with sub-area 8. Other three haplotypes were specific to this sub-area. In the 89 individuals from sub-area 7, thirty-five haplotypes were detected, sixteen of which were found only in a single specimens. In the 91 individuals from sub-area 8, thirty-six haplotypes were detected, twenty of which were found only in a single specimens. In the 188 individuals from sub-area 9, forty haplotypes were detected, fifteen of which were found only in the single specimens. One haplotype were shared among sub-areas 6, 7, 8 and 9. Twenty haplotypes were shared among three sub-areas in the eastern side of Japan (sub-areas 7, 8 and 9). Seven, eight and ten haplotypes were specific to sub-areas 7, 8 and 9, respectively. In the ENP region, the six individuals showed all different haplotypes. All of them were present in the three sub-areas in the eastern side of Japan (sub-areas 7, 8 and 9).

Homogeneity test

Temporal differences

Table 4 shows the results of the homogeneity test for the among surveys/sub-area and among periods/sub-area. No significant differences were found among surveys in a sub-area nor among periods within a survey in a sub-area.

Comparison among sub-areas

Table 5 shows the results of the homogeneity test among sub-areas. As expected both statistics Hst and Kst* separated clearly sub-area 6 (Korea) from three sub-areas in eastern side of Japan (7, 8 and 9). No significant differences were found among sub-areas 7, 8 and 9. The homogeneity test by Hst and Kst* between the ENP and sub-areas 7, 8 and 9 also showed no significant differences.

Inter-populational distances

Table 6 shows the inter-populational distances among sub-areas. The net inter-populational distance between sub-area 6 and eastern side of Japan ranged from 0.00692 to 0.00704. On the other hand, the net interpopulational distance among sub-areas in the eastern side of Japan ranged from negative value to 0.00001. Intrapopulational distances within sub-areas were almost equal to the interpopulational distances among sub-areas in the eastern side of Japan. An average of Kimura's net inter-populational distance between sub-area 6 and three sub-areas in the eastern side of Japan was 0.0070. The net inter-populational distance between eastern North Pacific and three sub-areas eastern side of Japan ranged from -0.00011 to -0.00003. The net distance between ENP and sub-area 6 was 0.00773.

DISCUSSION

In this study we extended a previous sequencing analysis on the western North Pacific minke whale. Sub-area 8 was examined for the first time and the sample sizes were increased for other sub-areas.

Genetic diversity

Nucleotide diversity was lower in the Korean minke whales (sub-area 6) than in minke whales from the eastern side of Japan. The low genetic diversity in the Korean sample is in agreement with the results of the microsatellite analysis (Abe *et al.* 1998), which found a lower heterozygosity in the Korean sample. The levels of mtDNA diversity found are well correlated with the population sizes of the J and O stocks, the former being substantial smaller that the O stock.

Nucleotide diversity in the North Atlantic minke whale was estimated at 0.0064 (Bakke *et al.,* 1996). Then this value is lower than North Pacific sub-areas 7, 8 and 9 but higher than sub-area 6 (Korea). Nucleotide diversity in the Antarctic minke whale (0.0159) is higher than in North Pacific and North Atlantic (Bakke *et al.*, 1996). These authors explained that the higher value of nucleotide diversity in the Antarctic than in the North Atlantic reflects a larger long-term effective population size of the Antarctic minke whale compared to the North Atlantic minke whale. In the Antarctic humpback whale

nucleotide diversity was larger ranging from 0.0238 to 0.0323 (Pastene *et al.*, 1997a). The range of nucleotide diversity in the North Pacific Bryde's whale (0.0077 to 0.0091) (Pastene *et al.*, 1997b) is similar to that of North Pacific minke whale of the eastern side of Japan.

Population structure

The result of the homogeneity test using sequencing analysis was consistent with that of the mtDNA RFLP analyses (Goto and Pastene, 1997a; 1997b; 1998) and with that of the microsatellite analysis using six loci (Abe *et al.*, 1998). This analysis discriminated clearly between Korean (sub-area 6) and eastern side of Japan (sub-areas 7, 8 and 9) minke whales. Also we could not find significant differences among sub-areas in the eastern side of Japan.

