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Challenges to Analysis of Ancestral Inference Using Mitochondrial DNA Hypervariable Region 1 SNP Typing

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Abstract

Current analyses of mtDNA with respect to Hispanics/Latinos and persons of African descent are underrepresented and researchers continue to lump them into monomorphic groups. Africans possess the most genetic diversity, thus it is a misnomer to continue delimiting "Africans" or "Blacks" simply as haplogroup L from sub-Saharan Africa, when North Africa (supra-Saharan Africa) is very much a part of the African genetic continuum. It has been established that New World Hispanics/Latinos generally represent persons with a combination of indigenous American and European ancestry or African, indigenous American and European ancestry. The latter is also true for many African Americans. Africans represent all known haplogroups and non-African populations are a subset of genotypes and haplogroups found naturally occurring on the African continent. We tested the mtDNA hypervariable region 1 of an Hispanic/Latino male of African descent and demonstrated via current genomic methods and a review of the recent scientific literature that haplogroup L2a is not restricted to sub-Saharan Africa and when found in supra-Saharan Africa is not present only due to chattel slavery or recent population movements, as is usually reported. We posit that trade across the Sahara in enslaved persons from other parts of Africa may account for some of the North African L2a signatures, however, natural selection and the fact that autochthonous Africans have been present on the African continent longer can account for greater African genetic diversity. Further testing of Hispanics/Latinos and Africans will greatly add to our knowledge of human genetic diversity and how it relates to human evolution, disease resistance, disease susceptibility and drug response.

Keywords: mtDNA, Hispanics/Latinos, sub-Saharan Africa, supra-Saharan Africa, African-specific Haplogroups, Haplogroup L2a, ancestry, maternal lineage, genotype, geogenetic origin, phenotype and evolutionary biology

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Introduction

Analyses of hypervariable regions of mitochondrial DNA (mtDNA) are currently being used in forensic analysis, human molecular genetics and evolutionary biology for biomedical data analysis, human migration studies and recovery operations in identifying deceased persons: ancient and modern. The mitochondrion is a 16,569 bp organelle which is circular, located outside of the nucleus and maternally inherited. It offers the researcher a unique view of an individual's molecular pin code via tracing their maternal ancestry by specifically examining hypervariable region 1 and hypervariable region 2 (HV1 and HV2) within the displacement-loop (D-Loop).This method of examination has been successful because mtDNA molecules exist in greater numbers per cell than nuclear DNA molecules, and boast a higher mutation rate over the nuclear genome [1]. Mitochondrial DNA, unlike autosomal DNA, does not undergo Mendelian inheritance, as only the mother passes clonal copies of her mitochondrial genome (mtgenome) to her progeny. These unique characteristics of mtDNA avoid recombination which is present in nuclear DNA [2]. Evolutionary studies of mtDNA have demonstrated that the oldest modern humans *(Homo sapiens)* originated within Africa and that subsequent African populations radiated out of Africa to populate the rest of the world [3, 4, 5, 6, 7]. This is better known as the Out of Africa hypothesis or the Recent African Origin model [7, 8]. Macro-hapologroup L has its origins in Africa, which suggest an African origin for *Homo sapiens* [9, 10]. Non-African populations exhibit less intra-mtDNA diversity than Africans, but do possess a portion of the mtgenome diversity prevalent among Africans [11, 12, 13, 14, 15]. In addition, genotype and phenotype variation found in Asia and Europe are subsets of African genomic and phenotypic diversity which had their genesis in Africa [16, 17, 18, 19, 20].

Most of world's ethnic groups have not been sampled, which leaves mtDNA databases incomplete [21]. Only an approximation can be made concerning specific ethnic origins; this is especially true with respect to New World populations of African descent [21, 22]. Thus, size and diversity of mtgenome databases continue to be a problem, as well as the number of samples that exist for specific ethnic groups [23, 24].

In addition, ethnic intermarriage, population movement, rape, genetic drift and colonization further complicate the possibility of pinpointing one's exact place of origin [10].

