Genomic evidence for population specific selection in Nilo-Saharan and Niger-Congo linguistic groups in Africa

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1.0 Abstract

Background: There are over 2000 genetically diverse ethno-linguistic groups in Africa that could help decipher human evolutionary history and the genetic basis of phenotypic variation. We have analysed 298 genomes from Niger-Congo populations from six sub-Saharan African countries (Uganda, Democratic Republic of Congo, Cameroon, Zambia, Ivory Coast, Guinea) and a Nilo-Saharan population from

Uganda. These samples were collected as part of the TrypanoGEN consortium project http://www.trypanogen.net.

Results: The population genetic structure of the 298 individuals revealed four clusters which correlated with ethno-linguistic group and geographical latitude, that is, West African Niger-Congo A, Central African Niger Congo, East African Niger-Congo B and the Nilo-Saharan.

We observed a spatial distribution of positive natural selection signatures in genes associated with AIDS, Tuberculosis, Malaria and Human African Trypanosomiasis among the TrypanoGEN samples. Having observed a marked difference between the Nilo-Saharan Lugbara and Niger-Congo populations, we identified four genes [APOBEC3G, TOP2B, CAPN9, LANCL2, (_iHS –log p > 3.0, Rsb –log p > 3.0, Fst > 0.1 bonferroni p > 1.8x10e4)], which are highly differentiated between the two ethnic groups and under positive selection in the Lugbara population.

Conclusion: The signatures that differentiate ethnically distinct populations provide information on the specific ecological adaptations with respect to disease history and susceptibility/ resistance; as demonstrated in this study where *APOBEG3G* is believed to be involved in the susceptibility of the Nilo-Saharan Lugbara population to Hepatitis B virus infection.

2.0 Background

The African continent's ethno-lingual groups have been classified into four major families, Afro-Asiatic, Nilo-Saharan, Niger-Congo, and Khoisan (Blench 2006). The Afro-Asiatic which includes the Semitic, Cushitic, and ancient Egyptian languages, is spoken predominantly by northern and eastern African pastoralists and agro-pastoralists; the Nilo-Saharan, which includes the Central Sudanic and Eastern

53 Sudanic (Nilotic) languages, is spoken predominantly by eastern and central Saharan
54 pastoralists; the Niger-Congo languages are subdivided into the Niger-Congo A in
55 West Africa and the Niger-Congo B or Bantu in Central, Southern and Eastern Africa
56 (Greenberg 1963; Lewis et al. 2009). Fourteen ancestral population clusters have been
57 identified amongst these groups that correlate with shared cultural and linguistic
58 affiliations (Tishkoff et al. 2009). These 14 ancestral populations break down further
59 into over 2000 ethnically diverse linguistic groups (Bryc et al. 2010; Tishkoff and
60 Williams 2002).
61 The diversity of ethno-linguistic groups can be used to study human evolutionary
62 history and the genetic basis of phenotypic variation (Tishkoff et al. 2009),
63 complementing studies of African genotype variations
64 (Tishkoff et al. 2009; Gurdasani et al. 2015; Busby et al. 2016; Patin et al. 2017)
65 which have contributed to the understanding of human origins and disease
66 susceptibility markers.
67 However, samples from sufficient individuals for population analysis have been
68 sequenced from relatively few African populations. The 1000 genome project
69 generated data from five Niger-Congo populations, The African Variome project
70 added Afro-Asiatic populations and there have been small scale studies of the
71 Khoisan hunter-gatherers (Kim et al. 2014; Mallick et al. 2016; Tishkoff et al. 2009;
72 Gurdasani et al. 2015). However no sequences of Nilotic populations have been
73 published to date although one previous study used 200,000 SNP loci to examine
74 genetic diversity of the Nilo-Saharan speaking population of southern Sudan
75 Darfurian and Nuba people (Dobon et al. 2015). In the present study we present the
76 first genome sequences of a Nilo-Saharan population and genome sequences from six
77 new Niger-Congo populations.

3.0 Results

3.1 Samples and sequencing

The samples used for this study were collected by the TrypanoGEN consortium and consisted of 298 individuals from 19 linguistic groups residents of Guinea, Ivory Coast, Cameroon, Democratic Republic of Congo, Uganda and Zambia (Table1). The DNA from the study participant's blood samples was extracted and genomes were sequenced on the Illumina 2500 at 10X coverge, except for the Zambia and Cameroon samples that were sequenced at 30X coverage.

Following mapping and SNP calling, we identified approximately 34.1 million single nucleotide polymorphisms (SNPs) and 5.3 million insertion/deletion polymorphisms (Table 2). We identified 2.02 million variants that did not have rsIDs and we hence consider them 'novel'. The SNPs had a transition-transversion ratio of 2.0 (Supplementary figure S1), implying good quality SNP calls (DePristo et al. 2011). Prior to population analysis, variants (SNPs and Indels) were filtered by removing loci with >10% missing data, MAF < 0.05 or Hardy Weinberg Equilibrium (HWE) P-value < 0.01. 13 individuals with > 10% SNP loci missing were removed from the data (Table 2). To our 298 samples, 504 additional samples from five African populations from the 1000 genomes project (Esan and Yoruba from Nigeria, Mende from Sierra Leone, Mandinka from Gambia and Luhya from Kenya), were included in some of our analyses.

3.2 Population stratification by Multiple Dimensional Scaling

Multiple Dimensional Scaling (MDS) implemented in Plink 1.9 was used to help visualise genetic distances between samples (Figure 1). All TrypanoGEN samples

clustered by country except those in Uganda, where the Nilo-Saharan Lugbara samples formed a distinct cluster from the Basoga samples. When the samples from the six TrypanoGEN and the four African 1000 genomes project countries were merged, five groups representing five major geographic groups were observed (Figure 1B): the Uganda Nilo-Saharan; East African Bantu speakers from Uganda and Kenya; Central African Bantu speakers from Cameroon, DRC and Zambia; Nigerian Niger-Congo A speakers (Esan and Yoruba);. West African Niger-Congo A speakers from the Ivory Coast, Gambia, Sierra Leone and Guinea. The African and European samples were very distinct (Figure 1C). Since all samples except Ugandan Bantu and Nilo-Saharan clustered by country by MDS, samples were grouped by country for subsequent analyses, except for the Uganda samples which were grouped by both country and linguistic group.

3.3 Population Admixture and differentiation

The amount of shared genetic ancestry within the samples was estimated using Admixture (Alexander et al. 2009). Admixture was run on 2-8 population clusters (K) in triplicate; with K=4, K=5 and K=6 having the lowest cross validation errors and hence the most probable numbers of ancestral components represented in the data (Supplementary Figure S2). At K=6 The Niger-Congo populations exhibited 17-60% admixture with minor ancestries, whilst the Ugandan Nilo-Saharan population had 7% admixture with Niger Congo ancestries (Figure 2A).

