Am. J. Hum. Genet. 43:134–143, 1988

Mitochondrial DNA Polymorphism among Five Asian Populations

S. Harihara,*[,1] N. Saitou,* M. Hirai,* T. Gojobori,† K. S. Park,‡ S. Misawa,§ S. B. Ellepola,‖ T. Ishida,# and K. Omoto*

*Department of Anthropology, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo; †National Institute of Genetics, Mishima, Shizuoka, Japan; ‡Department of Biology, Sung-Shin Women's University, Seoul; §Department of Legal Medicine, Institute of Community Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan; ‖Department of Medicine, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka; and #Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan

Summary

Mitochondrial DNA (mtDNA) polymorphisms were detected using 13 restriction enzymes on the total DNA obtained from blood samples of five Asian populations: Japanese and Ainu of northern Japan, Korean, Negrito (Aeta) of the Philippines, and Vedda of Sri Lanka. Of a total of 28 restriction-enzyme morphs detected, eight had not been reported previously. By combining the morphs, we were able to classify mtDNAs of 243 individuals into 20 mtDNA types. Phylogenetic analyses using maximum parsimony and genetic distance methods both showed that the Japanese, Ainu, and Korean populations were closely related to each other. Aeta was found to show a relatively close relationship to these three populations, confirming the conclusion from previous studies of blood markers. In contrast, Vedda was quite different from the other four populations.

Introduction

Polymorphism of mitochondrial DNA (mtDNA) detected by restriction enzymes has been analyzed in most major racial groups, and its usefulness in population genetic studies has been amply demonstrated (see, e.g., Brown 1980; Johnson et al. 1983; Cann et al. 1987). However, relatively little is known about the mtDNA types of the isolated indigenous groups of Asia whose racial origins have been in dispute. In the present paper we deal with three such groups: Ainu of northern Japan, Negrito of the Philippines, and Vedda of Sri Lanka (fig. 1).

All of these groups are known to have been hunter-gatherers until recent times and are considered to be truly indigenous groups of the areas they inhabit. Ainu and Negrito show morphological features markedly different from those of the surrounding mongoloid groups. Thus, in anthropological literatures they often have been considered to belong to the caucasoid and to the negroid or australoid groups, respectively. On the contrary, recent genetic studies using protein and antigen markers from blood indicated that their genetic affinities were closest to the east-Asian mongoloid groups (Misawa and Hayashida 1968; Omoto 1972, 1984). Our previous study on mtDNA polymorphism among Ainu and Japanese (Harihara et al. 1986) also supported the finding about Ainu.

However, since Ainu and Negrito today show considerable admixture with surrounding mongoloid populations, there had remained some reserve about the validity of the close genetic affinities to mongoloids. The origin of Vedda is also unknown, but a previous genetic study has suggested a link to mongoloids (Kirk et al. 1964).

In the present study the mtDNA types of the Ainu, Negrito, and Vedda groups are described, together with those of the Japanese and Korean groups, and

Received November 25, 1987; revision received March 7, 1988.

1. Present address and address for correspondence and reprints: Dr. Shinji Harihara, Department of Legal Medicine, Institute of Community Medicine, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

0002-9297/88/4302-0005$02.00

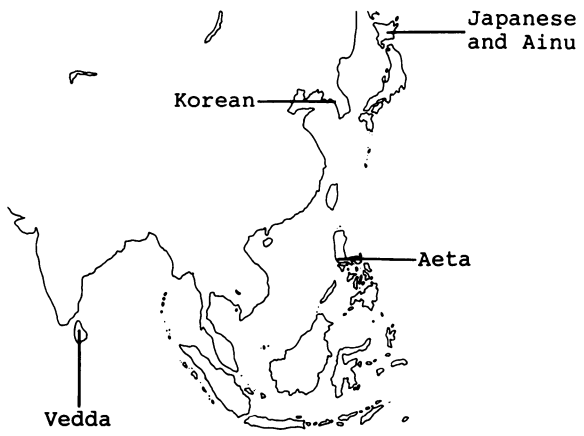


Figure 1 Five Asian populations of which samples were analyzed in this study.

used for analyses of genetic affinities of these populations. It was hoped that the maternally inherited mtDNA (Hutchison et al. 1974) may be relatively resistant to gene flow from outside, which is more likely to be due to migrant males than to migrant females.

Material and Methods

Blood samples were collected from five Asian populations. Samples of Ainu (48 individuals) and Japanese (74 individuals) were obtained in the Hidaka district of Hokkaido, northern Japan. These were the same samples as those used in the previous study by Harihara et al. (1986). Samples of 64 individuals of the Korean population were from Seoul. The Negrito (Aeta) samples (37 individuals) were collected on the Bataan Peninsula of Luzon in the Philippines. Twenty blood samples of Vedda were from Henanagawa, Sri Lanka.

Total DNA extracted from blood cells was digested with the following 13 restriction enzymes: *Ava*II, *Bam*HI, *Eco*RI, *Hin*cII, *Hin*dIII, *Kpn*I, *Hpa*I, *Msp*I, *Pst*I, *Pvu*II, *Sst*I, *Xba*I, and *Xho*I. For the Japanese, only five enzymes (*Ava*II, *Bam*HI, *Hin*cII, *Hpa*I, and *Pvu*II) were used. We did not use the other enzymes, because Horai et al. (1984) and Horai and Matsunaga (1986) had found a very low (if any) degree of polymorphism with them. Digested DNAs were separated on agarose gel (Hadler et al. 1983), and digestion patterns of mtDNA were detected by a hybridization technique (Southern 1975; Botchan et al. 1976). The probe used was purified mtDNA from either human placentas or cultured human cells which was nick-translated by the method of Rigby et al. (1977).

