FORENSIC SCIENCE JOURNAL SINCE 2002

Forensic Science Journal
2011;10(1):29-38
Available online at:fsjournal.cpu.edu.tw

The distribution of mitochondrial D-loop sequence variations in Taiwan populations

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Received: Feb 15, 2011 / Accepted: June 16 , 2011

Abstract

DNA polymorphisms within the mitochondrial D-loop are used commonly in forensic science for the purpose of human identification and genetic linkage. When using mitochondrial loci it is necessary to evaluate data based on variation within the population. We report on polymorphisms within the D-loop based on 363 members of the Taiwanese aboriginal population. A DNA fragment of approximately 980 bp was used for the analysis resulting in a total of 229 haplotypes, all of which were different from the rCRS. Within the total haplotypes there were 211 haplotypes that were specific to one of the nine tribes that comprise the Taiwanese aboriginal population with the remaining 18 haplotypes common between members of at least two tribes. Comparison of the sequence variations for the aboriginal and Taiwan Han populations, the specific haplotypes were 227 in 229 haplotypes and 144 in 146 haplotypes for the aboriginal population (363 samples) and Taiwan Han population (155 samples) respectively. Only 2 haplotypes were shared between these two populations cohabiting on the island of Taiwan. It inferred the maternal genetic homogeneity and, however, the genetic diversity for the aboriginal population. Comparison of these data to other population studies showed the greatest genetic distance between the UK population and the Saisiyat tribe (0.26219). The data indicate that the nine aboriginal tribes have distinct genetic origins compared to recent immigrations onto Taiwan and illustrate how inter-tribal variation occurs within a small isolated island population.

Keywords: forensic science, mitochondrial D-loop, Taiwan populations, genetic variations

Introduction

There are two major populations living in Taiwan, one being the Taiwan Han that arrived on the island relatively recently after Taiwan had been colonized previously by the aboriginal populations. The Taiwan Han constitutes approximately 95.94 % of the total population of Taiwan with the aboriginal constituting approximately 2.18 %. The remaining 1.88 % is of the foreign population [1]. The culture, language and custom are different between these populations. In recent times there was a large influx of the Han population from mainland China, especially after civil war in 1949. The

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migration of the aboriginal population onto Taiwan remains a matter of much study [2-7]. The aboriginal population comprises fourteen tribes, thirteen (Amis, Atayal, Bunun, Paiwan, Tsou, Puyuma, Rukai, Saisiat, Thao, Kavalan, Taroko, Sakizaya and Seediq) of them live in the mountains or on the eastern plain of Taiwan and only the Tao tribe (also called Yami) live on Orchid island, which is located to the southeast of Taiwan. The ancestral population of Tao tribe is thought to have migrated to Orchid island from Batan island in the north of Philippine archipelago about 800 years ago [6]. Based on the historic colonization of Taiwan it would be expected that there should be significant genetic differences between the aboriginal and the Taiwan Han populations.

The genetic variation between populations for the D-loop sequences of mitochondrial DNA (mtDNA) has been used widely in studies of population history, origin, migration or evolution [8-10]. The accumulation of databases of mtDNA D-loop aids in understanding further the migration of populations as well as in forensic applications [11-13]. The island of Taiwan has played a pivotal role in the early human colonization of South East Asia and the islands of the Pacific with most studies concentrating on the Taiwan Han population. A previous study has examined variation with the mtDNA D-loop based only on the Taiwan Han for maternal identification on forensic science [14], however the only analysis of the aboriginal population has been based on some parts of the D-loop and for limited aboriginal tribes [3, 4, 15]. Melton T et al. [3] used markers within the mtDNA to examine genetic lineages within some Polynesian and Asian populations to show that the DNA data were consistent with linguistic evidence for a Taiwanese origin for the proto-Polynesian expansion. However, only four tribes (Amis, Atayal, Bunun and Paiwan) were included in this paper. The further study of Melton T et al. [4] using sequence-specific oligonucleotide probes within the D-loop showed that the Taiwanese population was isolated from other Asian populations in recent history; this study was based on 82 aboriginal individuals from four tribes. Tajima A et al. [15] analyzed 180 members of the aboriginal population from nine tribes using parts of the D-loop (521 bp of the HV1 region and 42 bp of the HV1 downstream region) and concluded that there were eight monophyletic clusters. Most of the papers focused on the studies of the relationship between Austronesian and aboriginal population in Taiwan.