At K4 one European and three ancestral African populations were observed which corresponded to Nilo-Saharan, Niger-Congo-B (East African) and Niger-Congo-A (West African). At K5 a homogeneous group of seven samples emerged within the Zambia population with no admixture with other populations in our data set and were

also outliers on the MDS plot (Figure 1B). These seven were recorded as Soli/Chikunda speakers, which are Bantu languages but they had no admixture at (K=5 and K=6) with the other speakers of this language group from Zambia or any other group included in this study, suggesting that they were from a quite distinct population. At K6, a major group appeared that contributed ancestry to both East African Niger-Congo B and West African Niger-Congo A but does not correspond to any existing linguistic group.

The genetic variation within the populations that are part of the TrypanoGEN project was estimated using the pairwise F_{ST} (Wright 1949) (Figure 2B, supplementary fig S3). F_{ST} was relatively high between the Nilo-Shaharan Lugbara samples and the African Bantu populations (Figure 2B) except the East African Basoga (population mean $F_{ST} = 0.012$) and Luhya (population mean $F_{ST} = 0.011$), presumably due to the 30% admixture of Nilo-Saharan origin within these populations. The pattern of the observed genetic variation was consistent with the relative geographic distance from the Nilo-Saharan population (Figure 2C). In addition, a phylogenetic tree based on the genetic distances between populations (F_{ST}) showed clustering of populations by geographic region on the African continent (Figure 2D).

3.4 Population size over time and timing of population isolation.

The multiple sequentially Markovian coalescent (MSMC) was used to estimate population sizes over time and times at which populations became isolated (Figure 3). Effective population sizes (N_e) were relatively stable at around 13,000 in all populations tested from 100 thousand years ago (kya) until about 50kya when they started to decline reaching a nadir of about 8,000 about 13kya coinciding with the dry period at the end of the last ice age (Figure 3A, Supplementary table S6). All

153 population sizes increased rapidly thereafter but the Niger-Congo populations
154 increased to an N_e of around 200,000, whilst the Nilotic population only increased to
155 60,000. The Ugandan Bantu population was intermediate in N_e presumably due to
156 admixture with the Nilotics. This post glacial population increase was briefly reversed
157 in the Central and West African populations which suffered declines of 6-23%
158 between 1500 and 750 years ago before recovering to even higher levels at the present
159 time. This decline in N_e was not observed in the Ugandan Bantu population, although
160 the growth rate declined, and in the Nilotic population a decline was observed at a
161 later time point after 750 years ago.

162 Population separation data is less clear and may be more sensitive to admixture
163 (Figure 3B). The Guinea and Ivory Coast populations were the least admixed and
164 appeared panmictic until about 10kya, and had become isolated by about 3kya. The
165 Ugandan Bantu and Ugandan Nilotic appeared to begin separating from other
166 populations about 23 and 47kya, respectively and became isolated about 3kya but
167 these estimations may be confounded by admixture.
168
169 **3.5 Genome-wide screen for extended haplotypes under selection**
170 ***Signatures within population***
171 In order to identify alleles under selection pressure, we used the within population
172 Extended Haplotype Homozygosity (EHH) test (Sabeti et al. 2002). Similar patterns
173 of loci with extreme positive and negative iHS scores were observed across all groups
174 (Supplementary Figure S4A). The iHS values for all groups had an approximate
175 normal distribution (Supplementary Figure S5) implying that the sizes of iHS signals
176 from different SNPs in all the populations were comparable (Voight et al. 2006). The
177 mean number of loci with extreme positive and negative iHS score ($-\log p > 3$) from

all groups was 8,984, Guinea had the largest number of loci with extreme iHS score (11,401) and Zambia had the least (5,570) (Table 3, Supplementary Table S1). These extreme loci were classified by the Ensembl annotation of the nearest gene. Approximately 34% of these annotations were for protein coding genes; a mean of 3,058 SNPs in protein coding genes per population were associated with extreme iHS scores. Some protein coding genes with extreme iHS SNP loci were shared between different Countries whereas some occurred only in a single Country population (Supplementary Table S1, sheet 'ALLpop.protein_coding'). We observed strong iHS signatures in genes that have been previously identified in other African populations as being under strong selection (Voight et al. 2006; Gurdasani et al. 2015; Sabeti et al. 2007). These included ***SYT1,*** a synaptosomal protein implicated in Alzheimer's disease (Yoo et al. 2001) was found in all Country populations, ***LARGE*** a glycosylase involved in Lasser fever virus binding (Andersen et al. 2012) (Zambia, Cameroon, Ivory Coast), ***CDK5RAP2,*** a microcephaly gene controlling brain size (Bond et al. 2005) (Ugandan Bantu), ***NCOA1*** a transcriptional co-activator associated with Lymphoma (Guinea, Ivory Coast, DRC), ***SIGLEC12*** involved in immune responses (Crocker et al. 2007) (Zambia, Cameroon). Using the DAVID annotation (Huang et al. 2008) we observed that all of the Country populations had strong signals that have been implicated in communicable diseases such as HIV/AIDS, Malaria and Tuberculosis that have the highest burden on the African continent (Bhutta et al. 2014) (Table 4), suggesting an adaptive role of these genes to infection.

Having collected samples from Human African Trypanosomiasis endemic regions, we identified signatures that have been implicated in Trypanosome infection. These signatures were observed in genes overlapping the KEGG calcium signalling pathway (http://www.kegg.jp/)(Kanehisa et al. 2016); ***F2RL1*** (Guinea, Ivory Coast), ***GNA14***

(Zambia), ***GNAQ*** (Cameroon), ***GNAL*** (Guinea, Cameroon), ***GNAS*** (Zambia), identified mainly from mice studies (Grab 2009). The calcium signalling pathway regulates permeability of the blood brain barrier to trypanosome parasites during CNS disease (Nikolskaia et al. 2006). In addition, we observed signatures in genes overlapping the Mitogen-activated protein kinase MAPK pathway ***MAPK1*** (Cameroon), ***MAPK10*** (Ugandan Nilo-Saharan, DRC, Ugandan Bantu), ***MAPK9*** (Zambia); which is targeted by trypanosomatids in order to modulate the host's immune response (Soares-Silva et al. 2016). These host signalling pathways have been shown to play a role in host immunity against trypanosome infection in mice and cattle (Noyes et al. 2011).

Signatures unique to Nilo-Saharans

In order to determine which signatures are unique to the Nilo-Saharan Lugbara, we first ascertained which extreme iHS loci (-log p > 3) were common to the Nilo-Saharan and one or more Niger-Congo groups. We observed that approximately 15% of the protein coding gene associated extreme iHS SNPs of the Ugandan Bantu, DRC, Ivory Coast and Guinea groups were common with the Nilo-Saharan group, whereas Cameroon and Zambian groups had 2.7% in common (Table 3, supplementary figure S4B). 149 extreme SNPs associated with protein coding genes were unique to the Uganda Nilo-Saharan (Supplementary table S2). Using the PANTHER Gene ontology database (Thomas et al. 2003), we observed that these unique genes were mainly enriched for cellular and metabolic process proteins (approximately 50.8%) (Supplementary figure S6). Amongst these were SNPs associated with genes that have also been shown by other studies to be under positive selection including, ***APOBEC3G,*** which is involved in innate anti-viral immunity (Sawyer et al. 2004;

Zhang and Webb 2004), has protective alleles against HIV-1 in Biaka and Mbuti pygmies of Central African Republic and DRC respectively (Zhao et al. 2012); ***IFIH1*** (also called ***MDA5***) is a cytoplasmic RNA receptor that mediates antiviral responses by activating type I interferon signalling (Rice et al. 2014) but is also implicated in protection against type 1 diabetes ((Nejentsev et al. 2009; Fumagalli et al. 2010); ***OR2L13*** olfactory receptor involved in activation of signal transduction pathway for odorant recognition and discrimination (Sharon et al. 1999), and is associated with Diabetic nephropathy in African Americans (Bailey et al. 2014).

