8

Mitochondrial DNA of Human Remains at Man Bac

Ken-ichi Shinoda

Department of Anthropology, National Museum of Nature and Science, Japan

Up until as recently as twenty years ago the genetic affinities, including intra and inter-sample comparisons, of skeletal remains in archaeological contexts was the domain of morphologists, using a suite of metric and non-metric skeletal characteristics believed to have an underlying genetic basis. Studies of the genes themselves were restricted to explorations of genetic variation, amongst contemporary, or living, human populations with subsequent inferences about their past evolutionary history (Ingman et al., 2000; Forster, 2004). While the latter approach has provided a panoramic, broad-stroke picture of our evolutionary past, such straightforward retrospective projections of the modern genetic composition and distribution on to the past have a number of inherent limitations. Relatively short-term and local biological and cultural processes such as epidemics, conquests, and forced relocations are likely to have contributed to and complicated the modern-day genetic landscape. A logical complement to studies of living DNA is clearly an examination of ancient DNA in archaeologically recovered regional populations. Recent advances in molecular biological techniques have facilitated the recovery and analysis of DNA from ancient material, thus providing a direct means of studying the genetic composition of past populations.

Because of its special characteristics, including small size, matrilineal inheritance, high copy number, and fast mutation rate, the majority of ancient DNA analysis is on mitochondrial DNA (mtDNA) (Alzualde et al., 2006; Maca-Mayer et al., 2005). This recently acquired ability to analyse mtDNA from archaeological remains yields more accurate genetic information than can be obtained through the morphological study of bones. Such information, combined with the results of archaeological investigation, should allow us to put forth and test new theories concerning the formation and subsequent history of past populations (Casas et al., 2006; Adachi et al., 2008). Notwithstanding this, there is clearly still a role for traditional bioarchaeological approaches when investigating such issues as such as age-at-death, pathology, and nutritional status of archaeological skeletal material. However, it is desirable that molecular biological analyses are used in conjunction with conventional bioarchaeological and morphological techniques in all studies of human skeletons.

In the present study, DNA analysis was performed on human skeletal remains excavated from Man Bac, Vietnam from 1999 to 2007. The significance of this study

is increased, because of the lack of any previous DNA work on ancient human remains from Vietnam. The origin of the genetic diversity of human populations in Southeast Asia is still very controversial, despite the multidisciplinary approach of the research being used to address this question. Ancient DNA analysis can contribute to this debate by providing at least a piece of the genetic landscape at a precise time in the past, and so assists in shedding light on the origins of the genetic composition of present Southeast Asian populations.

MATERIALS AND METHODS

Of the skeletal sample excavated from 1999 to 2007, 35 well-preserved individuals were selected for DNA analysis. As tooth enamel forms a natural barrier to exogenous DNA contamination; and because DNA recovered from teeth appears to lack most of the inhibitors to the enzymatic amplification of ancient DNA (Woodward et al., 1994; Thomas et al., 2003) the majority of DNA samples were collected from teeth for this analysis. When teeth were not available, bone was used instead. A list of all the samples used in this study is presented in Table 8.1.

Authentication Methods

During analysis of ancient DNA samples it is necessary to exclude false positive results, that can arise because of postmortem damage and contamination with more recent DNA samples (Cooper and Poinar, 2000; Bandelt, 2005). 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.

DNA Extraction and Purification

Bone and tooth samples were dipped in a DNA-OFF solution for 10 min to eliminate contamination, rinsed several times with DNase/RNase-free distilled water and air dried. When the samples were completely dry they were pulverised in a mill (Multi-beads shocker MB400U; Yasui Kikai, Osaka, Japan).

DNA was extracted in 2 steps using a DNA extraction kit (Mo Bio Co.). The pulverised tooth or bone powder (0.3 g) was placed in a 15-ml conical tube and demineralised in 5 ml of 0.5 M ethylene diamine tetra-acetic acid (EDTA). The samples were rotated and incubated at 37°C for 12–15 h. After digestion with proteinase K (0.5 mg/ml), the resultant pellet was used for DNA extraction. The eluted DNA (approximately 50 µl) was amplified by PCR, without prior processing. DNA extraction was performed only once; if the subsequent PCR amplification was not successful, no further extraction was carried out.