The results of our sequencing analysis, which is considered to be more powerful than the RFLP analysis, provide no evidence for the occurrence of more than one stock in the eastern side of Japan and then we can not reject the hypothesis that the same O stock distribute in both coastal and offshore areas in the eastern side of Japan.

As regard to the six eastern North Pacific samples, each sample presented a different haplotype. However, all of those haplotypes were represented in sub-areas 7,8 and 9. In addition a homogeneity test showed no significant difference between this sample and the three sub-areas in the eastern side of Japan. Furthermore, the net inter-populational distances between eastern and western North Pacific was a negative value.

These results might be indicating that either:

1) There is no additional stock structure in the North Pacific.
2) Additional structure in the North Pacific exist but the level of genetic differentiation between putative stocks are very low and then the sequencing analysis of six samples was unable to detect such differences.

It should be noted, however, that we are still being pendent an evaluation of the statistical power of the genetic analysis. Method to estimate the power of the genetic analysis should be developed and applied in future.

As regard to the Korean samples, which was used as outgroup in this and previous studies, the Scientific Committee had noted the small sample size (30) and that the sample had been collected from a limited area over a short period (September – October, 1982) (IWC, 1999). In response to this, we have begun a cooperative genetic study on the minke whales in the Sea of Japan with Republic of Korea, in order to examine new genetic material from this region.

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ble 1. Number of samples collected during the JARPN surveys from 1994 to 1998 sub-area, year, month and sex. All these were examined for sequencing analysis cept sub-area 11.

ub-area	Year	Month										Total
		May		June		July		August		September		
		Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	
7	1996					1			15	2	13	31
	1997				2							2
	1998	7	49									56
8	1996						11		5			16
	1997					1	30					31
	1998	1	7	3	33							44
9	1994					2	6	1	8		4	21
	1995				14	5	56	4	21			100
	1997	7	20	5	35							67
11	1996							11	19			30
	Total	15	76	8	84	9	103	16	68	2	17	398

Table 2: Variable sites defining 61 North Pacific minke whale unique sequences (haplotypes) in the mtDNA control region. The column on the left is haplotype ID. The numbers above list the nucleotide position of the polymorphic sites from the 5 end of the mtDNA control region. On the right side of the table are the frequencies of the 61 haplotypes in the Sea of Japan (SA6: Sub-area 6), western North Pacific (SA7:Sub-area 7, SA8: Sub-area 8, SA9: Sub-area 9) and eastern North Pacific (ENP).