Nilo-Saharan versus Niger-Congo cross population signatures

There were 299 SNP with high F_{ST} (above 99th percentile) and XPEHH (Rsb –log p > 3) in the regions of protein coding genes that were also highly differentiated between the Nilo-Saharan and Niger-Congo populations (Supplementary table S3). We then compared SNP loci with derived alleles that are unique to the Nilo-Saharans and occur in highly differentiated genes (extreme Rsb, high Fst) between the Nilo-Saharan and Niger-Congo groups. From this we identified 12 genes (Table 5, Supplementary figure S8B) including the ***APOBEC3G*** gene that are highly differentiated between the Nilo-Saharan and Niger-Congo groups (mean F_{ST} 0.11, Rsb –log p 4.1). *APOBEC3G* also contains the SNP rs112077004, which was observed to be under positive selection in the Nilo-Saharans (Figure 4, Supplementary figure S9).

4. Discussion

We have analysed the genomes of 298 individuals from seven major groups of samples from six Sub-Saharan Africa Countries, investigating their admixture profile,

253 demographic histories and signatures of selection that differentiate the major
254 linguistic groups. The MDS analysis identified five major clusters: Nilo-Saharan, two
255 Niger-Congo A groups from Nigeria and West Africa and two groups of Niger-Congo
256 B (Bantu) speakers from Central and East Africa, which were consistent with
257 previous studies (Tishkoff et al. 2009; Gomez et al. 2014; Gurdasani et al. 2015). The
258 samples represented three of the five major linguistic groups in Africa. Afro-Asiatic
259 speakers are found across North and North-East Africa in regions adjacent to Nilo-
260 Sharan and Bantu speakers. Afro-Asiatic reference populations were not included in
261 this study and we are therefore not able to detect any admixture from this source.
262 However a SNP genotype based analysis of Nilotic populations indicated that Nilotic
263 populations only contain a trace of Afro-Asiatic ancestry and therefore our
264 observations on East African populations may not be significantly limited by the
265 absence of Afro-Asiatic data (Dobon et al. 2015).
266 **Admixture:** Niger-Congo speaking hunter-gathers are believed to have originated
267 from the Kordofanian speakers of the Nuba mountains of Sudan and then traversed
268 the Sahel to Mali (Figure 5). They then colonised the coast from Senegal to Nigeria
269 and Cameroon, over several thousand years forming multiple linguistic groups. The
270 Bantu (Niger-Congo-B) speaking people emerged as another linguistic group amongst
271 the greater than 60 Niger-Congo-A groups in the Nigeria/Cameroon region about
272 3,000 years ago. Bantu speaking peoples then spread South and East along savannah
273 corridors through the Congo basin and emerged in the Great Lakes region and spread
274 North to the Lake Victoria region and South down the East Side of Africa
275 (Grollemund et al. 2015; Patin et al. 2017). This rapid expansion is believed to have
276 been enabled by the development of agriculture and later enhanced by the acquisition
277 of iron tools (Tishkoff et al. 2009).

The admixture analysis at K=4 is consistent with this linguistic history and recent genetic analyses (Patin et al. 2017; Gurdasani et al. 2015) with three African Ancestral allele clusters (AAC) which can be interpreted as representing Niger-Congo A languages in West Africa, Niger-Congo B (Bantu) in Central and East Africa and Nilo-Saharan in Northern Uganda. The Niger-Congo-A speakers in extreme West Africa appear to have approximately 10% Nilo-Saharan ancestry, consistent with an ancestral relationship with Nilo-Saharans and this declines towards the East. The Bantu speakers are a mix of Niger-Congo-A and a distinct putative Bantu ancestral cluster that it at highest frequency in Nigeria and Cameroon, the Niger-Congo-A component is displaced by a Nilotic component with easterly latitude whilst the "Bantu" component remains constant. At K=5 a small AAC of 7 Bantu speakers from Zambia emerges, who evidently have a genetic heritage that does not match their self-declared linguistic affiliation, and may be of Khoisan descent. At K=6 a fourth major African AAC appears (green in Figure 2) with strongest representation in the Nigerian Yoruba and Esan then tapering off east and west into Central and West Africa. This does not correspond to any linguistic group and displaces the Niger-Congo-A ancestry to the east of Nigeria and Niger-Congo-B (Bantu) in Nigeria and to the West. This ancestral cluster could represent a secondary movement out of Nigeria of migrants who adopted their hosts language. One possible driver for such a migration, if it occurred, was the development of iron smelting which may have originated in Nigeria about 2,500 years ago (Vansina 2006). Irrespective of the true number of ancestral allele clusters there is evidence of back migration of people with Bantu ancestral alleles into West Africa as has been observed before (Gomez et al. 2014). This migration to the west was not accompanied by language expansion as it was to the east.

Population History: The estimates of current N_e obtained from our data with MCMS (Fig 3A) of around 200,000 in West and Central Africa and 57,000-125,000 in East Africa (Supplementary Table S6) was consistent with previous observations on other African samples using the same method (Schiffels and Durbin 2014) but ten times higher than the estimates of around 20,000 obtained from SNP chip genotype data (Shriner et al. 2014). The faster growth in the Niger-Congo A and B than the Nilotic populations appears to predate the Bantu expansion. The Niger-Congo A population was believed to be expanding through West Africa as the climate became wetter after 10kya, consistent with the separation times between the Guinea and Ivory Coast populations observed on the Cross-Coalescence Plot (Figure 3B). The Nilotics population developed a pastoralist economy probably after 6kya but their expansion into the tsetse belt may have been delayed by trypanosomiasis and other diseases until the cattle developed tolerance (Gifford-Gonzalez 2000) (Smetko et al. 2015) (Chritz et al. 2015) and the effective population size did not grow so fast as that of the Niger-Congo-A populations. The brief population decline dated at ~1340CE by MSMC coincides with the timing of the Black Death (1343-1353), however time resolution is low and the decrease was only observed at a single time point. There is evidence of abandonment of multiple large settlements throughout West Africa around the time of the Black Death and there is speculation that this was caused by the disease (Chouin 2015). The decrease at this time appears to have impacted the West and Central African Niger-Congo but not the East African populations. Both Bantu and Nilotic populations in East Africa were cattle keepers and pastoralists to varying degrees (Chritz et al. 2015) and the concomitant lower population density and mobile lifestyle may have made them less vulnerable than the more settled and urbanised West Africans to plague infection. The more recent decline in the Nilotic Lugbara effective

