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High-Resolution RFLP Analysisof Mtdna Variation of Southern Kaduna Asholio and Atakar and their Genetic Relationships to other African Populations

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ABSTRACT

The mtDNA variation of 40 individuals (Asholio and Atakar) from Southern Kaduna, in Northern Nigeria, were examined by high-resolution RFLP analysis. The resulting data were combined with published RFLP haplotype from sub-Saharan African populations and then were subjected to phylogenetic analysis to deduce the evolutionary relationships among them. All of the Asholio and Atakar mtDNA samples were found to belong to the major mtDNA lineage, macrohaplogroup L (defined by a*Hpa*I site at nucleotide position 3592), which is prevalent in sub-Saharan African populations. Additional sets of RFLPs subdivided macrohaplogroup L into two extended haplogroups—L1 and L2—both of which appeared in the Asholio and Atakar. Besides revealing the significant substructure of macrohaplogroup L in African populations, these data showed that the Asholio have one of the most ancient RFLP sublineages observed in African mtDNA and, thus, that they could represent one of the oldest human populations. In addition, the Asholio exhibited a set of related haplotypes that were positioned closest to the root of the human mtDNA phylogeny, suggesting that they, too, represent one of the most ancient African populations. Finally, the overall sequence divergence of 214 African RFLP haplotypes defined in both this and other studies was 0.364%, giving an estimated age, for all African mtDNAs, of 125,500–165,500 years before the present, a date that is concordant with all previous estimates derived from mtDNA and other genetic data, for the time of origin of modern humans in Africa.

Keywords: mtDNA, RFLP, Variation and Genetic relationship

INTRODUCTION

The Southern Kaduna Asholio and Atakar ethnic groupsmtDNA variation have not been analyzed by restriction analysis [1,2] In these studies, the mtDNA sequences of the Asholio were surveyed for variation, by means of six rarecuttingrestriction endonucleases (*Ava*II, *Bam*HI, *Hae*II,*Hin*cII, *Hpa*I, and *Msp*I) and Southern blotting. Althoughthis procedure screened only 2%–3% of themtDNA sequence for variation, it revealed that more than 90%–95% of the Asholio mtDNAs were characterized by a*Hpa*I site gain at nucleotide position (np) 3592. This markerwas subsequently found at very high frequencies inmtDNAs of other sub-Saharan African populations[3 4 5 6] but was not observed in populationsof non-African origin, with a few exceptions[8 34]. Furthermore, theAfrican mtDNAs defined by the *Hpa*I np-3592 site gainformed a group of related mtDNA haplotypes defined as haplogroup "L" which was found to be the most divergent of those identifiedin human populations from around the world[7].These findings contributed to the hypothesis of an African origin of modern humanmtDNAs [35 36 37], although other interpretations of these datahave also been put forward [9 10].Since 1981, relatively limited data have been collectedon the RFLP variation in Southern Kaduna populations. Studies that were conducted on other populations continued touse low-resolution (LR)–RFLP analysis with only the six rare cutting restriction endonucleases listed above[11 38]. These data provides information about populations but could not be entirely integrated withthose obtained, on other populations, by high-resolution(HR)–RFLP analysis [39 13].The value of using HR-RFLP analysis with African mtDNAs, for phylogenetic reconstructions of humanmtDNA, has been shown primarily by two studies ofAfrican populations [40 41]. In the first study, [42] conductedgenomic digestions of mtDNAs, by 12 enzymes (*Alu*I,*Ava*II, *Dde*I, *Fnu*DII, *Hae*III, *Hha*I, *Hin*fI, *Hpa*I, *Hpa*II,*Mbo*I, *Rsa*I, and *Taq*I), with the resulting restrictionfragments being resolved by PAGE. In the later study,the entire genome of African mtDNAs was PCR amplifiedin nine overlapping fragments, which were thensubjected to digestion by 14 enzymes (*Alu*I, *Ava*II,*Bam*HI, *Dde*I, *Hae*II, *Hae*III, *Hha*I, *Hin*cII, *Hin*fI, *Hpa*I,*Hpa*II, *Mbo*I, *Rsa*I, and *Taq*I), with the resulting restrictionfragments being resolved by agarose gel electrophoresis.Both of these studies revealed that Africanhaplotypes form the deepest branches of the