In order to try to complete this complicated genetic map of Taiwan we report on a comprehensive survey of the aboriginal populations of Taiwan using a 980 bp fragment that spans HV1 and HV2 using 363 samples from members of nine aboriginal tribes (Ami, Bunun, Puyuma, Tsou, Rukai, Paiwan, Saisiat, Atayal and Tao). The results were compared with the Taiwan Han, Asian and European populations to determine whether there is a need for a different database for the aboriginal populations and to assist in positioning these populations with the colonization of this part of the world, and further for forensic applications.

Materials and Methods

Sample Collections and DNA Preparations

Saliva samples from 363 healthy people were collected randomly, with informed consent, between 1997 and 2004. These samples were from nine aboriginal tribes including 80 Amis (AM), 38 Bunun (BU), 22 Puyuma (PU), 36 Tsou (TS), 20 Rukai (RU), 50 Paiwan (PA), 25 Saisiyat (SA) , 44 Atayal (AT) and 48 Tao (TA) individuals. The five tribes of Thao, Kavalan, Taruku, Sakizaya and Sediq were only recognized officially in 2001, 2002, 2004, 2007 and 2008 respectively and not included in this study. DNA was extracted from all the samples using the salt-chloroform method [16] and quantified with the Human DNA quantification kit (Applied Biosystems, Foster City, CA, USA).

PCR Amplification and DNA Sequencing

The primer pair L15996/H408 was used to amplify the mtD-loop DNA [17] (Table 1). The predicted size of the PCR product was approximately 1024 bp, including the primer sequences. PCR amplification was performed in 50 μL of reaction mixture, which contained 2 ng genomic DNA, reaction buffer (10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 % (w/v) gelatin), 200 μM dNTP, 2.5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems) and 0.15 μM each of primers. Amplifications were conducted in a 9700 thermal cycler (Applied Biosystems) with the following conditions: denatured at 95°C for 10 min, followed by 35 cycles of

94°C for 45 sec, 65°C for 45 sec and 72°C for 90 sec, and a further extension at 72°C for 30 min. The PCR product was analyzed on a 2 % agarose gel.

Cycle sequencing of the PCR products was also conducted in a 9700 thermal cycler (Applied Biosystems) with the following conditions: 25 cycles of 96°C for 30 sec, 50°C for 15 sec and 60°C for 4 min. Sequencing reactions were performed using the BigDye™ Terminator Kit (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit). In addition to the PCR primers, two primers (L16508 and H16517) were also designed for DNA sequencing (Table 1). The cycle sequencing products were separated using POP-4™ polymer (Applied Biosystems) and detected using an ABI 3130 DNA Analyzer.

Table 1. Primer sequences used in this study.

Primer name	Sequences (5'-3')
L15996	CTCCACCATTAGCACCCAAAGC
H408	CTGTTAAAAGTGCATACCGCCA
L16508	CTGTATCCGACATCTGGTTCC
H16517	GCTATTTAGGCTTTATGACC

Note: The primers of L15996 and H408 are synthesized according to the previous report of Vigilant L et al. [17].
The primers of L16508 and H16517 are designed according to the reported human mtDNA sequence [18].

Sequence Analysis

Excluding the primer sequences, a DNA fragment of approximate 980 bp was used for further analysis. All sequences were analyzed initially using the ABI Prism Sequencing Analysis Software (Version 2.1). These sequences were compared with the reference sequence of rCRS (Revised Cambridge Reference Sequence) [18, 19], and any divergent bases were recorded. Gene diversity was calculated by the Arlequin program [20] using the following equation $\hat{H}= [n/(n-1)](1-\sum_{i=1}^{k} p_i^2)$, where n is the sample size, k is the number of haplotypes, and P_i is the sample frequency of the i-th haplotype [21]. The genetic distance was estimated as $\hat{d} = n_d$ based on the pairwise difference method using the Arlequin program, where n_d is the number of observed substitutions between two DNA sequences [20, 22]. The phylogenetic tree was constructed by the Neighbor-joining method with the PHYLIP computer package [23].