Many scholars tend to view Africa as monomorphic and refer to "Africans" (sub-Saharan variants) solely as members of haplogroup L-and exclude other Africans (primarily supra-Saharan variants whom are considered European or Asian) who display different genetic signatures, which is usually explained away, as due to admixture and back mutation [25, 26, 27, 28]. This is a curious view when one considers that the Eurasian hapolgroups M and N, which gave rise to Europeans and Asians are of African origin and still exist within African populations [29, 30]. Some have argued that this is simply the result of early modern humans migrating back to Africa and contributing their genes to African populations [31, 32]. Keita and Kittles note that to date, no scientific evidence supports a monomorphic group leaving the African continent to differentiate, later returning back to Africa during the Pleistocene or Holocene [33]. However, an African origin of *Homo sapiens* clearly explicates why all of the mtDNA diversity found outside of Africa is extant on the African continent [34]. From an evolutionary geogenetic perspective this is logical, especially, if we consider that the majority of human history has taken place in Africa [34, 35]. Dunston posits that "Africa may be instructive to Europeans, but it may not be vice versa. Eleven hours of the total 12 hours on the evolutionary clock was spent in Africa. That's where the most human variation occurred. As it migrated out, the base was stillthere, the base of the diverse human group…" [36: 42]

We believe that until all known autochthonous African populations have been haplotyped, it is premature to claim with certainty the exact African ethnic origin of New World persons of African descent as some genetic ancestry testing companies offer [37, 38, 39, 40]. These companies use a statistical approximation of an individual's relationship to a known African haplotype/genotype and due to their limited database samples, yield a proposed "exact" ancestry match. African ethnic units are genetically vigorous and should not be viewed through a monomorphic lens [41].

We tested the mtDNA hypervariable region 1 of an individual who self-identified as a Hispanic/Latino of African descent. The subject, *CT 1972,* indicated that his mother is of African American and Native American descent and his father a Hispanic/Latino. He was assigned to haplogroup L2a.

This was not surprising based on the ethnic identification data that we were given, which also indicates that *CT 1972's* maternal lineage is of African geogenetic origin.

Sub-clades of hapolgroup L are the most common haplogroups found in Africa, as it is very widespread [42, 43]. The highest frequencies of L2a and L2b are reported in southeast Africa [44]. Due to its wide distribution, scholars have had difficulty locating where these haplogroups arose [44]. Salas et al. report that during the prehistoric period haplogroup L2a had a vast distribution throughout the African continent, which accounts for the difficulty in assessing an exact geographical origin [45]. Amongst African Americans, the L2 haplogroup is predominant at about 20% [44]. However, it is important to remember that although a haplogroup may have a high frequency in a particular population, this does not mean that one will not find the same haplogroup registering in lower frequencies in different populations [46].It has been documented that geographical regions in Africa, south of the Sahara,is where macrohaplogroup L is primarily located [47]. Thus, haplogroup L and its sub-clades are known as African specific, however, we would like to highlight the fact that haplogroup L also exist in supra-Saharan populations and non-African populations, most notably within European groups [48, 49]. Furthermore, Kivisild et al. asseverate the following concerning haplogroup L: "In the strict phylogenetic sense, the 'African-specific haplogroup L' would now be a misconception, as different subclades of haplotype L embrace all mtDNA haplogroups, both in Africa and outside" [50: 163].

Background

African biological diversity should be understood within an evolutionary geogenetic perspective [16]. This approach should also apply when we find African mtDNA lineages and other African genetic markers in European populations [51, 52, 53, 54], as non-African populations represent a subset of the genomic variation present in Africans [41]. Non-African groups as a subset of continental African genetic diversity unequivocally supports bottlenecks and founder effects that came about due to migrant Africans who radiated out of Africa to populate the rest of the world [55, 56].Furthermore, it is important to note that when comparing non-Africans and Africans, the genetic differences within populations are much smaller than the genetic variation inlying African populations [7, 41].

New World Hispanics/Latinos are derived from a combination of either European and indigenous American admixture or African, European and indigenous American admixture [57, 58, 59, 60]. They are a heterogeneous group and include persons from 25 countries [58]. Therefore, it was no surprise to discover a haplogroup L signature in our sample *CT 1972*. However, we cannot say with certainty the exact geographical location in Africa of *CT 1972*'s maternal lineage, although our findings do indicate a marker of haplogroup L2a. The mtgenome continues to surprise scholars, as several new haplogroups have recently been discovered in the Chad Basin in Africa, specifically sub-clades L3f2 and L3e5 [61]. Thus, it is imperative that researchers continue to increase their African and non-African samples in order to better understand modern human evolutionary genetics and the nature of disease [62].Madrigal and Barbujani, in commenting on New World populations, highlighted that "as members of their culture, geneticists, medical and epidemiological practitionershave learned to classify humans in their own culture-specific manner. However, they should not assume that their racial taxonomy is supported by genetic data" [63: 31]. They further argue that "therefore representing US Whites, African Americans, Hispanics and Amerindians as homogeneous, clearly distinct genetic populations, fails to acknowledge the large amount of gene flow that produced them, plus the high amount of variation found within their parental populations" [63: 31].

