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Molecular Genetic Analysis of Remains of a 2,000-Year-Old Human Population in China—and Its Relevance for the Origin of the Modern Japanese Population

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Summary

We extracted DNA from the human remains excavated from the Yixi site (~2,000 years before the present) in the Shandong peninsula of China and, through PCR amplification, determined nucleotide sequences of their mitochondrial D-loop regions. Nucleotide diversity of the ancient Yixi people was similar to those of modern populations. Modern humans in Asia and the circum-Pacific region are divided into six radiation groups, on the basis of the phylogenetic network constructed by means of 414 mtDNA types from 1,298 individuals. We compared the ancient Yixi people with the modern Asian and the circum-Pacific populations, using two indices: frequency distribution of the radiation groups and genetic distances among populations. Both revealed that the closest genetic relatedness is between the ancient Yixi people and the modern Taiwan Han Chinese. The Yixi people show closer genetic affinity with Mongolians, mainland Japanese, and Koreans than with Ainu and Ryukyu Japanese and less genetic resemblance with Jomon people and Yayoi people, their predecessors and contemporaries, respectively, in ancient Japan.

Introduction

The genetic relationship between human populations of the Japanese archipelago and the Eurasian continent has been the object of many studies. Archaeological discovery in the Japanese archipelago has revealed evidence of habitation as far back as the Lower Paleolithic period (Imamura 1996). The four main islands constituting the Japanese archipelago were connected with each other or with the Eurasian continent during the coldest period of the latest glacial epoch, ~18,000 years before the present (ybp) (UNESCO/IOC Western Pacific Subcommission 1995). The Jomon period, corresponding to the Neolithic period in Europe, started ~12,000 ybp. Both the Upper Paleolithic period and the Jomon period are characterized by a hunter-gatherer economy, although the traces of plant cultivation and large-scale settlement have recently been found in many Jomon sites, including Sannai-Maruyama and Uenohara sites (which are located at the northern end of Honshu island and at the southern end of Kyushu island, respectively). The following Yayoi period is characterized by agriculture, especially wet-rice cultivation.

In accordance with these archaeological features showing drastic changes from the Jomon period to the Yayoi period, anthropological studies have pointed out that the Yayoi people are morphologically divided into two types. One of them is characterized by shorter stature, relatively low and wide facial contours, rather square orbital openings, markedly depressed nasal root, and prominent glabella and superciliary arches; these features are also known to be characteristic of the indigenous Jomon people. The other type is just the opposite—higher stature, higher and narrower facial contours, higher orbital openings, much shallower nasal root, and remarkably flat glabella and superciliary arches. The latter type is observed mainly in the Yayoi people, whose remains have been excavated from sites in northern Kyushu (in the Japanese archipelago, Kyushu is the southwestern main island nearest to Korea and China). Therefore, it has been proposed that the Yayoi people in northern Kyushu might have been immigrants from the Eurasian continent to the Japanese archipelago (Kanaseki 1966).

During recent decades, a number of studies have reported the genetic structures of the modern Asian populations and have discussed their phylogenetic relationships (e.g., see Nei 1995; Omoto and Saitou 1997). However, the population history is still in controversy.

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Table 1

mtDNA Nucleotide Sequences of 2,000-Year-Old Human Remains from the Yixi Site

	Sequence[a]	
	MT1+MT4	MT3
	1111111111111111111111111	
	6666666666666666666666666	
	2222222222222222233333	111111112222
Source/	0122334566667778990112 6	455678991457
Sample	3713249624560489718419 72	602705050935
CRS	ATCCCCTCCCACCGCCCCTTTGCT	TCTCCGCTAACA
Yixi:		
01	G.....T.C....	.....-.G
02	...T.....C	.....G
03	...T.....C...T.	C..TT....-.G
04	.....C....	C.....-.G
05	.....A.C	.....T.G..G
06	...T.....T.....C	.T.....G
07	.C...T.....	.....G
08	...T.....T.....C	.....TG
09	..T.....C....	..C....C.-.G
10	...T.....	**********
11	.....C....	**********
12	....A.C.....CC...	**********
13	...T.....C...T.	**********
14	...T.....A..T.....*	.T.....G
15	.....T.....C...*	..C.....-.G
16	G.....T.C...*	.....-.G
17	.....A.....A.*	.T.....G
18	.....C..A.....*	.....A.....G
19	...T.....*	C.....G
20	G.....T.....T.C...*	**********
21	...T...TTT.T...TA.C..A.*	**********
22	...T.....T.....*	**********
23	.....C...*	**********
24	**********	.T.....G

[a] The notation of the CRS is used for numbering of bases. A dot (.) denotes that the nucleotide is identical to that in the CRS (Anderson et al. 1981), a dash (–) denotes a gap, and an asterisk (*) denotes that the identity of the nucleotide is unknown.

