

## **Genetic diversity and phylogeographic structure of West African village chickens based on a 920bp segment of the mtDNA D-loop.**

Noah E.T. Tor<sup>1,2\*</sup>, Ayotunde O. Adebambo<sup>2,3</sup>, Matthew Wheto<sup>2</sup>, Olajide M. Sogunle<sup>2</sup>, Christian O.N. Ikeobi<sup>2</sup>, Abdulhakeem B. Ajibike<sup>4,5</sup>, Adewumi O. Omotoso<sup>2,6</sup>, Kilpatrick Shems.<sup>7,8</sup>, Samuel Durosaro<sup>2</sup>, Emmanuel W. Mendy<sup>9</sup>, Opeyemi Oladejo.<sup>10</sup>, Ifeanyi Ubaka<sup>2</sup>.

<sup>1</sup>Department of Animal Breeding and Physiology, Federal University of Agriculture, P.M.B. 2373 Makurdi 970213, Benue State, Nigeria.

<sup>2</sup>Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

<sup>3</sup>Stabvida Genetics Laboratory, Monte de Caparica, Portugal

<sup>4</sup>Birbrair Laboratory, Department of Pathology, Federal University of Minas Gerais, Belo Horizonte, Brazil.

<sup>5</sup>Department of Animal Production Technology, Oyo State College of Agriculture and Technology, Igbo-Ora, Oyo State, Nigeria.

<sup>6</sup>Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.

<sup>7</sup>William V.S. Tubman University, Harper, Maryland, Liberia

<sup>8</sup>Ministry of Health, Karluway district 1 and 2, Maryland County, Liberia

<sup>9</sup>Ministry of Agriculture, Department of Livestock Services, Central River Region North Wassu, The Gambia.

<sup>10</sup>Department of Animal Science and Fisheries Management, Bowen University, Iwo, Osun State, Nigeria.

---

**Abstract:** The West African village chicken characterised by small body size, low productivity, disease tolerance and strong links to local traditions is spread extensively within the sub-region especially in the rural settings. However, not much is known about its genetic diversity and population structure. In this study, the maternal genetic diversity and population structure of these chicken populations were characterised using sequence analysis of a 920-bp fragment of the mitochondrial DNA D-loop region. A total of 127 genomic samples were taken from the village chickens from four country populations (Gambia, Togo, Liberia and Nigeria). The mtDNA D-loop region was amplified following standard PCR protocols, using the primer pair of AV1F2: 5'-AGGACTACGGCTTGAAAAGC-3' and 5'-TGCTTAAGGTTAATTACTGCTG-3' based on complete mtDNA chicken genome. Sequencing was done using Sanger's dideoxy chain termination method. In addition, 268 sequence samples harvested from Genbank representing *Gallus gallus* from Africa, Mediterranean and different regions of Asia, were included in the analysis, which brought the total sample size to 395. In all 24 polymorphic sites and 13 West African Chicken haplotypes (WAC1-13), were detected. Unbiased gene diversity ( $0.083 \pm 0.020$ ) and haplotype diversity ( $0.43 \pm 0.29$ ) further showed the genetic diversity of West African village chickens. The Analysis of Molecular Variance (AMOVA) revealed a maternal genetic sub-structuring of the populations ( $F_{ST}=0.213$ ), with WAC8 and 10 haplotypes restricted to Nigeria. Mismatch distribution, Tests of goodness-of-fit and selective neutrality all indicated conformity with sudden demographic expansion model for Gambia, Togo and Liberia, suggestive of purifying selection, founder effect or genetic drift. On the other hand, Nigeria was indicative of a population under neutral selection, multiple parentage or bottleneck. The Nigerian and Liberian populations were the most genetically distant (pairwise  $F_{ST}=0.234$ ). The study shows that the West African village chickens are genetically diverse; however, the Nigerian population was more diverse. The study concluded that understanding the genetic diversity of the African chicken can be exploited for genetic improvement.

**Key words:** village chicken; West Africa; genetic diversity; mitochondrial DNA; D-Loop.

---

Date of Submission: 29-03-2021

Date of Acceptance: 12-04-2021

---

### **I. Introduction**

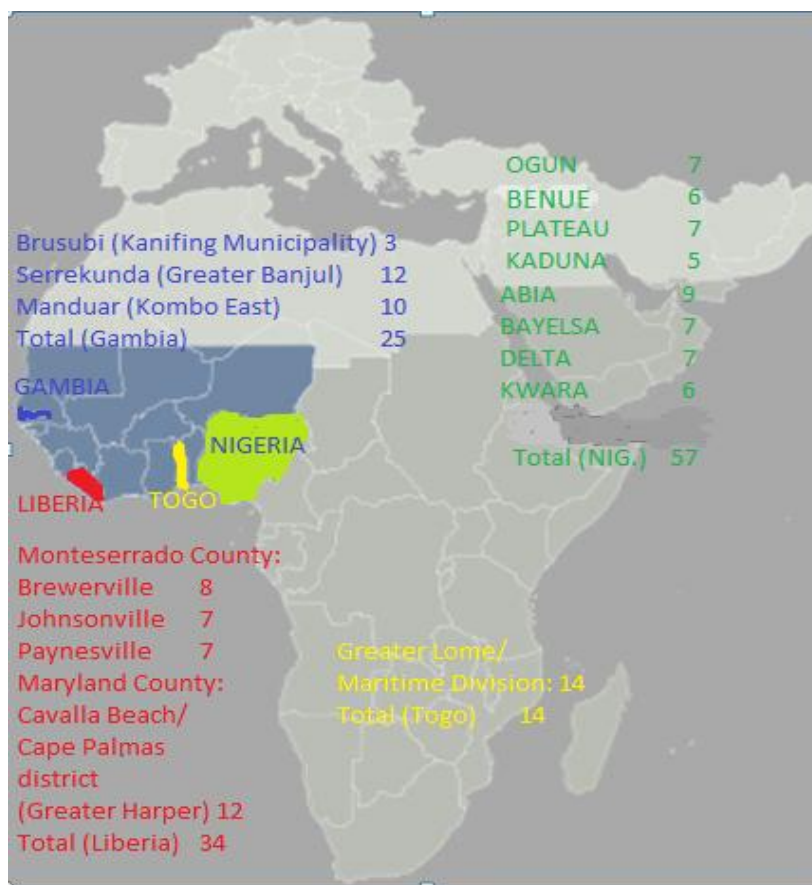
In West Africa, millions of people especially the rural ones depend on livestock production as a means of livelihood with over 80% of the sub region's livestock population in the hands of traditional or village based operators (Rikaterere and Luseba, 2010). The chicken is said to be the most abundant livestock species on the African continent and the most abundant species of domestic birds in the world (FAOSTAT, 2012). They have been reported to generally have small body size, slow growth rate and low productivity (Rudresh *et al.*, 2015;