Table 8.1 Changes in the nucleotides of mtDNA and the haplogroups observed in the samples from Man Bac.

Co No.	Sex	Age class	Sample	Mutations in the segments			
				HVR1 Sequence 121-238 (+16000)	HVR1 Sequence 209-402 (+16000)	HVR2 sequence 28-267	Haplogroup
99MB-3	M	18-20 years	Isolated tooth	N.E	N.D.	N.E	N.D.
01MB-9	F?	Young Adult	Isolated tooth	N.E	N.D.	N.E	N.D.
05MB-6		1-2 years	Fibula	N.E	223, 355, 362	N.E	D/G
05MB-9	F	Adult	Maxilla Right M3	N.E	256, 270	N.E	F
05MB-10		9 years	Costa fragment	N.E	N.D.	N.E	N.D.
05MB-11	M	Young Adult	Maxilla Left M3	N.E	N.D.	N.E	N.D.
05MB-12		3-4 years	Costa fragment	N.E	294, 296, 304	N.E	F
05MB-13		14-16 years	Costa fragment	N.E	223, 362	N.E	D/G
05MB-14		3-4 years	Mandible Right DM	N.E	N.D.	N.E	N.D.
05MB-15	M?	15-19 years	Costa fragment	N.E	209, 311	N.E	F
05MB-18		1-2 years	Isolated tooth	N.E	209, 311	N.E	F
05MB-19		Young Adult	Fibula	N.E	N.D.	N.E	N.D.
05MB-20	M	Adult	Mandible Right M3	N.E	223	N.E	N.D.
05MB-24		8 years	Costa fragment	N.E	223, 362	N.E	D/G
05MB-28	F	Adult	Fibula	N.E	N.D.	N.E	N.D.
05MB-31	M	Adult	Mandible Right M3	N.E	N.D.	N.E	N.D.
05MB-29		Adult	Mandible Right M3	N.D.	N.D.	N.D.	N.D.
05MB-34	F		Maxilla Right M2	C.R.S	311	N.D.	F
07MB H1-1		Child	Maxilla Left M3	184, 223	223, 318, 362	N.D.	D/G
07MB H1-4			Maxilla Left M3	N.D.	223, 284	150, 195	M*
07MB H1-5		Adult	Mandible Left M3	192, 223	223, 291, 362	150, 199	D/G
07MB H1-8		Adult	Mandible Left M3	N.D.	N.D.	N.D.	N.D.
07MB H2-1	M	Adult	Maxilla Right M3	223	223, 278, 362	150	D/G
07MB H2-5	F	Adult	Mandible Left M2	N.D.	N.D.	N.D.	N.D.
07MB H2-10	M	Adult	Mandible Left M3	N.D.	N.D.	N.D.	N.D.
07MB H2-11		Adult	Maxilla Right M3	N.D.	N.D.	N.D.	N.D.
07MB H2-12	F	Adult	Mandible Left M2	C.R.S	266G	N.D.	B5
07MB H2-18	M	Adolescent	Mandible Left M2	C.R.S	304, 335, 368	N.D.	F
07MB H2-19	M	Adult	Mandible Right M3	N.D.	N.D.	N.D.	N.D.
07MB H2-22	F	Adult	Maxilla Right M2	N.D.	N.D.	N.D.	N.D.
07MB H2-24	F	Adult	Maxilla Right M3	N.D.	217, 304, 311	N.D.	B4
07MB H2-27	M	Adult	Mandible Left M3	129, 162, 172	304	150, 152, 249d	F
07MB H2-30	M	Adult	Mandible Right M3	N.D.	232A, 249, 260, 304, 311	N.D.	F1b
07MB H2-32	M	Adult	Mandible Right M3	183, 189	311	N.D.	F

All polymorphic sites are numbered according to the revised Cambridge reference sequence (Andrews et al. 1999). C.R.S indicates that the sequence of the segment is identical to the revised Cambridge reference sequence, and diagnostic polymorphisms are emphasized by bold italic type. The suffix A and G indicates a transversion. N.D: Not Determined, N.E: Not Examined, Young adult: aged between 16 and 25 years. *denotes that the haplogroup status cannot be identified further.