Mitochondrial DNAs of all the individuals were classified into mtDNA types by combining the enzyme morphs for each individual. Genetic distances among mtDNA types were calculated by the method of Nei and Li (1979) and Nei and Tajima (1983). Genetic distances among populations were also calculated by the method of Nei and Li (1979). Phylogenetic trees were constructed by applying the unweighted pair-group method of analysis (UPGMA; Sokal and Sneath 1963) to the genetic distance data.

The phylogenetic relationship of mtDNA types was also depicted by the maximum parsimony method (Camin and Sokal 1965; Eck and Dayhoff 1966): an unrooted tree was constructed to connect all types by setting the total number of changes to a minimum.

Results

mtDNA Morphs for Each Restriction Enzyme

Variations in mtDNA digestion patterns were detected by *Ava*II, *Hin*cII, *Hpa*I, *Msp*I, *Pst*I, *Pvu*II, and *Xho*I. A total of 28 enzyme morphs were detected, 20 of which had been reported elsewhere (Denaro et al. 1981; Blanc et al. 1983; Johnson et al. 1983; Horai et al. 1984; Harihara et al. 1986; Horai and Matsunaga 1986). Eight newly found morphs (fig. 2) are as follows:

*Ava*II.—Three new morphs were found by using *Ava*II in the Korean, Aeta, and Vedda groups, and we called these *Ava*II morphs 24^{Korean}, 25^{Aeta}, and 26^{Vedda}, respectively (fig. 2*a*). Superscripts denote the origin of the samples.

*Ava*II morph 24^{Korean} was a site-gain variant, detected in one individual. In this morph, a 3.0-kb fragment of *Ava*II morph 1 (Johnson et al. 1983) was split into two fragments of almost the same size, 1.5 kb, and they could not be distinguished on the autoradiogram. The new site is at or near bp 14900 of Anderson et al.'s (1981) human mtDNA sequence, and the change is located in the coding region of cytochrome *b*.

In *Ava*II morph 25^{Aeta}, a 9.8-kb fragment of *Ava*II morph 1 became two fragments, one 6.6 kb and the other 3.2 kb. Double digests with *Sst*I and *Ava*II cut a 6.6-kb fragment into a 3.6-kb fragment and a 3.0-kb fragment, indicating that the extra site is lo-