humanmtDNA phylogeny and that African groups are the mostdivergent of all world populations.Several additional aspects of mtDNA variation in African populations were shown in the Chen et al. [43]study. First, two types of length polymorphisms wereobserved in some of the central-African mtDNAs(Mbuti [eastern] and Biaka [western] Pygmies), one of which was the COII/tRNALys intergenic 9-bp deletionthat formerly had been thought to occur only in Asian populations. This length polymorphism had previouslybeen noted in African individuals but had not been assignedto a particular haplogroup or mtDNA lineage[12]. In addition, western-Africanpopulations from Senegal were found to have haplotypesthat lacked the *Hpa*I np-3592 site gain and, thus,appeared not to belong to haplogroup L. Becausenearly all of these "non-L" haplotypes had the *Dde*Inp-10394 site gain, a polymorphism that was also present in almost every haplogroup L mtDNA, the datasuggested that the former haplotypes derived from thelatter, although the exact subhaplogroup (L1 or L2)from which these non-L haplotypes originated was notclear. Alternatively, non-L mtDNAs could have evolvedfrom haplotypes observed in Europeans, such as thosebelonging to haplogroups I–K [13],and could have been spread into Africa through populationcontractions/expansions since their origin. Suchambiguities clearly indicated that further analysis ofnon-L haplotypes was necessary in order to understandtheir phylogenetic relationships to other African and non-African mtDNAs.

MATERIALS AND METHODS

A total of 40 Southern kaduna individuals of the Northern Nigeria formedthe sample group for this study. Of this group, 20 were Asholio and 20 were Atakar ethnic groups. All of the mtDNA samples were analyzedfor mtDNA sequence variationThe individuals involved in this study were classified according to their ethnicity listed on either their identitydocuments or birth certificates. In the few rare cases inwhich this information was not available, both parentswere independently questioned about their own ancestrybefore their child was classified as belonging to eitherof the two groups. Fortunately, in all cases, the familyhistory furnished by both parents was identical, simplifyingthe classification of their children. Interpreters wereused in the interviews and counseling sessions, and carewas taken to verify the data and information gathered.Even though these two groups have been known to intermarryno such instance was recorded for these particular individuals.

Sample Acquisition and Preparation: After informed consent was obtained, buccal cells were collected from Asholio and Atakar individuals according to the protocol employed by Freeman *et al* [44]. 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.

Molecular Genetic Analysis: The entire mtDNA of each sample was subjected toHR-RFLP analysis using the primer pairs and PCR amplificationconditions described by Torroni et al. [13 14].This HR-RFLP analysis defined the *complete haplotype* for each individual. The evolutionary relationships among the Asholio and Atakar mtDNAs were further differentiated by the sequencing of both hypervariablesegments (HVS-I and HVS-II) of the CR of eachindividual, by methods described by Schurr et al. [37].Both hypervariable segments were PCR amplified by useof heavy-strand primer H15704 (np 15704–15723) andlight-strand primer L770 (np 770–751), whereas twodifferent sets of primers—H15978 (np 15997–16391)and reverse primer L16391 (np 163981 16483) for HVSI, H1 (np 1–17) and L48 (np 48–408), for HVSII were used for sequencing. The resulting data wereanalyzed by SEQUENCHER 3.0 software (Gene Codes).

Phylogenetic Analyses: The phylogenetic relationships between the mtDNAhaplotypes observed in the Asholio and Atakar and thosepreviously reported in the other sub-Saharan African populations [7] were inferred by parsimonyanalysis with PAUP 3.1.1 [16] and PAUP 4.0.2b [45]. The samples includedcomplete haplotypes of 62 Senegalese (AF01–AF24,AF26–AF36, AF45–AF59, AF64–AF65, and AF70–AF79), 17 Pygmy (AF25, AF37–AF44, AF60–AF63 and AF66–AF69), and 29 Asholio and Atakar (AF46, AF80- AF107). All dendrogramswere rooted from a chimpanzeehaplotype that was extrapolated from the whole mitochondrialgenome sequence presented by Horai et al.[19], by identification of all recognition sequences ofthe 14 enzymes used in the HR-RFLP analyses. The Africanhaplotypes were also midpoint rooted without anoutgroup.Maximum parsimony (MP) trees were generated via random addition of sequences, by the tree-bisection andreconnection(TBR) algorithm, with <10 MP trees beingsaved for each replication. Because of the numerous terminaltaxa in this data set, a large number of MP treeswere obtained, with 3,000 MP trees being generatedafter 1,388 replications. Although shorter trees couldexist, none were observed in this analysis. Strict and50%-majority-rule consensus trees encompassing allMPtrees were also obtained, to test the consistency of thebranching order in the MP trees. Similarly, the data weresubjected to bootstrap analysis to test the statistical supportfor the branch structure of the MP trees. All RFLPhaplotypes were bootstrapped through 10–500 replications,with resampling of characters (i.e., RFLPs) bythe TBR algorithm, with a 50%-majority-rule bootstrapconsensus tree being generated from the best trees savedafter each independent analysis. Genetic distance/neighbor-joining (NJ) trees were also generated by PAUP4.0.2b, on the basis of the mean character differencesof the haplotypes. These were also subjected to bootstrap analysis [16].Phylogenies of these sequenceswere obtained with the NJ [17] method present in