Amplification and Sequencing of HVR1 and HVR2

Figure 8.1 shows the structure of the mitochondrial genome and the analytical portion used for this study. Segments of hyper variable region (HVR) 1 (nucleotide positions 16121–16238 and 16209–16402, as per the revised Cambridge reference sequence; Andrews et al., 1999) and HVR 2 (nucleotide positions 128–267) of the D-loop region were sequenced. Because ancient DNA is usually degraded to fragments that are typically hundreds of base pairs in length, the PCR was designed to amplify specific segments of mtDNA less than 250 bps long. The distribution of mutations in the D-loop region is significantly nonrandom. The primer set was designed to include the most variable region.

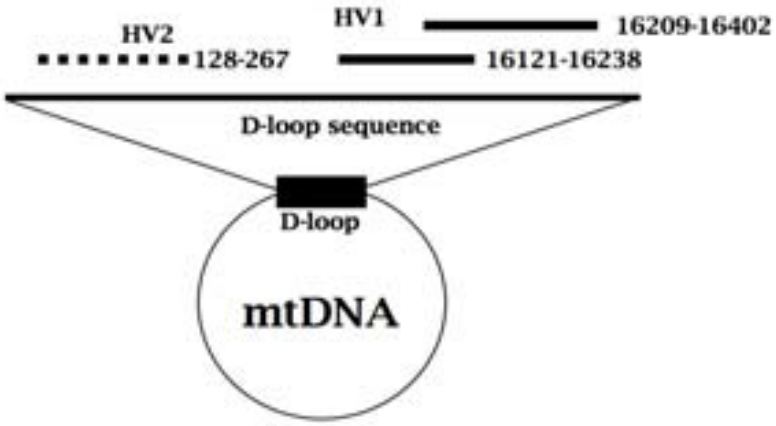

Figure 8.1 Map of human mitochondrion showing location of the D-loop region and analytical portion in this study.

Aliquots (2 μl) of the extracts were used as templates for PCR. Amplifications were carried out in a reaction mixture (total volume, 25 μl) containing 1 unit of Taq DNA polymerase (HotStarTaq™ DNA polymerase; Qiagen, Germany), 0.1 μM of each primer, and 100 μM of deoxyribo nucleoside triphosphates (dNTPs) in 1 × PCR buffer provided by the manufacturer. The PCR conditions were as follows: incubation at 95°C for 15 min; followed by 40 cycles of heat treatment at 94°C for 20 s; 50°C–56°C for 20 s; and 72°C for 15 s; and final extension at 72°C for 1 min.

The following primers were used to amplify HVR1 and HVR2.

L16120 5′-TTACTGCCAGCCACCATGAA-3′
H16239 5′-TGGCTTTGGAGTTGCAGTTG-3′
L16208 5′- CCCCATGCTTACAAGCAAG-3′
H16403 5′-TTGATTTCACGGAGGATGGTG-3′
L127 5′-AGCACCCTATGTCGCAGTAT-3′
H268 5′-GTTATGATGTCTGTGTGG-3′

The PCR products were subjected to agarose gel electrophoresis on a 1.5% gel and were recovered using a QIAEX II agarose gel extraction kit (Qiagen, Germany). Aliquots of the samples were prepared for sequencing on a BigDye cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The primers that were used in the PCR amplification were also used in the sequencing reaction. Sequencing was

performed in both directions so as to enable identification of polymorphisms or ambiguous bases using a single primer. The sequencing reactions were performed on a DNA Sequencer (ABI model no, 3130) equipped with SeqEd software.

Bases at CRS positions 16209 to 16402 contain the majority of phylogenetically variable sites and were consequently subjected to more amplification than were the outer regions. The HVR 1 portion of the D-loop region of our experiments overlaps 30 bases. This allows analysis of whether the DNA sources are different. Moreover, if the separate fragments of the D-loop region are well in line with modern mtDNA lineages from different branches of the mtDNA phylogeny, we can determine whether they may have been derived from some artificial recombinant or different DNA source.

Data Analysis

The nucleotide diversity and the mean number of pairwise differences between the mitochondrial D-loop sequences were computed using the Arlequin software package version 3.0 (Excoffier et al., 2005), considering Tamura and Nei distances and a gamma parameter value of 0.26 (Mayer et al., 1999). The differences between the Man Bac sample and other populations were also computed using the Arlequin software (Raymond and Rousset, 1995). Neighbour-joining (NJ) trees were constructed on the basis of the pairwise Fst values by using the Mega 3.0 program (Kumar et al., 2004) in order to study the relationships between the populations.

