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Molecular Genetic Analysis of 16th–18th Century Remains from Ishikobara Site, Nagano Prefecture, Japan

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Abstract In order to examine the genetic structure and kinship system of an Edo village, we investigated the ancient DNA recovered from the Ishikobara site in Nagano Prefecture, Japan. D-loop and coding region sequences of mitochondrial DNA (mtDNA) were retrieved from the samples of 12 bones and teeth. Ancient DNA was successfully amplified from 9 samples. Each mtDNA sequence was assigned to a relevant haplogroup using the known mtDNA database.

The mtDNA data of burials excavated at the site are presented for genetic comparison with another Edo population as well as for defining their respective internal variabilities. Maternally related individuals were identified and a haplotype comparison of these individuals provided additional background information on the local societies.

Taking the mtDNA data for the Ishikobara site and other Japanese populations, a phylogenetic analysis was performed in order to reveal the relationships among these populations. The results show that the genetic structures of the Edo villages resemble each other. Compared to the present-day Japanese population, these villages exhibit higher frequencies of haplogroups D4 and G. The loss of other haplogroups in the Edo populations is probably due to genetic drift or the small size of these populations.

Key words : Ancient DNA, Mitochondria, Edo period, Ishikobara site, Population genetics

Introduction

Recent advances in molecular biological techniques have facilitated the recovery and analysis of DNA from ancient materials, thereby enabling an objective approach to studying the genetic composition of past populations. There are various methods available for exploring the kinship relationships among excavated human burials. "Archaeogenetics," or the application of molecular genetics to the study of the human past, as Renfrew and Boyle (2000) note, is "a fast-growing field" since it offers a vastly superior discriminatory power over other methods. During the 1990s, a branch of archaeogenetics known as "biomolecular archaeology," which focuses on the study of ancient DNA derived from excavated materials, largely supplanted methods based on blood groups and other genetically determined markers derived from living populations (Renfrew, 2000).

Because of its special characteristics, including small size, matrilineal inheritance, high copy number, and fast mutation rate, DNA analysis of ancient materials is currently based mainly on mitochondrial DNA (mtDNA). This recently acquired ability to analyze mtDNA from archaeological remains yields more accurate genetic information than can be obtained through the morphological study of bones.

Although the molecular analyses of ancient bones has become one of the more frequently used methods in recent years, only one study has focused on the population genetics of archaeological sites dating from the Edo period of Japan (Adachi *et al*., 2004). In the present study, we analyzed the genetic structure and kinship system of an Edo village and the relationship between

the Edo population and the present-day Japanese population based on mtDNA data. However, it should be kept in mind that mtDNA data yields no information concerning the genetic connections along the paternal line. In the absence of additional independent lines of evidence, it is not possible to specify the biological relationships among excavated individuals beyond those that are described in this paper.

Materials and Methods

Archaeological site and specimens

The Ishikobara site, which is located in Nagano Prefecture, Japan, is a composite site that contains the ruins of Palaeolithic, Jomon, Yoyoi, and Edo period settlements. The approximate location of the site is shown in Figure 1. The site extends 130 m from north to south and 400 m from east to west. As part of a highway construction project, the site was excavated in 2000 and 2004 by the Center for Buried Cultural Properties of Nagano Prefecture. Only the site dating from the Edo period contained human skeletal remains.

As no cemetery inventory remains, the chronology of the Ishikobara site of the Edo period was determined by the design of burial coins. The dates of interment for these burials are estimated to range approximately from the 16th to 18th century. Twenty remains from 24 graves were selected for morphological study, based on the completeness of the remains. Age at death, sex, stature, and evidence of gross level pathology were determined for each excavated skeleton (Shigehara and Anezaki, 2006). These 20 individuals consisted of 8 males, 3 females, and 9 individuals of undetermined sex; their ages ranged from infants to mature adults. Twelve well-preserved samples were selected for further DNA