the MEGA X statistical package[18]. The evolutionary distance betweenpairs of CR sequences were estimated as *p* distances, theproportion (*p*) of nucleotide sites at which the pair ofsequences being compared differed, as calculated by dividing the number of nucleotide differences (*nd*) by thetotal number of nucleotides compared (*n*). All *p* distances were used with the NJ method, to produce phylogenetic trees. The NJ trees generated fromHVS-I sequences were rooted by use of either a chimpanzee[15] or the Neanderthal [20] sequence, and those generated from bothHVS-I and HVS-II data were rooted from only the chimpanzeesequence, because no HVS-II data were availablefor the Neanderthal mtDNA. Unrooted NJ trees weremidpoint rooted without an outgroup sequence. Thebranching structure of these NJ trees was, in turn, testedby bootstrap analysis, which produced bootstrap confidence-level estimates for each interior branch.

Sequence-Divergence Estimates: To calculate the genetic divergence of the West-African populations, as well as the divergence withinspecific haplogroups and their sublineages, we used theiterative maximum likelihood (ML) method of Nei and Tajima [20]. The interpopulation ML estimates wereobtained by taking a weighted average of the numberof individuals in each population. "Corrected" interpopulationvalues (Corr) were obtained by taking an average of two intrapopulation estimates and subtractingthat value from the uncorrected interpopulationvalue—that is, Corr = d 2 [(d 1 d)/2], where *x* and *y xy x y*are the two populations being analyzed. The samemethod was used for estimation of the divergence ofhaplogroups and their sublineages. When calculating thedivergence times of these haplogroups and of their sublineages,the mtDNA evolutionary rate of 2.2%–2.9%/1 million years was used[22].

RESULTS

A totalof 18 haplotypes were detected in the Asholio,and 13 haplotypes were found in the Atakar, only 2 ofwhich (i.e., AF85 and AF99) were shared between them (table 1). Of these West-African haplotypes, 77% clustered into haplogroup L, including 84% of the Asholio mtDNAs and 68% of the Atakar mtDNAs.Haplotypes belonging to haplogroup L have previously been observed in the western-African Senegalese[7 23] and Biaka and Mbuti Pygmy [7] populations, at veryhigh frequencies, as well as at similar frequencies in theBamileke of Cameroon [4] and invarious southern-African groups [11].

Table 1: HR mtDNA Haplotypes in Western-African Populations

HR HAPLOTYPE	STATUS HpaI 3592	Mbo1 10394	Asholio	Atakar	Total
AF46	+	+		1	1
AF80	-	+		2	2
AF81	-	+		1	1
AF82	-	+	1		1
AF83	-	+		2	2
AF84	-	-		1	1
AF85	-	+	2	1	3
AF86	-	+	1		1
AF87	+	+	1		1
AF88	+	+	1		1
AF89	+	+	1		1
AF90	+	+	1		1
AF91	+	-	1		1
AF92	+	-		3	3
AF93	+	+	1		1
AF94	+	+	1		1
AF95	+	+	1		1
AF96	+	+	2		2
AF97	+	+	1		1
AF98	+	+		2	2
AF99	+	+	1	1	2
AF100	+	+	1		1
AF101	+	+	1		1
AF102	+	+		1	1
AF103	+	+		1	1
AF104	+	+		1	1
AF105	+	+		3	3
AF106	+	+	1		1
AF107	+	+	1		1
Total			20	20	40