		10	20	30					
		11	1112222222	2222233334	44				
		11288922	3770001257	7889913670	66				
		6907002056	2344898032	4890812336	38	SA6	SA7	SA8	SA9	ENP
1	682KC001	ATTACTTCAC	CGTGCCATGT	CAGTGTAGCC	AA	18	3	1	2	
2	682KC002	....	....	.........T	..	2				
3	682KC004	-..G......	...A......	...CAC....	..	6				
4	682KC008	....	....	....	G.	1		1		
5	682KC013	-..G...T..	...A......	...CAC....	..	1				
6	796NP001	-..G...T..	...A.....C	...CAC...T	G.		4	1	9	
7	796NP048	-..G...T..	...A......	...CAC...T	G.		9	10	15	1
8	796NP049	-..G....G.	...A.T....	...CA....T	G.		3	1	3	
9	796NP050	-..G...T..	...A......	....A.....	G.		8	7	30	
10	796NP051	-..G......	.A.A.....C	...CA....T	G.		1			
11	796NP052	-..G...T..	...A......	...CA....T	G.		1	5	10	1
12	796NP053	-..G.C....	...A.....C	...CACG..T	G.		2	3	7	
13	796NP054	-..G.C.T..	...A.....C	...CAC...T	G.		7	8	4	
14	796NP056	-..G......	...A......	...CA.G..T	G.		4	6	11	1
15	796NP058	-..G...T..	...A.T....	...CA....T	G.		4	4	6	
16	796NP060	-..G......	...A......	....A.....	G.		6	4	11	
17	796NP061	-..G.C....	...A.....C	...CAC...T	G.		3	5	16	1
18	796NP063	-..G...TG.	...A.T....	...CA....T	G.		2	2	3	
19	796NP065	..CG.C....	...AT..C..	...CAC...T	G.		1	1	1	
20	796NP068	-..G.C...T	...A.....C	...CAC...T	G.		3			
21	796NP072	-..G...T..	...A.....C	...CA....T	G.		3		3	
22	796NP073	-..G......	...A......	...C.C....	G.		1			
23	796NP074	..CG.C....	...AT..C.C	...CAC...T	G.		1		1	
24	796NP075	-..G.C....	...A.T...C	...CACG..T	G.		1			
25	796NP076	..CG.C....	...A...C..	...CAC...T	G.		1	2	4	
26	796NP077	..CG.C....	...A.T.C..	...CAC...T	G.		1	1		
27	797NP068	-..G......	...A......	...CA.....	..		1		1	
28	798NP003	-..G..C...	...A....A.	...CA.....	G.		1			
29	798NP005	-..G......	...A......	...CAC...T	G.		1	4	5	
30	798NP006	-..G......	...A......	...CA.....	G.		3	1	9	1
31	798NP008	..CG.C....	....T..C..	...CAC...T	G.		2		2	
32	798NP009	-..G...T..	...A......	...CA....T	..		1			
33	798NP014	..CG.C....	...AT.T..C	...CAC...T	G.		1			
34	798NP016	-..G.C....	...A......	...CAC....	G.		2	4	3	
35	798NP025	-..G......	...A......	....A.....	GG		2	1		
36	798NP035	-..GT.C...	...A....A.	...CA.....	G.		3	3	1	
37	798NP041	-C.G...T..	...A......	...CA....T	G.		1		2	
38	798NP047	-..G..C...	...A......	...CAC...T	G.		1	1	1	
39	798NP050	-..G......	...A......	...CA....T	G.		1	2	5	1
40	896NP006	-..G...T..	...A......	...CAC....	G.			1		
41	896NP013	-..G......	..CA......	....A...T.	GG			1		
42	897NP072	....	....	..A..C....	..			1		
43	897NP080	..CG.C.T..	...A.....C	...CAC...T	G.			1		
44	897NP081	-..G......	...A......	...CA.G..T	GG			1		
45	897NP082	-..G...T..	...A......	...CA.G...	GG			2		
46	897NP086	-..G......	...A......	...CAC....	G.			1		
47	897NP088	-..GT.CT..	...A....A.	...CA.....	G.			1		
48	898NP060	....	....	..A.......	..			1	1	
49	898NP067	-..G.C.T..	...A.T...C	...CAC...T	G.			1	1	
50	898NP088	-..G......	...A......	.G..A.....	G.			1	1	
51	898NP091	-..G......	...A......	...CA.G...	G.			1	1	
52	995NP026	-..G...T..	...A......	....AC...T	G.				5	
53	995NP028	-..G...T..	...A......	....A.....	..				4	
54	995NP029	-..G......	...A......	...CA....T	-.				1	
55	995NP056	-..G...T..	T..A......	...CAC...T	G.				1	
56	995NP064	-......T..	...A.....C	...CA....T	G.				1	
57	995NP069	.CCG.C....	...A...C..	...CAC...T	G.				1	
58	995NP096	-..G.C....	...A.....C	....A....T	G.				1	
59	995NP099	-..G.C....	...A.....C	....AC...T	G.				2	
60	997NP001	-..G......	...A......	G...A....T	G.				2	
61	997NP013	-..G...T..	...A......	...CAC.C.T	G.				1	
		1222222222	2222222222	2222222222	22	28	89	91	188	6

ble 3: Nucleotide diversity in the North Pacific minke whale each sub-area.