This might be due to lack of direct genetic information about ancient people, because comparison using only modern humans is often affected by changes with the times, such as migration and random genetic drift. Genetic research on ancient populations is an absolute prerequisite for disclosing of past events and investigation of the population history.

To determine the genetic background of the ancient Chinese people, we extracted DNA from the 2,000-year-old human remains excavated in the Shandong peninsula of China and determined the nucleotide sequences of their mitochondrial D-loop regions. We also examined phylogenetic relationships among the modern and ancient Asian peoples, by constructing an mtDNA tree and evaluating nucleotide diversity among populations, to understand the genetic relationship of the peoples living in eastern Asia.

Material and Methods

Site and Samples

The Yixi site is located in Linzi, Shandong Province, China. Linzi was the capital of the Qi dynasty during the Spring-Autumn and Warring States period (770 B.C.–221 B.C.). This site has been dated to the period of the Han dynasty (206 B.C.–220 A.D.) in China, on the basis of the archaeological finds excavated. A total of 42 teeth (mainly third molars) and 16 bone samples were used for DNA analysis, by official permission authorized by the Cultural Relics Bureau of the People's Republic of China.

DNA Extraction and Purification

To destroy possible contaminant DNA on the remains' surfaces, bone and teeth samples were exposed to UV irradiation. After storage in a 0.5 M EDTA solution at 4°C for >1 mo, the samples, frozen in liquid nitrogen, were physically crushed by a Cryo-Press CP-100 (Microtech Nichion). For 36 teeth among the total of 58 samples, DNA extraction and purification were performed by the silica-based method (Boom et al. 1990; Höss and Pääbo 1993). Fine-powdered teeth were suspended in 1 ml of lysis buffer. Lysis buffer was prepared by mixing of 10 ml of 0.1 M Tris-HCl (pH 6.4), 12 g of guanidinium thiocyanate (GuSCN), 2.2 ml of 0.02 M EDTA, and 0.5 ml of Triton X-100. After being well shaken, the mixture was incubated at 65°C overnight, with occasional agitation by a touch mixer. After centrifugation for 5 min at 1,500 *g*, 500 μl of the supernatant was removed to another tube, and 500 μl of the lysis buffer was added to it. A 40-μl aliquot of 3% (w/v) silicon dioxide solution, stored at room temperature in the dark, was added, and the mixture was incubated for 15 min at room temperature. After centrifugation for a few seconds, the supernatant was removed, and the silica pellet was washed twice with 500 μl of 0.1 M Tris-HCl (pH 6.4) washing buffer containing 10 M GuSCN. Subsequently, the silica pellet was washed twice with 500 μl of 70% ethanol and once with acetone. After the pellet had been dried at 60°C in a heat block, ancient DNAs were eluted at 56°C in 65 μl of water (Ultraspec™; Biotech). A 50-μl portion was recovered and used as a template for DNA amplification by PCR. Lysis and washing buffers were used after preincubation with silica suspension.

For the remaining 22 samples (6 teeth and 16 bones), the EDTA/phenol method was used. The powdered samples were suspended in 10 ml of 0.5 M EDTA solution and then were extracted, twice, with $\frac{1}{2}$ vol of water-saturated phenol and chloroform/isoamyl alcohol (24:1). The aqueous phase was removed and precipitated