Materials and Methods

DNA Sample and Extraction

DNA was extracted from the buccal swab of a self-identified Hispanic/Latino of African descent, hereto identified as *CT- 1972,* using a phenol/chloroform/isoamyl alcohol (PCI 25:24:1) mixture. All samples were electrophoresed to observe quality and quantity of the extracted genomic DNA using a 1% agarose gel stained with Ethidium Bromide. Additionally, *CT 1972* completed a biographical sketch survey, which included: age, gender and maternal and paternal place of birth for three generations.

Amplification and sequencing of hypervariable region 1 of mitochondrial DNA

To generate double-stranded fragments of the HV1 region of mitochondrial DNA the forward primer FP-L15971 and the reverse primer RP-H16414 were used. The 5' end of each forward and reverse primer was engineered with a universal 18bp M13 adaptor sequence to facilitate subsequent sequence reactions. The M13 adaptor sequences were added by L. Strausbaugh group members at the University of Connecticut, Storrs. The final concentrations of reagents in the PCR mixture were 2ng of *CT-1972's* genomic DNA, 1U of AmpliTaq DNA® polymerase

(Applied Biosystems), 1X PCR Buffer (Applied Biosystems, Foster City, CA), 0.1uM each primer, 1.5mM $MgCl_2$, 0.2mM each dNTP and dH_2O to a final volume of 25uL. A negative sample was included which substituted the genomic DNA for dH_2O and contained all other reagents. Thermal cycling was conducted on a MyCycler Thermocycler (BioRad) and consisted of 94°C for 2 min followed by 25 cycles of 94°C for 45 sec, 52°C for 30 sec, 72°C for 1 min and 72°C for 5min.

PCR products were electrophoresed in a 1% agarose gel stained with Ethidium Bromide to assess the purity, quantity and size of the PCR product using 1Kb+ ladder (Invitrogen, Carlsbad CA). Immediately following product verification, PCR samples were purified using Qiaquick PCR purification columns (Qiagen, Valencia, CA). The manufacturer's protocol was strictly adhered to for re-suspension, except for the use of dH_2O rather than TE buffer.

All double-stranded fragments were sequenced bi-directionally at least twice, to confirm all base calls. The primers used were M13F for forward sequencing and M13R for reverse strands. Using the ABI3130 Capillary Sequencer, the ABI 377 Sequencing protocol was followed with the exception of preparing one-sixteenth of the recommended ABI amounts (Big Dye® Terminator v3.1 Cycle Sequencing Kit Protocol) which resulted in a final volume of 10uL. The sequencing product was then precipitated using 50uL of 95% ethanol and 2uL of 3M NaOAc. Precipitated pellets were reconstituted in 20uL of Hi-Di Formamide (Applied Biosystem, Foster City, CA). Sequence products were then separated electrophoretically using an ABI 3130 Capillary Sequencer.

Data Analysis and Bioinformatics

Sequences were extracted and base calls assigned by ABI Sequence Analysis software, while errors and N-calls were corrected using Chromas v1.2 software.

Corrected sequence was exported to FASTA file format for comparison to mtDNA rCRS (revised Cambridge reference sequence) using NCBI's Basic Local Alignment Search Tool. The haplotype of each sample was exported and organized using Microsoft Excel.

Haplogroup Assignment

Haplogroup L2a

Results

We successfully amplified mtDNA of region HV1 of the mtgenome. Our sample *CT 1972* was assigned Haplogroup L2a. The bioinformatic data yielded an alignment of range: 16044…16434 and length: 391bp and HV1: 322bp. The following mutations were observed: d16052, d1611, 16156C, 16181T, d16184, 16223T, d162277, 16278T 16286T, 16294T, 16309G, 16333W, 16384A, 16390A and 16418G.