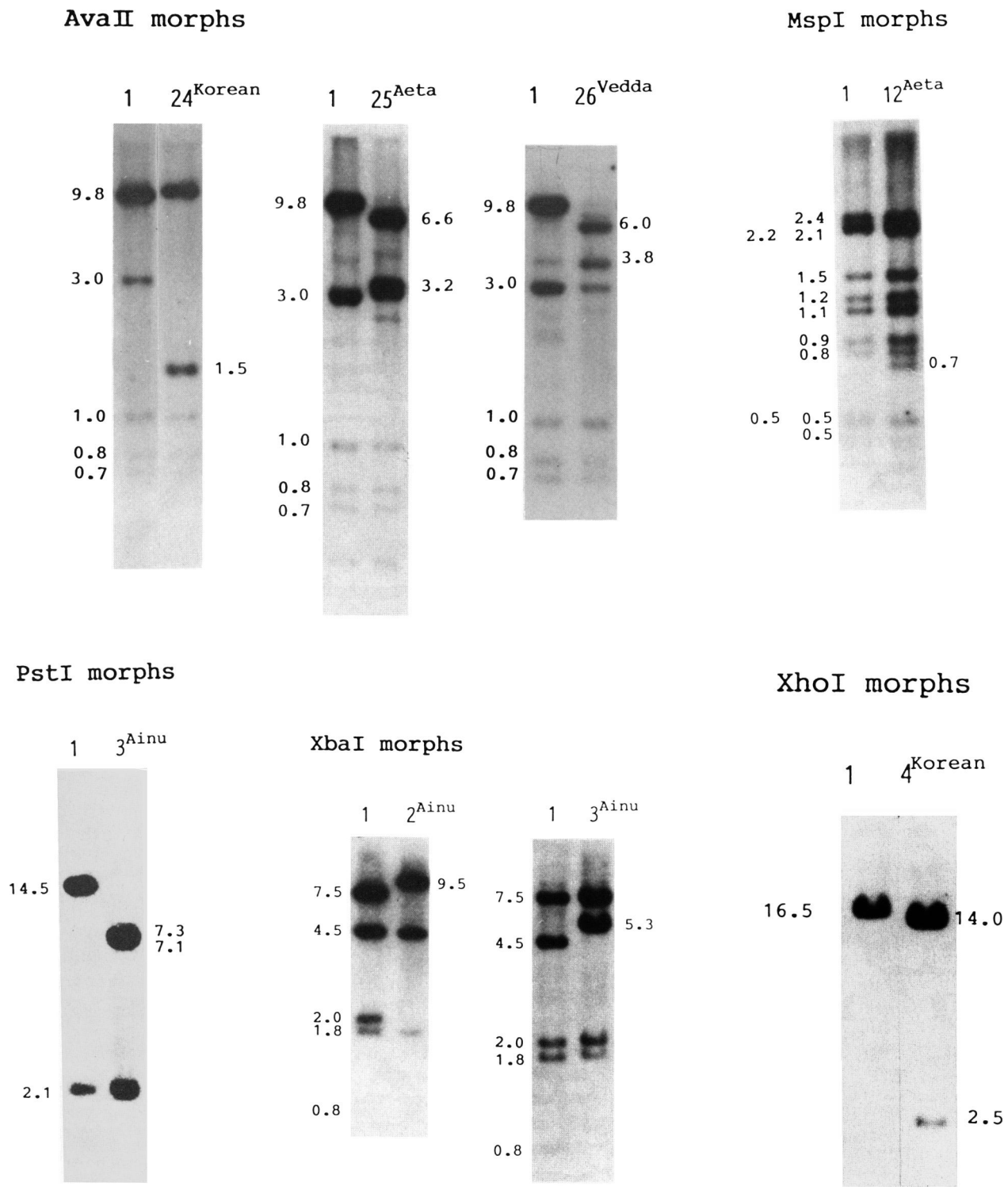


Figure 2 Autoradiogram of eight new mtDNA morphs detected in the present study. Morphs are denoted by numbers at top of lanes, and fragment sizes are designated in kilobases. *a*, *Ava*II morphs 1, 24^{Korean}, 25^{Aeta}, and 26^{Vedda}. *b*, *Msp*I morphs 1 and 12^{Aeta}. *c*, *Pst*I morphs 1 and 3^{Ainu}. *d*, *Xba*I morphs 1, 2^{Ainu}, and 3^{Ainu}. *e*, *Xho*I morphs 1 and 4^{Korean}.

cated approximately at bp 6000, in the coding region of cytochrome *c* oxidase subunit I.

*Ava*II morph 26^{Vedda} is also a site-gain variant, and two fragments (6.0 kb and 3.8 kb) were seen instead of the 9.8-kb fragment of *Ava*II morph 1. A 6-kb fragment became a 2.9-kb fragment and a 3.0-kb fragment after double digests with *Sst*I and *Ava*II, indicating that the extra site is located approximately at bp 6700, which is in the coding region of cytochrome *c* oxidase subunit I.

***Msp*I.**—A new morph was found in Aeta in the present study and was named *Msp*I morph 12^{Aeta} (fig. 2*b*). In this morph, a 0.7-kb fragment, which was not detected in *Msp*I morph 1 (Johnson et al. 1983), was generated without the splitting of any larger fragments. This seems to be the result of fusion of one of three 0.5-kb fragments of *Msp*I morph 1 and the adjacent 0.2-kb fragment, which could not be detected on the autoradiogram in the present study; and the site loss was located at bp 16453 in the noncoding region.

***Pst*I.**—*Pst*I morph 3^{Ainu} was newly found in Ainu (fig. 2*c*). This morph is generated by a site gain in the noncoding region, and the 14.5-kb fragment of *Pst*I morph 1 (Horai et al. 1984) was split into a 7.3-kb fragment and a 7.1-kb fragment. Double digests with *Eco*RI and *Pst*I generated three fragments (4.5 kb, 1.6 kb, and 1.1 kb) from the 7.3-kb fragment and two fragments (3.6 kb and 3.5 kb) from the 7.1-kb fragment, indicating that the mutation is located approximately at bp 16200 in the noncoding region.

***Xba*I.**—Horai et al. (1984) reported no variant *Xba*I morph in Japanese subjects, and all individuals were *Xba*I morph 1. In the present study, two new morphs were detected in Ainu (*Xba*I morphs 2^{Ainu} and 3^{Ainu}) (fig. 2*d*). In *Xba*I morph 2^{Ainu}, a site loss occurred at bp 10256 in the coding region of NADH dehydrogenase subunit 3 (Chomyn et al. 1985), and a 9.5-kb fragment was generated by fusion of a 7.5-kb fragment and a 2.0-kb fragment of *Xba*I morph 1. *Xba*I morph 3^{Ainu} is also a site-loss variant, the loss being located at bp 7440 in the coding region of cytochrome *c* oxidase subunit 1; and a 4.5-kb fragment and a 0.8-kb fragment fused to form a 5.3-kb fragment.

***Xho*I.**—In *Xho*I morph 4^{Korean} detected in the Korean group, two fragments, one of 14.0 kb and the other of 2.5 kb, could be seen instead of a 16.5-kb single fragment of *Xho*I morph 1 (Horai et al. 1984) (fig. 2*e*). Sizes of fragments generated by double digests with *Pst*I and *Xho*I were 8.5 kb, 3.5 kb, 2.5 kb, and 2.1 kb. The extra site was determined to be located approximately at bp 12500, in the coding region of NADH dehydrogenase subunit 5 (Chomyn et al. 1985).

The frequency distribution of enzyme morphs for each population is listed in table 1. Except for the dominant ones, the frequencies of most variant morphs are low in every population, whereas *Ava*II morphs 25^{Aeta} and 26^{Vedda} are relatively frequent in Aeta (10.8%) and Vedda (25.0%), respectively.

mtDNA Types Defined by Eight Enzymes in Five Asian Populations

When all enzyme morphs for each individual were combined, mtDNAs of 243 individuals of the five populations were classified into 20 mtDNA types; their distribution is listed in table 2. *Hpa*I morphs were excluded from this analysis, since *Hpa*I morphs 1 and 4 correspond to *Hinc*II morphs 1 and 3, respectively, and since *Hpa*I morph 2 is divided into *Hinc*II morphs 2 and 5. The reason for this is that (1) the recognition sequence of *Hpa*I (GTTAAC) is one of four recognition sequences of *Hinc*II and (2) there was no *Hinc*II morph that was divided into two *Hpa*I morphs in the present study. In other words, *Hpa*I happened to be not informative in the present study.

In every population, type 1 is the most frequent and the frequencies of other mtDNA types are low, except for type 19 in Vedda (25.0%).