A plus sign (+) denotes presence, and a minus sign (-)denotes absence

However, no comparable haplotypes havebeen observed in European, Middle Eastern, or Asian populations [24] [13] [14], with the exception of those groupswith a history of contact with African populations[25] [26] [27] [46]. Thus, these resultssubstantiate the African origin of this mtDNA lineage,as well as its widespread distribution, at varying frequencies, in all African populations.Consistent with a previous study of Khoisan mtDNA variation [28], none of the Asholio or the Atakar haplotypes exhibited any length polymorphismsin the COII/tRNALys intergenic region. Theseresults support the conclusion that deletion haplotypes from haplogroup L originated not in Khoisan populationsbut, instead, in ethnic groups of western and central Africa.

Phylogenetic Analysis of RFLP Haplotypes: The phylogenetic relationships among the mtDNAhaplotypes observed in the Asholio and Atakar andthose previously reported in the other sub-Saharan African populations were assessed by both MP and geneticdistance/NJ analysis. The MP tree of African mtDNAs is presented in figure 1. The statistical robustness of thebranches and subbranches of this tree are demonstratedby their preservation in 50%-majority-rule and strictconsensus trees.

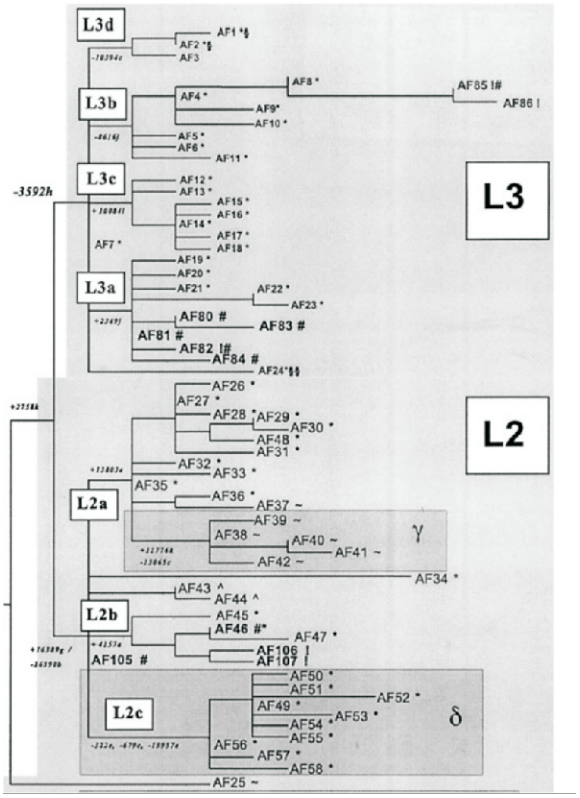

Figure 1:MP tree showing evolutionary relationships among 107 haplotypes (AF1–AF107) observed in 214 sub-Saharan Africans (Chen et al. 1995; present study). γ (Asholio) δ(Atakar). The tree is 200 steps in length, has consistency and retention indices (CI and RI) of .745 and .893, respectively, and is one of 3,000 MP trees generated by the TBR algorithm. It was rooted from a chimpanzee haplotype defined by sequence analysis (Horai et al. 1995). The RFLPs that define each of the major branches of the MP tree are indicated by the number of the first nucleotide of the respective recognition sites, according to the CR sequence (Anderson et al. 1981).

From the MP tree, it is clear that the African mtDNA phylogeny forms a successive series of branches, eachone occupied by clusters of related mtDNA haplotypes (fig. 1 and table 1), consistent with our previous report[7]. The overall phylogeny is divided intotwo major parts, the lower two-thirds having the *Hpa*Isite at np 3593 and the upper one-third lacking this site. The mtDNAs that havethe *Hpa*I np-3592 site as "haplogroup L" was designated [7] and had reported that it subdivides into two major branches—L1. The upper one-third of the mtDNA phylogeny encompasses mtDNAs that lack the np-3592 *Hpa*I site [45]. This was subsequently redefinedas "L3," by Watson et al [29]. When this nomenclatureis adopted, L3 is subdivided into four haplogroups:L3a, associated with a*Mbo*I np-2349 site gain; L3b,associated with a *Mbo*I np-8616 site loss; L3c, associatedwith a *Taq*I np 10084 site gain; and L3d, associated witha *Dde*I np-10394 site loss (fig. 1 and table 2).