328 population size is unexplained, but the catastrophic Rinderpest outbreak in the 1880's
329 and 1890's that killed up 90% of indigenous cattle, which lead to the depopulation of
330 the East African savannahs and may have ended the dominance of the Nilotic
331 speaking Maasai over the Bantu Kikuyu could have been a contributory factor (Mack
332 1970).
333 The Cross-Coalescence plots for comparison between populations other than the
334 Guinea and Ivory Coast Niger-Congo-A show long periods of separation. This is not
335 consistent with the Ugandan Bantu populations having separated from Niger-Congo-
336 A populations even more recently than the separation between Guinea and Ivory
337 Coast populations, and is presumably due to the extensive admixture with the Nilotics
338 observed in this population. The Central African cross-coalescence data also indicated
339 older separation times than linguistic evidence suggests (not shown) and although
340 there was less evidence of admixture in this population these data should be treated
341 with caution.
342 **Selective Sweeps:** We identified selective sweeps in genes that have been associated
343 with HIV/AIDS, Tuberculosis and Malaria. Given the high prevalence of these
344 infections on the continent (Bhutta et al. 2014), there is increased frequency of these
345 beneficial heritable traits hence positive natural selection. However not all these genes
346 occurred in all the populations demonstrating spatially varying selection probably due
347 to differing environmental pressures (Gillespie 1994; Thorne et al. 1998).
348 We identified signatures in genes that are involved in pathways implicated in
349 trypanosome infection: calcium signalling, (Grab et al. 2009; Nikolskaia et al. 2006),
350 the MAPK pathway (Noyes et al. 2011), *HPR*, *APOL1, IL6 and HLAG,* (Hardwick et
351 al. 2013; Genovese et al. 2010; Cooper et al. 2017; Courtin et al. 2013; 2007)
352 (Supplementary figure S10, Supplementary table S5). We only found evidence for

353 selection for the calcium signalling and MAPK pathway genes. This suggests that
354 HAT may have had a selective force in these populations.,
355 In order to determine signatures of selection unique to the Nilo-Saharan population,
356 we used a combination of linkage disequilibrium-based method (iHS and Rsb) and
357 population differentiation based method (F_{ST}) (2013a). Using this approach we
358 identified 12 loci associated with coding genes, which are unique to the Nilo-Saharan
359 Lugbara population and highly differentiated from the Niger-Congo population.
360 Among these was the variant associated with *APOBEC3G* that demonstrated
361 significant positive selection in the Lugbara Nilo-Saharan population. This protein is
362 involved in viral innate immunity (2003a), by inducing a high rate of dC to dU
363 mutations in the nascent reverse transcripts leading to the degradation of the viral
364 genome (2004c; 2004a). The Lugbara have relatively low prevalence of HIV (4%) in
365 comparison to the Basoga (6.4%) and Baganda (10.7%) Bantu groups of Uganda but
366 relatively high prevalence of Hepattis B suggesting that either *APOBEC3G* does not
367 control both these viruses or it has different effects on each (2011b)(2003b; 2003c)
368 (2009a; 2013b). (2017).
369 We also identified the missense variant rs10930046 (T/C) located in the *IFIH1* CDS,
370 which was unique to the Nilo-Saharan Lugbara and highly selected (iHS –log p-value
371 3.264). This gene is associated with up regulation of type I interferon signalling
372 occurring in a spectrum of human diseases (2014a) and is believed to be involved in
373 the suppression of Hepatitis B viral replication (2013d). Being a nonsynonymous
374 variant, rs10930046 could alter the functioning of IFIH1 and thus increase
375 susceptibility to HBV in the Lugbara population, something that could be tested by a
376 candidate gene study for DNA virus infections. Northern Uganda is considered to
377 have one of the highest prevalence of Hepatitis B virus in the world (2015a) which

has perhaps resulted in a unique adaption of the Lugbara Nilo-Saharan population to infection.

5. Conclusion

We have incorporated a Nilo-Saharan population into a analysis of genomic sequences of Niger-Congo populations for the first time and show extensive admixture between Nilo-Saharan ancestry and Niger-Congo B (Bantu) populations. We show evidence for signatures of selection the Nilo-Saharan population in genes associated with communicable diseases that have different prevalences from surrounding Bantu (Niger-Congo B) populations.

6. Materials and Methods

Ethical approval and sample collection

The samples used for this study are part of the TrypanoGEN biobank (Ilboudo et al. 2017), which describes ethics approval, recruitment, sample processing and the meta data collected. The ethical approval for the study was provided by the national ethics councils of the TrypanoGEN consortium countries involved in the sample collection which are: Uganda (HS 1344), Zambia (011-09-13), Democratic Republic of Congo (No 1/2013), Cameroon (2013/364/L/CNERSH/SP), Cote d'Ivoˆıre (2014/No 38/MSLS/CNER-dkn), and Guinea (1-22/04/2013). All the participants in the study were guided through the consent forms, and written consent was obtained to collect biological specimens. Peripheral blood was collected from the participants at the field sites, transported to reference laboratories from where DNA extraction was carried out using the Whole blood MidiKit (Qiagen). The DNA was quantified using the Qubit (Qiagen) and approximately 1µg was shipped from each country to the

University of Liverpool, UK except for Cameroon and Zambia from where DNA was shipped to Baylor College, USA.

6.1 Sequencing and SNP calling

The whole genome sequencing libraries were prepared using the Illumina Truseq PCR-free kit and sequencing done using the Illumina Hiseq2500. The samples from Guinea, Cote D'Ivoire, Uganda and DRC were sequenced to 10x coverage at the Center for Genomic Research at the University of Liverpool. The samples from Zambia and Cameroon were sequenced to 30X at the Baylor College of Medicine Sequencing Facility.

The sequenced reads were mapped onto the 1000 genomes project human_g1k_v37_decoy reference genome using BWA. The SNP calling on all the samples was done using the genome analysis tool kit GATK v3.4. The SNPs were then filtered by; a) removing loci with > 10% missing SNP, b) removing individuals with > 10% missing SNP loci and c) removing loci with Hardy Weinberg P value < 0.01. In addition, loci with MAF < 0.05 were also removed for the PCA and Admixture analysis. The variant annotation was done using snpEff (www.snpeff.sourceforge.net).

6.2 PCA analysis

The principal component analyses (PCA) were performed using Plink 1.9 and R v 3.2.1. Data were filtered using the following criteria: a) removing loci with > 10% missing SNP, b) removing individuals with > 10% missing SNP loci and c) removing loci with Hardy Weinberg P value < 0.01, removing loci with minor allele frequencies (MAF) < 0.05. SNP loci less than 2000bp apart were removed in order to reduce the

linkage disequilibrium (LD) between adjacent SNP. PCA was carried out for (i) all TrypanoGEN data , (ii) all TrypanoGEN data plus African 1000 genome data, (iii) all TrypanoGEN data including 50 European and all African 1000 genome data excluding African Caribbean in Barbados (ACB) and African Southwest USA (ASW) populations.

