Control Region Sequencing of Hyper Variable Segment II (HVSII) of Mitochondrial DNA and its Genetic Relationship in Asholio and Atakar Ethnic Group of Nigeria

Solomon A Y¹, Danborno S. B² and Timbuak J³

¹Department of Anatomy, Faculty of Basic Medical Sciences, Bingham University, Abuja-Keffi Rd, New Karu, Nigeria
²Department of Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Community Market, Zaira Nigeria, local 810211, Zaria, Nigeria
³Department of Anatomy, Faculty of Basic Medical Sciences, North-West University, Potchefstroom, Mahikeng, Vanderbijlpark, South Africa

Abstract

Africa contains the most genetically divergent group of continental populations and several studies have reported that African populations show a high degree of population stratification. In this regard, it is important to investigate the potential for population genetic structure or stratification of some ethnic groups. Nigeria is one of the West African countries that took part in the human history since the dawn of modern man. The population of Nigeria is composed of Asholio and Atakar ethnic groups in the southern part of Kaduna state. DNA genetic marker such as HVII region of the mitochondrial DNA of a sample population of Asholio and Atakar ethnic group was used to get population genetic parameters. Buccal cells of 40 unrelated male individuals, 20 from each ethnic group was extracted using the protocol describe by Bioneer AccuPrep® Genomic DNA extraction kit. DNA samples extracted were analyzed and HVS-II sequences were amplified and purified. Sequencing for the light strand was done followed by sequence alignment, restriction fragment length polymorphism (RFLP) and single nucleotide polymorphism (SNP) analysis. Nucleotide positions 73-340 for HVS-II were compared to the revised Cambridge Reference Sequence (rCRS) and 40 haplotypes were observed with haplotypic diversity of 0.9431 for Asholio and 0.9560 for Atakar. A total of 80 polymorphic sites characterized the haplotypes. All of the haplotypes found have been described in other West African populations of the world. The haplotypes frequencies were used to calculate FST. Mitochondrial DNA haplogroups present in Africans were represented in the two ethnic groups. The genetic diversity of Asholio was 0.9143 and Atakar, 0.9145. The calculation of FST (0.003) for the two ethnic groups suggests no difference between them. In order to understand the expansion of the haplotypes of mitochondrial DNA in West Africa, the studied population was compared with neighbor populations. Some African ethnic groups were grouped and the Asholio and Atakar ethnic groups were next to the west African populations. Therefore we can suggest that the populations geographically related and those with the same language (West Africa) are genetically similar, corroborating the fact that these mtDNA marker can be used in the inquiry of the recent history of a population.

Keywords: mtDNA, HVS-II, Control region, Asholio, Atakar and Variation.

INTRODUCTION

Models of Homo sapiens origin and dispersion have been proposed and combated. One of the dispersion models of Man is called multiregional. In this model Homo erectus left Africa scattering to Old World regions. These regional populations then slowly evolved into the modern man [1]. Africa is inhabited by populations that show high levels of genetic diversity compared to most other continental populations today and it is thought to be the ancestral home of modern humans. African populations have the largest number of population specific autosomal, X-chromosomal and mitochondrial DNA haplotypes with non-African populations having only a subset of the genetic diversity present in Africa [2]. Estimates of FST (the classic measure of population subdivision) from mitochondrial DNA are much higher in Africa than other populations, as summarized by Tishkoff et al., [3]. In addition, analyses from studies based on autosomal SNPs, STRPs or Alu elements show higher FST values for African populations [2-4]. Recent
studies of world populations based on large genomic data also reported significant population structure among the African groups [5, 6]. However, given the cultural and linguistic diversity of African populations (with over 2000 distinct ethnic groups and languages), these studies have typically included only a handful of African populations indicating that most African populations have not been studied. As previously noted, most existing genetic data on African populations have come from a few countries that are relatively economically developed and/or with key research or medical centers [1]. Availability of more genetic data from sub Saharan Africa will clearly be useful in our understanding of population structure, demographic history and the efforts to map disease-causing genes. Linguistic inclinations, anthropometric records and geographical background of human populations are known to provide a historical basis for human evolution and variation, as well as the reasons underlying such changes [14]. The diversity exhibited by Homo sapiens arose during their processes of dispersal into their present regions, thus, the subsets of variation tend to be associated with particular geographic areas and populations [15]. Human variation has been measured using simple visual characters like size, form and skin colour leading to the conclusion that sharing one or more features is an indication of common descent [16], but advances in the science of genetics have revealed greater distinctions [17]. Thus, anthropological genetics, a comparatively new discipline makes attempt at answering questions that concern human origin and variation using methods and theories of genetics [18].