Table 2: mtDNA Haplogroup Distribution in African Populations

HAPLOGROUP	DEFINING POLYMORPHISM	Asholio (n=20)	Atakar (n=20)	NO. % IN Mandenkalu (n=60)[a]	Wolof (n=20)[a]
L	+HpaI 3592, + DdeI 10394	5(10)	6(11)	44(73)	14(70)
L1	+*Hin*fI 10806, 2 *Rsa*I 2758		…	17(28)	4(20)
L1a	+*Alu*I 4310	…	…	1(2)	…
L1b1	+*Mbo*I 2349	…	1(3)	13(22)	4(20)
L3	-*Hpa*I 3592	7(16)	10(32)	16(27)	6(30)
L3a	+*Mbo*I 2349	2(5)	9(29)	4(7)	1(5)
L3b	-*Mbo*I 8616	5(12)	1(3)	7(12)	2(10)

[a] Data are from Chen et al. [7]

L1, L2, and L3 each encompass a sequence diversitythat is approximately equivalent to those of other continent- specific haplogroups [21 43 23]. Therefore,it is reasonable to delineate these as haplogroups and todelineate the combination of L1 and L2, delineated by the presence of the *Hpa*I site at np 3592, as a macrohaplogroup, "L." This then relegates L1a, L1b,L2a–L2c, and L3a–L3d to the level of subhaplogroups.Of our L3 subhaplogroups (i.e., L3a–L3d), L3a and L3bare identical to similarly named groupings recognized by Watson et al. [29].Within the African MP phylogeny (fig. 1), the west- African Asholio and Atakar samples cluster together in L2. Within L2ais predominantly Asholio and L2c is predominantly Atakar. To establish the reliability of the major branches ofour African mtDNA MP tree, we subjected the MP treeto a bootstrap analysis. Because of the large number oftaxa (haplotypes) and character states (restriction-site variants) in our data set, the 50%-majority-rule bootstraptree was unable to provide confidence values fora number of the internal branches. To facilitate the analysis,we reduced the number of taxa from each of the major clusters seen in figure 1, eliminating most of thehaplotype-specific restriction-site variants.

Sequence Divergence of African Haplogroups: On the basis of intrapopulation ML calculations,we estimated the divergence times for all major clustersof mtDNAs thus far observed in African populations (table 3). The sequence-divergence
value for all African HR haplotypes is 0.364%, an estimatethat gives a maximum age, for the most recentcommon ancestor (MRCA), of »126,000–166,000 years

Table 3

Sequence Divergence and Divergence Time of African mtDNA Haplogroups

Haplogroup	No. of Haplotypes	No. of Subjects	Sequence Divergence (%)	Divergence Time (YBP)
All African	107	214	.364	125,500–165,500
L	76	164	.356	122,800–161,800
L1	40	98	.328	113,100–149,100
L1a	20	60	.265	91,400–120,500
$L1a_1$	10	32	.166	57,200–75,500
$L1a_2$	9	27	.119	41,000–18,600
L1b	19	36	.214	73,800–97,300
$L1b_1$	11	21	.041	14,100–18,600
$L1b_2$	8	15	.246	84,800–111,800
L2	36	66	.171	59,000–77,700
L2a	17	29	.113	39,000–51,400
L2b	5	9	.072	24,800–32,700
L2c	10	22	.052	17,900–23,600
L3	31	50	.227	78,300–103,200
L3a	10	17	.120	41,400–54,500
L3b	9	17	.225	77,600–102,300
L3c	7	9	.082	28,300–37,300
L3d	3	5	.051	17,600–23,200

before the present (YBP). Similarly, macrohaplogroup L shows a sequence divergence of 0.356%, which givesa divergence time of 123,000–162,000 YBP. These findingsare concordant with earlier estimates of AfricanmtDNA divergence, which gave coalescence values of110,000–160,000 YBP [21 45 16 19], and confirm that macrohaplogroup L is the most divergent of all haplogroups in human populations [7]. These data further

supportthe hypothesis that, in being the oldest mtDNAs inhuman populations, these African haplotypes form theroot of the modern human mtDNA phylogeny.

Genetic Divergence of African Populations: The intra- and interpopulation sequence divergenceswithin and between African populations are shown in table 4.