The haplogroup status of mtDNA was tentatively assigned on the basis of a search for HVR1 motif specific to a haplogroup and by matching or almost matching the values with the mtDNA haplotypes in the global database. The haplogroup status was further characterised on the basis of other specific mutations in the HVR2 motif.

RESULTS AND DISCUSSION

Table 8.1 shows the list of materials that we used for this study. Bone and teeth samples belonging to 35 individuals were collected. Because of the generally poor quality of the mtDNA extracted from ancient materials, it was not possible to amplify all of the samples. Table 8.1 also shows the results of PCR amplification. Suspected false positive results stemming from contamination with contemporary DNA and other questionable data were omitted from this study and resulted in 34 out of 70 (approximately 49% success rate) PCR amplifications being successfully analysed. It is known from past studies that the success rate of DNA analysis of ancient human remains is between 60%–80% at best, even when well-preserved samples are used. Our results may suggest that the preservation conditions of DNA in the Man Bac samples are poor. In general, hot and humid conditions are unfavourable for the preservation of DNA in human skeletal remains; the possibility of finding well-preserved DNA in a tropical region such as Vietnam is low. However, the present experiment proved that sufficient amounts of DNA are retained in some human samples, even though the efficiency of analysis may be poor. For this reason it was decided that there was value in continuing the experiments to obtain more detailed data on the human skeletal remains from Man Bac.

Comparison with the revised Cambridge Reference Sequence for this region enabled the identification of 16 mitochondrial haplotypes that were defined on the basis of 21 segregating sites (Table 8.1). One of the main purposes of studying 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. Since mtDNA is maternally inherited, the observation that the studied individuals shared the same haplotype suggests the possibility of a maternal relationship. Our mtDNA analysis has revealed some biological links. Kinship ties were defined among 4 out of 19 individuals. Among the samples analysed here, there may be related individuals from several generations. However, most individuals did not share the same haplotype, which could be due to the absence of close matrilineal relationships at this site.

Table 8.2 summarises the results of sequence analysis calculated from the HVR 1 region. The value of gene diversities, mean number of pairwise differences, and nucleotide diversity are presented, and can sometimes reflect relationships between populations. To clarify the genetic characteristics of the Man Bac sample, the mtDNA data were compared with the previous work on ancient Japanese and contemporary aboriginal Formosan populations (Table 8.2). The Man Bac series shows higher values of these parameters compared with ancient Japanese and is practically identical with aboriginal Formosan. It is known that the contemporary northern Vietnamese population possesses high genetic diversity and a large number of unique haplotypes (Irwin et al., 2008). It is possible that this tendency goes back to ancient times. However, in ancient DNA analysis it is necessary to take into account the possibility that the original sequences have changed due to the ageing of the DNA. Therefore, it is risky to regard as authentic all the base sequences determined in the investigation under discussion. It should be appreciated, in advance, that it is inevitable that such a limitation will occur in analyses of the scarce DNA that remains in ancient samples.

Table 8.2 mtDNA HVR I haplotype diversity indices for the Man Bac site and other populations.

Site	n	Gene diversity	Nucleotide diversity	Mean number of pairwise differences
Man Bac	19	1.00 +/- 0.017	0.020 +/- 0.011	3.673 +/- 1.943
Jomon (Kanto)	67	0.91 +/- 0.024	0.018 +/- 0.010	3.429 +/- 1.775
Yayoi (Kuma-Nishioda)	31	0.85 +/- 0.051	0.012 +/- 0.007	2.241 +/- 1.268
Aboriginal Formosan	28	1.00 +/- 0.010	0.022 +/- 0.012	4.161 +/- 2.133

Population references: Kanto Jomon (Shinoda and Kanai, 1999; Shinoda, 2003), Yayoi (Shinoda, 2004), Aboriginal Formosean (Tajima et al., 2003).