Discussion

We ascertained that sample *CT 1972* is part of hapologroup L and specifically is of the L2a subclade. Barkhan and Soodyall have observed that "most L2 mtDNA types are part of subHG L2a, and have a widespread distribution in Africa" [43: 142]. Our finding is not unique when examining previously published data from New World Hispanic/Latino populations, which often have a combination of European and indigenous American ancestry or African, indigenous American and European biogenetic material [64, 65]. Research has shown that the majority of Hispanics/Latinos of African ancestry tend to be of eastern coast Hispanic/Latino origin, who are from Dominican, Cuban and Puerto Rican groups [66, 67]. More research among Hispanic/Latino populations is necessary, especially when we consider the fact that the Federal Bureau of Investigation (FBI) has included all Hispanic/Latino samples into a single database for convenience [66]. It is of import to point out that not all persons labeled Hispanic/Latino have the same ancestral background [66]. "Unfortunately, it is still common to find geneticists naively treating the polymorphic genes in a typological manner" [68: 623]. The FBI's lack of sensitivity to the diverse geogenetic origins of Hispanics/Latinos will ultimately lead to false assumptions with respect to attributed statistics [58, 66].

"Thus, categorizing Hispanics within racial categories Black or White could confer upon them social-patterned experiences and exposures similar to those of non-Hispanic Blacks, thus affecting their health negatively and leading to disease, disability, and death" [58; 380].Overall, incomplete databases fail to accurately represent current demographics and there needs to be a sincere effort to correct these shortcomings. Hispanic/Latino populations in the USA are now counted as the largest ethnic minority group and accurate and current genetic data is necessary for medical genetics and genetics research [69].

Most academic publications and genetics literature continue to propagate outmoded anthropological typology and taxonomy of what constitutes an "African" or "Black" due to blatant "unjustifiable generalization" [68: 622] by using West African/ Central African phenotypes and genetic samples solely as proxies to represent the whole African continent [70]. According to Wolpoff and Caspari: "Typological thinking is part of our cultural heritage, a part of our mind-set, it is the way most of us organize the world. And, even if we 'know' not to apply it to biology, it seeps in anyway" [71: 317]. Keita and Kittles surmise that "the definition of African is clearly socially constructed and not developed logically from biogeography" [33: 539]. Natural selection, genetic drift, mutation, gene flow, cultural selection and environmental factors account for the biological variation found amongst human populations [7].

Conclusions and Prospects

In commenting on the Human Genome Project (HGP) Molnar reports that: "Given the data we now possess, it appears that two to three-fifths of the world's population have been excluded from the sample pool. The composite genome produced by the HGP from several U.S. individuals could more aptly be called, 'the Western nation genome project' " [72: 163].

Due to changes in diet, vulnerability to climatic fluctuations, demographic shifts and exposure to harmful diseases, several unique adaptations have developed on the African continent. These adaptations are of import as they are indicative of ongoing phenotypic and genetic changesthat are constantly evolving, along with random genetic drift [7]. DNA studies support a Neo-Darwinian paradigm for African natural selection, along with a fission of populations within Africa. Africa is the motherland of humanity and has produced two of the world's first civilizations, namely the states of ancient Kush (Nubia) and ancient Egypt [73, 74].

However, regarding human evolution, many scholars continue to naively believe and perpetuate the ideas of Coon, who maintained that: "If Africa was the cradle of mankind, it was only an indifferent kindergarten. Europe and Asia were our principal schools" [75: 656].

A hint at future research is the work of Mao et al. [76] whom are using ancestry informative markers (AIMs) within the genome to ascertain ancestral origin in admixed groups, such as, persons of Hispanic/Latino descent. However, we may have to proceed cautiously with the AIMs technology, as there appears to be limitations concerning the application of genetic models to human data. "The most basic models of population genetics, for example, assume not only that populations, but generations also, are discretely bounded" [77: 3]. Due to distribution amongst humans and other structures that affect breeding populations, some geneticists are convinced that "we thus face the danger that out theoretical models may either be too simplistic to account for natural phenomena or too complex to be usable" [77: 4]. Jackson has suggested that geneticists, anthropologists and physicians employ a non-typological approach in interpreting human genetic data through the use of ethnogenetic layering [79, 80]. Her technique is defined as "an alternative bioanthropological strategy for identifying genetic and non-genetic sub-structuring within and between geographical groups…" [79: 217]. Similarly, Benn Torres and Kittles evince that "genetic ancestry is potentially more useful for efficient analyses of the genetics of complex diseases and pharmacogenetics than the traditional racial classifications" [78: 358].