Kaya and Yildiz, 2008), besides their traits of adaptability, scientific, economic interest, cultural-historical value and strong links to local traditions (Mtileni *et al.*, 2011). In the developing world like Africa, the dearth of information has made the question of what and how to conserve, develop and select among local breeds most difficult to answer (FAO, 2007a). These animal populations are thought to face genetic erosion, which could lead to the loss of genetic variability (Palacios *et al.*, 2016). It has been posited that the main threats to animal genetic resources (AnGR) are massive production, importation of exotic birds and indiscriminate breeding (Hillel *et al.*, 2003; FAO, 2007b; and FAOSTAT, 2017). To alleviate these challenges, the FAO (2011a) suggested the molecular characterization of animal genetic resources as a short to medium-term panacea, as there is a general dearth of molecular genetic information on indigenous animal genetic resources in Africa. This shortage of genetic information has led to the abandonment of the development of local breeds in preference to the introduction of germplasm from exotic ones, about which there is generally more information (FAO, 2011b). This introgression of exotic alleles is said to adversely threaten the genetic diversity of the local stock (FAO, 2007b). Therefore, the goal of efficient and sustainable genetic management of AnGR via diversity characterisation is not yet achieved, which calls for the commitment of more resources (FAO, 2007b). Apart from the inadequacy or poor utilisation of within-country genetic information, where they exist at all, there is greater inadequacy or in some cases a total lack of trans-boundary standardisation in order to enrich the results for interpretation in an international context (FAO, 2011b). Much of the results on evolutionary origins and genetic diversity of chickens from the continent have been based within single countries, such as Egypt (Osman *et al.*, 2016; Soltan *et al.*, 2016 and Ramadan *et al.*, 2012), Chad (Hassaballah *et al.*, 2015), South Africa (Mtileni *et al.*, 2011), Nigeria (Adebambo *et al.*, 2010) and Zimbabwe (Muchadeyi *et al.*, 2008). Only a few, Sudan and South Sudan (Wani *et al.*, 2014), Cameroon, Ghana, Cote d'Ivoire, Benin and Morocco (Leroy *et al.*, 2012) and Kenya, Ethiopia, Sudan and Uganda (Mwacharo *et al.*, 2011), had data from more than a country. The work of Leroy *et al.* (2012) though spanning a large geographical area of North, West and Central Africa, had only three entries from West Africa (Benin, Ghana and Cote d'Ivoire) one each from the other sub-regions and was not based on mtDNA but microsatellite marker analysis. Nigeria, consisting of 47% of West Africa's human population (World Bank, 2018), was conspicuously missing, Leroy *et al.* (2012) observed that restricting the study to Benin, Ghana and Cote d'Ivoire, did not result in a typical breed structure, but a South-West to North-West gradient was observed, thus indicating the need for the inclusion of more countries in the sub-region in similar studies. There is therefore the need to include Nigeria in a trans-border comparative characterisation study. Also, no work currently exists on the West African village chicken to the magnitude of that of Mwacharo *et al.* (2011) on the East African Chicken, covering as much as four countries in a single sub-region. This study therefore sought to characterise the genetic diversity of chicken populations from four West African countries namely Gambia, Togo, Liberia and Nigeria, using partial sequence of the mitochondrial DNA D-loop region as a molecular tool. West Africa is the region of Africa, lying west of an imagined North-South axis close to 10° East longitude (Speth, 2010). According to the United Nations scheme of African regions, the region includes 18 countries, inclusive of the Island of Saint Helena, a British overseas territory in the South Atlantic Ocean and Mauritania which withdrew from Economic Community Of West African States (ECOWAS) in 1999 (Seddon, 2004; Branine, 2011). West Africa's population accounts for 30% of Africa's and is expected to increase from roughly 367 million people in 2017 to around 570 million by 2035 (ISS, 2017). Similarly, Africa's population is thought to be the fastest growing and youngest in the world. The boundary of West Africa is defined by the Atlantic Ocean to the South and West, the Sahara desert in the North, and the Lake Chad, Benue trough and Cameroonian mountains to the East (Ham, 2009; Speth, 2010).

The FAO (2011b) has recognized the mtDNA among other genetic markers as most advanced and informative for molecular characterization and biodiversity studies. The farm animal breeds developed in genetic isolation in the technologically-advanced countries are generally characterised by clear-cut phenotypic features, unlike the ones in the developing countries that most often correspond to local populations that differ only gradually across geographical zones.

## **II. Materials And Methods**

A total of 127 blood samples from four countries (Gambia, 25; Togo, 14; Liberia, 34 and Nigeria, 54) were collected to represent the village chicken population of West Africa. The number was distributed across two or more agro-climatic/vegetation zones in each country, where possible. To minimize the possibility of the chickens being related, the sampling was purposely done in at least two different regions of at least 100km apart, except in Togo where all the samples were from different locations within one region (Maritime region). Figure 1 shows the number and sampling locations within each country. In addition to the 127 West African samples, 268 chicken mtDNA sequences were downloaded from Genbank, representing major Asian centres of chicken domestication, Europe and Africa. This brought the total number of samples used in this study to 395 chickens (Table 1).



**Figure 1:** Map of the study area showing a breakdown of the sampling locations.

**Table 1:** Details of the samples downloaded from Genbank.

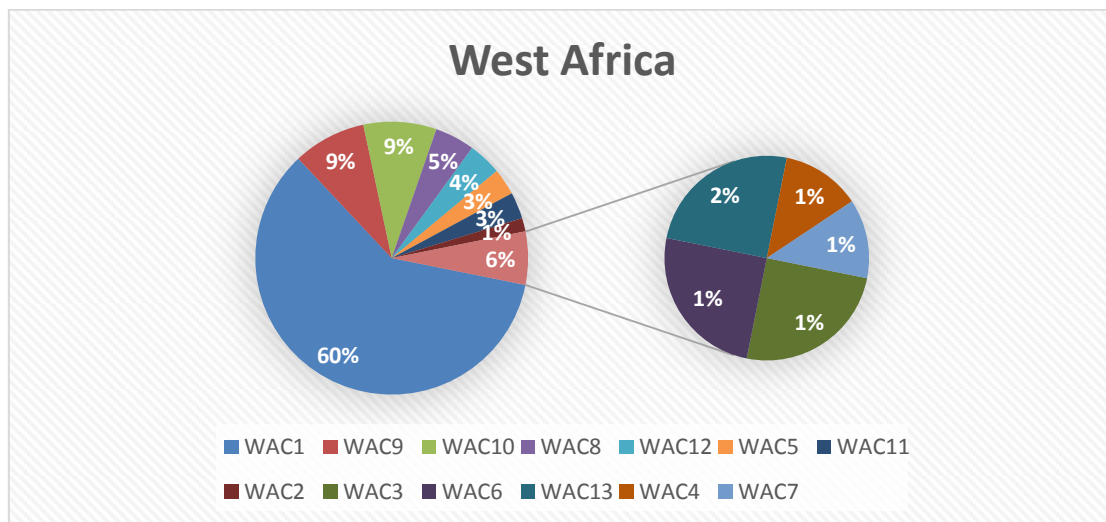
Source	Number of Sequences	Genbank Accession number	Reference
Egypt	18	AB829473-AB829490	Osman <i>et al.</i> (2016)
Kenya	30	EU095192-EU095163	Mwacharo <i>et al.</i> (2011)
China	23	AB098666-AB098664 KY3008169-KY308150	Komiyama <i>et al.</i> (2003) Gao <i>et al.</i> (2017)
South Korea	20	HQ836363-HQ836343	Cho <i>et al.</i> (2010)
Pakistan	40	MH094617-MH094656	Nisar <i>et al.</i> (2018)
India	21	KF411029-KF411009	Ghosh <i>et al.</i> (2013)
Mainland South-East Asia (MSEA): Thailand (10), Vietnam (1)	11	AB009441-AB009444 KC817527-KC817533	Miyake <i>et al.</i> (1997) Pramual <i>et al.</i> (2013)
Mediterranean Italy (3), Spain (4), Malta (1)	8	LK391757-LK391764	Ceccobelli <i>et al.</i> (2015)
Island South-East Asia (ISEA): Indonesia (54), Philippines (38), Fiji Island (5)	97	KX642436-KX643040	Herrera <i>et al.</i> (2017)
Number of samples downloaded from Genbank = 268; Total samples used for this study = 395			