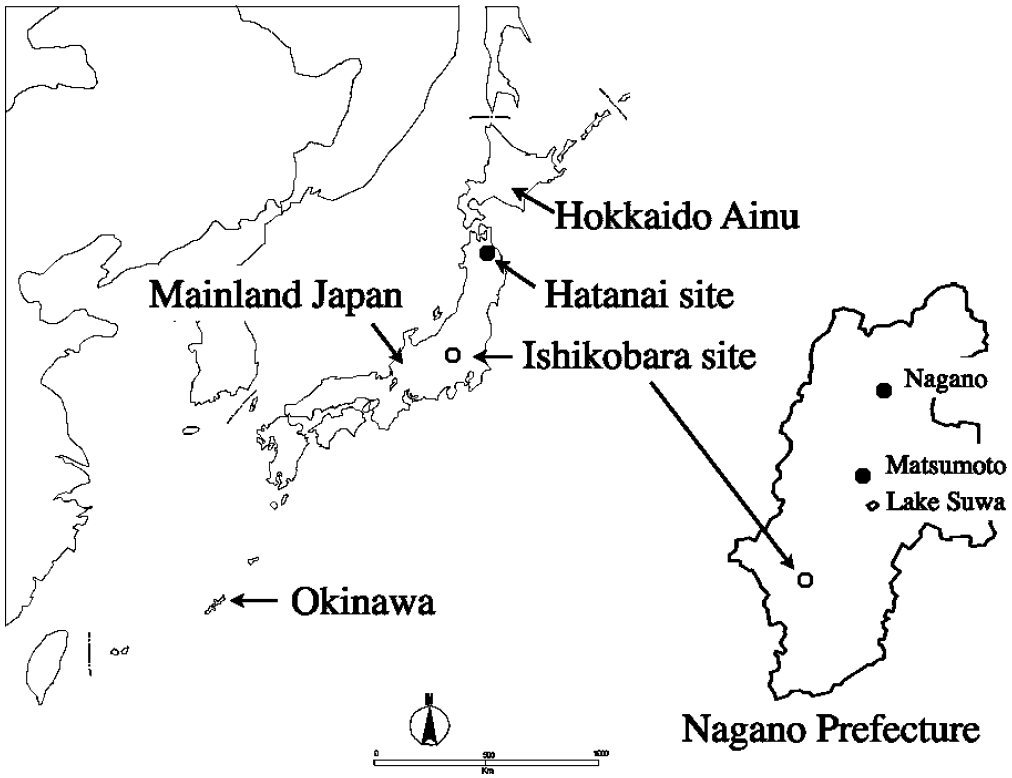

Fig. 1. Map of Japan. Arrows indicate the locations of the populations analyzed in this study. Open and closed circles represent the Ishikobara and Hatanai sites, respectively.

Table 1. Samples used for ancient DNA extraction.

No.	Code name	Sex	Age	DNA source
1	SM07	unknown	mature	tooth
2	SM08	male	old	femur
3	SM09	male?	adult	coxa
4	SM13	female	adult	tooth
5	SM14	male	adult	tooth
6	SM15	female	adult	femur
7	SM16	male?	adult	tooth
8	SM19	male	mature	tooth
9	SM20	unknown	unknown	femur
10	SM22	unknown	adult	tooth
11	SM23	unknown	juvenile	tooth
12	SH05	male	adult	tooth

analysis.

It is suggested that tooth enamel forms a natural barrier to exogenous DNA contamination; furthermore, the DNA recovered from teeth appears to lack most of the inhibitors to the enzymatic amplification of ancient DNA (Woodward *et al*., 1994). Therefore, in many cases, teeth samples were used in the present analysis. When teeth samples were not available, cortical bones were used. A list of all the samples used in this study is presented in Table 1.

Authentication methods

When ancient DNA is analyzed, it is necessary to exclude false positive results that stem from contamination with contemporary DNA. In order to ensure the accuracy and reliability of results, standard contamination precautions, such as separation of pre- and post-PCR experimental areas, use of disposable laboratory ware and filter-plugged pipette tips, treatment with DNA contamination removal solution (DNA-OFF$_{TM}$; TaKaRa, Otsu, Japan), UV irradiation of equipment and benches, negative extraction controls, and negative PCR controls, were employed in the present study. Other rigorous authentication methods were employed throughout the DNA-based analyses as described elsewhere (Shinoda *et al*., 2006). Bone or tooth preparation, DNA extraction, and PCR amplification were carried out in a physically separated room of a laboratory dedicated to the study of ancient DNA.