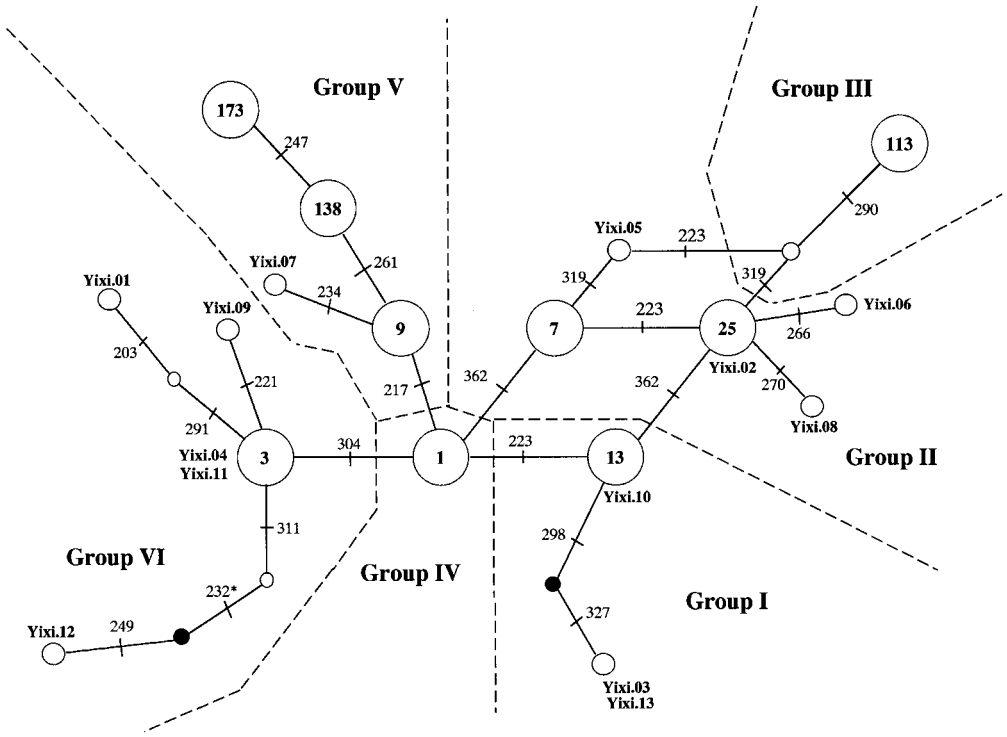

Figure 1 mtDNA network. Smaller unblackened circles represent distinct mtDNA types found in modern humans, blackened circles represent types absent in the mtDNA database, and larger unblackened circles represent backbone types 1, 3, 7, 9, 13, 25, 113, 138, and 173. The numbers on the branches denote nucleotide positions (according to the CRS; note that the prefix "16" has been omitted) where substitutions have occurred. Reticulations in the network indicate the existence of incompatible nucleotide configurations.

with 2 vol of cold ethanol at −20°C. The precipitate was redissolved in 2 ml of water. After 0.5 ml of 10 M lithium chloride solution was added and the mixture was kept on ice for 30 min, the supernatant was removed by centrifugation and was precipitated with 2 vol of cold ethanol. After the precipitate was dissolved in 0.1 ml of water, the DNA extracts were further purified by the silica-based method described above. To verify that no contamination had occurred during DNA extraction, we used, as blank controls, the extracts that did not have remains.

PCR Direct Sequencing of mtDNA

We used three primer sets designed within the mitochondrial D-loop region, one of which (MT4) was newly designed in the present study and the remaining two of which (MT1 and MT3) were from Kurosaki et al. (1993). The MT1 and MT4 regions are in hypervariable region I, whereas MT3 region is in hypervariable region II. The nucleotide sequences correspond to positions 16190–16208 and 16422–16403, positions 90–109 and 326–307, and positions 16135–16154 and 16366–16347 of the Cambridge Reference Sequence (CRS) (Anderson et al. 1981) for MT1, MT3, and MT4, respectively. Ancient DNAs were amplified by the hot-start PCR technique using anti-*Taq* DNA polymerase antibody (*Taq*Start Antibody; Clontech). DNA amplification was performed in 20, 40, and 40 μl of reaction mixture containing 100 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 500 mM KCl, 200 M of each dNTP, 2 units of Ampli*Taq* DNA polymerase (Perkin-Elmer), and 5, 10, and 20 pmol of the PCR primers for MT1, MT3, and MT4, respectively. Forty cycles of PCR were performed as follows: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s. Infrequently, second-round PCR amplification was necessary, in which the reaction was performed under ordinary conditions without anti-*Taq* antibody. To verify the reliability of the results obtained, we repeated the experiments, along with negative control experiments without template DNAs.

DNAs amplified by PCR were purified by means of an S-300 spin column (Pharmacia) and were used as sequencing templates. Nucleotide sequences of both strands were determined by means of the FS *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied BioSystems), according to the manufacturer's instructions, by an Applied BioSystems 373S DNA sequencer.