Phylogeny of mtDNA Types

The phylogeny of 20 mtDNA types is shown as an unrooted tree obtained by the maximum parsimony method (fig. 3). All variant mtDNA types can be derived from the most frequent one (type 1) through one or two changes. A hypothetical type, X, is needed to connect types 1 and 16. The variant enzyme morph in type X is either *Ava*II morph 17 or *Xho*I morph 4. A total of 20 lines are needed to connect 21 mtDNA types. The tree in figure 3 is one of 15 possible ways of connection that are equally parsimonious. In one alternative way, for example, types 7 and 8 and types 6 and 20 are connected whereas the line connecting types 4 and 8 and that connecting types 7 and 20 are cut.

Johnson et al. (1983) reported mtDNA polymorphism among Caucasians, Orientals (mainly Chinese), Bantu, Bushman, and American Indian. They used five restriction enzymes, four of which—*Ava*II, *Bam*HI, *Hpa*I, and *Msp*I—were also used in the present study. When results obtained in the present

Table 1

Number of Individuals and Percentage of Enzyme Morphs for Each Population

Morph	Reference[a]	No. (%) Japanese (N = 74)	Ainu (N = 48)	Korean (N = 64)	Aeta (N = 37)	Vedda (N = 20)	Total (N = 243)
*Ava*II:							
1	3	66 (89.2)	48 (100.0)	64 (96.9)	33 (89.2)	13 (65.0)	224 (92.2)
2	3	1 (1.4)	0	1 (1.6)	0	0	2 (.8)
3	3	0	0	0	0	1 (5.0)	1 (.4)
5	3	0	0	0	0	1 (5.0)	1 (.4)
10	3	3 (4.1)	0	0	0	0	3 (1.2)
12	5	1 (1.4)	0	0	0	0	1 (.4)
14	6	1 (1.4)	0	0	0	0	1 (.4)
24^{Korean}	7	0	0	1 (1.6)	0	0	1 (.4)
25^{Aeta}	7	0	0	0	4 (10.8)	0	4 (1.6)
26^{Vedda}	7	0	0	0	0	5 (25.0)	5 (2.1)
*Hin*cII:							
2	2	64 (86.5)	46 (95.8)	56 (87.5)	37 (100.0)	20 (100.0)	223 (91.8)
1	2	5 (6.8)	1 (2.1)	4 (6.3)	0	0	10 (4.1)
3	2	3 (4.1)	1 (2.1)	1 (1.6)	0	0	5 (2.1)
5	2	2 (2.7)	0	3 (4.7)	0	0	5 (2.1)
*Hpa*I:							
2	1	66 (89.2)	46 (95.8)	59 (92.2)	37 (100.0)	20 (100.0)	228 (93.8)
1	1	5 (6.8)	1 (2.1)	4 (6.3)	0	0	10 (4.1)
4	1	3 (4.1)	1 (2.1)	1 (1.6)	0	0	5 (2.1)
*Msp*I:							
1	3		48 (100.0)	64 (100.0)	35 (94.6)	20 (100.0)	
12^{Aeta}	7		0	0	2 (5.4)	0	
*Pst*I:							
1	4		47 (97.9)	62 (96.9)	37 (100.0)	20 (100.0)	
2	4		0	2 (3.1)	0	0	
3^{Ainu}	7		1 (2.1)	0	0	0	
*Pvu*II:							
1	4	73 (98.6)	48 (100.0)	64 (100.0)	37 (100.0)	20 (100.0)	242 (99.6)
3	6	1 (1.4)	0	0	0	0	1 (.4)
*Xba*I:							
1	4		45 (93.8)	64 (100.0)	37 (100.0)	20 (100.0)	
2^{Ainu}	7		2 (4.2)	0	0	0	
3^{Ainu}	7		1 (2.1)	0	0	0	
*Xho*I:							
1	4		48 (100.0)	63 (98.4)	37 (100.0)	20 (100.0)	
4^{Korean}	7		0	1 (1.6)	0	0	

[a] 1 = Denaro et al. (1981); 2 = Blanc et al. (1983); 3 = Johnson et al. (1983); 4 = Horai et al. (1984); 5 = Horai and Matsunaga (1986); 6 = Harihara et al. (1986); 7 = present study.

study are combined with their data on variation in mtDNAs' patterns of digestion by the four restriction enzymes, a total of 37 mtDNA types (named mtDNA types 1–37) are produced for the 10 populations. Note that this numbering system is different from that of table 2.

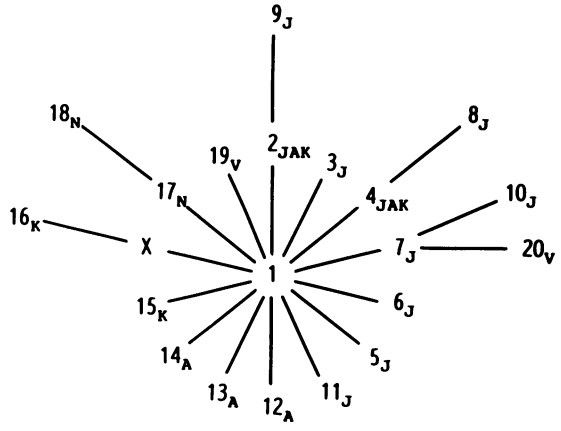
Genetic distances for these mtDNA types were calculated, and a UPGMA tree was constructed (fig. 4). In this tree, two main clusters (A and B) are depicted if three types (16, 19, and 37) that are apart from the others are excluded. Cluster A consists of mtDNA types found in non-African populations, except for type 1 (which is the most frequent) and type 2. On the other hand, there are four subclusters (1, 2, 3, and 4) in cluster B, and two of them (subclusters 2 and 4) consist of African mtDNA types exclusively. The other two subclusters (1 and 3) contain mtDNA types found in the non-African populations. Al-