Table 4

Sequence Divergence Within and Between African Populations

	Sequence Divergence (%)					
	(*N* =20)	(*N* =20)	Mandenkalu (*N* = 60)	Wolof (*N* = 20)	Mbuti Pygmies (*N* = 22)	Biaka Pygmies (*N* = 17)
Asholio	.320	.334	.366	.368	.378	.400
Atakar	.036	.277	.300	.298	.315	.353
Mandenkalu	.069	.024	.274	.275	.294	.332
Wolof	.076	.028	.007	.263	.283	.369
Mbuti Pygmies	.098	.056	.036	.032	.241	.371
Biaka Pygmies	.068	.043	.024	.067	.080	.342

NOTE.—Intrapopulation sequence-divergence values are on the diagonal (*underlined*), whereas the raw and corrected interpopulation sequence-divergence values are above and below the diagonal, respectively.

The Biaka Pygmies have the highest intrapopulationsequence divergence (0.342%), followed by the Asholio (0.320%) and then the Atakar (0.277%).In addition, all the interpopulation sequence-divergence values between Asholio and other African populationsare higher than those between the Atakar andother African populations. Thus, the Asholio and Atakar are close to other African populations. This finding is consistent both with [30] and with the Atakar being moreclosely related to the Negroid populations of west Africa, who, in turn, show greater affinities with populations from western and central Africa.These same relationships are seen quite clearly in theNJ tree based on these ML estimates (fig. 2). In this tree, the Asholio and Atakar clearly cluster more closelyto other Bantu-speaking Senegalese groups—the Mandelakuand Wolof.

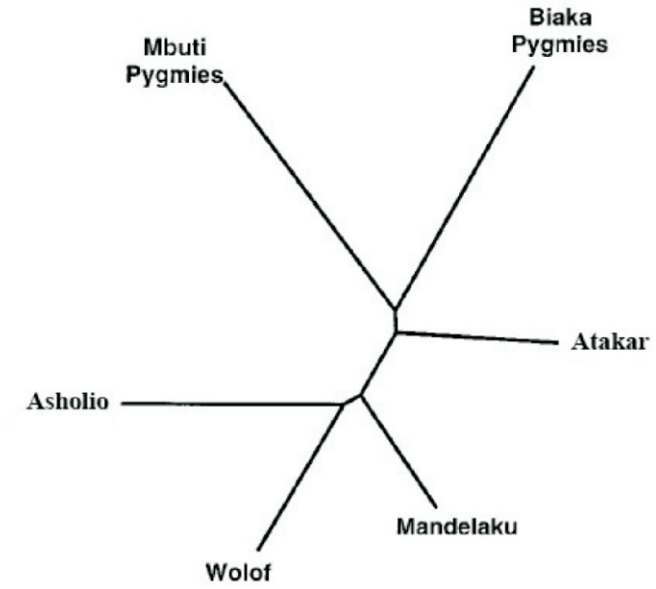

Figure 2:NJ tree of ML genetic distances for major Africanpopulations

DISCUSSION

Studies of African mtDNA sequence variation thathave used HR-RFLP analysis [8]have confirmed the ubiquity of macrohaplogroup L in African populations. Moreover, it's documented that macrohaplogroup L is the oldest African and human mitochondrial lineage, encompassing a sequencediversity of 0.364% and having an estimated age»126,000–165,000 YBP; as such, it must be consideredthe root of the human mtDNA phylogeny, from whichall other haplogroups evolved. Macrohaplogroup L isfurther subdivided into two major haplogroups: L1 and L2 (figs. 1 and table 1). Haplogroup L1 is themost ancient of African haplogroups, and it is furthersubdivided into subhaplogroups L1a and L1b, with L1a