Extraction and purification of DNA

The bone and tooth samples were dipped in a contamination removal solution for 5 min, rinsed several times with DNase-/RNase-free distilled water, and allowed to air dry. When the samples were completely dry, they were pulverized in a mill (Multi-beads Shocker MB400U; Yasui Kikai, Osaka, Japan).

DNA was extracted in 2 steps using a GENECLEAN kit for Ancient DNA (Bio 101 Co.). The pulverized tooth or bone (powder) was placed in a 15-ml conical tube, and 5 ml of an overnight soaking solution was added for proteinase K digestion. The samples were rotated and incubated at 37°C for 12–15 h. The supernatant was used for DNA extraction with the kit. Approximately 100 μl of the extracted DNA solution was obtained. The eluted DNA was amplified by PCR without further processing. DNA extraction was performed only once; if the subsequent PCR amplification was not successful, no further extraction was carried out.

Amplification and sequencing of HVR 1, HVR 2, and coding region 10360–10485

A segment of hypervariable region (HVR) 1 (nucleotide positions 16209 to 16402, relative to the revised Cambridge reference sequence; Andrews *et al*., 1999), HVR 2 (128–267), and a segment of the coding region (10360–10485) that covers a part of the NADH dehydrogenase 3 and tRNA^{Arg} genes, were sequenced for all samples. The mtDNA sequence can be tentatively assigned to respective haplogroups according to specific mutation observed in the HVR 1 region. Further characterization of the haplogroup status was based on other specific mutations in the HVR 2 and the coding region. This coding region sequence includes sites 10398 and 10400 that are among the defining sites for macrohaplogroups M and N (Quintana-Marci *et al*., 1999). Detection of this coding region provides us with a firmer basis for haplogroup assignment. If the haplogroup assignments derived from HVR 1 and coding region sequences did not accord with each other, the results were rejected. The primers

Table 2. Sequences of the mtDNA primers and the annealing temperature of each primer set used in this study.

HV1 primer	annealing 50°C
L16208	5′-TGTAAAACGACGGCCAGTCCTTTTACCCCTACCATGAG-3′
H16403	5′-AACAGCTATGACCATGATTGATTTCACGGAGGATGG-3′
HV2 primer	annealing 46°C
L127	5′-TGTAAAACGACGGCCAGTAGCACCCTATGTCGCAGTAT-3′
H268	5′-AACAGCTATGACCATGTGTTATGATGTCTGTGTGG-3′
mtDNA 10360–10485 primer	annealing 50°C
L10360	5′-TGTAAAACGACGGCCAGTCCCCATGCTTACAAGCAAG-3′
H10485	5′-AACAGCTATGACCATGATTGATTTCACGGAGGATGG-3′
APLP 5178 D/G	annealing 50°C
5178C	5′-GTCGCACCTGAAGCAAGC-3
5178A	5′-TGATCAACGCACCTGAAACAAGA -3′
D/G-R	5′-ATTGCAAAAAGCAGGTTAGCG -3′
APLP 3010 D4	annealing 50°C
3010G	5′-ATTGGATCAGGACTTCCCG-3′
3010A	5′-GCTACATGGATCAGGACAACCCA-3′
D4R	5′-GATCACGTAGGACTTTAATCG-3′

used to amplify the regions described above are listed in Table 2.

Five-microliter aliquots of the extracts were used as the templates for PCR. Amplifications were carried out in a total reaction volume of 50 μl containing one unit of Taq DNA polymerase (HotStarTaq$_{TM}$ DNA polymerase; QIAGEN), 0.2 μM of each primer, and 200 μM of dNTPs in 1× PCR buffer provided by the manufacture. The conditions for PCR were as follows: incubation at 95°C for 15 min; 40 cycles at 94°C for 20 s, 46°C–56°C for 20 s, 72°C for 15 s; and final extension at 72°C for 1 min.