MtDNA haplogroups show geographic specificity within Asia (Kivisild et al., 2002; Li et al., 2007; Soares et al., 2008), therefore to determine the genetic characteristics of the Man Bac sample their mtDNA data was compared with that of populations in geographically related areas (see Table 8.3). All the haplogroups known to occur in Southeast and Northeast Asian populations, i.e, D, G, B, and F were detected in the Man Bac sample. However, most of the haplogroups that were found at Man Bac are dominant in Southeast Asian populations, except D and G,

which are dominant in East Asia. The results of our phylogenetic analysis based on the Fst values show that the Man Bac population shares a relationship with south China, although they are divergent (Figure 8.2). An exact test of differentiation revealed that differences between Man Bac and other populations are statistically significant, except between north China, south China, and north Vietnam.

Table 8.3 Estimated frequencies of the mtDNA haplogroups among regional populations.

Haplogroup	Northern China (n = 125)	Southern China (n = 78)	Mainland Japan (n = 1312)	North Vietnam (n = 187)	South Vietnam (n=35)	Taiwan Aborigine (n = 640)	Philippines (n=59)	Man Bac (n = 19)
D4	35.2	14.1	32.6	34.0		1.5		33.2(D/G)
D5	6.4	5.1	4.8	2.7	14.3(D/G)	4.8	5.1(D/G)	
G	5.6	1.3	6.9	1.6		0.0		0.0
M7a	0.0	0.0	7.5	24.2	2.9	0.0	1.7	0.0
M7b	2.4	7.7	4.8	4.7	11.4	9.0	6.8	0.0
M7c	2.4	2.6	0.8	0.5	0.0	9.0	6.8	0.0
M8	6.4	2.6	1.4	0.0	2.9	0.0	0.0	0.0
M9	3.2	0.0	0.0	2.1	0.0	11.4	20.3	0.0
M10	3.2	2.6	1.3	0.0	2.9	0.4	1.7	0.0
CZ	1.6	0.0	1.8	0.3	0.0	0.0	0.0	0.0
A	4.0	0.0	6.9	8.0	0.0	0.0	0.0	8.0
B4	9.6	25.6	7.7	11.7	22.9	17.1	33.9	5.6
B5	1.6	1.3	4.3	2.4	5.7	5.9	5.1	5.6
F	7.2	23.1	5.3	2.1	11.4	26.7	6.8	50.0
N9a	3.2	1.3	4.6	0.3	5.7	1.2	0.0	0.0
N9b	0.0	0.0	2.1	4.3	0.0	0.0	0.0	0.0
Y	1.6	0.0	0.4	0.5	0.0	1.4	0.0	0.0
R	1.6	2.6	0.1	0.0	17.1	2.9	3.4	0.0
Other	4.8	10.1	6.7	0.5	2.8	8.7	8.4	5.6

The references for the population: Northern Chinese (Yao et al., 2002); Southern Chinese (Yao et al., 2002); Mainland Japanese (Tanaka et al., 2004); South Vietnam (Oota et al., 2002); Philippine (Tajima et al., 2004); Taiwan aborigines (Trejaut et al., 2005).

The distribution of mtDNA haplogroups among these areas will provide some suggestions about the population history of Man Bac. Haplogroups F and B are dominant in contemporary Southeast Asian populations; in contrast, the frequency of haplogroups D and G is relatively high in East Asian populations. It is noteworthy that both haplogroups appear in high proportions in the Man Bac series. It is suggested that southward population expansion during prehistoric times resulted in an admixture between these migrants and the local or indigenous Southeast Asian population in the region, leading to the formation of the basic genetic pattern seen in the modern northern Vietnamese population.

CONCLUSION

Inferences based on the results of the DNA analysis carried out here are limited due to the limited number of DNA sequences that could be successfully determined. Nonetheless, the establishment of kin relationships among numerous individuals buried in a single site provides extremely valuable information regarding the past social structure of the community. Furthermore, if it were possible to collect DNA data from the people inhabiting a single region over a prolonged period, it may be