6.3 Population Admixture

The population ancestry of each individual was obtained using Admixture 1.23 (Alexander et al., 2009) on the filtered PLINK .bed files on the same TrypanoGEN, one thousand genome African and European population data sets analysed by PCA. Admixture was run on K1 to K8 for which three replicates were done for each run. The Admixture plots were drawn using the R tool 'strplot' (Ramasamy et al. 2014).

6.4 Genetic diversity: Fst

The genetic diversity due to difference in allele frequency among populations was analysed by the inter-population Wright's Fst (Wright, 1951) in PLINKv1.9. The Fst estimates were made between TrypanoGEN (UGN, UGB, DRC, CIV, GUI) and one thousand genome African (LWK, YRI, ESN, MSL, GWD) populations. The F_{ST} dendrogram was generated using Fitch in Phylip3.685 (1993). The geographic distance matrix between populations was calculated based on their global position system (GPS) coordinates (2011a).

6.5 Population History.

Population sizes and divergence times were calculated using MSMC (Schiffels and Durbin 2014). Since PCA and Admixture analysis had indicated little difference

between linguistic groups in each country with the exception of the Ugandan Bantu and Nilotic populations, samples from each country with highest coverage were analysed together except for Uganda where Bantu and Nilotic samples were analysed as separate populations. For population size estimates output from 3 independent runs each using 8 different haplotypes were combined. Using 8 haplotypes rather than 4 gives higher resolution at more recent time points. For estimates of relative cross coalescence rate, three replicate runs were done, each using 2 different samples (4 haplotypes) from each pairwise comparison between populations. Results presented are the means of the replicates.

6.6 Signatures of selection

The estimation of haplotypes was carried out by Phasing of the genotyped SNPs using SHAPEIT v2.2 software (Delaneau et al., 2013). The extended haplotype homozygosity (EHH) was then analysed using the R software package *rehh* (Gautier et al., 2012). Two main EHH derived statistics were calculated from the phased haplotype data, that is, intra-population integrated haplotype Score (iHS) (Voight et al., 2006) and inter-population Rsb (Tang et al, 2007). Bedtools v2.26.0 was used to identify the intersection of the Fst and Rsb loci.

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The authors declare no competing interests.

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581

582 **Tables**

583 **Table 1.** Table showing the Ethnic groups and number of individual from each
584 Country that were used for Whole genome sequencing
585

Country	District(s)	Ethno-linguistic group(s)	No. of samples	Branch	Family
Uganda (UGN)	Maracha	Lugbara	50	Central Sudanic	Nilo-Saharan
Uganda (UGB)	Iganga	Basoga	33	Bantu	Niger-Congo B
Zambia (ZAM)	Chama, Rufunsa	Soli/Chikunda (28), Tumbuka (14), Bemba(8)	41	Bantu	

Democratic Republic of Congo (DRC)	Bandundu	Ngongo, Songo, Yansi, Mbala	50	Bantu	
Cameroon (CAM)	Campo, Fontem, Bipindi	Bamilike(6), Mundani(8)	26	Bantoid	
		Ngoumba(12)		Bantu	
Ivory Coast (CIV)	Bonon, Sinfra	Baoule (11),	50	Kwa	Niger Congo A
		More (12), Senoufo (4),		Gur	
		Gouro (21), Malinke (1), Koyaka (1)		Mande	
Guinea (GUI)	Forecariah, Boffa, Dubreka	Soussou	48	Mande	

Table 2. The number of SNPs and Indels obtained from the mapping and variant calling pipeline. The SNPs were filtered for HWE, MAF and missing genotypes

* *Identified 2,023,049 SNPs without rsIDs {Total SNPs (30,591,165) – SNPs with rsIDs (28,568,116)}*

Table 3. Extreme iHS loci that overlap with the UGN population

Population	Number	SNPs before filtering	SNPs after filtering	Indels before filtering	Indels after filtering
CIV	50	18,780,913	16,066,827	3,069,408	1,583,594
DRC	50	19,188,537	16,449,696	3,146,802	1,626,826
GUI	48	18,831,834	16,075,002	3,063,080	1,579,352
UGB	33	17,671,306	14,987,699	2,889,915	1,426,646
UGN	50	18,986,243	15,598,629	3,130,979	1,536,490
CAM	26	17,183,994	14,579,603	3,283,543	1,539,459
ZAM	41	18,232,386	15,548,110	3,448,501	1,651,467
Total	298	**34,116,333**	**30,591,165**	**5,336,622**	**3,166,196**

CIV=Côte d'Ivoire, DRC= democratic republic of Congo, GUI= Guinea, UGB= Uganda Bantu, UGN= Uganda Nilotic, CAM = Cameroun, ZAM= Zambia,

Pop	Extreme iHS SNPs (–log p > 3.0)	Extreme iHS SNPS associated with protein coding genes	Extreme iHS SNPs overlapping with UGN
UGN	8454	2613	2613
UGB	9617	3326	512
DRC	10037	3790	535
ZAM	5570	1990	86
CIV	10129	3541	534
CAM	7686	2597	82
GUI	11401	3741	382