Table 2

Distribution of Radiation Groups in Yixi and Jomon Individuals

Radiation Group and Ancient Individual(s)	Sharing Populations (No. of Individuals Shared) in DNA Database
I:	
Yixi-10	Turk (1), Ainu (4), mainland Japanese (2), Korean (2), Ryukyu Japanese (7), Cantonese (3), Mongolian (1), Siberian Altai (1), Native American (2), Indonesian (2), aboriginal Taiwanese (2), Filipino (1), Melanesian (1), Polynesian (1)
Yixi-03 and Yixi-13	Korean (1), Taiwan Han Chinese (2), Cantonese (1), Mongolian (5), Native American (1), Polynesian (1)
II:	
Yixi-02	Turk (1), Ainu (1), mainland Japanese (15), Koreans (11), Ryukyu Japanese (1), Taiwan Han Chinese (3), Asian (1), Mongolian (6), Native American (1), aboriginal Taiwanese (1), Melanesian (8)
Yixi-05	(Unique)
Yixi-06	(Unique)
Yixi-08	(Unique)
III:	
(None)	
IV:	
(None)	
V:	
Yixi-07	Indonesian (1)
VI:	
Yixi-04 and Yixi-11	Turk (2), Taiwan Han Chinese (5), Cantonese (1), Mongolian (4), Native American (1), Indonesian (1), Filipino (1), Polynesian (3)
Yixi-01	(Unique)
Yixi-09	Mainland Japanese (1)
Yixi-12	Korean (1), Mongolian (3)

Some sequences were determined by means of primers end labeled with [γ-^{32}P]-ATP by T4 polynucleotide kinase. Samples with conflicting nucleotide sequences between strands or between regions overlapping with each other were excluded from the subsequent data analysis (in this study, there were three samples whose nucleotide sequences of both strands obtained were not completely complementary).

Phylogenetic Analyses

We compared mtDNA sequences of a total of 1,298 individual samples retrieved from the DDBJ/EMBL/GenBank international nucleotide-sequence database: 45 Turks (Comas et al. 1996), 5 Australian Aborigines (Vigilant et al. 1991; Betty et al. 1996), 51 Ainu, 64 South Koreans, 50 Ryukyu Japanese (Horai et al. 1996), 62 mainland Japanese (Vigilant et al. 1991; Horai et al. 1996), 66 Taiwan Han Chinese (Horai et al. 1996), 20 Cantonese (Betty et al. 1996), 17 Asians (Vigilant et al. 1991), 101 Mongolians (Kolman et al. 1996), 17 Altai of central Siberia (Shields et al. 1993), 353 New World Native Americans (Vigilant et al. 1991; Ward et al. 1991, 1993; Shields et al. 1993; Batista et al. 1995; Kolman et al. 1995), 65 Indonesians (Vigilant et al. 1991; Redd et al. 1995; Sykes et al. 1995), 35 aboriginal Taiwanese (Sykes et al. 1995), 36 Filipinos (Vigilant et al. 1991; Sykes et al. 1995), 104 Melanesians (Vigilant et al. 1991; Sykes et al. 1995; Redd et al. 1995), 5 Micronesians (Sykes et al. 1995), 16 Kapongamrangi (Sykes et al. 1995), and 186 Polynesians (Vigilant et al. 1991; Sykes et al. 1995; Redd et al. 1995). After identical sequences from these data were combined, 414 distinct mtDNA sequences (types) were obtained. In addition, sequence data on 5 Jomon (Horai et al. 1989, 1991), 14 Yayoi (Oota et al. 1995), and 28 Pygmies (Vigilant et al. 1991) were used. Phylogenetic networks of mtDNA types were constructed by means of the network methods of Excoffier and Langaney (1989) and Bandelt et al. (1995). Nucleotide diversity is the average number of nucleotide differences, per site, between populations and is equivalent to heterozygosity at the nucleotide level, for a randomly mating population (Nei 1987). Distance values for calculation of nucleotide diversity were estimated by means of the program CLUSTAL W (Thompson et al. 1994).

Results

For the total of 58 samples, we obtained 23 and 16 nucleotide sequences, for hypervariable regions I and II, respectively. There is a cytosine tract in the hypervariable region I from CRS positions 16184–16193. Since heteroplasmy due to length variation in this region has been reported (Bendall and Sykes 1995), nucleotide sequences excluding this tract were used for phylogenetic analysis. There were a total of 36 phylogenetically informative sites, which are presented in table 1.