Results and Discussion

Sequence variations of the aboriginal samples

All the 363 samples from the aboriginal tribes were successfully amplified and sequenced, producing PCR products with the expected size of about 980 bp; this is excluding the primer sequences. The 363 sequences fell into 229 haplotypes and all were different from the rCRS. Alignment of the 229 sequences indicated that there were 142 variable sites, including insertions, deletions, transitions and transversions. The probability of match was in the order of 0.0017. The analysis of all the variable sites, number of haplotypes and their diversity for each tribe based on the Arlequin program is shown in Table 2. The ratios of haplotypes and specific haplotypes are provided, as the number of samples for each tribe is different. A higher ratio of haplotype and gene diversity represents greater sequence variations within the tribe. The TS tribe exhibited the greatest sequence variation with a ratio of haplotype of 0.94 and gene diversity 0.9889 (± 0.0096), with the least variation within the SA tribe showing a ratio of haplotypes of 0.48 and gene diversity of 0.8667 (± 0.0410). The TS tribe had higher specific haplotype ratio (0.94), and it was suggested resulting from its distribution in the remote mountain area and the forbidding marriage with the individual having maternal relationship [24-25]. In addition, the variation observed corresponded with historic and geographic isolation as the TA tribe had higher specific haplotype ratio (0.94) and was an isolated tribe on the Orchid islands. Gene diversity for the total aboriginal population (0.9942 ± 0.0008) was slightly higher than that of each of the tribes, indicating that intra-tribe marriage was more frequent than marriage between different tribes.

Within the 229 haplotypes there was a total of 211 haplotypes specific for one of the nine tribes with the other 18 haplotypes (excluding haplotypes 7 and 16 in Table 3) shared between at least two tribes. Fifteen of the haplotypes were shared by two tribes, two haplotypes were shared by three tribes and one haplotype by four tribes. In line with the previous findings for the TA tribe, there were only 2 haplotypes (5 and 12) shared by this tribe with the other tribes, supporting its genetic isolation from the other aboriginal tribes of Taiwan (Table 3).

The genetic distance between the nine tribes was estimated using the Arlequin program, and a phylogenetic tree was constructed by the Neighbor-joining method with the PHYLIP computer package (Figure 1). Based on these results the aboriginal population with the least genetic distance (0.02563) were the RU and PA tribes, and the greatest was between the SA and PU tribes (0.19863) (Table 4). The multiple branches for the nine tribes in the phylogenetic tree (Figure 1) are in line with a previous report [5] inferring that the distribution of variations within the mitochondrial D-loop support the nine aboriginal tribes having different genetic origins [2-5].

Table 2. Sequence analysis of the mtDNA D-loop sequences for the aboriginal and Taiwan Han populations.

Population	Sample no.	Variable site	Haplotypes (specific haplotypes)	Ratio of haplotype(ratio of specific haplotype)	Gene diversity
Aboriginal	363	142	229(227)	0.63(0.99)	0.9942 ± 0.0008
AM	80	56	59(55)	0.74(0.93)	0.9744 ± 0.0085
BU	38	53	29(26)	0.76(0.90)	0.9459 ± 0.0213
PU	22	49	16(11)	0.73(0.69)	0.9307 ± 0.0327
TS	36	63	34(32)	0.94(0.94)	0.9889 ± 0.0096
RU	20	39	12(9)	0.60(0.75)	0.9105 ± 0.0452
PA	50	61	32(25)	0.64(0.78)	0.9559 ± 0.0151
SA	25	40	12(6)	0.48(0.50)	0.8667 ± 0.0410
AT	44	51	25(20)	0.57(0.80)	0.9281 ± 0.0250
TA	48	50	32(30)	0.67(0.94)	0.9441 ± 0.0187
TW	155	168	146(144)	0.94(0.99)	0.9989 ± 0.0009
Aboriginal+TW	518	215	373	0.72	0.9971 ± 0.0004

Note: The nine aboriginal tribes are Amis (AM), Bunun (BU), Puyuma (PU), Tsou (TS), Rukai (RU), Paiwan (PA), Saisiyat (SA), Atayal (AT) and Tao (TA). TW is for Taiwan Han population. The ratio of each haplotype was determine by the number of haplotypes divided by sample number. The ratio of a specific haplotype was determined by the numbe of specific haplotypes divided by total number of haplotypes.