No matter how many clusters, clines or dendrograms one creates, the end result will be the same, if you want to demonstrate differences between so called "races" [15]. However, this does not translate into a biological reality [19] "Race" is a poor proxy for human genotypes, since 99.9 per cent of human genome sequences are identical [81, 82]. Two points are of import here, namely, how the raw data was originally entered into an algorithm, and second, that many geneticistsbelieve that the "presumptive differences among the groups being highlighted are supposed to be genetic" [70: 132]. One would be hard pressed to find any geneticist that would deny the fact that when you eliminate so-called typological "groups" from an algorithm, you will quite clearly notice that Europeans and Asians are a subset of Africans.

Continental groupings depicting charts and diagrams to buttress imagined isolated and divergent evolutionary patterns amongst humans, simply reifies supposed human races [83].Marks makes a salient point: "It thus makes little sense to ask whether Africans are more *closely related* to Europeans or to Asians, if both of the latter are *subsumed* within the African gene pool. Nevertheless, population geneticists still commonly assign individual genotypes *a priori* into races, and ask their computers about the genetic relationshipsamong the races they have constructed" [83: 358].Thus, geneticists and other researchers need to collaborate with anthropologists, linguists, historians and physicians to better understand African genetic diversity and how it relates to human variation, disease resistance, disease susceptibility and drug response [36]. This will subsequently lead to alternative ways of pinpointing deleterious genes and result in new medical therapies for the Occident and the rest of the world, and especially for African's, who are inordinately suffering from pandemic and rampant diseases throughout the continent [62].

Feldman et al. sum up the import and responsibility of getting it right, concerning genomic research, especially with respect to Hispanics/Latinos and African Americans:

The assignment of a racial classification to an individual hides the bio-logical information that is needed for intelligent therapeutic and dia-gnostic decisions. A person classified as "Black" or "Hispanic" by so-cial convention could have any mixture of ancestries, as defined by continent of origin. Confusing race and ancestry could be potentiallydevastating for medical practice. Other attempts to classify people in-to broad genetic groups based on the frequency of specific genes for,say, drug–metabolizing enzymes, are also likely to be poor predictors of medical outcome. As with racial groupings, the overall variation in the frequencies of such genes between groups is likely to be less than that within each social group [69: 374].

Our understanding of the human genome has but one overall truth that we cannot ignore, primarily, that modern anatomically humans are members of a lone evolutionary line originating from Africa. And "human differences are mostly superficial changes which took place in the blinking of an eye in terms of our whole evolutionary history" [84: 16]. Duster points out that"recent research in medicine and genetics makes it even more crucial to resist actively the temptation to deploy racial categories as if immutable in nature and society" [85: 1050]. We are hopeful that with increased sampling of Africans and non-Africans that our knowledge of the mtgenome will continue to increase and yield information beneficial to humankind.

Lastly, we concur with Chakravarti, when he suggest that "an even clearer, and unbiased, picture of humanity's genetic diversity and relationships would emerge if geneticists focused on individuals instead of populations" [21: 381].

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References

Bar, W., et al., Guidelines for Mitochondrial DNA Typing, Vox Sang. 79 (2000) 121-125.

Pakendorf, B. and M. Stoneking, Mitochondrial DNA and Human Evolution,Ann. Rev. Genomics Hum. Genet.6 (2005) 165-183.

Jobling, M.A., et al., Human Evolutionary Genetics, 2nd edition, New York: Garland Science,2013.

Behar, D.M. et al., The Dawn of Human Matrilineal Diversity, Am. J. Hum. Genet. 82 (May 2008) 1130-1140.

Scheinfeldt, L.B., et al., Working toward a synthesis of archaeological, linguistic, and genetic data for inferring African population history, PNAS 107, suppl. 2 (11 May 2010) 8931-8938.

Vigilant, L. et al., African Populations and the Evolution of Human Mitochondrial DNA, Science 253 (5027) (27 September 1991) 503-1507.

Futuyma, D.J., Evolution, 3rd edition, Sunderland, MA: Sinauer Associates, Inc., 2013.

Tishkoff, S.,et al.,The Genetic Structure and History of Africans and African Americans, Science324 (22 May 2009) 1035-1044.

Wallace, D.C., et al., mtDNA Variation, Climatic Adaptation, Degenerative Diseases, and Longevity, Cold Spring Harb Symp Quant Biol 68 (2003) 471-478.