From an evolutionary view point, such relationships spread across the world’s genetic map have led to efforts on illuminating the origin and dispersal of anatomically modern man across the world [19] Postulations based on developed models have shown different origins for man, out of which the Recent African origin (RAO) also known as the “out of Africa” model asserts a common descent for all populations from an anatomically modern Homo sapiens ancestor [20, 17]. This makes the African continent, the ancestral home of all humans today. Reconstructing the history of the West African population is considered complex [15], this is due to short and long migration events within the region [14]. One of the earliest indications of West Atlantic occupation by modern humans goes back to about 40 KYA [12], but subsequent changes in the climatic conditions resulted in the significant movement of these occupants [21].

The dearth of archeological evidence (attributable to differences in sea level which may have buried such artifacts) for reconstructing the past has also contributed to this situation, as such language groups were used to genetically classify populations into groups of common descent [22]. This is evident in the fact that cultures may spread without attendant spread of genes, but languages are not easily acquired in later life than other cultural transformations [23]. The recent development of genetic tools has proven that linguistic groups within Africa share common gene pools [24] which have become useful in probing phylogenies. The genetic variation in modern man occurred during the events of early migration into new territories, with concomitant localization of these variations to particular regions [25, 26]. Studies are ongoing to understand the past events involving population expansion, contraction, genetic drift and substructure. Some of these studies employ genetic methods to probe the human genome to investigate and analyse single nucleotide polymorphisms (SNPs) in conjunction with restriction fragment length polymorphism (RFLP) techniques obtained from the hypervariable region of the d-loop [13] [27]. These studies have demonstrated that human mtDNA is geographically structured and may be classified into groups of related haplotypes [13, 28].

SUBJECTS AND METHODS

Subject Sampling

mtDNA yielding specimen (buccal cells) for this study was obtained from the Asholio and Atakar found within the Kaura Local Government Areas of Kaduna State (Figure 1). Collection of specimen was done in randomly selected Secondary Schools from villages where each of the ethnic groups predominates. The villages sampled are: Manchok, where the Asholio subjects will be obtained while Mayit village where the Atakad subjects will be obtained. Twenty unrelated males (n=20 from each ethnic groups) were enrolled for this study. Certification of ethnicities was based on information given by the participants on both parental sides, whose ancestors were known to belong exclusively to a specific ethnic group for at least the last two generations. This information was corroborated by community elders from each of the villages where the participants was drawn.

METHODS

Buccal cells collection was done according to the protocol employed by [29]. For each participant, sterile cotton tipped applicators (swabs) was used to scrape off the cells of the oral mucosa, for 30 seconds and in-between their gum and cheek for 30 seconds. The ends of the swabs was cut, air-dried at room temperature and placed in 15 ml plastic tubes. After the swab, each subject were asked to rinse his mouth with the provided 10 ml of clean water. The water was swished vigorously in the mouth of the subjects for one minute and then poured into 50 ml collection tubes. The tubes was transferred to portable cooling chambers (flasks) containing ice so that the samples was preserved at 4°C prior to transportation and subsequent extraction.
DNA Extraction, purification and amplification of genomic DNA was carried out at the DNA Laboratory Kaduna. The equipment and laboratory wares (tips and tubes) were autoclaved to prevent contamination.