Table 3. The individual haplotypes and the number of samples shared between the aboriginal tribes of Taiwan and with Taiwan Han population.

Tribe / Haplotype	AM	BU	PU	TS	RU	PA	SA	AT	TA	TW
1	6		1							
2	1						1			
3	1		1							
4	2			1						
5	2								1	
6	1	1					1	10		
7	1									1
8		4					1			
9		1				1				
10			3		5	2				
11			1			1				
12			1			2			1	
13			1	1						
14					1	1				
15					2	1				
16						7				1
17						1		2		
18							1	2		
19							3	1		
20							4	1		

Note: The nine aboriginal tribes are Amis (AM), Bunun (BU), Puyuma (PU), Tsou (TS), Rukai (RU), Paiwan (PA), Saisiyat (SA), Atayal (AT) and Tao (TA). TW is for Taiwan Han population.

Table 4. Genetic distance between the Asian and Caucasian populations in this study.

	AM	**BU**	**PU**	**TS**	**RU**	**PA**	**SA**	**AT**	**TA**	**TW**	CN	AU	JP	DE	UK	FR
AM	0															
BU	0.06360	0														
PU	0.08973	0.12746	0													
TS	0.04081	0.02767	0.07745	0												
RU	0.11008	0.13312	0.06581	0.07481	0											
PA	0.07945	0.08653	0.06070	0.04862	0.02563	0										
SA	0.12057	0.05781	0.19863	0.08802	0.13829	0.08473	0									
AT	0.17831	0.17097	0.14894	0.14124	0.08262	0.14927	0.18460	0								
TA	0.06393	0.08462	0.13648	0.07397	0.10874	0.10250	0.09520	0.15421	0							
TW	0.04520	0.06686	0.04038	0.01052	0.05208	0.05670	0.12745	0.10462	0.08615	0						
CN	0.09742	0.09899	0.08034	0.04100	0.04628	0.06580	0.14864	0.11052	0.12307	0.02881	0					
AU	0.20311	0.19517	0.17844	0.14188	0.15020	0.17325	0.25260	0.16164	0.21873	0.11508	0.07869	0				
JP	0.11790	0.11715	0.06937	0.05858	0.04801	0.08243	0.15868	0.10279	0.13376	0.03376	0.01982	0.10189	0			
DE	0.16809	0.15604	0.15763	0.11625	0.17349	0.17803	0.22811	0.16991	0.19943	0.10429	0.12438	0.04544	0.14627	0		
UK	0.21203	0.20367	0.17904	0.14850	0.15860	0.18601	0.26219	0.16473	0.22342	0.12233	0.09012	-0.00165	0.10739	0.04577	0	
FR	0.19058	0.17818	0.16718	0.12195	0.14164	0.15456	0.23848	0.16080	0.20599	0.09951	0.06331	-0.00022	0.08513	0.04516	0.00105	0

Note: The nine aboriginal tribes used in this study are Amis (AM), Bunun (BU), Puyuma (PU), Tsou (TS), Rukai (RU), Paiwan (PA), Saisiyat (SA), Atayal (AT) and Tao (TA). TW is for Taiwan Han population. The other populations are Mainland China Han (CN), Australia (AU), France (FR), Germany (DE), Japan (JP) and United Kingdom (UK).

Comparison of the sequence variations for the aboriginal and Taiwan Han populations

Sequence variations between the aboriginal and Taiwan Han populations were analyzed by comparing the 363 aboriginal samples obtained in this study with those of the Taiwan Han population (TW) (155 samples) reported previously by our laboratory [14]. Within the total of 518 samples, 215 variable sites and 373 haplotypes were observed. While the number of specific haplotypes for the aboriginal population was 227 out of 229, 144 specific haplotypes out of a total of 146 were observed in the TW population (Table 2). Only 2 haplotypes (7 and 16) were shared by both of the populations (Table 3). TW shared one haplotype with the tribes of AM and PA respectively. It inferred the maternal genetic homogeneity and, however, the genetic diversity for the aboriginal population. There were only 142 variable sites for the 363 aboriginal samples, however a much greater number of variable sites (168 from 155 samples) was noted for the TW population (Table 2). The number of variations when comparing the aboriginal data to the rCRS ranged from 6 to 16, compared to 5 to 18 for the Taiwan Han populations.