Gibbons, A., African's Deep Genetic Roots Reveal Their Evolutionary Story Science 324 (01 May 2009) 575.

Kidd, K.K., et al., A global survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus,Hum. Genet. 103 (1998) 211-227.

Barbujani, G. and D.B. Goldstein, Africans and Asians Abroad: Genetic Diversity in Europe,Ann. Rev. Genomics Hum. Genet. 5 (2004) 119-150.

Marshall, E., DNA Studies Challenge the Meaning of Race,Science282 (23 October 2008) 654-655.

Long, J.C., Human Genetic Variation: The Mechanisms and Results of Microevolution, a paperpresented at the session:"Exploring the Nature of Human Biological Diversity: Myth v. Reality"at the American Anthropological Association (AAA) 2003 Annual Meeting 21 November 2003 in Chicago, IL.

Bolnick, D.A., Individual Ancestry Inference and the Reification of Race as a Biological Phenomenon, pp. 70-85. In Koenig, B., et al., eds., Revisiting Race in a Genomic Age, New Brunswick, New Jersey: Rutgers University Press, 2008.

Kittles, R.A. and S.O.Y. Keita, Interpreting African Genetic Diversity, African Archaeological Review 16 (2) (1999) 87-91.

Strachan, T. and A. P. Read, Human Molecular Genetics 3, 4th ed., London: GarlandScience, 2010.

Frigi, S. et al., Ancient Local Evolution of African mtDNA Haplogroups in Tunisian Berber Populations, Human Biology82 (4)(August 2010) 367-384.

Pääbo, S., The mosaic that is our genome, Nature 421 (23 January 2003) 409-412.

Pääbo, S., The Human Genome and Our View of Ourselves, Science 29 (16 February 2001) 1219-1220.

Chakravarti, A., Kinship: Race relations,Nature457(22 January 2009) 380-381.

Ely, B., et al.,African-American mitochondrial DNAs often match mtDNAs found in multiple African ethnic groups, BMC Biology4 (2006) 34-47.

Ng, P.C., et al., Individual Genomes Instead of Race for Personalized Medicine, Clinical Pharmacology & Therapeutics, 84 (3) (September 2008) 306-309.

MacEachern. S., Genes, Tribes and African History, Current Anthropology41(3) (June 2000) 357-384.

Keita, S.O.Y., Biocultural Emergence of the Amazigh (Berbers) in Africa: Comment on Frigi et al.,Human Biology 82 (4) (August 2010) 385-393.

Cavalli-Sforza, L.L., et al., The History and Geography of Human Genes, Princeton, NJ: Princeton University Press, 2004.

MacEachern, S., Where in Africa does Africa start? Identity, genetics and African studies from the Sahara to Darfur, Journal of Social Archaeology 7 (3) (2007) 393-412.

Forster, P. and V. Romano, Timing of a Back Migration in Africa, Science 316 (6 April 2007) 50-53.

Schurr, T. et al., Genetic Diversity in Modern African Populations and Its Use for Reconstructing Ancient and Modern Movements, pp 169-207 in Reed, David M., ed., Biomolecular Archaeology: Genetic Approaches to the Past, Carbondale, IL: Board of Trustees, Southern Illinois University, 2005.

Lee, C., et al., Inferring ethnicity from mitochondrial DNA sequence, BMC Proceedings 5, Suppl 2, S11 (2011) 1-14.

Sanchez-Mazas, A. and E.S. Poloni, Genetic Diversity in Africa, pp. 444-451. In Cooper, D.N. and H. Kehrer-Sawatzki, eds., Handbook of Human Molecular Evolution, Vol. 1, England: West Sussex, John Wiley & Sons, Ltd, 2008.

Turchi, C. et al., Polymorphisms of mtDNA control region in Tunisian and Moroccan populations; An enrichment of forensic mtDNA databases with Northern Africa data, Forensic Science International: Genetics 3 (2009) 66-172.

Keita, S.O.Y. and R. A. Kittles, The Persistence of Racial Thinking and the Myth of Racial Divergence, American Anthropologist 99 (3) (September 1997) 534-544.

Chen, Y.S., et al., Analysis of mtDNA Variation in African Populations Reveals the Most Ancient of All Human Continent-Specific Haplogroups, Am. J. Hum. Genet. 57 (1995) 133-149.