DNA was isolated from the FTA-classic cards following the protocol recommended by the manufacturer ([www.whatman.com](http://www.whatman.com)). The Polymerase Chain Reaction (PCR) to amplify the mtDNA D-loop and Sanger sequencing of the resultant products was done at Stab Vida Genetics Laboratory in Monte de Caparica, Portugal. 950 base pair fragment of the mtDNA D-loop region was amplified by PCR using a primer pair of AV1F2: 5'-AGGACTACGGCTTGAAAAGC-3' and 5'-TGCTTAAGGTTAATTAC TGCTG-3' (Nishibori *et al.*, 2001). All 127 sequences of West African village chickens were viewed and edited using BioEdit 7.0 (Hall, 1999) and MEGA 7 (Kumar, *et al.*, 2016). Noises in the sequences were removed, and the sequences were truncated to a uniform fragment length of 920 base pairs. Multiple alignments of all sequences to detect nucleotide variations were conducted with ClustalW software hosted in MEGA 7. The sequence variation and by implication the population indices including number of segregation (polymorphic) sites (s), number of haplotypes, average number of nucleotide and pairwise genetic distance were evaluated with DnaSP V.5.10 software (Librado and Rozas, 2009). The haplotypic diversity, its sampling variance and standard deviation were estimated using Nei (1987) method implemented in Arlequin 3.5.1.3 software (Excoffier *et al.*, 2005). The mean

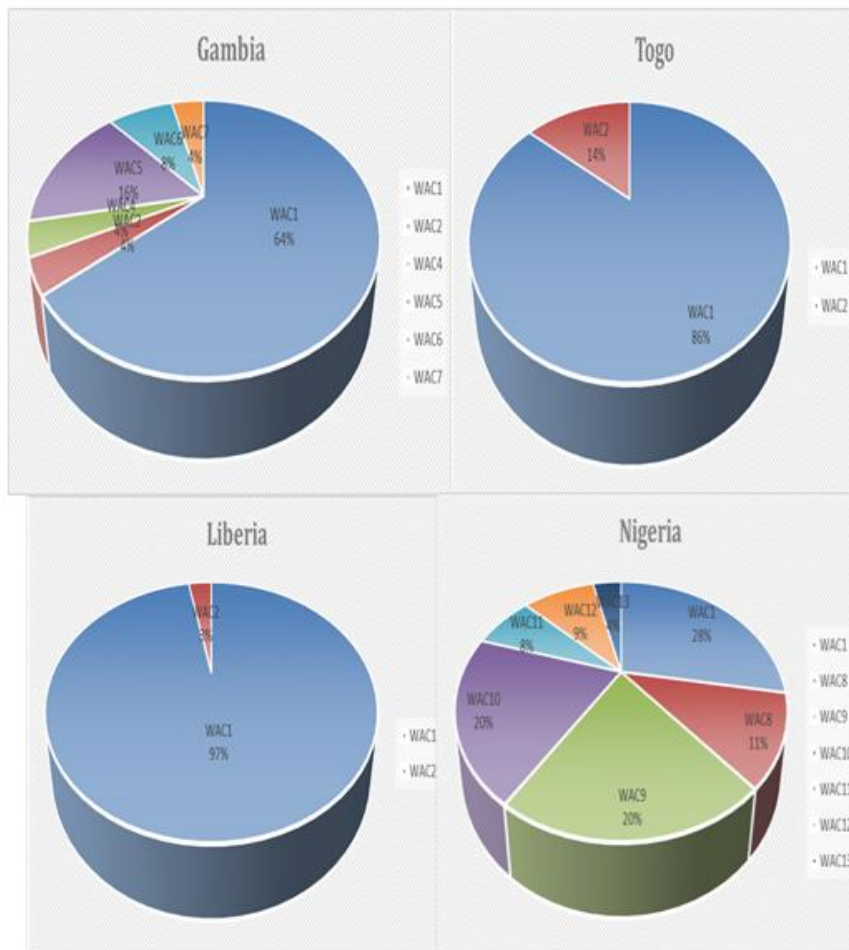
number of pairwise differences ( $\pi$ ), Nucleotide diversity ( $\hat{\pi}_n$ ) and standard deviations were estimated using methods proposed by Tajima (1983, 1993) in Arlequin 3.5.1.3 (Excoffier *et al.*, 2005). Theta estimators ( $\hat{\theta}_H$ ,  $S$  and  $k$ ) and Mismatch distribution were evaluated in Arlequin 3.5.1.3. Arlequin was also used to calculate the sum of squares deviations ( $SSD$ ) between the observed and expected mismatch, raggedness index ( $r$ ) (Harpending, 1994), Tajima's test of selective neutrality ( $D$ ) statistic (Tajima (1989)) and the maternal analysis of molecular variance (AMOVA).

### III. Results

The analysis of West African village chickens from four countries; Gambia (n=25), Togo (n=14), Liberia (n=34) and Nigeria (n=57), based on a 920 base-pair fragment of the mtDNA produced 13 haplotypes (WAC 1-13) from 24 polymorphic (segregating) sites. Figures 2 and 3 show a summary of the relative percentage occurrence of the 13 observed haplotypes in West Africa and their geographic distributions. WAC1 did not only exhibit the widest geographic spread, it also had the highest frequency of occurrence (59.8%). It was found and dominated in all the four populations, It was distantly followed by WAC9 and 10 with a frequencies of 8.7% each. Furthermore, three of the largest haplotypes (WAC 8-10) with combined frequency of about 23% occurred only in Nigeria,



**Figure 2:** Summary of percentage occurrence of the 13 haplotypes observed in the mtDNA D-loop of 127 sampled West African village chickens.