The amplification of the hypervariable region (HVI) was performed according to the protocol described by Budowle et al. [30] with the following primer pairs:

HVII C1 (L048): - (5'-CTC ACG GGA GCT CTC CAT GC-3') and D1 (H408): - (5'-CTG TTA AAA GTG CAT ACC GCC A-3'). Which amplified the region between nucleotides 73 and 340, i.e 260 bp.

Agarose gel electrophoresis was carried out to get the DNA bands of interest. After verifying the presence of DNA through the gel, the amplicons was purified to remove excess dNTPs and unbound primers using ExoSAP DNAse. The PCR amplicons was digested for 10-18 hours at 37 °C with the appropriate restriction enzymes (MboI and HpaI).

Molecular Genetic Analysis
All intra population diversity analyses was done using DNAsp 5.10 [31], XLSTAT 4.06 v13 and Arlequin 3.513 package [32, 24]. The distance matrix was used to measure genetic distances of the study populations. The amount of variation within each study population was determined using the Nei’s gene/haplotype diversity. Tajima’s D [33] and Fu’s F was used to test for any departure from neutrality. mtDNA genealogies were constructed using median-joining network approaches (available in the Network 4.6 program, from the Fluxus Engineering Web site) [34]. AMOVA was used to test the significance of their genetic diversity. Phylogenetic analysis was performed utilising the nucleotide sequence data generated in the study.

RESULT AND DISCUSSION
The extracted DNA were purified and quantified using NanoVue spectrophotometer (GE healthcare) as shown in Table-1. The absorbance ratio (A260/A280) of the DNA was calculated showing high DNA yield from the sample amplified. The HVS-II segments of the control regions of the 40 individuals extracted were amplified. The process utilized primer sets that produced 240 bp fragments for HVS II Figure-1.

![Image of Agarose Gel](image_url)

**Fig-1:** PCR amplicons of HVS II visualized on a 2% agarose gel. First lane M: Molecular DNA genetic marker, Lane +: POSITIVE control, Lane - : NEGATIVE control, Lane 1-13 are the PCR products

| Table-1: Yields and quality of DNA from buccal swabs |
|-----------------------------------------------------|
| **HVS II**                                         |
| Average DNA yield per swab and range (in parenthesis) in µg | 3.82 (1.35-7.1) |
| Mean OD260/OD280 ratio ± SD and range (in parenthesis) | 1.85±0.17* (1.27-1.82) |

*P< 0.001
Fig-2: Representative mtDNA sequence chromatogram for HVS II. Nucleotide peaks intensities and reading positions are colour coded to aid identification of sequenced nucleotide bases

Table-2: Mutation sites observed in mtDNA control region sequences with respect to rCRS

| SAMPLE | HAPLOGROUP | HVI (nt 73 – nt 340) | ASHOLIO | ATAKAR |
|--------|------------|----------------------|----------|---------|
| V1     | L          | 73 150 228 263 315   | 1        |         |
| V2     | L          | 73 150 263 315.1     | 1        |         |
| V3     | L0         | 204 263 309.1 315    | 1        |         |
| V4     | L0a        | 73 146 152 195 309.1 315.1 | 1 |         |
| V5     | L0a        | 73 151 152 263 315.1 | 1        |         |
| V6     | L1         | 73 152 182 1896 195 247 263 297 316 | 1 |         |
| V7     | L1         | 73 150 263 315.1     | 1        |         |
| V8     | L1         | 73 143 150 263 309.1 315.1 | 1 |         |
| V9     | L1         | 263 309.1 315.1      | 1        |         |
| V10    | L1         | 118 146 152 263 298 315.1 | 1 |         |
| V11    | L1         | 73 150 195 263 295 315.1 316 | 1 |         |
| V12    | L1b        | 73 146 152 263 315.1 | 1        |         |
| V13    | L1b        | 263 309.1 315.1      | 1        |         |
| V14    | L1b1       | 73 152 263 295 309.1 315.1 | 1 |         |
| V15    | L1b1       | 73 263 309.1 309.2 315.1 | 1 |         |
| V16    | L1c        | 73 152 263 315.1     | 1        |         |
| V17    | L2a        | 73 152 263 315.1     | 1        |         |
| V18    | L2a        | 73 143 146 152 182 1896 195 263 309.1 315.1 | 1 |         |
| V19    | L2a        | 73 263 315.1         | 1        |         |
| V20    | L2a1       | 73 150 263 315.1     | 1        |         |
| V21    | L3a        | 73 143 150 152 153 189 263 309.1 315.1 | 1 |         |
| V22    | L3b        | 73 143 146 152 182 189 195 263 315.1     | 1        |         |
| V23    | L3b        | 73 152 263 309.1 309.2 31.1    | 1        |         |
| V24    | L3c        | 73 152 1827 1857 195 247 263 309.1 315.1 | 1 |         |
| V25    | L3e        | 73 263 315.1         | 1        |         |