The PCR products were filtered using Centricon-100 spin columns (Amicon), and the filtrates were prepared for sequencing using 21M13 and M13 forward and reverse primers and a BigDye Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). All sequencing reactions were analyzed using a model 310 DNA Sequencer with SeqEd software, and the sequence of each region that did not contain primer regions was determined and compared with the revised Cambridge reference sequence (CRS).

Amplified Product-Length Polymorphisms (APLP) analysis

Several methods are available for the detection of single nucleotide polymorphisms (SNPs). Restriction fragment length polymorphism (RFLP) analysis is one of the most widely used techniques for detecting known mutation sites in the coding regions of mtDNA. Recently, a simple and rapid APLP method has been developed for the analysis of mtDNA polymorphisms (Umetsu *et al*., 2001, 2005). The principle of this method is based on the attachment of a non-complementary sequence to the 5′- end of 1 of 2 allele-specific primers. The use of such primers permits the amplification of 2 differently sized products distinguishing two alleles. Using APLP, diagnostic polymorphic sites can be examined directly; therefore, ambiguity with respect to the actual mutation site, which is one of the problems associated with the RFLP method caused by insufficient enzymic digestion, can be avoided.

The primers used for the analyses in the present study are listed in Table 2. The constitution of the PCR reaction mixture was the same as that described above. The thermal conditions used were as follows: incubation at 95°C for 15 min; 40 cycles at 94°C for 10 s, 50°C for 10 s, 72°C for 5 s; and final extension at 72°C for 1 min. Each region was examined independently using the monoplex PCR method in order to maximize the robustness of the PCR. One-microliter aliquots of the PCR products were separated by electrophoresis on an 8-cm native polyacrylamide gel (10% T, 5% C) containing 1× TBE buffer (pH 8.0) with a running buffer of 0.5×

TBE (pH 8.0). DNA bands were detected by UV irradiation after staining with ethidium bromide.

Data analysis

With advances in our understanding of global mtDNA phylogeny in recent years, control and coding region motifs have been identified for a majority of the major haplogroups and their subhaplogroups (e.g., Macaulay *et al*., 1999; Quintana-Murci *et al*., 1999; Alves-Silva *et al*., 2000; Kivisild *et al*., 2002; Yao *et al*., 2002, 2003; Kong *et al*., 2003; Maruyama *et al*., 2003; Tanaka *et al*., 2004). Consequently, an understanding of the structure of the mtDNA data and assigning the mtDNA haplogroup to a position in phylogenetic trees has been simplified.

Therefore, we assigned each mtDNA to haplogroups according to the HVR 1, HVR 2, and coding region data, using the data and classification tree described. Since several segments of the same mtDNA were analyzed independently, we took particular precautions to avoid artificial recombination caused by potential sample crossover. After assigning the mtDNAs to relevant haplogroups, we classified them further into maternal lines based on the mutations observed in the control and coding regions.

Nucleotide diversity and the mean number of pairwise differences of mitochondrial D-loop sequences were computed with the Arlequin version 3.0 program (Excoffier *et al*., 2005), using Tamura and Nei distances and a gamma parameter value of 0.26 (Mayer *et al*., 1999). Population differentiation (Raymond and Rousset, 1995) between the Ishikobara site and comparative populations was also computed using the Arlequin program. Neighbor-joining (NJ) trees based on pairwise Fst were constructed using the Mega 3.0 program (Kumar *et al*., 2004) in order to study the relationships between the populations.

Results

Among the 12 individuals considered in this study, ancient DNA was successfully amplified from 9 samples. The remaining 3 samples either failed to yield a product on amplification or produced several ambiguous positions during the sequencing reaction, possibly due to misincorporations by the DNA polymerase during PCR. Therefore, the success rate was 75%. Usually, with well-preserved sample, the success rate lies between 50% and 70%; thus, our results are reasonably high compared with those obtained for other ancient materials. This can be attributed to the fact that the bones were well preserved and that these samples are comparatively young in terms of ancient DNA.