**Figure 3:** Geographic distribution of the 13 haplotypes observed in the mtDNA D-loop of the sampled West African chicken populations

Table 2 shows the nucleotide polymorphism and haplotype distribution patterns of the sampled chickens, thirteen of the sites were singleton polymorphic while the remaining 11 were parsimony informative sites. In all 36 transitions (10: G↔A and 26: T↔C) and 3 transversions were observed (1:A↔C; 1:A↔T and 1:G↔C). No insertions and deletions (*Indels*) were detected in the sequences. While Results for the standard and molecular diversity indices of West African chickens are contained on table 3. The nucleotide (gene) diversity ranged from 0.002 (Liberia) – 0.28 (Nigeria) with a mean regional value of 0.083.

**Table 2:** Observed nucleotide Polymorphisms and haplotype distribution patterns of the sampled chicken populations

Ref	11112222222223333346888 589900123344691244437899 245727081416655703411901	Population				
		Gambia	Togo	Liberia	Nigeria	N
H_1(WAC1)	.....C.....	16	12	33	15	76
H_2(WAC2)	.....T.C.....	1	-	1	-	2
H_3(WAC3)	.....C....G.....	-	2	-	-	2
H_4(WAC4)	.....C.....T..ATC	1	-	-	-	1
H_5(WAC5)	.....C..C.....	4	-	-	-	4
H_6(WAC6)	.....C.....T.....	2	-	-	-	2
H_7(WAC7)	...T....C.....	1	-	-	-	1
H_8(WAC8)	C..T.T..TT..TC.C....CA...	-	-	-	6	6
H_9(WAC9)	....G...GC.G...C...A...	-	-	-	11	11
H_10(WAC10)	C..AT..TT..TC.C.G...CA...	-	-	-	11	11
H_11(WAC11)	.C.....C.....	-	-	-	4	4
H_12(WAC12)	.....C.....G.....	-	-	-	5	5
H_13(WAC13)	.....G...C...T.....	-	-	-	2	2

**Table 3:** Standard and molecular diversity indices from mtDNA of sampled West African village chicken populations:

	Gambia	Togo	Liberia	Nigeria	Mean
Number of Sequences	25	14	34	54	31.75±14.70
Number of Haplotypes	6	2	2	7	4.25±13.00
Haplotypes diversity (Hd)	0.58±0.11	0.26±0.14	0.06±0.05	0.83±0.02	0.43±0.29
Nucleotide diversity	0.04±0.03	0.01±0.01	0.002±0.006	0.28±0.15	0.083±0.02
Mean number of pairwise differences	0.90±0.64	0.26±0.31	0.06±0.13	6.64±3.18	1.97±1.13
Sum of square frequency	0.45	0.76	0.74	0.19	0.53±0.70
Number of observed transitions	5	1	1	19	6.5±7.40
Number of observed transversions	2	0	0	0	0.5±0.87
Number of substitutions	7	1	1	19	7±7.35
Number of observed indels	0	0	0	0	0
Number of polymorphic sites	7	1	1	19	7.0±7.35
$\theta_H$	1.02±0.45	0.27±0.19	0.05±0.05	3.86±0.67	1.30±1.5
$\theta_k$	2.18±0.85	0.37±0.08	0.27±0.06	1.92±0.83	1.18±0.87
$\theta_s$	1.85±0.89	0.31±0.31	0.24±0.24	4.17±1.45	1.65±1.60
$\theta_\pi$	0.90±0.72	0.26±0.35	0.06±0.15	6.64±3.53	1.96±2.7
C (%)	37.33	37.50	37.38	38.04	37.56
T (%)	29.33	29.17	29.29	28.63	29.11
A (%)	25.00	24.40	25.00	24.07	24.62
G (%)	8.34	8.93	8.33	9.26	8.71

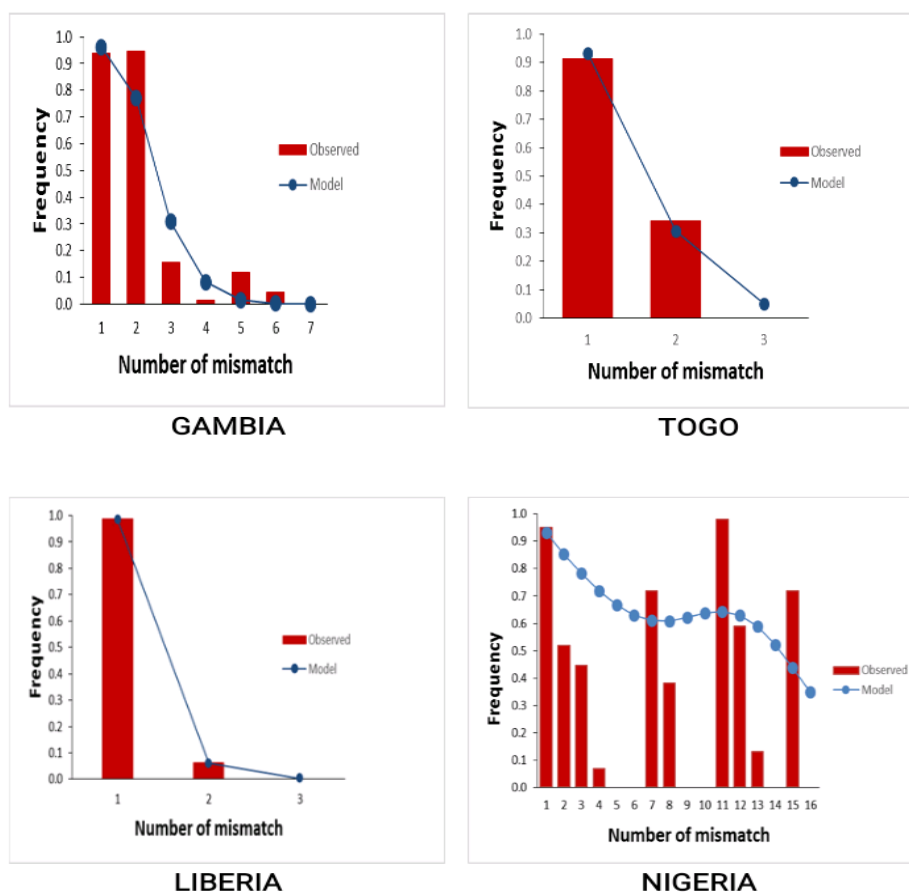
$\theta_H$ : Theta value based on expected homozygosity;  $\theta_k$ : Theta value based on number of alleles;  $\theta_s$ : Theta value based on number of segregating sites;  $\theta_\pi$ : Theta value based on the average number of pairwise differences; C: Cytosine; T: Thymine ; A: Adenine; G: Guanine

Table 4 shows the mismatch distribution indices of the mtDNA of sampled West African village chicken populations, while the mismatch distribution is represented on Figure 4. Distribution models for all sampled populations were half bell uni-modal, except that of Nigeria.