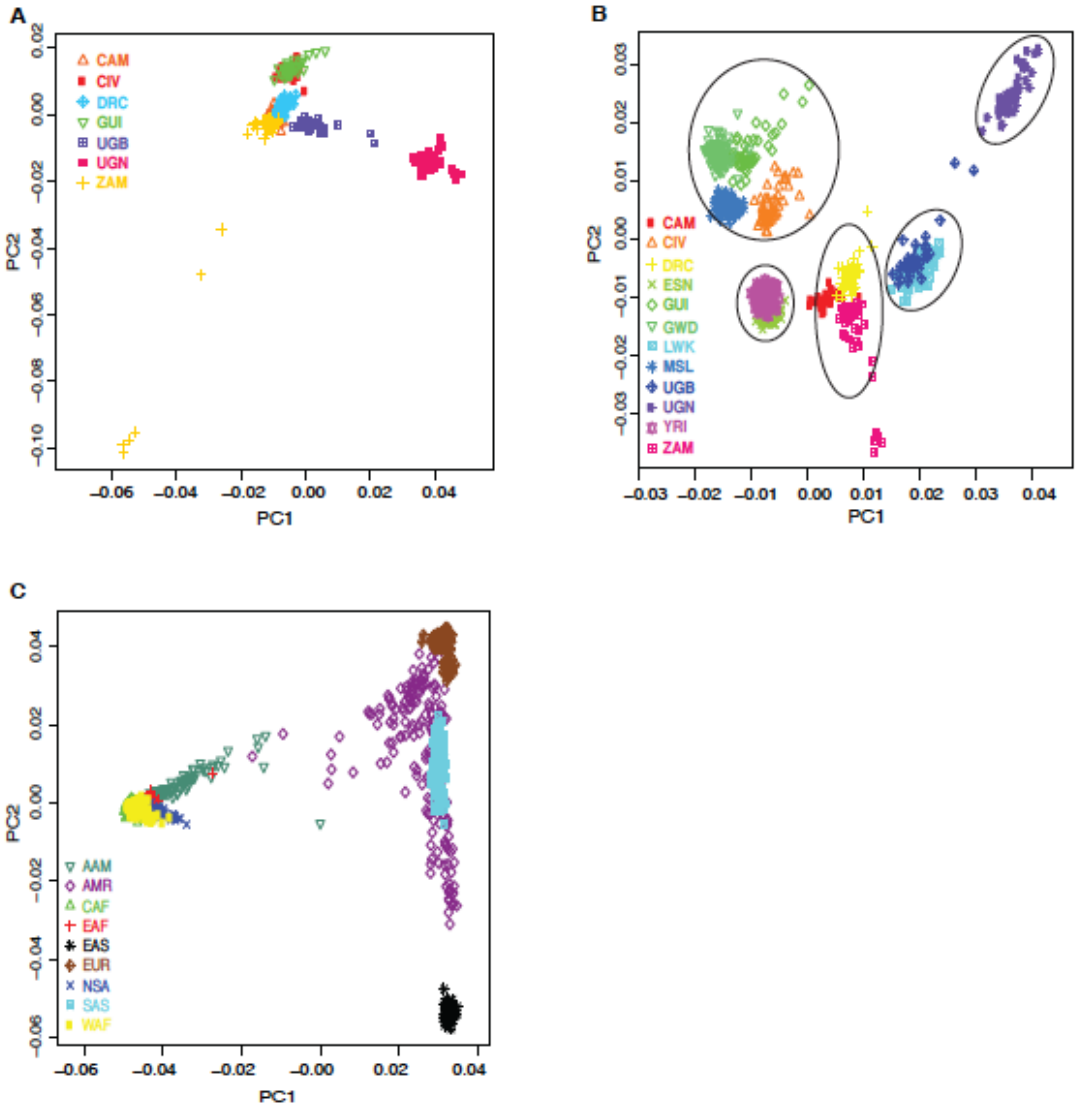
598 **Table 4**. DAVID (Huang et al., 2009) analysis of Genes that are highly selected within TrypanoGEN population and associated with HIV,
599 Tuberculosis, and Malaria. The Fisher's exact test *P*-values indicate significant gene enrichment in the associated disease (1991; 2010; 2001;
600 2015b; 2014b; 2004b; 2009b; 2016c; 2015c; 2005a; 2013c)
601

Gene	Chr	Populations affected	Associated Disease	P value	Reference
HLA-DRB1	6p21.32	ZAM,CAM,CIV,DRC,UGB	HIV/TB/Malaria	1.63E-09	Ranasinghe et al., 2013, Hill et al., 1991
NLRP1	17p13.2	ZAM,CIV,DRC,GUI,UGB	HIV	2.42E-07	Pontillo et al., 2010
VPRBP	3p10.6	UGB,CIV,DRC,GUI	HIV	2.42E-07	Zhang et al, 2001
TRIM5	11p15.4	UGN,CAM,GUI	HIV	7.30E-07	Deng et al., 2015
ANKRD30A	10p11.21	DRC,CIV	HIV	2.42E-07	Meyerson et al., 2014
HLA-A	6p22.1	ZAM,CAM	HIV/TB	4.70E-06	Louie et al., 2004
HLA-DQA1	6p21.32	UGB,DRC	HIV/TB	4.70E-06	Louie et al., 2004
HLA-DQB1	6p21.32	UGB,DRC	HIV/TB	4.70E-06	Louie et al., 2004
KIR3DL1	19q13.42	UGN,CIV	Malaria	1.63E-09	Taniguchi et al., 2009, Norman et al., 2013
CD36	7q21.11	UGN,CAM,CIV	Malaria	1.55E-06	Hsieh et al., 2016
DDC	7p12.2	UGB,DRC,GUI	Malaria	1.63E-09	Manjurano et al., 2015
HBE1	11p15.4	UGB,CAM,DRC	Malaria	5.48E-07	Patrinos et al., 2005
ADCY9	16p13.3	UGN,CIV	Malaria	5.48E-07	Maiga et al., 2013

602 **Table 5**. Genes that are highly differentiated between the Nilo-Saharan and Trypanogen Niger congo populations that contain SNPs
603 unique to UGN population
604

Chr	Gene	Position of Unique SNP	UGN unique SNP	iHS pvalue	Differentiated loci	Mean Fst	Bonferroni pvalue	Rsb pvalue
22	APOBEC3G	22:39453783	rs112077004	4.002	rs5757467	0.110	8.17E-23	4.116
3	TOP2B	3:25670166	rs11712723	3.000	rs6786520	0.115	4.80E-11	4.151
1	CAPN9	1:230886378	rs113802713	3.362	rs16852681	0.105	1.89E-08	5.632
7	LANCL2	7:55476708	rs62457872	3.019	rs3807360	0.109	1.89E-08	3.269
3	NEK4	3:52762698	rs11130321	3.101	rs6445535	0.111	1.51E-04	6.871
20	GDAP1L1	20:42907542	rs1884607	3.187	rs4810417	0.114	0.003070493	3.931
2	NBAS	2:15527280	rs6723183	3.763	rs4668447	0.123	0.053555484	3.020
3	PBRM1	3:52698560	rs12488527	3.130	rs2878632	0.113	0.053555484	6.742
17	ZPBP2	17:38031164	rs11658278	3.115	rs9903250	0.113	0.053555484	3.384
12	MGAT4C	12:86435551	rs11513957	3.134	rs1502802	0.113	0.060467781	3.520
11	FAT3	11:92291634	rs675654	3.063	rs2852859	0.112	0.061504234	3.582
9	MEGF9	9:123462573	rs75959206	3.046	rs1530370	0.113	0.061504234	4.958

605 **Figures**



606

607 **Figure 1**. Principal component analysis (PCA) of the sequenced TrypanoGEN
608 samples, Guinea (GUI), Ivory Coast (CIV), Cameroon (CAM), Democratic Republic
609 of Congo (DRC), Uganda (Nilotics, UGN, Bantu, UGB) and Zambia (ZAM), (**A**); **B**,
610 TrypanoGEN and selected 1000 genomes African samples Nigeria (ESN, YRI),
611 Sierra Leone (MSL), Gambia (GWD), Kenya (LWK); **C,** 1000 genomes samples
612 from Africa and the rest of the world. AAM, African Americans; AMR, indigenous
613 Americans; CAF, Central Africa; EAF, East Africa; EAS, East Asia; EUR, Europe;
614 NSA, Nilo-Saharan; SAS, South Asia; WAF, West Africa;
615