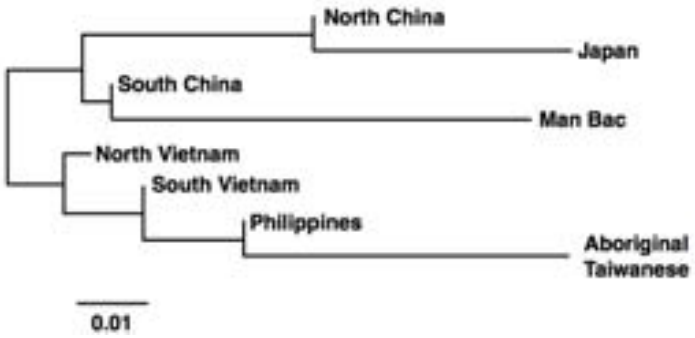

Figure 8.2 Neighbor joining tree based on the Fst values determined for 8 populations.

possible to deduce the movement of groups and their population dynamics. Since hot and humid conditions are unfavourable to the preservation of DNA, the possibilities are low for finding well-preserved DNA in a region like tropical Vietnam. However, the present study demonstrates the possibility that sufficient amounts of DNA are retained in human skeletal samples from tropical regions. It is very important that ancient DNA work continues in this region of the world.

SUMMARY

Man Bac is one of the largest neolithic sites in Vietnam. Due to its geographical and chronological position, the site is thought to play an important role in the evolution of modern-day Vietnamese. To investigate the genetic composition of the Man Bac community and to address questions regarding their potential genetic relationships with other Asian populations at a molecular level, we analysed HVR1 and HVR2 of mitochondrial DNA (mtDNA) from 35 samples excavated from this site. Some 34 out of 70 PCR amplifications were successfully analysed. The distribution of mtDNA haplotypes at the site indicated the existence of a number of different maternal lineages. The mtDNA sequence can be tentatively assigned to respective haplogroups according to specific mutations observed in the HVR 1 and 2 regions. The Man Bac sample showed affinities to Southeast and East Asian populations. The frequencies of these haplogroups indicates that a southward population expansion during the ancient past resulted in the admixture of these people with an indigenous Southeast Asian population and led to the formation of the basic pattern seen in modern northern Vietnamese.

LITERATURE CITED

Adachi N, Shinoda K, Umetsu K, Matsumura H. 2008. Mitochondrial DNA analysis of Jomon skeletons from the Funadomari site, Hokkaido, and its implication for the origins of native American. Am J Phys Anthropol 138;255-265.

Alzualde A, Izagirre N, Alonso S, Albarran C, Azkarate A, de la Rua C. 2006. Insight into the "isolation" of the Basques: mtDNA lineages from the historical site of Aldaieta (6th-7th centuries AD). Am J Phys Anthropol 130:394-404.

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nature Genet 23: 147.

Bandelt HJ. 2005. Mosaic of ancient mitochondrial DNA: positive indicators of nonauthenticity. Europe. J Hum Genet 13:1106-1112.

Casas MJ, Hagelberg E, Fregel R, Larruga JM, Gonzalez AM. 2006. Human mitochondrial DNA diversity in an archaeological site in al-Andalus: Genetic impact of migrations from north Africa in medieval Spain. Am J Phys Anthropol 131:539-551.

Cooper A, Poinar HN. 2000. Ancient DNA: Do it right or not at all. Science 289:1139.

Excoffier L, Laval G, Schneider S. 2005. Arlequin ver.3.0: An integrated software packages for population genetics data analysis. Evol Bioinform Online 1.

Forster P. 2004. Ice Ages and the mitochondrial DNA chronology of human dispersals: a review. Phil Trans R Soc Lond B 359:255-264.

Ingman M, Kaessmann H, Paabo S, Gyllensten U. 2000. Mitochondrial genome variation and the origin of modern humans. Nature 408:708-713.

Irwin J, Saunier JL, Strouss KM, Diegoli TM, Strurk KA, O'Callaghan JE, Paintner CD, Hohoff C, Brinkmann B, Parsons TJ. 2008. Mitochondrial control region sequences from a Vietnamese population sample. Int J Legal Med 122:257-259.

Kivisild T, Tolk H-V,Parik J, Wang Y, Papiha SS, Bandelt HJ, Villems R. 2002. The emerging limbs and twigs of the East Asian mtDNA tree. Mol Biol Evol 19:1737-1751.

Kumar S, Tamura K, Nei M. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinformatics 5:150-163.