**Table 4:** Mismatch distribution indices of the mtDNA of sampled West African village chicken populations

	Gambia	Togo	Liberia	Nigeria	Mean
Mismatch observed mean	0.09	0.26	0.06	6.64	1.76
Mismatch observed variance	1.31	0.20	0.06	24.41	6.50
T	0.80	0.33	3.00	12.43	4.14
$\theta_0$	0.00	0.00	0.00	0.00	0.00
$\theta_1$	99999.0	99999.0	0.07	10.79	50002.21
Sum of square deviation	0.014	0.001	0.00	0.058	0.02
$P$ (Sim. $SSD \geq Obs. SSD$ )	0.190	0.670	0.17	0.050	0.27
Harpending's Raggedness index	0.135	0.293	0.782	0.115	0.33
$P$ (Sim. $Rag. \geq Obs. Rag.$ )	0.320	0.430	0.880	0.010	0.41
Tajima's $D$	-1.590	-0.341	-1.138	1.85	-0.30
$P$ (Sim. $D < Obs. D$ )	0.045	0.228	0.027	0.94	0.31
Fu's $F_s$	-2.23	0.19	-1.32	7.25	0.97
$P$ (Sim. $F_s < Obs. F_s$ )	0.037	0.32	0.05	0.99	0.35

T: time of expansion;  $\theta_0$  and  $\theta_1$ : mutation parameters



**Figure 4:** Mismatch distributions of West African village chicken populations

The result for the analysis of molecular variance (AMOVA) of the maternal genetic structure of the West African village chickens is indicated on Table 5.

**Table 5:** Analysis of molecular variance within and among West African village chicken populations using mtDNA sequence.

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	$F_{ST}$
Among populations	3	41.575	0.41796	21.36	0.21356
Within Populations	123	189.318	1.53917	78.64	
<b>Total</b>	<b>126</b>	<b>230.913</b>	<b>1.95713</b>		

$F_{ST}$ : Fixation index

Results of the Pairwise difference between the populations (Table 6) showed that the most divergent populations were Nigeria and Liberia,

**Table 6:** Population among the pairwise  $F_{ST}$  populations:

	Gambia	Togo	Liberia	Nigeria
Gambia	0.00			
Togo	0.04344	0.00		
Liberia	0.07228*	0.11676	0.00	
Nigeria	0.18974*	0.16655*	0.23417*	0.00

\* $P=0.05$

#### IV. Discussion

All observed populations were found to be polymorphic with the number of haplotypes ranging from 2 in Togo and Liberia to 7 in Nigeria. The restriction of three major West African haplotypes to Nigeria is suggestive of phylogeographic substructuring. The thirteen haplotypes observed in this study fell within the range previously reported on African chickens with implication that the populations are polymorphic at the mtDNA hypervariable region. This number was comparable to those reported on South Africa chickens (13; Mtileni *et al.*, 2011) and Sudan and South Sudan chicken breeds (14; Wani *et al.*, 2014) but higher than some records on Egyptian indigenous chicken (7; Eltanany and Hemeda, 2016). On the contrary, the haplotype number of 13 was below earlier observations on Nigerian village chickens (35; Adebambo *et al.*, 2010 and 31; Ajibike *et al.*, 2017), Chadian indigenous village chickens (20; Hassaballah *et al.*, 2015), East African domestic chickens (41; Mwacharo *et al.*, 2011), and Egyptian native chickens (18; Osman *et al.*, 2016). The mean haplotype diversity (heterozygosity) obtained from Nigeria ( $0.83 \pm 0.02$ ) and Gambia ( $0.58 \pm 0.11$ ) in this study were higher than previous reports on Nigerian chickens ( $0.4217 \pm 0.0419$ , Adebambo *et al.*, 2010;  $0.39 \pm 0.07$ , Ajibike *et al.*, 2017). Similarly, the Nigerian value was higher while that of the Gambia was comparable to observations on village chickens elsewhere in Africa, Egypt, ( $0.81 \pm 0.03$ , Osman *et al.*, 2016;  $0.608 \pm 0.09$ , Eltanany and Hemeda, 2016); East Africa,  $0.638 \pm 0.024$  (Mwacharo *et al.*, 2011); Sudan and South Sudan,  $0.577$  (Wani *et al.*, 2014); Chad,  $0.541 \pm 0.044$  (Hassaballah *et al.*, 2015); South Africa,  $0.54-0.88$  (Mtileni *et al.*, 2011), and beyond, Vietnam ( $0.62-0.94$ ; Cuc *et al.*, 2011), Korea ( $0.59-0.82$ ; Hoque *et al.*, 2013) and Laos ( $0.5183-0.8798$ ; Kawabe *et al.*, 2014). This may imply that the Nigerian chickens were comparatively more heterozygotic while the Liberian ( $0.06 \pm 0.05$ ) and Togolese ( $0.26 \pm 0.14$ ) were less. Also higher haplotypic values amongst the Nigerian, Gambian, North African/Arabian and Asian countries, could be expectedly indicative that the Liberian and Togolese chickens were comparatively younger or more recent on the evolutionary time scale of the domestic chickens. Savolainen *et al.* (2002) observed that ancient populations were more likely to be genetically more diverse than their derived or recent counterparts. In addition haplotype diversities might be positively influenced by small sample size and the unique breeding histories of the chickens (Joshi *et al.*, 2013), as well as natural selection (Yap *et al.*, 2011).

The nucleotide diversity value ( $0.083 \pm 0.02$ ) from this study was higher than the observations of Osman *et al.* (2016) and Eltanany and Hemeda (2016) (Egypt,  $0.0045 \pm 0.0013$  and  $0.004 \pm 0.001$  respectively); Mwacharo *et al.* (2011) (East Africa,  $0.00745 \pm 0.00042$ ); Wani *et al.* (2014) (The Sudans,  $0.00282$ ); Hassaballah *et al.* (2015) (Chad,  $0.00312 \pm 0.00049$ ), Adebambo *et al.* (2010) (Nigeria,  $0.00157 \pm 0.0137$ ), Oka *et al.* (2007) (Japan,  $0.001020-0.001623$ ) and Kawabe *et al.* (2014) (Laos,  $0.0007706-0.014519$ ). This result is also indicative that the West African chickens are genetically diverse. The relatively lower haplotype ( $0.43 \pm 0.29$ ) and higher nucleotide diversities of the sampled West African chicken populations could be inferred to be evidence of purifying selection (Yap *et al.*, 2011), higher mutation rates (Komiyama *et al.*, 2003) and multiple maternal ancestry (Silva *et al.*, 2008). It is noteworthy that for both genetic diversity indices, Nigerian chicken showed higher values than the regional averages, suggestive of greater influence of a possible multiple maternal ancestry (Silva *et al.*, 2008). It is only among the Nigerian samples that there appeared a scenario of more than one major haplotype leading to greater genetic diversity.