Table 2

Number of Individuals and Percentage of mtDNA Types for Each Population

mtDNA Type	No. (%)					
	Japanese (N = 74)	Ainu (N = 48)	Korean (N = 64)	Aeta (N = 37)	Vedda (N = 20)	Total (N =243)
1	57 (77.0)	42 (87.5)	52 (81.3)	33 (89.2)	13 (65.0)	197 (81.7)
2 (*Hin*cII 1)	4 (5.4)	1 (2.1)	4 (6.3)	0	0	9 (3.7)
3 (*Ava*II 10)	3 (4.1)	0	0	0	0	3 (1.2)
4 (*Hin*cII 3)	2 (2.7)	1 (2.1)	1 (1.6)	0	0	4 (1.6)
5 (*Hin*cII 5)	2 (2.7)	0	3 (4.1)	0	0	5 (2.1)
6 (*Ava*II 2)	1 (1.4)	0	1 (1.6)	0	0	2 (.8)
7 (*Ava*II 3)	1 (1.4)	0	0	0	1 (5.0)	2 (.8)
8 (*Ava*II 3, *Hin*cII 3)	1 (1.4)	0	0	0	0	1 (.4)
9 (*Ava*II 12, *Hin*cII 1)	1 (1.4)	0	0	0	0	1 (.4)
10 (*Ava*II 14)	1 (1.4)	0	0	0	0	1 (.4)
11 (*Pvu*II 3)	1 (1.4)	0	0	0	0	1 (.4)
12 (*Xba*I 2^{Ainu})	0	2 (4.2)	0	0	0	2 (.8)
13 (*Pst*I 3^{Ainu})	0	1 (2.1)	0	0	0	1 (.4)
14 (*Xba*I 3^{Ainu})	0	1 (2.1)	0	0	0	1 (.4)
15 (*Pst*I 2)	0	0	2 (3.1)	0	0	2 (.8)
16 (*Ava*II 24^{Korean}, *Xho*I 4^{Korean})	0	0	1 (1.6)	0	0	1 (.4)
17 (*Ava*II 25^{Aeta})	0	0	0	2 (5.4)	0	2 (.8)
18 (*Ava*II 25^{Aeta}, *Msp*I 12^{Aeta})	0	0	0	2 (5.4)	0	2 (.8)
19 (*Ava*II 26^{Vedda})	0	0	0	0	5 (25.0)	5 (2.1)
20 (*Ava*II 5)	0	0	0	0	1 (5.0)	1 (.8)

NOTE.—mtDNA types are classified by combining the enzyme morphs for each individual. Enzyme morphs of the most frequent type, type 1, are *Ava*II morph 1, *Hin*cII morph 2, *Msp*I morph 1, *Pst*I morph 1, *Pvu*II morph 1, *Xba*I morph 1, and *Xho*I morph 1. Variant enzyme morphs in each type are listed in parentheses. We assumed that no variation could be detected in the Japanese by *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I, *Msp*I, *Pst*I, *Xba*I, and *Xho*I.



though two main clusters (A and B) are observable in figure 4, the phylogenetic relationships of the populations are not clear.

Therefore, we also applied the maximum parsimony method to the same data set. Figure 5 is one of the possible maximum parsimony trees. Four hypothetical mtDNA types (X_1, X_2, X_3, and X_4) are necessary to complete the tree. All mtDNA types can be divided into two main groups separated by a dashed line. One of these main groups is for the mtDNA types detected only in African populations (Bantu and Bushman), and we call this the African group; these results confirm those of Johnson et al. (1983). The other (non-African) group contains all of the mtDNA types found in the non-African popula-

Figure 3 Possible maximum parsimony unrooted tree for the phylogeny of 20 mtDNA types listed in table 2. Subscripts by the numbers of each of the mtDNA types indicate the populations from which the individuals were sampled. Type X is hypothesized to complete the phylogenetic tree.