Figure 1 shows a phylogenetic network for nine back-

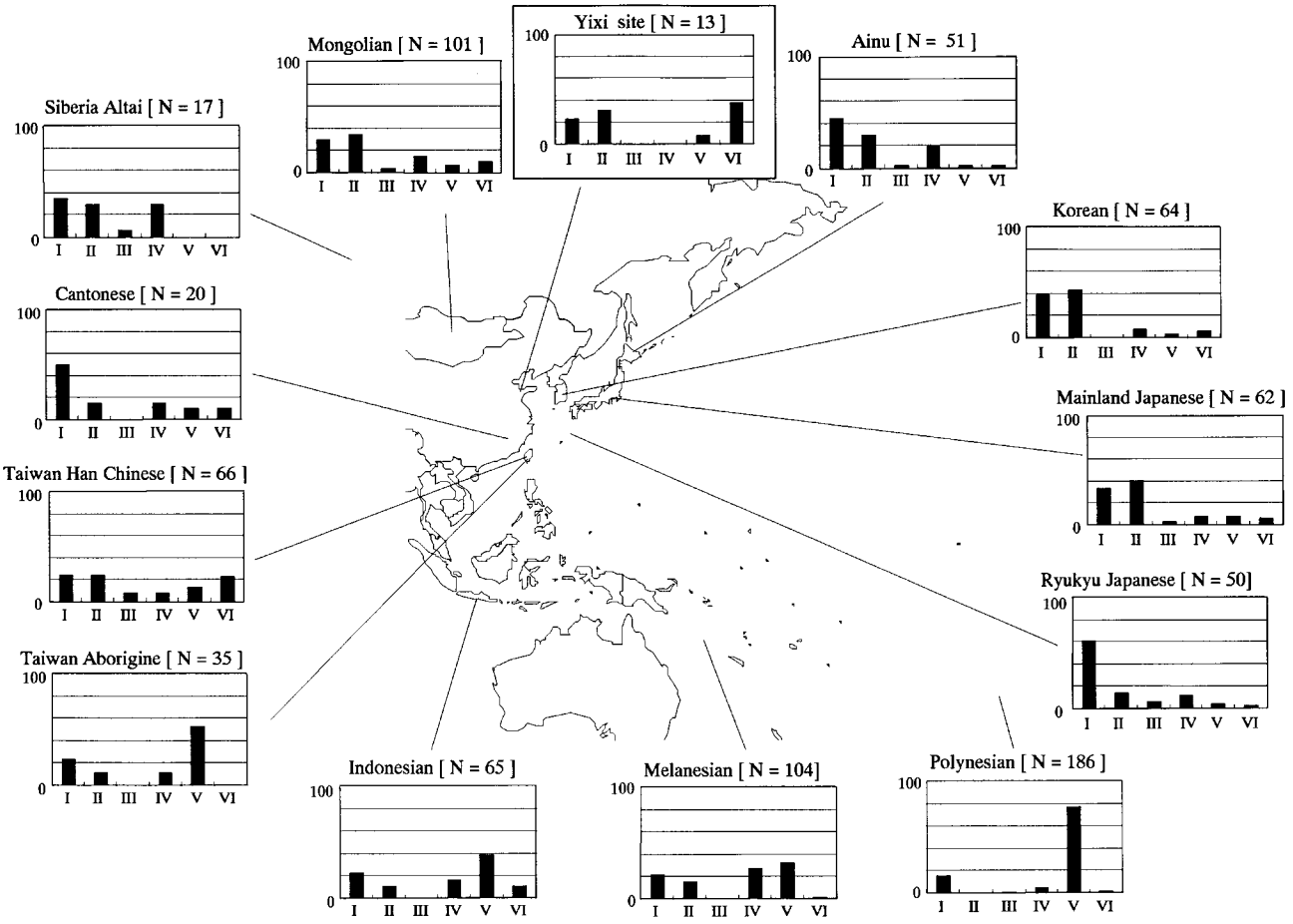

Figure 2 Geographic distribution (in %) of six radiation groups in the eastern Eurasian continent and Oceania

bone mtDNA types (types 1, 3, 7, 9, 13, 25, 113, 138, and 173), which were deduced from the huge phylogenetic tree constructed by use of 185 nucleotides of MT1 and MT4 regions from 414 mtDNA types of 1,298 modern humans in Asia and the circum-Pacific region (H.O., S.U., and N.S., unpublished data). The backbone type is defined as a center of a star having >10 descendant types. On the basis of the starlike clusters, we identified six radiation groups. Group I, including type 13, was characterized by C/T transition at CRS position 16223, without T/C transition at position 16362. Group II includes types 7 and 25 and was characterized by T/C transition at CRS position 16362, without G/A transition at position 16319. Group III was characterized by three nucleotide substitutions, at CRS positions 16223, 16319, and 16362. Group V was characterized by T/C transition at CRS position 16217, without substitutions at positions 16223 and 16362. Group VI was characterized by T/C transition at CRS position 16304, without substitutions at positions 16223 and 16362.