Population	Nucleotide diversity	SE
Sub-area 6	0.00457	0.00090
Sub-area 7	0.00879	0.00048
Sub-area 8	0.00832	0.00050
Sub-area 9	0.00727	0.00028
ENP	0.00493	0.00104

ble 4: Results of the homogeneity test for the year/sub-area groups. Above diagonal: Hst(a) and K-st*(b) lues, below diagonal: probabilities. In parenthesis shows sample size. '96NP,SA7' means JARPN survey 1996 in sub-area 7. The doted block show the comparison between different survey seasons within same b-area.

Hst

	96NP,SA7 (31)	98NP,SA7 (56)	96NP,SA8 (16)	97NP,SA8 (31)	98NP,SA8 (44)	94NP,SA9 (21)	95NP,SA9 (100)	97NP,SA9 (67)
6NP,SA7		-0.0035	-0.0075	-0.0005	-0.0047	0.0024	0.0006	-0.0011
8NP,SA7	0.868		-0.0032	0.0013	-0.0028	-0.0017	0.0011	-0.0018
6NP,SA8	0.966	0.797		0.0008	-0.0062	-0.0009	0.0005	0.001
7NP,SA8	0.523	0.319	0.429		0.0048	0.0059	0.003	-0.0013
8NP,SA8	0.916	0.819	0.896	0.106		0.0034	0.0012	-0.0002
4NP,SA9	0.312	0.637	0.515	0.126	0.232		-0.0008	-0.0015
5NP,SA9	0.358	0.234	0.368	0.110	0.253	0.555		-0.0012
7NP,SA9	0.587	0.747	0.359	0.623	0.475	0.630	0.726	

Kst*

	96NP,SA7 (31)	98NP,SA7 (56)	96NP,SA8 (16)	97NP,SA8 (31)	98NP,SA8 (44)	94NP,SA9 (21)	95NP,SA9 (100)	97NP,SA9 (67)
6NP,SA7		0.001	-0.0056	0.0001	-0.002	0.0009	0.0032	0.0028
8NP,SA7	0.357		-0.0051	0.0006	-0.0048	-0.0057	-0.0001	-0.0005
6NP,SA8	0.736	0.863		-0.0039	-0.0072	-0.0088	-0.0013	-0.0006
7NP,SA8	0.427	0.379	0.676		0.0012	0.0011	0.0051	0.0011
8NP,SA8	0.564	0.932	0.875	0.344		-0.0024	-0.0005	-0.0019
4NP,SA9	0.375	0.917	0.792	0.366	0.600		-0.0019	-0.0027
5NP,SA9	0.139	0.394	0.569	0.076	0.467	0.655		-0.0022
7NP,SA9	0.211	0.480	0.456	0.311	0.649	0.653	0.829	

Table 5: Results of the homogeneity test between Sub-areas. Above diagonal: Hst(a) and K-st*(b) values, below diagonal: probabilities. Sample size in parenthesis.

(a) Hst

	Sub-area 6 (28)	Sub-area 7 (89)	Sub-area 8 (91)	Sub-area 9 (188)
Sub-area 6		0.0855	0.0902	0.0585
Sub-area 7	0.0000		-0.0019	0.0012
Sub-area 8	0.0000	0.9109		0.0015
Sub-area 9	0.0000	0.1383	0.0979	

(b) Kst*

	Sub-area 6 (28)	Sub-area 7 (89)	Sub-area 8 (91)	Sub-area 9 (188)
Sub-area 6		0.1667	0.1736	0.1207
Sub-area 7	0.0000		-0.0024	0.0010
Sub-area 8	0.0000	0.9095		0.0019
Sub-area 9	0.0000	0.2292	0.1186	

Table 6: Net interpopulational distance among sub-areas in the North Pacific.