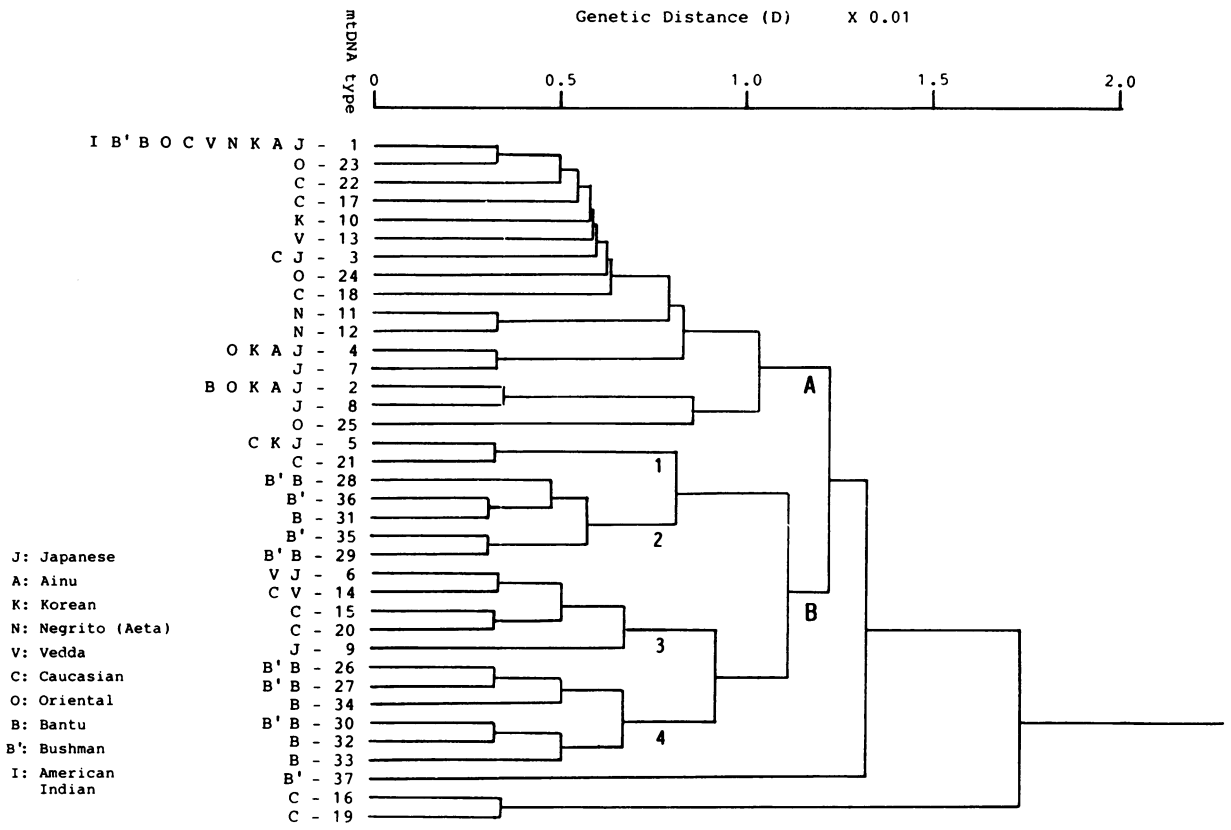
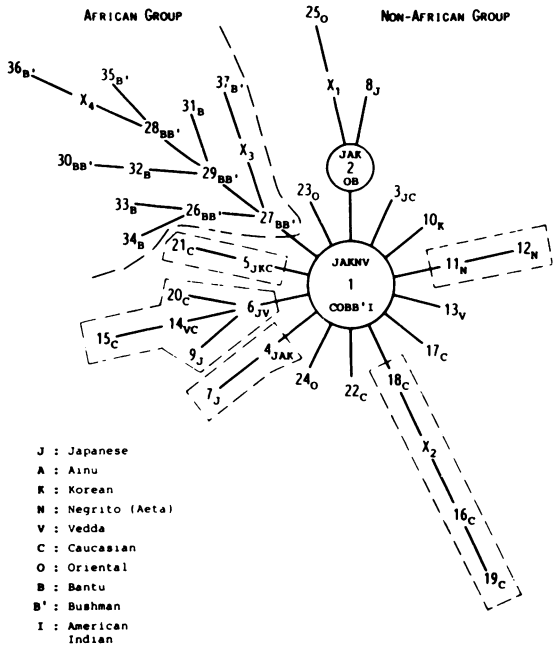


Figure 4 UPGMA phylogenetic tree based on genetic distances for 37 mtDNA types in 10 human populations. Letters at the left side of the number of each mtDNA type indicate the populations from which the individuals were sampled. Note that this numbering system is different from that of table 2 and fig. 3.



tions, and only types 1 and 2, which are circled in figure 5, contain some African individuals. Types 1 and 2 of the present study correspond to mtDNA types 1 and 8 of Johnson et al. (1983), respectively. There are five clusters in this group (boxed by dashed lines; see fig. 5), all radiating from type 1. One cluster contains two mtDNA types (11 and 12) found only in Negritos, whereas mtDNA types found only in Caucasians (16, 18, and 19) make one cluster with a hypothetical type X_2. On the other hand, the other three clusters contain mtDNA types found in more

Figure 5 Possible maximum parsimony tree for the phylogeny of 37 mtDNA types listed in 10 populations. Subscripts by the numbers of each of the mtDNA types indicate the populations from which the individuals were sampled. Four hypothetical types are needed to complete the tree: types X_1 (6-1-1-1), X_2 (9-3-2-4 or 9-1-2-4), X_3 (1-1-3-2 or 1-1-3-3), and X_4 (5-1-3-2) (figures in parentheses are enzyme morphs listed in the following order: *Ava*II, *Bam*HI, *Hpa*I, and *Msp*I). Groupings of mtDNA types are indicated by lines, and six subgroups are indicated by dashed lines.

Table 3

Nucleotide Diversities of Five Asian Populations and Genetic Distances among The Five Populations

	Japanese	Korean	Ainu	Aeta	Vedda
Japanese	.00086				
	(.00019)				
Korean	.000000	.00065			
	(.000127)	(.00017)			
Ainu	.000006	.000004	.00041		
	(.000122)	(.000114)	(.00015)		
Aeta	.000029	.000026	.000019	.00050	
	(.000157)	(.000151)	(.000145)	(.00022)	
Vedda	.000091	.000100	.000096	.000111	.00110
	(.000214)	(.000213)	(.000210)	(.000232)	(.00031)

NOTE.—On the diagonal are the nucleotide diversities; and under the diagonal are the genetic distances. Figures in parentheses are SEs.

than one population, though one of them (cluster of types 4 and 7) is for only Asian populations (Japanese, Ainu, and Korean). The remaining two clusters (one for types 5 and 21 and the other for types 6, 9, 14, 15, and 20) contain individuals from the Japanese, Korean, Vedda, and Caucasian groups. These two clusters correspond to subclusters 1 and 3 of cluster B of the UPGMA tree (see fig. 4). Interestingly, mtDNA types in these two clusters can be related to those of the African group in some alternative maximum parsimony trees. Connections between types 5 and 29 and between types 6 and 26 are possible, and mtDNA types belonging to the two clusters can be connected to mtDNA types detected only in the African populations.