To reveal the genealogy of the mtDNA sequences of the Yixi people, we attached their sequence data to the network tree obtained on the basis of modern human data. Unfortunately, the data on the MT3 region in modern humans are fewer. We were thus obliged to use nucleotide sequences of MT1 and MT4 regions alone as the longest sequence available. A total of 11 mtDNA types from 13 Yixi individuals (Yixi-01–Yixi-13) are presented in table 1; 4 types are unique to the Yixi, whereas the remaining 7 types are shared with modern human populations, and 4 of these 7 are shared with more than five modern human populations (fig. 1 and table 2). Thus, 9 of the 13 Yixi individuals share their mtDNA types with modern humans from populations covering the entire circum-Pacific region.

Figure 2 shows the frequency distributions of six radiation groups in the ancient Yixi and in 12 modern human populations. Group I was found in all the 13 populations. Groups II, V, and VI, however, had distributions that were unique to each population. For Mongolians, Siberian Altai, Ainu, Koreans, mainland Japanese, and Taiwan Han Chinese, the frequencies of group

Table 3

Mean Pairwise Comparisons Within and Between Populations

	Genetic Distance from[a]							
Population	Yixi ($n = 13$)	Mainland Japanese ($n = 62$)	Korean ($n = 64$)	Ainu ($n = 51$)	Ryukyu Japanese ($n = 50$)	Taiwan Han Chinese ($n = 66$)	Mongolian ($n = 101$)	Pygmy ($n = 28$)
Yixi	2.035							
Mainland Japanese	.066	2.131						
Korean	.097	0	2.069					
Ainu	.154	.075	.077	2.274				
Ryukyu Japanese	.129	.029	.038	.072	1.844			
Taiwan Han Chinese	0	.031	.058	.125	.102	2.561		
Mongolian	.023	.014	.016	.113	.077	.024	2.456	
Pygmy	1.44	1.44	1.39	1.46	1.39	1.38	1.38	3.028

[a] All values have been multiplied by 100. Genetic distances (D_A) between populations are below the diagonal and have been calculated according to the following formula: $D_A = D_{XY} - [(D_X + D_Y)/2]$, where D_{XY} is the pairwise mean between populations X and Y and where D_X (D_Y) is the pairwise mean within population X (population Y) and is given on the diagonal. The values given as "0" contain negative-value estimations.

II were >20%, whereas those for Indonesians, aboriginal Taiwanese, Melanesians, and Polynesians were <20%. Mongolians, Koreans, and mainland Japanese showed particularly high frequencies of group II (34%, 44%, and 40%, respectively). Group V had the highest frequency in Polynesians (77%). High frequency for group V was also observed in aboriginal Taiwanese (53%) and Indonesians (39%). Thus, the frequencies of group II were higher in the northern populations than in the southern populations of the western circum-Pacific, whereas those of group V were just the opposite.

Three, four, and one Yixi individuals belonged to groups I, II, and V, respectively. No individuals were classified as belonging to groups III and IV, which were the most major radiation groups for Native Americans (59%) and Europeans (81%), respectively. The most characteristic feature of the Yixi people is that 5 (38%) of 13 individuals belonged to group VI. Modern human populations showed relatively low frequency for group VI, except for modern Taiwan Han Chinese, in which this frequency was >20%. In Siberian Altai, aboriginal Taiwanese, and Micronesians, there were no individuals who belonged to group VI, and the frequency was <10% in Ainu, Koreans, mainland Japanese, Ryukyu Japanese, and Melanesians. Distribution frequencies for group II suggest similarity between the Yixi people and northern Asian populations. We also compared the frequency distributions of radiation groups by using the 151-bp MT4 information alone, from 23 Yixi samples. One informative site (CRS position 16362) was lost, and there was no distinction either among mtDNA types 1 and 7 or among mtDNA types 13 and 25. However, the radiation group characteristic of the Yixi people and Taiwan Han Chinese—that is, group VI—remained distinguishable, and its distribution frequency was high (i.e., 9 [39%] of 23).

Table 3 presents the values of nucleotide diversity within and between populations. The highest value of nucleotide diversity within circum-Pacific populations was observed in Taiwan Han Chinese (2.6%). The Yixi people had a value of 2.0%. Among the seven Asian populations, the values for nucleotide diversity within populations varied within the range of 1.8%–2.6% (that of Pygmies was 3.0%). Between the Yixi people and the seven modern human populations, there was no significant within-population difference in nucleotide diversity. Between populations, the lowest value in net nucleotide substitutions, 0, was found between the Yixi people and Taiwan Han Chinese, suggesting that the closest genetic relationship is between them; the second lowest value for difference from the Yixi population was observed in Mongolians (0.023), followed by mainland Japanese (0.066) and Koreans (0.097). The lowest values of difference from modern mainland Japanese were in Koreans (0.00), Mongolians (0.014), Ryukyu Japanese (0.029), and Taiwan Han Chinese (0.031). When the 154-bp MT4-site information alone was used, one informative site (CRS position 16362) was lost, but 10 additional samples (23 samples in total) were available for comparison. Between the Yixi people and other populations, the lowest value in net nucleotide substitutions was observed in Taiwan Han Chinese (0.000), as in the case when the 185-bp MT1+MT4 region from 13 Yixi individuals was used for comparison.