Our data indicate that of the nine tribes, TS has the closest genetic distance to TW, with SA having the furthest to TW (Table 4 and Figure 1). These data indicate that even within a small island, that the aboriginal tribes have a distinct genetic heritage and distinct to TW, supporting the necessity to establish separate mtDNA databases for the aboriginal tribes.

Comparison of the sequence variations for the Asian and Caucasian populations

Sequences of Asian and Caucasian populations reported by other laboratories were included for comparison in this study. The Asian populations (totally 723 samples) were represented by the Taiwan aboriginal tribes from this study (363 samples), 155 Taiwan Han (TW), 105 Mainland China Han (CN) [26] and 100 Japan (JP) [27] samples; the Caucasian populations

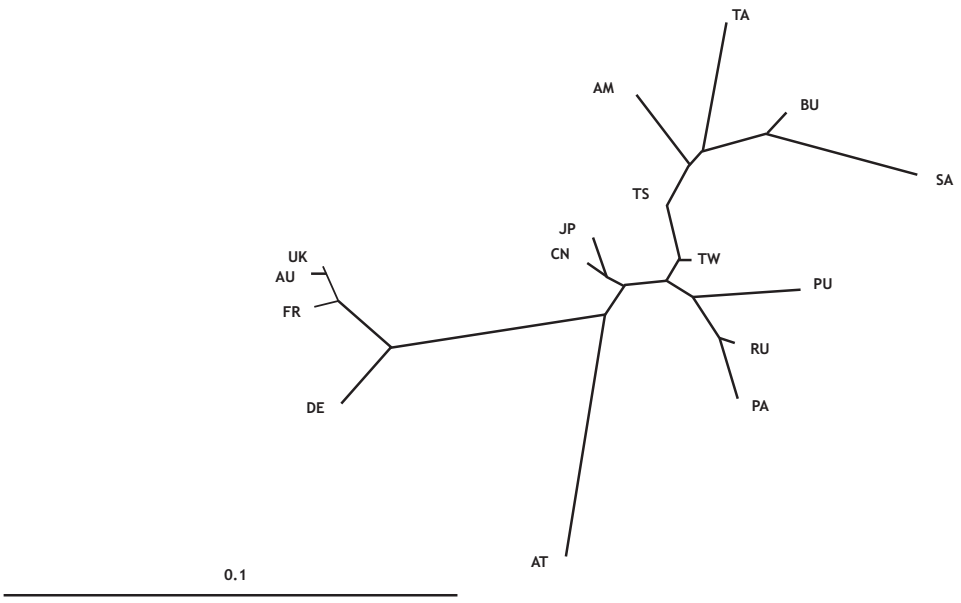

Fig.1 A neighbor-joining tree was constructed using the PHYLIP computer package for the Asian and Caucasian populations. The European populations are from Australia (AU), France (FR), Germany (DE) and the United Kingdom (UK). The Asian populations are from Mainland China Han (CN), Japan (JP) and the populations on Taiwan used in this study.

(451 samples) included those from 200 Germany (DE) [28], the 100 United Kingdom (UK) [29], 101 Australia (AU) [30] and 50 France (FR) [31] samples. Variable sites of the Asian populations ranged from 3 to 19 for each sequence comparing with the HV1 and HV2 of mtDNA D-loop region, and the average was 10.01. For the Caucasian populations, it ranged from 0 to 19 and the average was 7.01. Figure 2 showed the distribution of the variable sites. The most genetic distance was between UK and the SA tribe (0.26219), and as expected the AU and UK showed the closest genetic distance (Table 4).