Sequencing analysis of hypervariable region I (16209–16402) of mtDNA

Comparison of the mitochondrial DNA sequence of this region enabled the identification of 4 mitochondrial haplotypes that were defined on the basis of 7 segregating sites. Table 3 shows the positions at which the sequences of the Ishikobara Edo population differed from the reference sequence described by Andrews *et al*. (1999).

Sequencing analysis of hypervariable region II (143–267) of mtDNA

The base sequences in 124 base pairs of DNA were determined in which mutations were observed in 8 portions in total. Nucleotide change at position 263 was observed in all specimens. As seen in Table 4, 2 out of 8 successfully amplified individuals had unique haplotypes, and the remaining individuals could be classified into 3 haplotype groups.

Sequencing analysis of the coding region (10360–10485) of mtDNA

Of the 12 specimens examined, we were unable to obtain PCR products from 6 specimens. Consequently, only 6 specimens were analyzed in this region (Table 5). According to the defining sites for macrohaplogroups M and N, 1 individual belongs to macrohaplogroup N and other individuals belong to macrohaplogroup M.

Results of APLP analysis

Based on diagnostic sequence variations in the

Table 3. Segregating sites in the mtDNA HV1 region (16209–16402) of the Ishikobara samples

```
consensus TACAGCAATCAACCCTCAACTATCACACATCAACTGCAACTCCAAAGCCACCCCTCACCC
SM07.seq  ·····T·····
SM09.seq  ·····T·····
SM13.seq  ·····T·····
SM14.seq  ·····T·····
SM15.seq  ·····T·····
SM16.seq  ·····C·····T·····
SM19.seq  ·····T·····
SM20.seq  ·····T·····
SM23.seq  ·····T·····
consensus ACTAGGATACCAACAAACCTACCCACCCTTAACAGTACATAGTACATAAAGCCATTTACC
SM07.seq  ····························
SM09.seq  ·····T·····A·····
SM13.seq  ····························
SM14.seq  ····························
SM15.seq  ····························
SM16.seq  ·····G·····
SM19.seq  ····························
SM20.seq  ····························
SM23.seq  ····························
consensus GTACATAGCACATTACAGTCAAATCCCTTCTCGTCCCCATGGATGACCCCCCTCAGATAG
SM07.seq  ·····C·····
SM09.seq  ··G···C·····
SM13.seq  ·····C·····
SM14.seq  ·····C·····
SM15.seq  ··G···C·····
SM16.seq  ·····C·····
SM19.seq  ·····C·····
SM20.seq  ··G···C·····
SM23.seq  ·····C·····
consensus GGGTCCCTTGACCA
SM07.seq  ··············
SM09.seq  ··············
SM13.seq  ··············
SM14.seq  ··············
SM15.seq  ··············
SM16.seq  ··············
SM19.seq  ··············
SM20.seq  ··············
SM23.seq  ··············
```

A total of 7 segregating sites were observed, shown as differences from the human consensus sequence. The base number of each site in the control region is assigned by the reference sequence (Andrews *et al*., 1999). Dots indicate a match to the reference sequence.

mitochondrial control region, only 2 individuals (SM09 of haplogroup A and SM16 of haplogroup B) can be assigned in this study. Therefore, we analyzed other samples by using APLP analysis. In the present study, 2 SNPs in the coding region that detected haplogroup D4 and G were analyzed using the monoplex APLP method. Finally, 4 haplogroups were detected in the ancient samples tested, with haplogroup D4 being the most prevalent. The observed nucleotide changes in the coding and noncoding regions of mtDNA and the haplogroup of each individual are shown in Table 6.