Disotell, T. R., Molecular Anthropology and Race,Annals of the New York Academy of Science 925 (2000) 9-24.

Wheelright, J., Genes, Race, and Medicine; Human, Study Thyself,Discover26 (3) (March 2005) 38-45.

Shriver, M.D. and R.A. Kittles, Genetic ancestry and the search for personalized genetic histories, Nature Reviews Genetics5 (August 2004) 611-618.

Bolnick, D.A., et al., The Science Business of Genetic Ancestry Testing, Science318 (19 October 2007) 399.

Soo-Jin Lee, S. et al., The Illusive Gold Standard in Genetic Ancestry Testing,Science325 (03 July 2009) 399-400.

Elliot, C. and P. Brodwin, Identity and genetic ancestry testing,BMJ (21-28 December 2002) 1469-1471.

Jackson, F.L., HLA Diversity within the Context of General Human Heterogeneity: Anthropological Perspectives, Transplantation Proceedings 21 (6) (December 1989): 3869-3871.

Torroni, A. et al., Do the Four Clades of the mtDNA Haplogroup L2 Evolve at Different Rates? Am. J. Hum. Genet. 69 (2001) 1348-1356.

Barkhan, D. and H. Soodyall, Mitochondrial DNA (mtDNA) and Y chromosome DNA variation in African populations, pp. 139-187. In Soodyall, H., ed., The Prehistory of Africa, South Africa: Johannesburg & Cape Town, Jonathan Ball Publishers, 2006.

Wells, S., Deep Ancestry: Inside the Genographic Project, Washington, DC: National Geographic, 2006.

Salas, A., et al., The African Diaspora: Mitochondrial DNA and the Atlantic Slave Trade, Am. J. Hum. Genet. 74 (2004) 454-465.

Suhl, J.A., Mining the Full Information Content of Mitochondrial DNA, Unpublished Diss., University of Connecticut, 2008.

Gonder, M.K., et al., Whole-mtDNA Genome Sequence Analysis of Ancient African Lineages, Mol. Bio. Evol. 24 (3) (2007) 757-768.

Malyarchuck, B. A., et al., Reconstructing the phylogeny of African mitochondrial DNA lineages in Slavs, European Journal of Human Genetics 16 (2008) 1091-1096.

Turchi, C., et al., Polymorphisms of mtDNA control region in Tunisian and Moroccan populations; An enrichment of forensic mtDNA databases with Northern Africa data, Forensic Science International: Genetics 3 (2009) 166-172.

Kivisild, T., et al., The World mtDNA Phylogeny, Nucleic Acids and Molecular Biology 18 (2006) 149-179.

Pereira, L., et al., Linking the sub-Saharan and west Eurasian gene pools: maternal and paternal heritage of the Tuareg nomads from the African Sahel, European Journal of Human Genetics 18 (8) (August 2010) 915-923.

Arnaiz-Villena, A., et al., HLA genes in Macedonians and the sub-Saharan origin of the Greeks, Tissue Antigens 57 (2001) 118-127.

Sanchez-Mazas, A., et al., The molecular determination of HLA-Cw alleles in the Mandenka (West Africa) reveals a close relationship between Africans and Europeans, Tissue Antigens 56 (2000) 303-312.

Arnaiz-Villena, A., et al., Population genetic relationships between Mediterranean populations determined by HLA allele distribution and a historic perspective, Tissue Antigens 60 (2002) 111-121.

Underhill, P. A. and T. Kivisild, Use of Y Chromosome and Mitochondrial DNA Population Structure in Tracing Human Migrations, Ann. Rev. Genet. 41 (2007) 539 564.

Reed, F.A. and S.A. Tishkoff, African human diversity, origins and migrations, Current Opinion in Genetics & Development 16 (2006) 597-605.

Saunier, J.L. et al., Mitochondrial control region sequences from a U.S. 'Hispanic' population sample, Forensic Science International: Genetics2 (2008) e19-e-23.

Borrell, L.N., Racial Identity Among Hispanics: Implications for Health and Well-Being, American Journal of Public Health 95 (3) (March 2005) 379-381.

Tang, H. et al., Recent Genetic Selection in the Ancestral Admixture of Puerto Ricans, Am. J. Hum. Genet. 81 (September 2007) 626-633.

Abe, K., Identities and Racism of Puerto Rican Migrants in New York City: An Introductory Essay, Transforming Anthropology 14 (1) (2006) 83-88.