The clustering of the European and Asian populations is as expected (Figure 1) and if also true for

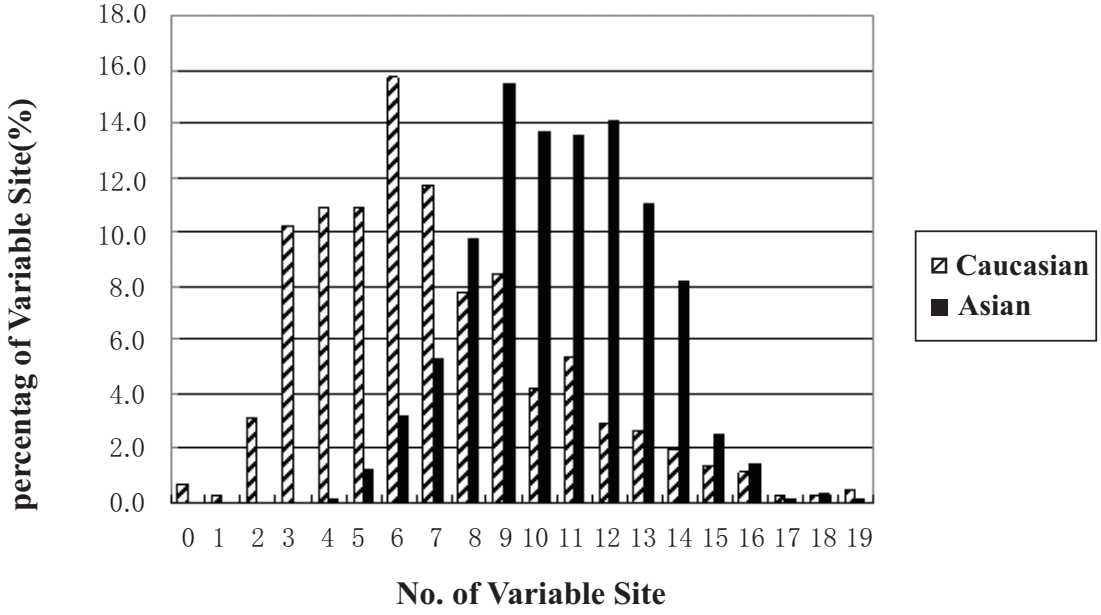

Fig.2 Comparison of the variable sites for Asian and Caucasian populations with rCRS.

the Taiwan tribes, these data would further support the tribes having distinct genetic origins to the Taiwan Han with the Atayal tribe being least related to any of the other tribes.

Conclusions

In this study, sequences of mitochondrial D-loop for Taiwan aboriginal population were analyzed and compared with the Taiwan Han, Mainland China Han and Caucasian populations for forensic purposes. Distribution variations and different genetic distance were observed between the tribes or populations. Database of mtDNA D-loop sequences for aboriginal population was established, not only being of value in forensic science but further adding to the genetic heritage of Taiwan.

Acknowledgements

This study was partially supported by the grants No. 99-1301-05-0501 from Ministry of Justice and NSC96-2320-B-015-002-MY2 from National Science Council, Taiwan, ROC.

References

1. Department of Household Registration, Ministry of The Interior, Taiwan, ROC http://www.moi.gov.tw/news_detail.aspx?sn=4156. Assessed in April 2010.
2. Bellwood P. The Austronesian dispersal and the origin of languages. Sci. Am. 1991; 7:70-75.
3. Melton T, Peterson R, Redd AJ, Saha N, Sofro AS, Martinson J, Stoneking M. Polynesian genetic affinities with southeast Asian populations as identified by mtDNA analysis. Am. J. Hum. Genet.