Discussion

One of the main purposes of studying the specimens from ancient burial sites is to clarify whether the human remains belong to unrelated individuals or to members of a single family or limited number of families. The recently acquired technical ability to analyze DNA from ar-

Table 4. Segregating sites in the mtDNA HV2 (143–267) region of the Ishikobara samples.

```
concensus GCCTCATCCTATTATTTATCGCACCTACGTTCAATATTACAGGCGAACATACTTACTAAAGTGTG
SM07.seq ····························································
SM09.seq ·············G··························································
SM13.seq ····························································
SM14.seq ·············C··························································
SM15.seq ····························································
SM16.seq ····························································
SM19.seq ·············C··························································
SM20.seq ····························································
concensus TTAATTAATTAATGCTTGTAGGACATAATAATAACAATTGAATGTCTGCACAGCCACTTT
SM07.seq ····························································
SM09.seq ····························································
SM13.seq ····························································
SM14.seq ····························································
SM15.seq ····························································
SM16.seq ····························································
SM19.seq ····························································
SM20.seq ····························································
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A total of 8 segregating sites were observed, shown as differences from the human consensus sequence. The base number of each site in the control region is assigned by the reference sequence (Andrews *et al*., 1999). Dots indicate a match to the reference sequence.

Table 5. Segregating sites in the mtDNA coding region (10360–10485) of the Ishikobara samples.

```
concensus GTCTGGCCTATGAGTGACTACAAAAAGGATTAGACTGAACCGAATTGGTATATAGTTTAAACAAA
SM07.seq ····························G···························
SM13.seq ····························G···························
SM14.seq ····························G·T·········C···············
SM16.seq ····························································
SM19.seq ····························G·T·········C···············
SM22.seq ····························G·T·········C···············
consensus ACGAATGATTTCGACTCATTAAATTATGATAATCATATTTACCAAATGCCCCTCATTTAC
SM07.seq ····························································
SM13.seq ····························································
SM14.seq ····························································
SM16.seq ····························································
SM19.seq ····························································
SM22.seq ····························································
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A total of 3 segregating sites were observed, shown as differences from the human consensus sequence. The base number of each site in the control region is assigned by the reference sequence (Andrews *et al*., 1999). Dots indicate a match to the reference sequence.

chaeological remains using PCR provides us with more accurate genetical information than can be obtained through the morphological study of skeletal remains. In this study, partial genealogical reconstruction was achieved using maternally inherited mtDNA in a sample of 9 human skeletal remains excavated from the Ishikobara site.

The amount of data that can be gained using ancient DNA analysis depends almost entirely on the quality of DNA that has been preserved in the sampled materials. A few intensive population genetic studies have been conducted using biparental or paternal genetic systems (Duder *et al*., 2003; Keyser-Tracqui *et al*., 2003; Ricaut *et al*., 2004). These studies were able to reach convincing conclusions regarding the kinship relationship of burials based on a range of analyses conducted on nuclear and mtDNA preserved in excavated bones. While it would be ideal to be able to formulate precisely conceived and worded hypotheses, our study differed fundamentally in the quality of DNA and lack of textual informa-

Table 6. Nucleotide changes and haplogroups observed in the Ishikobara site analyzed in the present study.

Code Number	Haplo-group	16209–16402 (16000+)	128–267	10382–10465 (10000+)	APLA analysis D/G 5178	D4 3010
SM07	G	223,262	CRS	398	C	G
SM08	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
SM09	A	223,290,319,335,362	153,235	N.D.	—	—
SM13	G	223,362	CRS	398	C	G
SM14	D4	223,362	152	398,400,410	A	A
SM15	D4	223,335,362	194	N.D.	A	A
SM16	B4	217,284	199,202,207	CRS	—	—
SM19	D4	223,362	152	398,400,410	A	A
SM20	D4	223,335,362	194	N.D.	A	A
SM22	N.D.	N.D.	N.D.	398,400,410	N.D.	N.D.
SM23	D4	223,362	N.D.	N.D.	A	G
SH05	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

All polymorphic sites are numbered according to the revised Cambridge Reference Sequence (Andrews *et al*., 1999). CRS denotes that the sequence of the segment is identical to the revised Cambridge Reference Sequence, and N.D. indicates "Not Determined." "—" indicates "not examined."

tion. Though, such studies are still rare, efforts should be made to implement them.