Table-3: Haplotypes and segregating sites compared to the Cambridge reference sequence
Table 4: HVS-II Sequence Polymorphisms defining mtDNA Haplotypes

| HAPLOGROUP | HVS-II POLYMORPHISM MOTIF | ASHIOLO | ATAKAR | Hpsd | Mbdel |
|------------|---------------------------|---------|--------|------|-------|
| L0a1       | 73T 150C 228A 263G 315T | 1       | 3      | +    | -     |
| L0b1       | 73A G150C 195G 263T 312C | 2       | +      | -    | -     |
| L0c1       | 204C 263C 309T 315G       | 1       | +      | -    | -     |
| L0c2       | 73T 146A 152G 195C 309A 345C | 1     | +      | -    | -     |
| L1a        | 73C 151T 152T 263G 295   | 1       | +      | -    | -     |
| L1b1       | 63T 152G 182T 386A 189C 195T 247A 263C 297G 316G | 1       | +      | -    | -     |
| L1c        | 73T 150G 195G 263G 295T 315T 316C | 1     | +      | -    | -     |
| L1d        | 73A 146C 152T 263G 315A   | 1       | +      | -    | -     |
| L2a        | 263C 309A 315A            | 1       | +      | -    | -     |
| L2b        | 263C 309A 315A            | 1       | +      | -    | -     |
| L2c        | 263C 309A 315A            | 1       | +      | -    | -     |
| L2d        | 263C 309A 315A            | 1       | +      | -    | -     |
| L3a        | 263C 309A 315A            | 1       | +      | -    | -     |
| L3b        | 263C 309A 315A            | 1       | +      | -    | -     |
| L3c        | 263C 309A 315A            | 1       | +      | -    | -     |
| L3d        | 263C 309A 315A            | 1       | +      | -    | -     |

Transitions are indicated by the nucleotide position followed by a nucleotide and transversions are indicated by a nucleotide prefix and suffix. A plus (+) indicates a site gain, and a minus (-) indicates a loss of restriction site for the restriction enzymes.

Table 5: HVS-II diversity indices for the Ashio and Atakar ethnic groups

| Population | n | Segregating sites (S) | Haplotypes (h) | Haplotype diversity (HDI) | Sd (HDI) | Are pairwise differences (K) | Nucleotide diversity(n) | Sd(n) |
|------------|---|-----------------------|----------------|--------------------------|---------|-----------------------------|-------------------------|-------|
| Ashio | 20 | 62 | 13 | 0.9431 | 0.020 | 9.45 | 0.024 | 0.006 |
| Atakar | 20 | 46 | 14 | 0.9640 | 0.015 | 7.56 | 0.030 | 0.010 |

Table 6: AMOVA table showing analysis of populations grouped according to major language groups

| Source of variation | D.F | Sum of squares | Components of variation | Percentage of variation |
|---------------------|-----|---------------|-------------------------|------------------------|
| Among groups        | 2   | 52.432        | 0.20746*                | 4.56                   |
| Among populations within groups | 8   | 69.654        | 0.13513                 | 2.41                   |
| Within populations  | 221 | 1634.51       | 4.96502                 | 92.53                  |
| Total               | 231 | 1923.43       | 5.30761                 |                        |