being the older of the two (table 2). L1a is further subdividedinto lineages L1a1 and L1a2 (table 1). Haplogroup L2 is considerably younger than haplogroupL1, being approximately half the age of the latter.It is subdivided into three subhaplogroups: L2a, L2b,and L2c. L2a contains the core population-specific haplotypes(g) of the Mbuti Pygmies, whereas L2c containsthe core haplotypes (d) of the Bantu-speaking Senegalese.The existence of four distinct L3 subhaplogroups[7] also has been confirmed. Of these,L3a and L3b are the oldest, whereas L3c and L3d areof more recent origin. In addition, L3 mtDNAs havebeen found to be much more closely related to thosefrom L2 than either is to L1, by RFLP sequence analysis. These findingssuggest that haplogroups L2 and L3 have a morerecent origin than does haplogroup L1 and that the splitof L3 from L2 happened relatively soon after L2 split from L1 (figs. 1 and table 4) Not only do the mtDNAs of these two populationsform distinct clusters by parsimony analysis (figs. 1 and 2), but they branch separately in the NJ treeof RFLP genetic distances (fig. 2). The distinction between the two Pygmy populations is further demonstratedby the significantly greater sequence diversity of the Biaka Pygmies (0.342%) relative to that of the MbutiPygmies (0.241%). This difference suggests that theBiaka Pygmies arose before the Mbuti Pygmies and represent one of the oldest African populations,whereas the Mbuti Pygmies appear to have arisen independentlyand more recently. This conclusion is consistentwith the nuclear genetic data reported by Cavalli- Sforza et al. [31] and with the different linguisticaffiliations of the two populations, with the Mbutispeaking a Nilo-Saharan language and with the Biakaspeaking a Niger-Kordofarian language [32]. The estimates of the age of the African population and its haplogroups are also comparable to estimatesmade by other investigators. In our studies, the antiquityof African mtDNAs has been demonstrated by meansof a sequence-divergence time of 125,500–165,500 YBP[7]. Similarly, the coalescencetime for the MRCA, as calculated by Watson etal. [29] on the basis of their L1 cluster, was 111,000 ± 5,700 YBP; that estimated by Horai et al. [15] onthe basis of whole mtDNA sequences was 143,000 ± 18,000 YBP; and that estimated by Cann et al. [12]on the basis of RFLP haplotype data was 120,000 YBP.

By contrast, the age of haplogroup L2 was muchyounger, being 59,000–77,700 YBP by our analysis,56,000 ± 3,000 YBP according to Watson et al. [29],and 70,333 ± 25,710 YBP according to Graven et al. [23]. Finally, the age of haplogroup L3 is78,300–103,200 YBP by our analysis, 60,000 ± 2,400YBP according to Watson et al. [29], and 66,321 ± 24,965 YBP according to Graven et al. [23]. Thus, all of the most recent studies show consistent trends in the diversity and ages of the major clusters of mtDNAsin African populations.The other interesting question raised both by our analyses[33]and by that of Watson et al. [29] is the origin and dispersal of haplogroupL3 mtDNAs. These haplotypes are widely dispersedin eastern Africa but are less prevalent in western and southern Africa also Watson et al. [29 33]. Moreover, there are several differentsubhaplogroups within L3 andthese appear to be differentially distributed in these populations. On the basis of the continental distributionand age that they reported, Watson et al. [29] proposed that a subset of L2 and L3 mtDNAsmoved out of eastern Africa to found the Eurasian populations»60,000 YBP.Since the subhaplogroups of L3 are the most likely precursors of modern European and Asian mtDNA haplotypes[7 29], theirsequence variation and age are of considerable importancein the determination of the timing and processby which these mtDNAs were dispersed out of Africa.In this regard, subhaplogroups L3a and L3b appear to be the oldest of the L3 subhaplogroups, dating to41,400–54,500 YBP and 77,600–102,300 YBP, respectively.These estimates are somewhat similar to the60,000 5 3,200 YBP and 44,000 5 3,000 YBP coalescence times that Watson et al. [29] calculated forthe analogous subhaplogroups. In contrast, subhaplogroupsL3c and L3d have somewhat younger divergence times—28,300–37,300 YBP and 17,600–23,200 YBP, respectively suggesting that they emerged afterthe evolution of L3a and L3b.

CONCLUSION

In conclusion the Asholio and Atakar are genetically related and it is possible that mtDNA subhaplogroups L3a and L3d arose in sub-Saharan Africa and then moved upward into eastern Africaand out of eastern Africa into the Middle East, to yieldAsian macrohaplogroup M and European haplogroupU. Such a hypothesis is supported by recent studies ofeastern-African populations, which have revealed an unusuallyhigh percentage of L3 mtDNAs [29 44]. Therefore, it ispossible that subhaplogroups L3a and L3d radiated out of west Africa, to give rise to European and Asian mtDNAs. If so, then west Africa may still harbor theprogenitor haplotypes from which European and AsianmtDNAs were derived.

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