Discussion

We compared the genetic relatedness between the ancient Yixi people and the modern Asian populations by two methods using mtDNA sequences; one is the frequency-distribution comparison of radiation groups on the phylogenetic network, and the other is the mean

Table 4

Distribution of Radiation Groups in Jomon Individuals

Jomon Individual(s)[a]	Sharing Populations (No. of Individuals Shared) in DNA Database	Radiation Group
URAWA-1a (5,900 ybp)	Indonesian (1), aboriginal Taiwanese (1), Filipino (6), Micronesian (2)	II
TODA-1a (6,000 ybp), TAKA-5b (3,000 ybp), TAKA-21b (3,000 ybp)	Yixi-02, Turk (1), Ainu (1), mainland Japanese (15), Korean (11), Ryukyu Japanese (1), Taiwan Han Chinese (3), Asian (1), Mongolian (6), Native American (1), aboriginal Taiwanese (1), Melanesian (8)	II
TAKA-13b (3,000 ybp)	Yixi-10, Turk (1), Ainu (4), mainland Japanese (2),Korean (2), Ryukyu (7), Cantonese (3), Mongolian (1), Siberian Altai (1), Native American (2), Indonesian (2), aboriginal Taiwanese (2), Filipino (1), Melanesian (1), Polynesian (1)	I

[a] Lowercase letters appended to numerals denote the geographic source: a = Saitama Prefecture in mainland Japan (Horai et al. 1989), and b = Hokkaido Island in Japan (Horai et al. 1991).

pairwise sequence comparison based on the nucleotide diversity among populations. Both show the closest genetic affinity between the Yixi people and the modern Taiwan Han Chinese.

We added the sequence data from the Jomon people (Horai et al. 1989, 1991) within the phylogenetic tree of the modern human data. All of the five Jomon individuals belonged to either of group I or group II; 1 Jomon individual shared mtDNA type with 10 individuals from Southeast Asia and Oceania, and the remaining 4 Jomon individuals shared mtDNA type with 7 Yixi and 90 circum-Pacific individuals (see table 4). This means that the Jomon people shared mtDNA types with individuals from all of the circum-Pacific populations, including the 2,000-year-old Chinese population.

Figure 3 shows the phylogenetic network constructed by use of the mtDNA sequences of (*a*) the Yayoi people from the Takuta-Nishibun site of northern Kyushu, Ja-