Our mtDNA analysis has, however, revealed some biological links. Kinship ties were defined among 6 out of 9 individuals. As shown in Table 6, 3 individuals had unique haplotypes, and the remaining individuals could be classified into 3 haplotype groups. The spatial distributions of the defined haplotypes are shown in Figure 2. Based on the patterned distribution in space of the tombs and the variation in the composition and quality of the burial goods (Hirabayashi *et al*. 2006), the excavated individuals have been interpreted as being members of the same social class.

Since mtDNA is maternally inherited, the observation that the studied individuals shared the same haplotype suggests the possibility of a maternal relationship. A number of individuals were found to share the same haplotype in several instances, which could be due to the close matrilineal relationships at this site. The Ishikobara cemetery was used for approximately 200 years—from the 16th to 18th century. Therefore, among the samples analyzed here, there may be related individuals from several generations.

When we perform genetic analysis for a specific site, we have to bear in mind the possibility that kinship may alter a population's true gene frequencies. In order to avoid the distortion of haplotype frequencies due to kinship, it is advisable to detect the possible family relationships present at a site and to remove the bias they cause (Vernesi *et al*., 2004). In the situation in which a certain haplotype is represented by 2 or more individuals interred in the same burial, we should consider the multiple samples as representing a single individual. However, in this study, certain lineages exhibited a differential distribution in the cemetery and none of haplotypes were concentrated in specific burials (Figure 2). Thus, we could not rule out the possibility of bias stemming from kinship. This bias entails certain risks that reduce the value of the standard deviation.

For the comparison with another Edo population, we added 30 published sequences obtained for samples from the Hatanai site of Aomori Prefecture (Adachi *et al*., 2004). In order to estimate the relationship between populations, genetic diversities at the segment of HVR 1 (nucleotide position 16209 to 16366,) and HVR 2 (143–267), relative to the revised CRS, were calculated. An exact test of differentiation revealed that these differences were not statistically significant ($P=0.216\pm0.0309$). Both populations have high frequencies of haplogroup D4 and the population pairwise Fst was not exceptionally large (Table

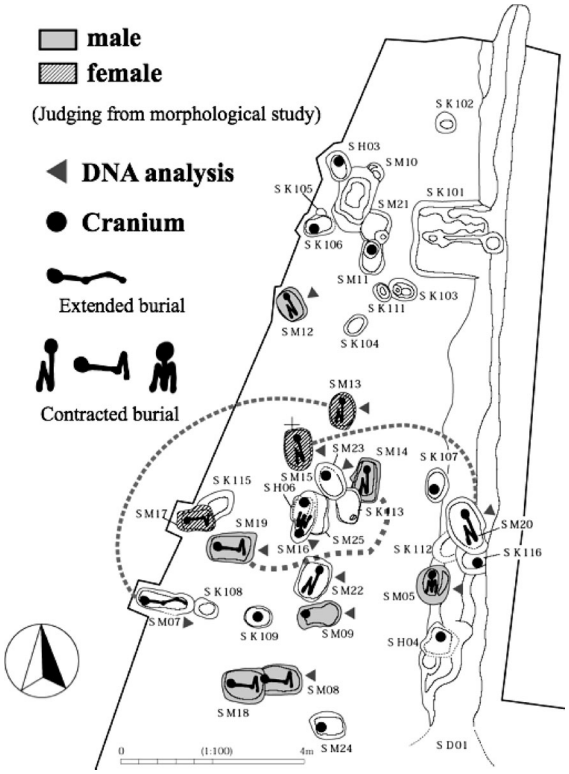

Fig. 2. Map of the Ishikobara cemetery (after Hirabayashi *et al*. 2006). Dashed line indicates the individuals with same mtDNA haplotype.

Table 7. mtDNA HV 1 and HV2 haplotype diversity parameters for the Ishikobara and Hatanai sites.

	n	k	Pw	p	Pairwise Fst
Ishikobara	8	5	3.857±2.165	0.012±0.009	0.216±0.031
Hatakai	30	15	6.292±3.071	0.020±0.011	

n, sample size; k, number of different sequences; Pw, mean number of pairwise differences; p, nucleotide diversity.