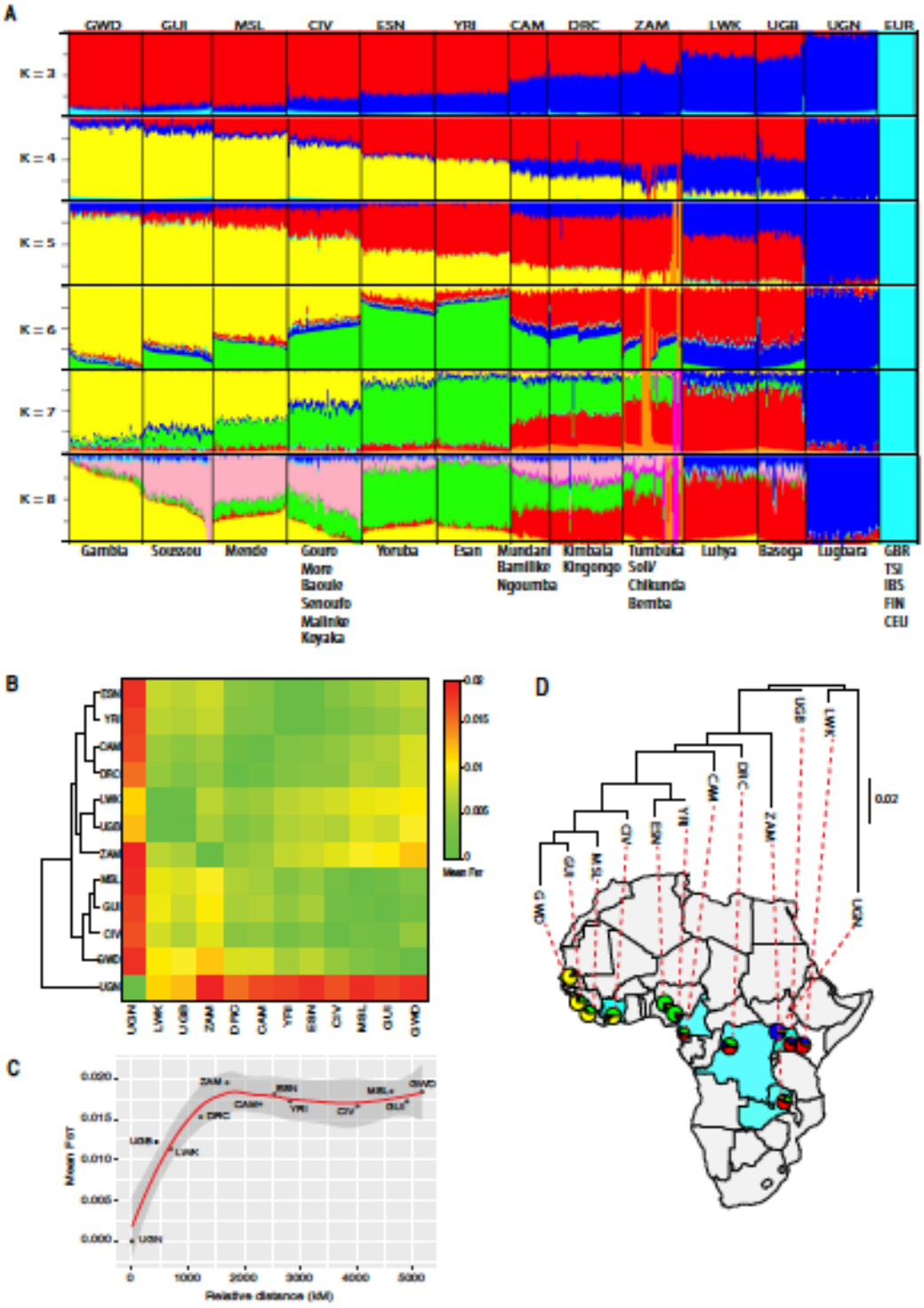
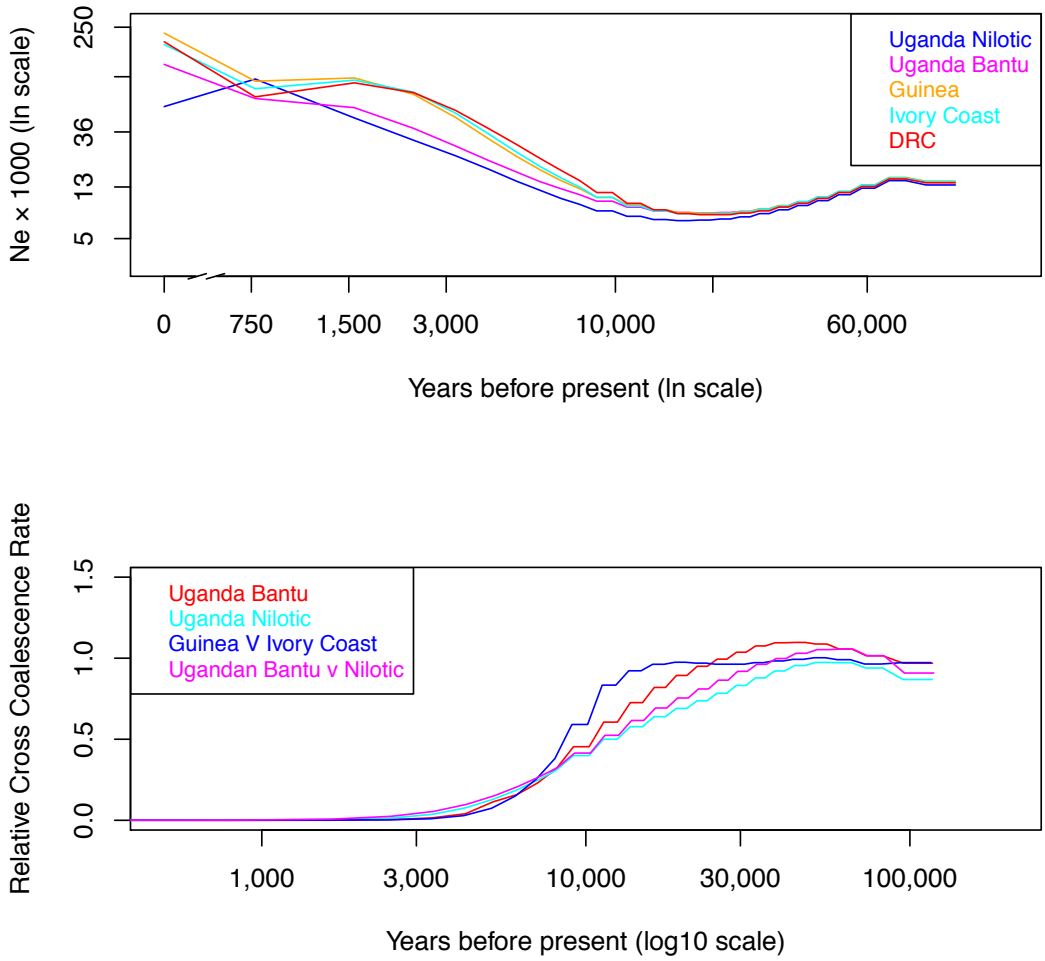


Figure 2. Genetic admixture and diversity between TrypanoGEN and selected 1000 genome populations. **A**. Admixture plot of the K populations of the TrypanoGEN, 1000 genome African and European populations. **B**. Heatmap of mean Fst between TrypanoGEN and 1000 genome African populations. **C**. Polynomial regression plot of the mean Fst against the relative geographical distance of the African Niger-Congo populations from the Uganda Nilotic population. **D**. Phylogeographic plot of the mean Fst distances on the Trypanogen populations and selected 1000 kgenome African populations; the pie charts represent the population sample size and admixture.