The negative Tajima's D (for The Gambia, Togo and Liberia) is suggestive of population expansion or purifying selection (Tajima, 1996), indicating that such populations are not at equilibrium nor experiencing random (neutral) selection. Due to the effect of these factors (purifying selection, population expansion and selective sweep), Hahn *et al.* (2002) observed that such populations tended to have excess of rare alleles. This is in tandem with the mismatch distributions (Figure 4) which were uni-modal (half bell shaped), for the three populations, with predominant allele types being of high and low frequencies. The excess of low frequency alleles was a clear indication of departure from equilibrium occasioned by recent or past population expansion (Tajima, 1996). On the other, the positive Tajima D value of Nigerian chickens was a sign that the population was at neutral equilibrium or experiencing random selection. Such populations tend to be devoid of or low on rare alleles (Hahn *et al.*, 2002), as seen on the mismatch distribution graph of Nigerian chickens. The bimodal distribution (one and half bells shape) shows a complete absence of low frequency alleles and only very few high frequency ones. A vast majority of the alleles are of medium frequencies indicative of a population at neutral equilibrium, undergoing random selection and free of sudden expansions whether in the recent or distant past (Tajima, 1996).

To validate the results of the mismatch distribution and Tajima's D, sum of square deviations (SSD) (Schneider and Excoffier, 1999) and Harpending's raggedness index ( $r$ ) (Harpending, 1994) were further used to test the hypothesis that the observed data fit the sudden expansion model. The non-significant values for SSD signified that the data did not deviate from the expected under the model for expansion while a non-significant  $r$  value indicated population expansion (Rogers and Harpending, 1992; Rogers, 1995; Jobling *et al.*, 2004). In other words, non-significant SSD and  $r$  were evidences that such populations conformed to an estimated sudden demographic model which in this case was that of population expansion (since  $t > 0$  and  $\theta_1 > \theta_0$ ), the alternative



being the model for stationary population (if  $t = 0$  or  $\theta_1 = \theta_0$ ). It could therefore be inferred that chicken populations of Gambia, Togo and Liberia were experiencing or had experienced sudden population expansions and therefore not at neutral equilibrium. On the contrary, the Nigerian population with both positive SSD and  $r$  value could be said to be a departure from the model of sudden demographic changes whether that of expansion or stationary, indicating that it was at neutral equilibrium (Jobling *et al.*, 2004). Hence the SSD and  $r$  results were consistent with those of Tajima's  $D$ . The negative values of Fu's  $F_s$  for Gambia and Liberia were consistent with population expansion, and the positive value for Nigeria was suggestive of neutrality (Okello *et al.*, 2005; Joshi *et al.*, 2013), all agree with results from Tajima's  $D$ . The rather positive  $F_s$  for Togo (0.19) and the overall mean (0.97) indicative of departure from population expansion was inconsistent with findings from Tajima's  $D$ , but was not statistically significant ( $p > 0.05$ ).

The result for the analysis of molecular variance (AMOVA) of the maternal genetic structure of the West African village chickens might be considered a further indication of the genetic diversity of the West African chicken populations. The variance component due to groups (21.36%) was much higher than values earlier reported from Nigeria by Ajibike *et al.* (2017) (2.70%) and Adebambo *et al.* (2010) (2.68%) respectively. The difference might be due to the wider geographic area covered in this study, well beyond Nigeria, leading to a lower gene flow which could accelerate the process of differentiation (Joshi *et al.*, 2013). The relatively higher between group molecular variance component observed in this study was also indicative of some form of differentiation or sub-structuring of the chicken population of West Africa (Eltanany and Hemeda, 2016), agreeing with earlier submissions from haplotype spread and the bimodal mismatch distribution for Nigeria. Similarly, the  $F_{ST}$  value of 0.213 was concordant with genetic differentiation or subdivision among the populations (Frankham *et al.*, 2010; Holsinger and Weir, 2009).

That the Nigerian and Liberian populations were the most divergent agrees with results of molecular diversity and mismatch distribution. This might be due to several factors such as differences in size, geographic distance, population, government policies on animal improvement among others. The prolonged Liberian civil war (*TheGaurdian*, 2003) followed by outbreak of the deadly Ebola disease (WHO, 2019), could have both drastically reduced the chicken population size and the number of people and animals moving to that country under the auspices of trade. It had been widely observed that dispersal of the chicken was closely associated with human activities (Osman *et al.*, 2016; Eltanany and Hemeda, 2016; Mwacharo *et al.*, 2013; 2011). This could imply that the current village chicken population might likely be products of an adverse founder effect, resulting to heterozygosity deficiency (Chen *et al.*, 2017).

## V. Conclusion

The findings from this study showed that West African chickens were genetically diverse, with the Nigerian population showing higher diversity. Also the Nigerian chicken population was at neutral equilibrium while chickens from Togo, Liberia and Gambia had experienced sudden population expansions. The Nigerian and Liberian chicken populations were the most genetically distant of the sampled populations in the sub-region. The study also revealed the phylogeographic sub-structuring of the West African village chicken populations, with three major haplotypes restricted to Nigeria, being the first such report from the sub-region using mtDNA.

## VI. Recommendation

Findings from this study could be exploited for genetic improvement of the West African village chicken through breeding. It may also be necessary to carry out similar studies using longer segments of the mtDNA genome, over a greater geographic area of the West African region, in order to better understand the genetic diversity and evolutionary origins of its village chickens.

## References

- [1]. Adebambo, A. O., Mobegi, V. A., Mwacharo, J. M., Oladejo, B. M., Adewale, R. A., Ilori, L. O., Makanjuola, B. O., Afolayan, O., Bjornstad, G., Jianlin, H. and Hanotte, O. (2010). Lack of phylogeographic structure in Nigerian village chickens revealed by mitochondrial DNA D-loop sequence analysis. *International Journal of Poultry Science* 9: 503 – 507.
- [2]. Ajibike, A.B., Adeleye, O.O., Ilori, B.M., Osinbowale, D.A., Adeniyi, O.A., Durosaro, S.O., Sanda, A.J., Adebambo, O.A. and Adebambo, A.O. (2017). Genetic diversity, phylogeographic structure and effect of selection at the mitochondrial hypervariable region of Nigerian chicken populations. *Journal of Genetics* 96(2) 959-968.
- [3]. Branine, M. (2011). *Managing Across Cultures: Concepts, Policies and Practices*. p. 437. SAGE Publications Ltd, London.
- [4]. Ceccobelli, S., Di Lorenzo, P., Lancioni, H., Monteagudo Ibanez, L.V.V., Tejedor, M.T.T., Castellini, C., Landi, V., Martinez Martinez, A., Delgado Bermejo, J.V.V., Vega Pla, J.L.L., Leon Jurado, J.M.M., Garcia, N., Attard, G., Grimal, A., Stojanovic, S., Kume, K., Panella, F., Weigend, S. and Lasagna, E. (2015). Genetic diversity and phylogeographic structure of Mediterranean chicken breeds assessed with microsatellites and mitochondrial DNA. *Livestock Science* 175: 27–3628
- [5]. Chen, J., Glemin, S. and Lascoux, M. (2017). Genetic diversity and the efficacy of purifying selection across plant and animal species. *Molecular Biology and Evolution* 34(6):1417-1428.
- [6]. Cho, C.Y., Lee, P.Y., Ko, U.K., Kim, H.K., Park, M.N. and Yeon, S.H. (2010). mtDNA D-loop showing the multiple maternal origins of Korean native chicken. Unpublished. Direct submission (23-DEC-2010) to Genbank.
- [7]. Cuc, N. T. K., Simianer, H., Groeneveld, L.F. and Weigend, S. (2011). Multiple maternal lineages of Vietnamese local chickens inferred by mitochondrial D-loop sequences. *Asian-Australian Journal of Animal Science* 24:155-161.