Three mtDNA types (16, 19, and 37) that were outside clusters A and B in figure 4 seem to fit more reasonably in figure 5. Types 16 and 19 found in Caucasians belong to the non-African group, and together with type 18 they form a cluster for Caucasians, whereas type 37 found in Bantu belongs to the African group.

Nucleotide Diversity of Each Population and Population Phylogeny

Nucleotide diversities for the five populations examined in the present study and genetic distances among these populations are presented in table 3. The largest value (0.00110 ± 0.00031) of nucleotide diversity was for the Vedda, and the smallest (0.00041 ± 0.00015) was for Ainu.

On the basis of genetic distances, a UPGMA tree for the five Asian populations was constructed (fig. 6). In this tree, the distance between the Japanese and Korean populations is the smallest, and Ainu is clustered next to them. Aeta are not far away from these three mongoloid populations and make one cluster with the other three populations. On the other hand, Vedda is located quite apart from the other four populations. The genetic distance between Vedda and the remaining four populations is more than four times larger than that between Aeta and the other three Asian populations.

Discussion

In the present study, 12 presumably population-specific mtDNA morphs were detected in total. Also, Brega et al. (1986) have reported six distinct morphs among the Tharu of Nepal, morphs that have not been found in other populations, including those of the present study. These population-specific mtDNA

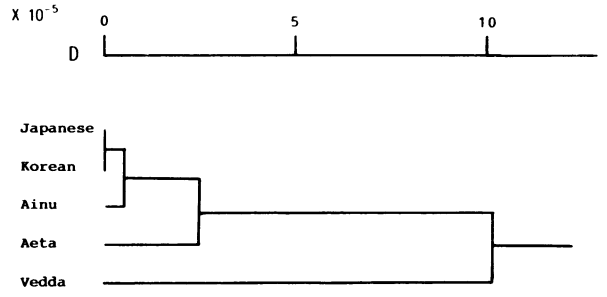


Figure 6 Phylogenetic tree based on genetic distances (listed in table 3) for five Asian populations.

types may be useful for elucidating problems of racial admixture.

*Hpa*I morph 1 (*Hin*cII morph 1) has been detected mainly in mongoloid populations: Orientals (mainly Chinese) (Denaro et al. 1981; Blanc et al. 1983), American Indians (Wallace et al. 1985), Japanese and Ainu (Harihara et al. 1986), the Tharu of Nepal (Brega et al. 1986), and Koreans (present study). Although it also has been found in low frequency in Bantu (Denaro et al. 1981), this morph can be used as a marker for mongoloid populations. *Hpa*I morph 4 (*Hin*cII morph 3) has been detected only in the mongoloid populations of east Asia: Orientals (mainly Chinese) (Denaro et al. 1981; Blanc et al. 1983), Japanese and Ainu (Harihara et al. 1986), and Koreans (present study). This morph can also be used as a mongoloid marker.

Among the variant morphs found in the present study, *Ava*II morphs 25^{Aeta} and 26^{Vedda} were relatively frequent in Aeta and Vedda, respectively. This may be due to the effect of random genetic drift, which likely has occurred in these small and isolated populations. A similar finding was reported for the American Indian by Wallace et al. (1985), who attributed the cause to the small number of female founders.

The maximum parsimony tree (fig. 5) seems to depict phylogeny of mtDNA types more appropriately than does the UPGMA tree (fig. 4). All mtDNA types could be divided into two main groups, African and non-African. However, we should be cautious when a phylogeny of mtDNA types is used for inferring population phylogeny, because a substantial part of the divergence between mtDNA types is thought to be attributable to ancient polymorphism, which existed well before the divergence of populations (Horai et al. 1986; Saitou and Omoto 1987).

The values of nucleotide diversity are lower by one order than those obtained by Cann et al. (1987). This might be due to differences between our study and theirs with regard to the restriction enzymes used. Another probable cause for this discrepancy is that Cann et al. (1987) may overestimate the values, since they used polyacrylamide gel for electrophoresis of digested mtDNA fragments and detected DNA conformational mutations (Singh et al. 1987).

The Negrito's relatively close affinity to mongoloid populations in the present study (see fig. 6) is consistent with the results of previous genetic studies (Omoto et al. 1978; Matsumoto et al. 1979; Horai et al. 1981; Omoto 1981, 1984). We computed genetic distances from Johnson et al.'s (1983) data and the present data and found that distances between two African populations and non-African populations are much greater than those among non-African populations (data not shown); thus the Negrito's affinity with the African populations seems to be quite low. The Vedda's phylogenetic location in the present study may suggest a long isolation of this population. A genetic study of Vedda and Sinhalese by means of blood markers also suggested this tendency (Ellepola and Wikramanayake 1986). However, since Vedda individuals today may be considerably mixed with surrounding populations, and since no mtDNA data are available for the neighboring Sinhalese and Tamils, we reserve any definite conclusions. We also note that the SE of the genetic distance is much larger than the genetic distance itself (table 3). Further studies, using other populations and additional restriction enzymes, will be necessary to clarify the evolution and phylogeny of the Asian populations.

Acknowledgments

This study was supported by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan.

References

Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457–465.

Blanc, H., K. H. Chen, M. A. D'Amore, and D. C. Wallace. 1983. Amino acid change associated with the major polymorphic *Hin*cII site of Oriental and Caucasian mitochondrial DNAs. Am. J. Hum. Genet. 35:167–176.