1995; 57:403-414.
4. Melton T, Clifford S, Martinson J, Batzer M, Stoneking M. Genetic evidence for the proto-Austronesian homeland in Asia: mtDNA and nuclear DNA variation in Taiwanese aboriginal tribes. Am. J. Hum. Genet. 1998; 63:1807-1823.
5. Lin M, Chu CC, Lee HL, Chang SL, Ohashi J, Tokunaga K Akaza T, Juji T. Heterogeneity of Taiwan's indigenous population: possible relation to prehistoric Mongoloid dispersals. Tissue Antigens 2000; 55:1-9.
6. de Beauclair. Three genealogical stories from Botel Tobago. Bulletin of the Institute of Ethnology. 1959; 7:105-140.
7. Su B, Jin L, Underhill P, Martinson J, Saha N, McGarvey ST, Shriver MD, Chu J, Oefner P, Chakraborty R, Deka R. Polynesian origins: insights from the Y chromosome. Proc. Natl. Acad. Sci. 2000; 97(15):8225-8228.
8. Cann RL, Stoneking M. Wilson AC. Mitochondrial DNA and human evolution. Nature 1987; 325:31-36.
9. Martínez-Bouzas C, Castro A, Fernández-Fernández I, Rodríguez-Filgueira JL, de Pancorbo MM. Genetic structure of autochthonous Basques through analysis of the HVI and HVII regions of mitochondrial DNA. International Congress S. 2003; 1239:495-499.
10. van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum. Mutat. 2009; 30(2):E386-394.
11. Nilsson M, Andre´asson-Jansson H, Ingman M, Allen M. Evaluation of mitochondrial DNA coding region assays for increased discrimination in forensic analysis. Forensic Sci. Int. Genet. 2008; 2(1):1-8.
12. Lertrit P, Poolsuwan S, Thosarat R, Sanpachudayan T, Boonyarit H, Chinpaisal C, Suktitipat B. Genetic history of southeast Asian populations as revealed by ancient and modern human mitochondrial DNA analysis. Am. J. Phys. Anthropol. 2008; 137:425-440.
13. Allard MW, Wilson MR, Monoson KL, Budowle B. Control region sequences for east Asian individuals in the Scientific Working Group on DNA Analysis Methods forensic mtDNA data set. Legal Medicine (Japan) 2004; 6(1):11-24.
14. Tsai LC, Lin CY, Lee CI, Chang JG, Linacre A, Goodwin W. Sequence polymorphism of mitochondrial D-loop DNA in the Taiwan Han population. Forensic Sci. Int. 2001; 119:239-247.
15. Tajima A, Sun CS, Pan IH, Ishida T, Saitou N, Horai S. Mitochondrial DNA polymorphisms in nine aboriginal groups of Taiwan: implications for the population history of aboriginal Taiwanese. Hum. Genet. 2003; 113:24-33.
16. Mullenbach R, Lagoda PJL, Welter C. An efficient salt-chloroform extraction of DNA from blood and tissues. Trend Genet. 1989; 5:391.
17. Vigilant L, Pennington R, Harpending H, Kocher TD, Wilson AC. Mitochondrial DNA sequence in single hairs from a southern African population. Proc. Natl. Acad. Sci. USA 1989; 86:9350-9254.
18. Anderson S, Bankier AT, Barrell BG., de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreuer PH, Smith AJH, Staden R, Young IG. Sequence and organization of the human mitochondrial genome. Nature 1981; 290:457-465.
19. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM. Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. 1999; 23:147.
20. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinform. Online 2005; 1:47-50.
21. Nei M. Molecular Evolutionary Genetics. Columbia University Press, New York, NY, USA, 1987; pp180.
22. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 1992; 131:479-491.
23. Felsenstein J. PHYLIP (Phylogeny Inference Package) Version 3.5c, University of Washington, 1986.
24. Lin YT. Tsou people. National Museum of Prehistory (Chinese), Taipei, ROC, 2004.
25. Taiwan Indigenous Culture Park: Introducing the Tsou tribe. 2010 http://www.tacp.gov.tw/home02_3aspx?ID=$3101&IDK=2&EXEC=L
26. Rao L, Wu MY, Liang WB, Zhang L. Sequence polymorphisms of the mitochondrial DNA control region in 105 Chinese Han population. J. Forensic Sci. 2003; 48:891-895.
27. Seo Y, Stradmann-Bellinghausen B, Rittner C, Takahama K, Schneider M. Sequence polymorphism

of mitochondrial DNA control region in Japanese. Forensic Sci. Int. 1998; 79:155-164.
28. Lutz S, Weisser HJ, Heizmann J, Pollak S. Location and frequency of polymorphic positions in the mtDNA control region of individuals from Germany. Int. J. Legal. Med. 1998; 111:67-77.
29. Piercy R, Sullivan KM, Benson N, Gill P. The application of mitochondrial DNA typing to the study of white Caucasian genetic identification. Int. J. Legal Med. 1993; 106:85-90.
30. Parson W, Parsons TJ, Scheithauer R. Holland MM. Population data for 101 Austrian Caucasian mitochondrial DNA d-loop sequence: Application of mtDNA sequence analysis to a forensic case. Int. J. Legal Med. 1998; 111:124-132.
31. Rousselet F, Mangin P. Mitochondrial DNA polymorphisms:a study of 50 French Caucasian individuals and application to forensic casework. Int. J. Legal Med. 1998; 111:292-298.