- [8]. Eltanany, M.A and Hemeda, S.A. 2016. Deeper insight into maternal genetic assessments and demographic history for Egyptian indigenous chicken populations using mtDNA analysis. *Journal of Advance Research*, 7, 615-623.
- [9]. Excoffier, L., Laval, G. and Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47 - 50.
- [10]. Excoffier, L. and Lischer, H. E. L. (2010). ARLEQUIN suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Eco. Res.* 10:564–567.
- [11]. Food and Agriculture Organisation (FAO) (2007a). Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. FOA, Rome, Italy.
- [12]. Food and Agriculture Organisation (FAO) (2007b). The state of the world's animal genetic resources for food and agriculture, edited by Rischkowsky, B. and Pilling, D. Rome, Italy.
- [13]. Food and Agriculture Organisation (FAO) (2011a). Draft guidelines on molecular genetic characterization of animal genetic resources. Item 5.2 of the Provisional agenda, commission on genetic resources for food and agriculture, Thirteenth regular session, Rome, 18-22 July 2011.
- [14]. Food and Agriculture Organisation (FAO) (2011b). Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9, Rome, Italy.
- [15]. Frankham, R., Ballou, J.D., Briscoe, D.A. (2010). Introduction to conservation genetics. Second Ed. Cambridge University press, UK.
- [16]. FAOSTAT (2012). Food and Agriculture Organisation Statistical Yearbook 2012. FAO, Rome, Italy. <http://faostat.fao.org/>. Accessed August 23, 2012
- [17]. FAOSTAT (2017). [www.fao.org/faostat/en/data/fao](http://www.fao.org/faostat/en/data/fao). Accessed March 12, 2018.
- [18]. Frankham, R., Ballou, J.D., Briscoe, D.A. (2010). Introduction to conservation genetics. Second Ed. Cambridge University press, UK.
- [19]. Hahn, M.W., Rausher, M.D. and Cunningham, C.W. (2002). Distinguishing between selection and population expansion experimental lineage of bacteriophage T7. *Genetics* 161:11-20.
- [20]. Ham, A. (2009). West Africa, p. 79. Lonely Planet. UK.
- [21]. Harpending, H. C. (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology* 66:591-600.
- [22]. Hassaballah, K., Zeuh, V., Lawal, R.A., Hanotte, O. and Sembene, M. (2015). Diversity and Origin of Indigenous Village Chickens (*Gallus gallus*) from Chad, Central Africa. *Advances in Bioscience and Biotechnology*, 6, 592-600.
- [23]. Herrera, M.B., Thomson, V.A., Wadley, J.J., Piper, P.J., Sulandari, S., Dharmayanthi, A.B., Kraitsek, S., Gongora, J., and Austin, J.J. (2017). East African origins for Madagascan chickens as indicated by mitochondrial DNA. *Royal Society Open Science* 4: 160787
- [24]. Hillel, J., Groenen, M. A. M., Tixier-Boichard, M., Korol, A. B., David, L., Kirzhner, V. M., Burke, T., Barre-Dirie, A., Crooijmans, R. P. M. A., Elo, K., Feldman, M. W., Freidlin, P. J., Maki-Tanila, A., Ortwijn, M., Thomson, P., Vignal, A., Wimmers, K. and Weigend, S. (2003). Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. *Genetics, Selection, Evolution*. 35: 533 – 557
- [25]. Holsinger, K. E. and Weir, B.S. (2009). Genetics in geographically structured populations: defining, estimating and interpreting FST. *Nature Reviews Genetics*. 10 (9): 639–650.
- [26]. Hoque, M. R., Choi, N.R., Sultana, H., Kang, B.S., Heo, K.N., Hong, S.K., Jo, C. and Lee, J.H. (2013). Phylogenetic analysis of a privately-owned Korean native chicken population using mtDNA d-loop variations. *Asian-Australian Journal of Animal Science* 26:157-162.
- [27]. ISS Today (2017). Africa's population boom a burden or opportunity? By Julia Bello-Schlunemann, an online publication of the International Institute of Security Studies, South Africa, 15 may 2017. Retrieved from <https://issafrika.org/iss-today> on 13th March 2018.
- [28]. Jobling, M.A., Hurles, M.E. and Tyler-smith, C. (2004). *Human Evolutionary Genetics*. Garland science, New York/ Abingdon, UK.
- [29]. Joshi, J., Salar, R. K., Banerjee, P., Upasna, S., Tantia, M. S. and Vijh, R. K. (2013). Genetic Variation and Phylogenetic Relationships of Indian Buffaloes of Uttar Pradesh. *Asian Australasian Journal of Animal Science* 26 (9): 1229–1236
- [30]. Kawabe, K. Worawut, R., Taura, S., Shimogiri, T., Nishida, T. and Okamoto, S. (2014). Genetic Diversity of mtDNA D-loop Polymorphisms in Laotian Native Fowl Populations. *Asian Australasian Journal of Animal Science* 27 (1): 19-23
- [31]. Kaya, M. and Yildiz, M.A. (2008). Genetic Diversity among Turkish Native Chickens Denizli and Gerze, estimated by Microsatellite Markers. *Biochemical Genetics*, 46:480-491.
- [32]. Komiyama, T., Ikeo, K. and Gojobori, T. (2003). Where is the origin of the Japanese gamecocks? *Gene* 317: 195 – 202.
- [33]. Leroy, G., Kayang, B.B., Youssao, I.A.K., Yapi-Gnaoré, C.V., Osei-Amponsah, R., Loukou, N.E., Jean-Claude, F., Benabdeljelil, K., Bed'hom, B., Tixier-Boichard, M. and Rognon, X. (2012). Gene diversity, agro ecological structure and introgression patterns among village chicken populations across North, West and Central Africa. *BMC Genetics*, 13:34-45.
- [34]. Librado, P. and Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451 - 1452.
- [35]. Miyake, T. (1997). *Gallus gallus* mitochondrial DNA for D-loop region. Published Only in Database. Direct Submission (02-OCT-1997).
- [36]. Mtileni, B. J., Muchadeyi, F. C., Maiwashe, A., Groeneveld, E., Groeneveld, L. F., Dzama, K. and Weigend, S. (2011). Genetic diversity and conservation of South African indigenous chicken populations. *Journal Animal Breeding Genetics*. 128: 209 – 218
- [37]. Muchadeyi, F. C., Eding, H., Simianer, H., Wollny, C. B. A., Groeneveld, E. and Weigend, S. (2008). Mitochondrial DNA D-loop sequences suggest a Southeast Asian and Indian origin of Zimbabwean village chickens. *Animal Genetics* 39: 615 – 622.
- [38]. Mwacharo, J. M., Bjornstad, G., Mobegi, V., Nomura, K., Hanada, H., Amano, T., Jianlin, H and Hanotte, O. (2011). Mitochondrial DNA reveals multiple introductions of domestic chickens in East Africa. *Molecular Phylogenetics of Evolution* 58:374–382. Nei, M. (1987). *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- [39]. Nisar, A., Waheed, A., Khan, S., Feng, X. and Shah, A.H. (2018). Population structure, genetic diversity and phylogenetic analysis of different rural and commercial chickens of Pakistan using complete sequence of mtDNA D-loop. *Mitochondrial DNA Mapping Sequence Annals*, 1-8. In press. Publication Status: Available-Online prior to print, PUBMED 30231799. Direct Submission
- [40]. Nishibori, M., Hayashi, T., Tsudzuki, M., Yamamoto, Y., Yasue, H. (2001). Complete sequence of the Japanese quail (*Coturnix japonica*) mitochondrial genome and its genetic relationship with related species. *Animal Genetics* 32:380–5.
- [41]. Oka, T., Ino, Y., Nomura, K., Kawashima, S., Kuwayama, T., Hanada, H., Amano, T., Takada, M., Takahata, N., Hayashi, Y., Akishinonomiya, F. (2007). Analysis of mtDNA sequences shows Japanese native chickens have multiple origins. *Animal Genetics* 38, 287–293.