*P < 0.001 Fixation indices: F_{ST} (the variance among subpopulations relative to the total variance): 0.0423, F_{SC} (the variance among subpopulations within groups): 0.0343, F_{CT} (the variance among groups relative to the total variance): 0.0216

AMOVA describes the partitioning of genetic variation (the average distance between randomly chosen haplotypes or alleles) into within and among population components. The results of the analysis are given in Table-6. The major language group model was adopted and the analysis reveals little variation among the populations (4.56 %) and among-populations within groups (2.41 %). The greatest amount of the variation (92.5 %) was found within the populations. The low fixation indices (FSC: 0.0343, FST: 0.0423 and FCT: 0.0216) further demonstrates this little variation. The fixation indices and the variance values here are statistically significant (P<0.001) based on the probability of observing the same or lower measure for each statistics.

Fig 3: Neighbour-Joining (NJ) tree showing the relationship between the study population and other ethnic groups
The Neighbour-Joining (NJ) tree (Figure 3) was constructed for the study populations based on allele frequencies converted into Kimura-2p distances. This method uses the stepwise addition and star decomposition model rather than the cluster analysis to produce the nodes on a tree as against the taxa. The extracted tree is un-rooted and it consists of five major branches indicative of existing genetic variation. The clusters conform to the geographical pattern of an existing genetic differentiation. The hierarchical topology of the tree in Figure 3 suggests the absence of an evolutionary root and branch lengths are less informative. The Asholio and Atakar forming a monophyletic clade, appear more closely related. They both exhibit a shorter genetic distance with other west African ethnic group like Kanuri than the more distant Yoruba node.

Table-5 compares this study’s diversity values with those from [36, 37]. Their high haplotype diversities closely matched those from other West African populations. The study populations had lower haplotype diversities than other West African populations, with the Atakar having the least haplotype diversity. The Tajima’s D statistic showed negative values for all the populations. Compared to the West African groups, the Bajju had higher number of sequence segregating sites. Generally, the populations from the present study and the comparative populations appear to share similar characteristic. During man migration to new geographic regions, genetic mutations were occurring and by selection developing into new haplogroups. Each haplogroup behaves as an independent evolutionary lineage. Climatic and environmental conditions of the different regions of the world contributed to the spread or establishment, by drift or gene flow of these haplogroups. The older the haplogroup, the greater its geographical distribution. Part of the haplogroups has a geographical distribution restricted to some parts of the globe, allowing the construction of their geographical or ethnic origin. Men, when they left their genetic markers, migrated to different continental regions about 60,000 years ago. These marks are still possible to be seen today by the gradient of allelic frequency distribution [38]. From phyleogeographic studies, and the mapping of the frequencies of genetic markers, a picture could be created that suggests where and when the ancestors of modern man have moved. These migratory flows a small group of Africans left descendants to occupy the furthest points of land [38]. This study investigated the major haplogroup types, the presence of a genetic structure, statistical relationship between the ethnic groups, language family, geography and genetics and also gene flow among the Asholio and Atakar ethnic groups. In order to understand the expansion of populations in West Africa, mitochondrial DNA haplotypes from Asholio and Atakar ethnic groups were analyzed. Macrohaplogroup L of African populations was found, however, the genetic distances measured by FST between the two ethnic groups was 0.003, that is, they are similar. These results suggest that there is no great difference between the two ethnic groups in southern Kaduna by mitochondrial DNA analysis.

**CONCLUSION**

The following deductions can be made about the maternal line genetic composition of the population groups included in the study. First, various levels of admixture from West African groups are present in the Asholio and Atakar groups. Secondly, the Asholio group is similar to the Atakar group and might represent remnants of another extinct hunter-gatherer group that were displaced by the Asholio-speaking expansions and became associated with the Atakar. Thirdly, there is a distance-based genetic relationship between the Asholio and Atakar groups. Fourth, the haplogroup distribution between the Asholio and Atakar groups is similar, this clusters with, and is similar to other West African ethnic groups.