625

626 **Figure 3.** Population sizes and cross-coalescence rates compiled by MSMC. **A**
627 Effective population sizes for each population since 75kya. The Ugandan Bantu and
628 Nilotic populations have grown continuously but at a slower rate than the West and
629 Central African populations. These latter populations experienced and declines of 6-
630 23% between 1500 and 800 years ago. **B** Cross-coalescence rates for pairs of
631 populations. At 1.0 populations are panmictic and at 0.0 there is no gene flow. The
632 Guinea and Ivory Coast populations were panmictic until about 10 kya and then
633 became separated by 3kya. Other populations appear to have separated more
634 gradually but these may be confounded by admixture.

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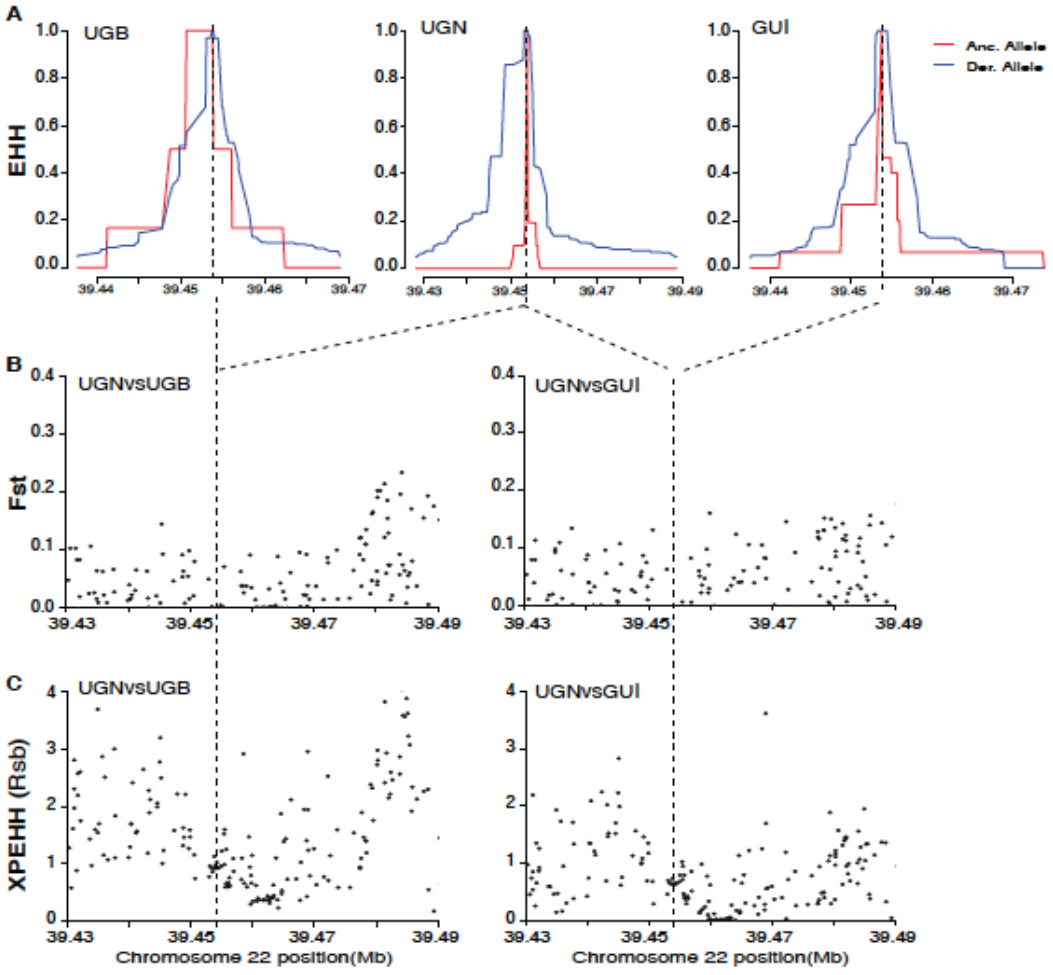

Figure 4. Illustration of signatures unique to the Uganda Nilotic population. Signal of positive selection within the *APOBEC3G* gene on Chromosome 22 at the rs112077004 loci of the Uganda Nilo-saharan Lugbara population, in comparison with the Niger-Congo B populations of Uganda (UGB) and Niger-Congo A population of Guinea (GUI). **A**. The calculated site specific extended haplotype homozygosity (EHH) within a population. **B**. Between population Fst analysis. **C.** Across population (XPEHH) analysis.

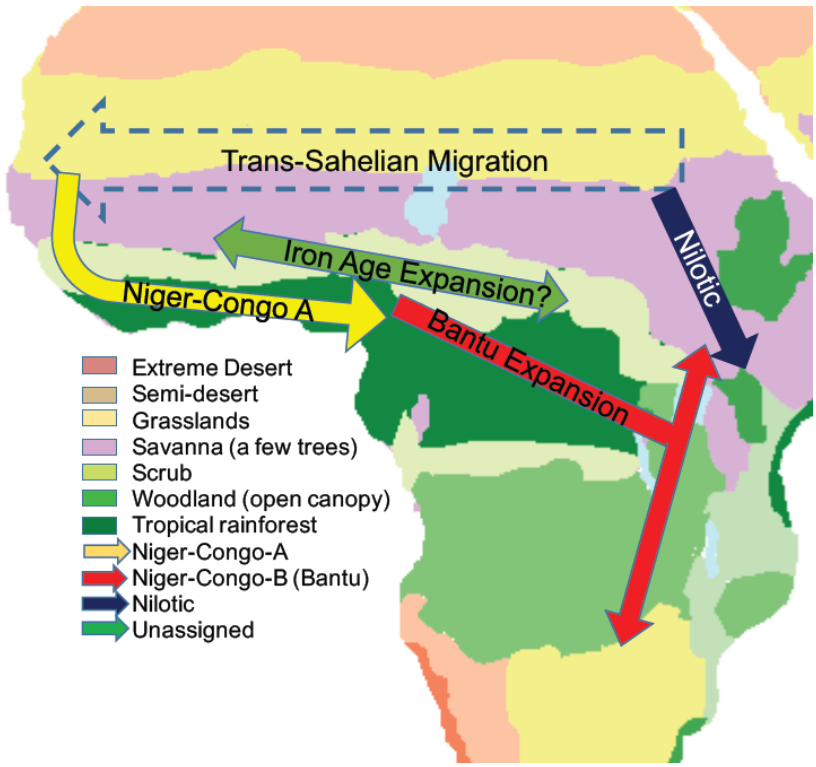

Figure 5. Migrations of Niger-Congo speakers. Map colours show vegetation coverage approximately 10kya (Adams, 1998). Colours for linguistic groups as for fig 4. Blue Nilo-Saharan; Yellow, Niger-Congo A; Red, Niger-Congo-B (Bantu); Green putative expansion of an ancestral group out of modern Nigeria. Blue dotted arrow, suspected route of proto-Niger-Congo-A speakers from Nuba mountains of Sudan to Senegal across the Sahel (1966) when it was much wetter than at present.