Li H, Cal X, Winograd-Cort ER, Wen B, Cheng X, Qin Z, Liu W, Liu Y, Pan S, Qian J, Tan C-C, Jin L. 2007. Mitochondrial DNA diversity and population differentiation on Southern East Asia. Am J Phys Anthropol 134:481-488.

Maca-Mayer N, Cabrera VM, Arnay M, Flores C, Fregel R, Gonzalez AM, Larruga JM. 2005. Mitochondrial DNA diversity in 17th–18th century remains from Tenerife (Canary Islands). Am J Phys Anthropol 127:418-426.

Mayer, S, Weiss G, von Haeseler A. 1999. Pattern of nucleotide substitution and rate of heterogeneity in hyper variable region I and II of human mtDNA. Genetics 152:1103-1110.

Oota H, Kitano T, Jin F, Yuasa I, Wang L, Ueda S, Saitou N, Stoneking M. (2002) Extreme mtDNA homogeneity in continental Asian populations. Am J Phys Anthropol 118:146–153.

Raymond M, Rousset F. 1995. An exact test of population differentiation. Evolution 49:1280-1283.

Shinoda K, Kanai S. 1999.Intracemetery genetic analysis at the Nakazuma Jomon site in Japan by Mitochondrial DNA sequencing. Anthropol Sci 107:129-140.

Shinoda K. 2003. DNA analysis of the Jomon skeletal remains excavated from Shimo-Ohta shell midden, Chiba prefecture. Report for Sohnan Research Institute for Cultural Properties 50:201-205. (In Japanese.)

Shinoda K, 2004. Ancient DNA analysis of skeletal samples recovered from the Kuma-Nishioda Yayoi site. Bull Natl Sci Mus Tokyo D 30:1-8.

Shinoda K. Adachi N, Guillen S, Shimada I. 2006 Mitochondrial DNA analysis of ancient Peruvian highlanders. Am J Phys Anthropol 131: 98-107.

Soares P, Trejaut JA, Loo J-H, Hill C, Momina M, Lee C-L, Chen Y-M, Hudjashov G, Forster P, Macaulay V, Bulbeck D, Oppenheimer S, Lin M, Richards M. 2008. Climate change and postglacial human dispersals in Southeast Asia. Mol Biol Evol 25:1209-1218.

Tajima A, Sun C S, Pan IH, Ishida T, Saitou N, Horai S. 2003. Mitochondrial DNA polymorphisms in nine aboriginal groups of Taiwan: Implications for the population history of aboriginal Taiwanese. Hum Genet 113: 24–33.

Tajima A, Hayami M, Tokunaga K, Juji T, Matsuo M, Marzuki S, Omoto K, Horai S. 2004. Genetic origins of the Ainu inferred from combined DNA analyses. J Hum Genet 49:187-193.

Trejaut JA, Kivisild T, Loo JH, Lee CL, He CL, Hsu CJ, Lee ZY, Lin M. 2005. Traces of archaic mitochondrial lineages persist in Austronesian-speaking Formosan populations. PLoS Biol 3:e247.

Tanaka M, Cabrera VM, González AM, Larruga JM, Takeyasu T, Fuku N, Guo LJ, Hirose R, Fujita Y, Kurata M, Shinoda K, Umetsu K, Yamada Y, Oshida Y, Sato Y, Hattori N, Mizuno Y, Arai Y, Hirose N, Ohta S, Ogawa O, Tanaka Y, Kawamori R, Shamoto-Nagai M, Maruyama W, Shimokata H, Suzuki R, Shimodaira H. 2004. Mitochondrial genome variation in eastern Asia and the peopling of Japan. Genome Res 14: 1832–1850.

Thomas M, Gilbert P, Willerslev E, Hansen AJ, Barnes I, Rudbeck L, Lynnerup N, Cooper A. 2003. Distribution patterns of postmortem damage in human mitochondrial DNA. Am J Hum Genet 72:32-47.

Woodward SR, King MJ, Chiu NM, Kuchar ML, Griggs CW. 1994. Amplification of ancient nuclear DNA from teeth and soft tissues. PCR Method Appl 3:244–247.

Yao, YG, Kong QP, Bandelt HJ, Kivisild T, Zhang YP. 2002. Phylogenetic differentiation of mitochondrial DNA in Han Chinese. Am J Hum Genet 70:635–651.