	Sub-area 6 (28)	Sub-area 7 (89)	Sub-area 8 (91)	Sub-area 9 (188)	ENP (6)
Sub-area 6		0.00695	0.00704	0.00692	0.00773
Sub-area 7			-0.00005	0.00000	-0.00007
Sub-area 8				0.00001	-0.00011
Sub-area 9					-0.00003

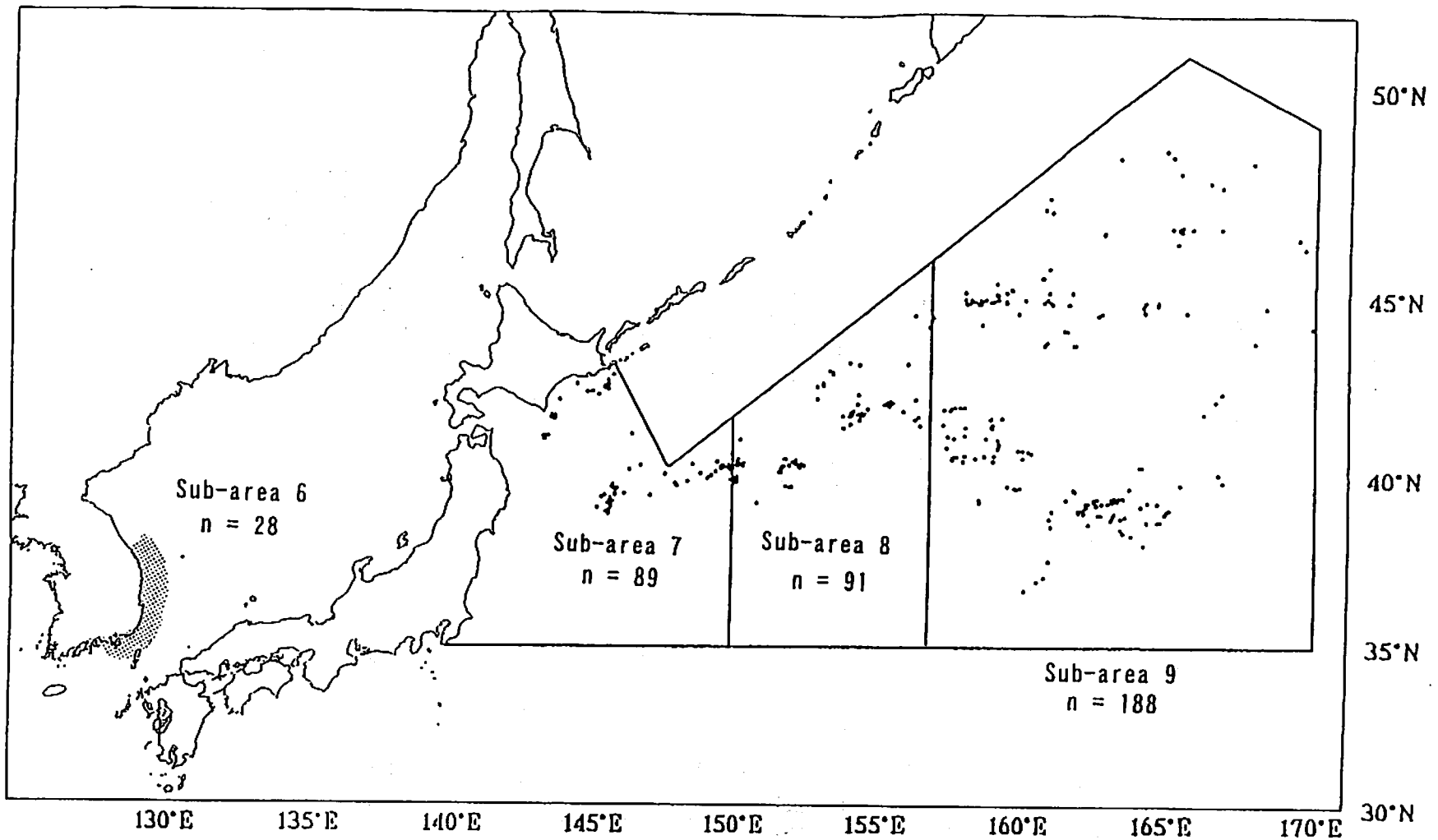

Fig. 1. Geographical localities and sample size used in the sequencing analysis of the mtDNA control region. Figures indicate sample size by locality.