Černy, V. et al., A Bidirectional Corridor in the Sahel-Sudan Belt and the Distinctive Features of the Chad Basin Populations: A History Revealed by the mitochondrial DNA Genome, Annals of Human Genetics 71 (2007) 433-452.

Campbell, M.C. and S.A. Tishkoff, The Evolution of Human Genetics and Phenotypic Variation in Africa, Curr. Biol. 20 (23 February 2010) R166-R173.

Madrigal, L. and G. Barbujani, Partitioning of Genetic Variation in Human Populations and the Concept of Race, pp.19-37.In Crawford, M., ed., Anthropological Genetics: Theory, Methods and Applications, Cambridge, UK: Cambridge University Press, 2007.

Martinez Cruzado, J.C., Some important research contributions of Genetics to the study of Population History and Anthropology in Puerto Rico: An Interview with Dr. Juan Carlos Martinez Cruzado, Dept. of Biology, University of Puerto Rico at Mayaguez, Delaware Review of Latin American Studies 1 (2) 2005 1-2.

Allard, M. W. et al., Evaluation of Variation in Control Region Sequences for Hispanic Individuals in the SWGDAM mtDNA Data Set, J Forensic Sci 51 (3) (May 2006) 566-573.

O'Connor, C.O., A Novel Forensic Approach to DNA Database Construction and Population Genetic Analysis, Unpublished Diss., University of Connecticut, 2008.

Bertoni, B., et al., Admixture in Hispanics: Distribution of Ancestral Population Contributions in the Continental United States, Human Biology 75 (1) (Feb 2003) 111.

Marks, J., Human Evolution: Human Biological Diversity, pp. 620-624.In Middleton, J. and J. C. Miller, eds., New Encyclopedia of Africa, Vol. 2 (Dakar-Hydrology), Detroit: Charles Scribner's Sons Publishers, 2007.

Feldman, M.W., et al., A genetic melting-pot, Nature 424 (24 July 2003): 374.

Marks, J., What It Means To Be 98% Chimpanzee: Apes, People, and Their Genes, Berkeley: University of California Press, 2003.

Wolpoff, M. and R. Caspari, Race and Human Evolution: A False Attraction, New York: Simon & Schuster, 1997.

Molnar, S., Human Variation: Races, Types, and Ethnic Groups, 6th ed., New Jersey: Prentice Hall, 2006.

Derricourt, R., The ancient Nile Valley, pp. 88-94. In Oliver, R. and M. Crowder, eds.,

The Cambridge Encyclopedia of Africa, Cambridge: Cambridge University Press, 1981.

Connah, G., Holocene Africa, pp. 351-391. In Scarre, C., ed., The Human Past: World Prehistory & the Development of Human Societies, London: Thames & Hudson, 2009.

Coon, C.S., The Origin of Races, 5th printing, New York: Alfred Knopf, 1967.

Mao, X., et al., A Genomewide Admixture Mapping Panel for Hispanic/Latino Populations, Am J. Hum. Genet. 80 (June 2007) 1171-1178.

Reid, R.M., Human Population Genetics, Minneapolis, Minnesota: Burgess Publishing Company, 1978.

Benn Torres, J. and R. A. Kittles, The Relationship Between "Race" and Genetics in Biomedical Research, Current Hypertension Reports 9 (2007) 196-201.

Jackson, F.L., Human genetic variation and health: new assessment approaches based on ethnogenetic layering,British Medical Bulletin 69 (2004) 215-235.

Jackson, F.L., Ethnogenetic layering (EL): an alternative to the traditional race model in human variation and health disparity studies, Annals of Human Biology 35(2) (March-April 2008) 121-144.

Marks, J., Why I Am Not a Scientist: Anthropology and Modern Knowledge, Berkeley: University of California Press, 2009.

Keita, S.O.Y., et al., Conceptualizing human variation,Nature Genetics Supplement 36 (11) (November 2004) S17-S20.

Marks, J., The Legacy of Serological Studies in American Physical Anthropology, Hist. Phil. Life Sci. 18 (1996) 345-362.

Stringer, C. and R. McKie, African Exodus: The Origins of Modern Humanity, New York, New York: Henry Holt and Company, Inc., 1996.

Duster, T., Race and Reification in Science, Science 307 (18 February 2005) 1050-1051.