7). Although it is common in modern Japanese, both villages shared the same haplotype (SM07,13 of the Ishikobara and HN 34,40 of the Hatanai site). Therefore, it may safely be assumed that these 2 villages shared a similar genetic structure. However, at the nucleotide level, the diversities for the Ishikobara and Hatanai sites are 1.23±0.008 and 2.01±0.011, respectively. These values are almost of the same magnitude as that for the East Asian population in general (1.36%; Horai *et al*., 1996), indicating a high level of DNA variation in the villages of the Edo period.

In order to elucidate the biological relationships between the Ishikobara site and other populations, its haplogroup frequencies were compared with the haplogroup data for the Hatanai site and present-day populations of Japan obtained from the published data (Horai *et al*., 1996; Tanaka *et al*., 2004; Umetsu *et al*., 2005). The historical samples exhibit higher frequencies of haplogroups D4 and G compared with the present day populations; this pattern could be due to the small sample size used in the present study or

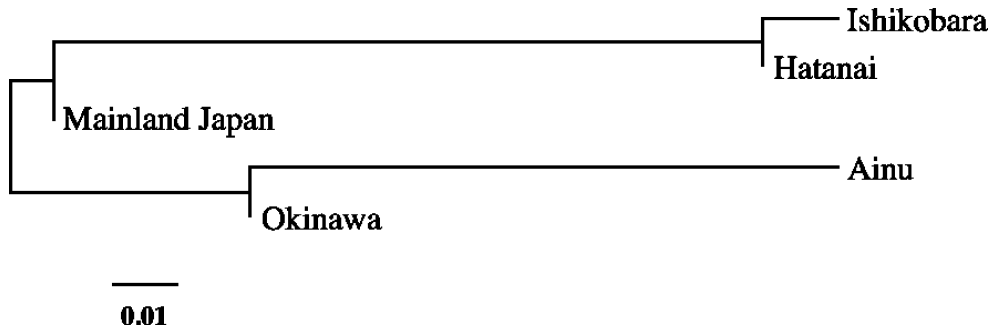

Fig. 3. Neighbor-joining tree based on the Fst values between 5 populations.

to the close relationships among the inhabitants of the site. An exact test of differentiation revealed that differences among the examined populations are statistically significant except between the Ishikobara and Hatanai samples ($P=0.441\pm0.044$). In order to assess the relationship between the Ishikobara population and other populations, an NJ tree (Figure 3) was constructed based on population pairwise Fst values (Table 8). The Ishikobara site was found to be clustered with the Hatanai site. These 2 populations exhibit small genetic distances from each other. The modern Japanese population belongs to the same cluster, although it is divergent. The most distant population, by a considerable margin, is that of Ainu, whereas the Okinawa population occupied an intermediate position.

It is generally agreed that immigrants from the Asian continent had a significant genetic effect on the development of the modern Japanese population, and that they intermixed with the native Jomon people to form the mainland Japanese. This influence is, however, less pronounced in Ainu and Okinawans (Hanihara, 1991). However, there is still some controversy as to when the admixing process in mainland Japan came to an end. Thus, the fact that the local Edo populations fall into the same cluster as the modern Japanese provides partial support for the "dual structure model" of Japanese population history, and suggests that the admixing process continued throughout the 16th to 18th centuries.

We indicate in this study that the intensive analysis of particular archaeological sites using molecular biological methods could yield valuable information for the reconstruction of relationships both within and among ancient communities. The Ishikobara site has close affinities with the Hatanai site of the same period, which to some extent suggests that the genetic characteristics of the Edo period were relatively uniform. Though tantalizing, these conclusions must be regarded as tentative as they are based on small sample sizes and an inadequate understanding of the genetic processes and variabilities of pertinent regional populations. Further analyses of more archaeological sites dating from the Edo period will lead us toward a more precise genetic characterization of these societies.

Table 8. Pairwise Fst values between each pair of populations.

	Ishikobara	Mainland Japan	Ainu	Okinawa
Mainland Japan	0.07440			
Ainu	0.27436	0.07517		
Okinawa	0.12675	0.00602	0.07479	
Hatanai	0.00801	0.06876	0.24316	0.11970

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