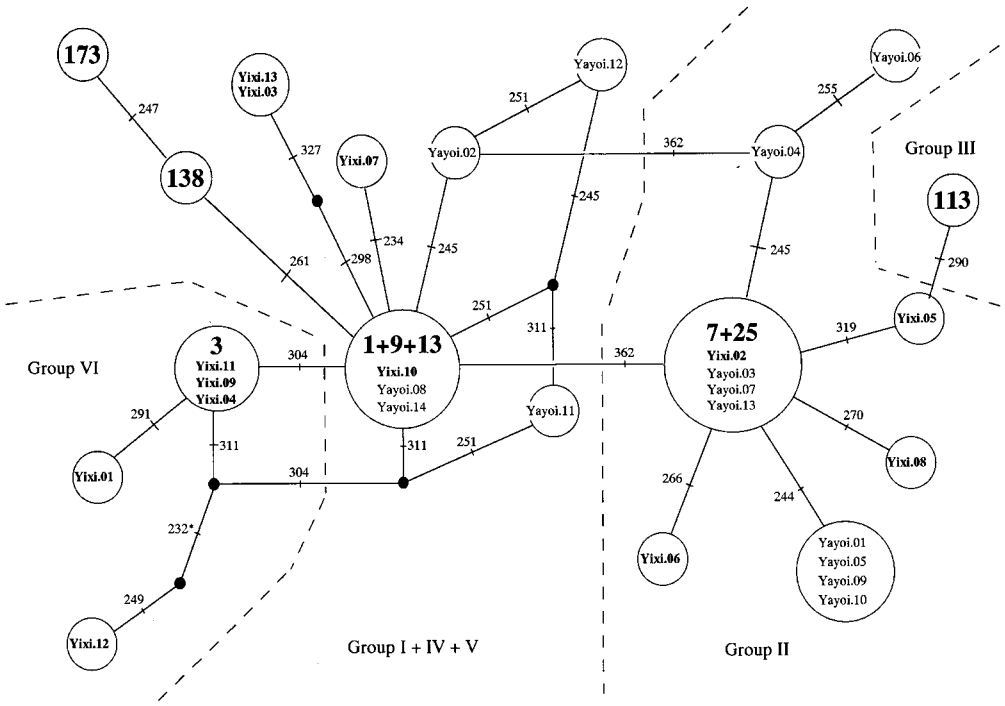

Figure 3 mtDNA network among ancient Japanese and ancient Chinese individuals. The network was constructed by use of the 154-bp MT-1 nucleotide sequences of 14 individuals from the Takuta-Nishibun Yayoi site, 13 individuals from the Yixi Han site, and 9 backbone mtDNA types. Completely closed circles represent absent types (circle size is not significant). The numbers on the branches denote nucleotide positions (according to the CRS; note that the prefix "16" has been omitted) where substitutions have occurred. The positions with and without an asterisk (*) are sites of substitutions by transversion and by transition, respectively. Reticulations in the network indicate the existence of incompatible nucleotide configurations.

pan (Oota et al. 1995), (*b*) the Yixi people (present study), and (*c*) the nine backbone types (H.O., S.U., and N.S., unpublished data). Since only shorter sequences (154-bp MT1) were available for comparison, two informative sites—CRS positions 16217 and 16223—were lost. Therefore, mtDNA background types 1, 9, and 13 also were indistinguishable, and mtDNA background types 7 and 25 were indistinguishable; and the network therefore was split into only four radiation groups (groups I+IV+V, group II, group III, and group VI). No individuals belonging to group III were found in either the Yayoi people or the Yixi people. Of the 14 Yayoi individuals, 5 were included within groups I+IV+V and 9 were included within group II; however, neither any Yayoi people from the Takuta-Nishibun site nor any Jomon people were included within group VI.

On the basis of morphometric data, Hanihara (1991) claimed that large-scale migration from the continent to the Japanese archipelago occurred during the transition period from Jomon to Yayoi—that is, ~2,000 ybp—and that, subsequently, gene flow by immigrants significantly contributed to the genetic structure of the mainland Japanese. Present data seem to show less contribution from the 2,000-year-old Yixi population to the genetic structure of the modern mainland Japanese.

Hanihara (1991) also proposed the dual-structure model, one of the hybridization theories, which supposes two events of major migration for the genetic constitution of modern Japanese—that of the Jomon ancestors and that of the Yayoi immigrants. This model claims (1) that the indigenous Jomon people and the Yayoi immigrants admixed with each other gradually, (2) that Ainu and Ryukyu Japanese are direct descendants of the Jomon people, and (3) that the Jomon people originated from Southeast Asia, whereas the Yayoi immigrants originated from northern Asia. Horai and his colleagues concluded that the modern Japanese are divided into two groups and that the mtDNA of the Jomon people and that of Ainu belong to the same group (Horai and Matsunaga 1986; Horai et al. 1991). These investigators also estimated the divergence time between the indigenous and immigrant groups as being 125,000 ybp and, moreover, claimed, as evidence, that the Jomon people and Southeast Asians share identical mtDNA sequence (Horai et al. 1989, 1991). As Oota et al. (1995) subsequently explained, however, such an extended divergence time merely means that genetic polymorphisms existed in the ancestral population of the Jomon people, because the Jomon period started only ~12,000 ybp. Furthermore, four of the five Jomon individuals reported by Horai et al. shared mtDNA types with many individuals from northern Asia. The three mtDNA types found in the Jomon individuals were included within groups I and II, which have higher frequencies in eastern Asia than in Southeast Asia. In addition, no Jomon individuals were found to belong to group V, a group that is characteristic of Southeast Asian and Oceanic populations; thus, there seems to be no strong connection between them.

The present study shows the closest genetic relatedness between the 2,000-year-old population in Shandong peninsula and the modern Taiwan Han Chinese but shows less genetic resemblance between the 2,000-year-old populations from northern Kyushu (Japan) and the Shandong peninsula (China). Genetic information on the earlier people of the continent holds the key to solution of the question of the origin of the Jomon/Yayoi people and, consequently, of human history in the Japanese archipelago.

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Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

DDBJ/EMBL/GenBank, http://www.ddbj.nig.ac.jp/Welcome-e.html (for nucleotide sequences [AB021466–AB021489])

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