**REFERENCE**

1. Goldstein, D. B. E., & Chikhi, L. (2002). Human migrations and population structure: what we know and why it matters. *Annual Revised Genomics of Human Genetics*, 3:129-52.
2. Currat, M. E., & Excoffier, L. (2004). Modern humans did not admix with Neanderthals during their range expansion into Europe. *PLoS Biology*, 2:2264–2274.
3. Cavalli-Sforza, L. L., Menozzi, P., & Piazza, A. (1994). The history and geography of human genes. Princeton, Princeton University Press. 93.
4. Harpending, H. E., & Rogers, A. (2000). Genetic perspectives on human origins and differentiation. *Annual Revised Genomes of Human Genetics*.1:361–385.
5. Liu, H., Prugnolle, F., Manica, A., & Balloux, F. (2002). A geographically explicit genetic model of worldwide human-settlement history. *Current. Opinion on Genetic Development.*, 12(6):675-82
6. Forster, P. E., & Matsumura, S. (2005). Did Early Human Go North or South. *Science.*, 38:965-966.
7. Portin, P. (2007). Evolution of man in the light of molecular genetics; a review. Part I. Our evolutionary history and genomes. *Hereditas*, 144(3):80-95.
8. Underhill, P., Shen, P., Lin, A. A., Jin, L., Passarino, G., Yang, W. H., Kaufman, E., Bonné-Tamir, B., Bertranpetit, J., Franchalacci, P., Ibrahim, M., Jenkins, T., Kidd, J. R., Mehd, S. Q., Seielstad, M. T., Wells, R. S., Piazza, A., Davis, R. W., Feldman M. W., Cavalli-Sforza, L. L., & Oefner, P. J. (2000). Y chromosome sequence variation and the history of human populations. *Nature Genetics*, 26:358–361.
9. Ennafaa, H., Cabrera, V., Abu-Amero, K. A., González, A. M., Amor, M. B., Bouhara, R., Dzimiri, N., Elgaaïed, A. B., & Larruga, J. M.
(2009). Mitochondrial DNA haplogroup H structure in North Africa. BMC Genetics, 10:8.

10. [10] Thangaraj, K., Chaubey, G., Kivisild, T., Reddy, A.G., Singh, V. K., Rasalkar, A. A., Singh, L. (2005). Reconstructing the origin of Andaman Islanders. Science, 5724:996.

11. Hurles, M. E., Nicholson, J. Bosch, E., Renfrew, C., Sykes, B. C., & Jobling, M. A. (2002). Y chromosomal evidence for the origins of oceanic-speaking peoples. Genetics, 160:289-303.

12. Wood, E. T., Stover, D. A., Ehret, C., Destro-Bisol, G., Spedini, G., McLeod, H., Louie, L., Bamshad, M., Strassmann, B. I., & Soodyall, H. (2005). Contrasting patterns of Y chromosome and mtDNA variation in Africa: evidence for sex-biased demographic processes. European Journal of Human Genetics, 13:867-876.

13. Chen, J. T., Sokal, R. R., & Ruhlen, M. (1995). Worldwide analysis of genetic and linguistic relationships of human-populations. Human Biology, 67:595-612.

14. Tishkoff, S. A., & Gonder, M. K. (2007). In: Anthropological genetics: Theory Methods and applications. Ed Crawford MH Cambridge UK. Cambridge University Press, 141-186.

15. Rosa, A., Brehm, A., Kivisild, T., Metspalu, E., & Villems, R. (2004). Mtdna profile of West Africa Guineans: towards a better understanding of the Senegambia region. Annals of Human Genetics, 68:340-352.