- [42]. Okello, J. B. A., Nyakana, S. Masembe, C. Siegismund, H.R. and Arctander, P. (2005). Mitochondrial DNA variation of the common hippopotamus: evidence for a recent population expansion. *Heredity* 95:206 - 215
- [43]. Osman, S.A.M., Yonezawa, T. and Nishibori, M. (2016). Origin and genetic diversity of Egyptian native chickens based on complete sequence of mitochondrial DNA D-loop region1. *Poultry Science* 95:1248–1256.
- [44]. Palacios, E.Y.; Álvarez, L.; Muñoz, J. (2016). Genetic diversity of Creole hens of the Colombian southwest. *Archivos de Zootecnia*, vol. 65 (249), pp. 73-78.
- [45]. Pramual, P., Meeyen, K., Wongpakam, K. and Klinhom, U. (2013). Genetic diversity of Thai native chicken inferred from mitochondrial DNA sequences. Unpublished. Direct Submission (22-MAR-2013).
- [46]. Ramadan, S., Kayang, B.B., Inoue, E., Nirasawa, K., Hayakawa, H., Ito, S. and Inoue-Murayama, M. (2012). Evaluation of genetic diversity and conservation priorities for Egyptian chickens. *Open Journal of Animal Sciences*. 2 (3): 183-190.
- [47]. Rikaterere, D. and Luseba, D. (2010). *Ethnoveterinary Botanical Medicine: Herbal Medicines for Animal Health*, CRC Press, 450pp.
- [48]. Rogers, A. R. (1995). Genetic evidence for a Pleistocene population expansion. *Evolution* 49:608-615.
- [49]. Rogers, A.R. and Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* 9(3): 552-569.
- [50]. Rudresh, B.H., Murthy, H.N.N., Jayashankar, M.R., Nagaraj, C.S., Kotresh, A.M., Byregowda, S.M. (2015). Microsatellite based genetic diversity study in indigenous chicken ecotypes of Karnataka, *Veterinary World* 8(8): 970-976.
- [51]. Savolainen, P., Zhang, Y.P., Luo, J., Lundeberg, J. and Leitner, T. (2002). "Genetic evidence for an East Asian origin of domestic dogs," *Science*, vol. 298, no. 5598, pp. 1610–1613.
- [52]. Schneider, S., and Excoffier, L. (1999). Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152:1079-1089.
- [53]. Seddon, D. (2004). *A Political and Economic Dictionary of the Middle East*. Routledge Publishers, London.
- [54]. Silva, P., Guan, X., Ho-Shing, O., Jones, J., Xu, J., Hui, D., Notter, D., Smith, E., (2008). Mitochondrial DNA-based analysis of genetic variation and relatedness among Sri Lankan indigenous chickens and the Ceylon Jungle Fowl (*Gallus lafayetti*). *Animal Genetics* 40:1–9.
- [55]. Soltan, M. E., Enab, A.A., Abou-Elwela, E.M. and Farrag, S.A. (2016). Genetic characterization of a local Egyptian chicken strain norfa using microsatellite markers, 9th International Poultry Conference – Proceeding. 7-10 November 2016, Hurghada, Red Sea – Egypt. P.188-202.
- [56]. Speth, P. (2010). *Impacts of Global Change on the Hydrological Cycle in West and Northwest Africa*, p. 33. Springer.
- [57]. Tajima, F. (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics* 105: 437-460.
- [58]. Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123:585-595.
- [59]. Tajima, F. (1996). The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. *Genetics* 143: 1457-1465
- [60]. TheGuardian.com 24 July 2003. "Peacekeepers 'rushing' to Liberia". Retrieved July 14, 2019.
- [61]. Wani, C.E., Ibrahim, A. Y., Muntasir E. I., and Hassan H. M. (2014). Molecular Characterization of Sudanese and Southern Sudanese Chicken Breeds Using mtDNA D-Loop. *Genetics Research International* Volume 2014 Article ID: 928420, 8 pages.
- [62]. World Bank, (2018). <http://www.worldbank.org/en/country/nigeria/overview>. Accessed 17 March 2018.10:00 GMT
- [63]. World Health Organisation, (2019). Ebola in Liberia: Misery and despair tempered by some good reasons for hope. <https://www.who.int/csr/disease/ebo>. Retrieved July 14<sup>th</sup> 2019. 23.44 GMT
- [64]. Yap, F.C., Yan, Y.J., Loon, K.T., Zhen, J.L.N., Kamau, N.W. and Kumaran, J.V. (2011). Phylogenetic analysis of different breeds of domestic chickens in selected areas of Peninsular Malaysia inferred from partial cytochrome b gene information and RAPD Markers. *Animal Biotechnology* 21(4): 226-240.

Noah E.T. Tor, et. al. "Genetic diversity and phylogeographic structure of West African village chickens based on a 920bp segment of the mtDNA D-loop." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 14(4), 2021, pp. 26-36.