Botchan, M., W. Topp, and J. Sambrook. 1976. The arrangement of simian virus 40 sequences in the DNA of transformed cells. Cell 9:269–287.

Brega, A., R. Gardella, O. Semino, G. Morpurgo, G. B. Astaldi Ricotti, D. C. Wallace, and A. S. Santachiara-Benerecetti. 1986. Genetic studies on the Tharu population of Nepal: restriction endonuclease polymorphisms of mitochondrial DNA. Am. J. Hum. Genet. 39:502–512.

Brown, W. M. 1980. Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. Proc. Natl. Acad. Sci. USA 77:3605–3609.

Camin, J. H., and R. R. Sokal. 1965. A method for deducing branching sequences in phylogeny. Evolution 19: 311–326.

Cann, R. L., M. Stoneking, and A. C. Wilson. 1987. Mitochondrial DNA and human evolution. Nature 325:31–36.

Chomyn, A., P. Marriottini, M. W. J. Cleeter, C. I. Ragan, A. Matsuno-Yagi, Y. Hatefi, R. F. Doolittle, and G. Attardi. 1985. Six unidentified reading frames of human mitochondrial DNA encode components of the respiratory-chain NADH dehydrogenase. Nature 314:592–597.

Denaro, M., H. Blanc, M. J. Johnson, K. H. Chen, E. Wilmsen, L. L. Cavalli-Sforza, and D. C. Wallace. 1981. Ethnic variation in *Hpa*I endonuclease cleavage patterns of human mitochondrial DNA. Proc. Natl. Acad. Sci. USA 78:5768–5772.

Eck, R. V., and M. O. Dayhoff. 1966. Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, MD.

Ellepola, S. B., and E. R. Wikramanayake. 1986. A genetic study of the Veddas and the Sinhalese. Ceylon J. Med. Sci. 29:1–21.

Hadler, H. I., B. Dimitrijevic, and R. Mahalingam. 1983. Mitochondrial DNA and nuclear DNA from normal rat liver have a common sequence. Proc. Natl. Acad. Sci. USA 80:6495–6499.

Harihara, S., M. Hirai, and K. Omoto. 1986. Mitochondrial DNA polymorphism in Japanese living in Hokkaido. Jpn. J. Hum. Genet. 31:73–83.

Horai, S., T. Gojobori, and E. Matsunaga. 1984. Mitochondrial DNA polymorphism in Japanese. I. Analysis with restriction enzymes of six base pair recognition. Hum. Genet. 68:324–332.

———. 1986. Distinct clustering of mitochondrial DNA types among Japanese, Caucasians and Negroes. Jpn. J. Genet. 61:271–275.

Horai, S., and E. Matsunaga. 1986. Mitochondrial DNA polymorphism in Japanese. II. Analysis with restriction enzymes of four or five base pair recognition. Hum. Genet. 72:105–117.

Horai, S., K. Omoto, T. Juji, H. Sonozaki, H. Mitsui, S. Misawa, J. S. Sumpaico, and A. S. Mercado. 1981. The HLA antigens of two Negrito populations in the Philippines. Tissue Antigens 17:343–348.

Hutchison, C. A. III, J. E. Newbold, S. S. Potter, and M. H. Edgell. 1974. Maternal inheritance of mammalian mitochondrial DNA. Nature 251:536–538.

Johnson, M. J., D. C. Wallace, S. D. Ferris, M. C. Rattazzi, and L. L. Cavalli-Sforza. 1983. Radiation of human mitochondrial DNA types analyzed by restriction endonuclease cleavage patterns. J. Mol. Evol. 19:255–271.

Kirk, R. L., W. C. Parker, and A. G. Bearn. 1964. The distribution of the transferrin variants D_1 and D_{Chi} in various populations. Acta Genet. 14:41–51.

Matsumoto, H., T. Miyazaki, K. Omoto, S. Misawa, S. Harada, M. Hirai, J. S. Sumpaico, P. M. Medado, and H. Ogonuki. 1979. Population genetic studies of the Philippine Negritos. II. Gm and Km allotypes of three population groups. Am. J. Hum. Genet. 31:70–76.

Misawa, S., and Y. Hayashida. 1968. On the blood groups among the Ainu in Shizunai, Hokkaido. Proc. Japan Acad. 44:83–88.

Nei, M., and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.

Nei, M., and F. Tajima. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction site data. Genetics 105:207–217.

Omoto, K. 1972. Polymorphism and genetic affinities of the Ainu of Hokkaido. Hum. Biol. Oceania 1:278–288.

———. 1981. The genetic origins of the Philippine Negritos. Curr. Anthropol. 22:421–422.

———. 1984. The Negritos: genetic origins and microevolution. Acta Anthropogenetica 8:137–147.

Omoto, K., S. Misawa, S. Harada, J. S. Sumpaico, P. M. Medado, and H. Ogonuki. 1978. Population genetic studies of the Philippine Negritos. I. A pilot survey of red cell enzyme and serum protein groups. Am. J. Hum. Genet. 30:190–201.

Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. 113:237–251.

Saitou, N., and K. Omoto. 1987. Time and place of human origins from mtDNA data. Nature 327:288.

Singh, G., N. Neckelman, and D. C. Wallace. 1987. Conformational mutations in human mitochondrial DNA. Nature 329:270–272.

Sokal, R. R., and P. H. A. Sneath. 1963. Principles of numerical taxonomy. W. H. Freeman, San Francisco.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

Wallace, D. C., K. Garrison, and W. C. Knowler. 1985. Dramatic founder effects in Amerindian mitochondrial DNAs. Am. J. Phys. Anthropol. 68:149–155.