16. Molnar, S. (1998). Human Variation: races, types and ethnic groups. Prentice Hall Upper Saddle River, NJ 07458:34.

17. Relethford, J. H. (2008). Genetic evidence and the modern human origins debate. Heredity, 100(6):555-563.

18. Crawford, M. H. (2007). Anthropological genetics: Theory, Methods and Applications. Ed Crawford M. H. 1-16. University press Cambridge.

19. Saccone, C., Pesole, G., Shisà, E., & Preparata, G. (1992). Time and biosequences: a contribution to the origin of modern man. Human Evolution, 7(2):37-46.

20. [20] Ramachandran S., Deshpande, O., Roseman, C. C., Rosenberg, N. A., Feldman, M. W., & Cavalli-Sforza, L. L. (2005). Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. Proceedings of the National Academy of Sciences of the USA, 102(44):15942-15947.

21. Aumassip, G., Ferhat, N., Hedddouche, A., & Vernet, R. (1994). Le milieu saharien aux temps préhistoriques. In Milieux, hommes et techniques du Sahara préhistorique. Problèmes actuels. Paris: L’Harmattan: 9-29.

22. Brown, K., & Ogilvie, S. (2009). Concise encyclopedia of languages of the world. Elsevier Ltd., The Boulevard, Langford Lane, Kidlington, Oxford, OX5 1GB, UK Butler, J. (2005). Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers. 2nd Edition. London. Elsevier Academic Press.

23. Bellwood, P. (2001). Early agriculturalists population diasporas? Farming, languages and genes. Annual Review in Anthropology, 30:181-207.

24. Excoffier, L., Smouse, P., & Quattro, J. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131(2):479.

25. Ingman, M., Kaessmann, H., Paabo, S., & Gyllensten, U. (2000). Mitochondrial genome variation and the origin of modern humans. Nature, 408:08-713.

26. Atkinson, Q. D., Gray, R. D., & Drummond, A. J. (2009). Bayesian coalescent inference of major human mitochondrial DNA haplogroup expansions in Africa. Proceedings of the Royal Society of Biological Sciences, 276:367-373.

27. Salas, A., Richards, M., Lareu, M. V., Scozzari R., Coppa, A., Torroni, A., Macaulay, V., & Carracedo, A. (2004). The African diaspora: mitochondrial DNA and the Atlantic slave trade. American Journal of Human Genetics, 74:454-465.

28. Wallace, D. C. (2007). Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. Annual Review of Biochemistry, 76:781-821.

29. Freeman, B., Powell, J., Ball, D., Hill, L., Craig, L., & Plomin, R. (1997). DNA by mail: An inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. Behavioural Genetics, 27:251-257.

30. Budowle, B., Allard, M. W., Wilson, M. R., & Chakraborty, R. (2003). Forensics and Mitochondrial DNA: Applications, Debates, and Foundations. Annual Revised Genomics of Human Genetics., 4:119-141.

31. Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25:1451-1452.

32. Schneider, S., Roessli, D., & Excoffier, L. (2000). Arlequin version 3.5: A software for population genetics data analysis. http://anthropologie.unige.ch/arlequin.

33. Tajima, K. (1989). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution. 10:512-526

34. Bandelt, H. J., Forster P., Sykes B. C., & Richards, M.B. (1995). Mitochondrial portraits of human populations using median networks. Genetics, 141:743-753.

35. Allard, M. W., Polanskey, D., Miller, K, Wilson, M. R., Monson, K. L., & Budowle, B. (2005).
Characterization of human control region sequences of the African American SWGDAM forensic mtDNA data set. *Forensic Science International*. 148:169–179.

36. Timbuak, J. A., Adebisi, S. S., Nok, A. J., Danborno, S. B. (2015). Study of mitochondrial DNA variability in four ethnic groups within the southern part of kaduna state, Nigeria. *Genetics and molecular biology*. 38(3):485-485.

37. Wallace, D. C. (2007). Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. *Annual Review of Biochemistry*, 76:781–821.

38. Campbell, M. C., & Tishkoff, S. A. (2008). African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. *Annu. Rev. Genomics Hum. Genet.*, 9, 403–433.