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

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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±0.020) and haplotype diversity (0.43±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 (FST=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.*

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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 *mt*DNA 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^{o} East longitude (Speth, 2010). According to the United Nations scheme of African regions, the region includes 18 countries, inclusive of the Island of Saint Hellena, 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 fasted 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).

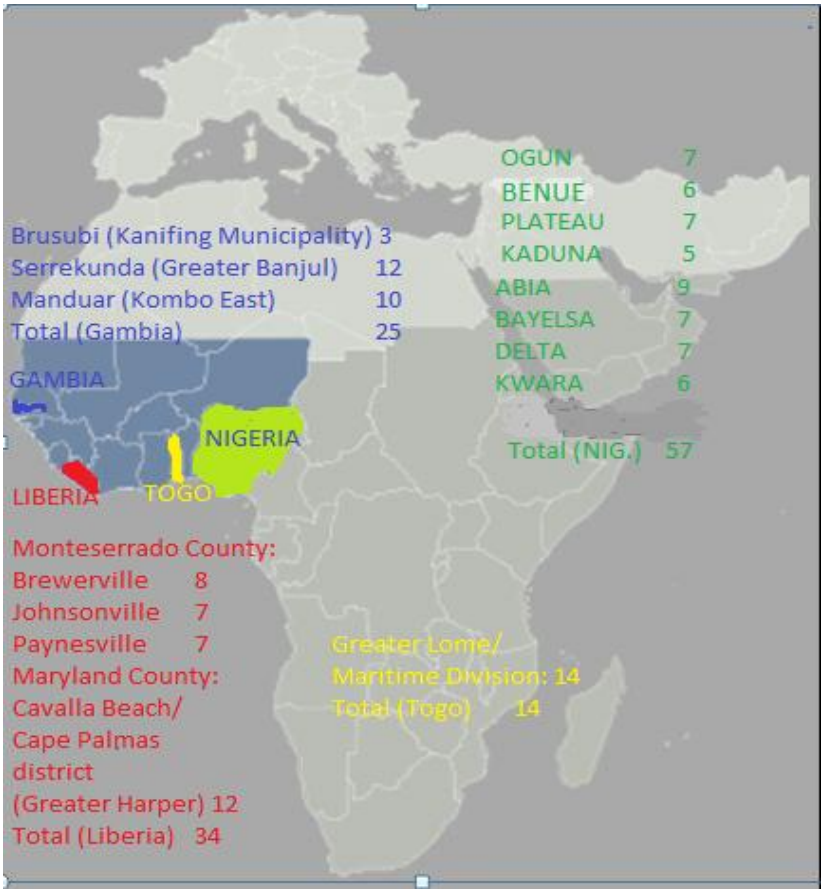

Figure 1: Map of the study area showing a breakdown of the sampling locations.

Table 1: Details of the samples downloaded from Genbank.

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

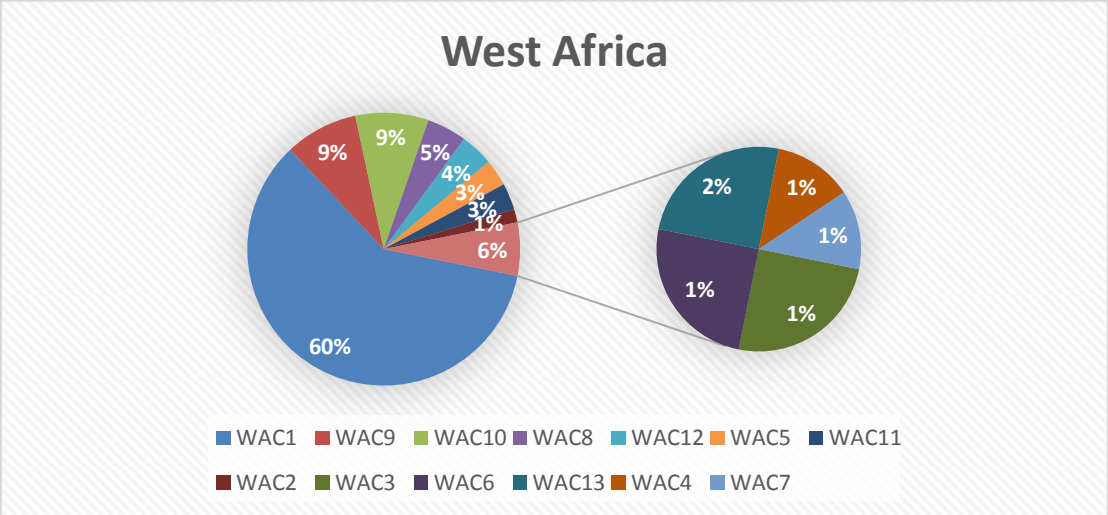

Figure 2: Summary of percentage occurrence of the 13 haplotypes observed in the mtDNAD-loop of 127 sampled West African village chickens.

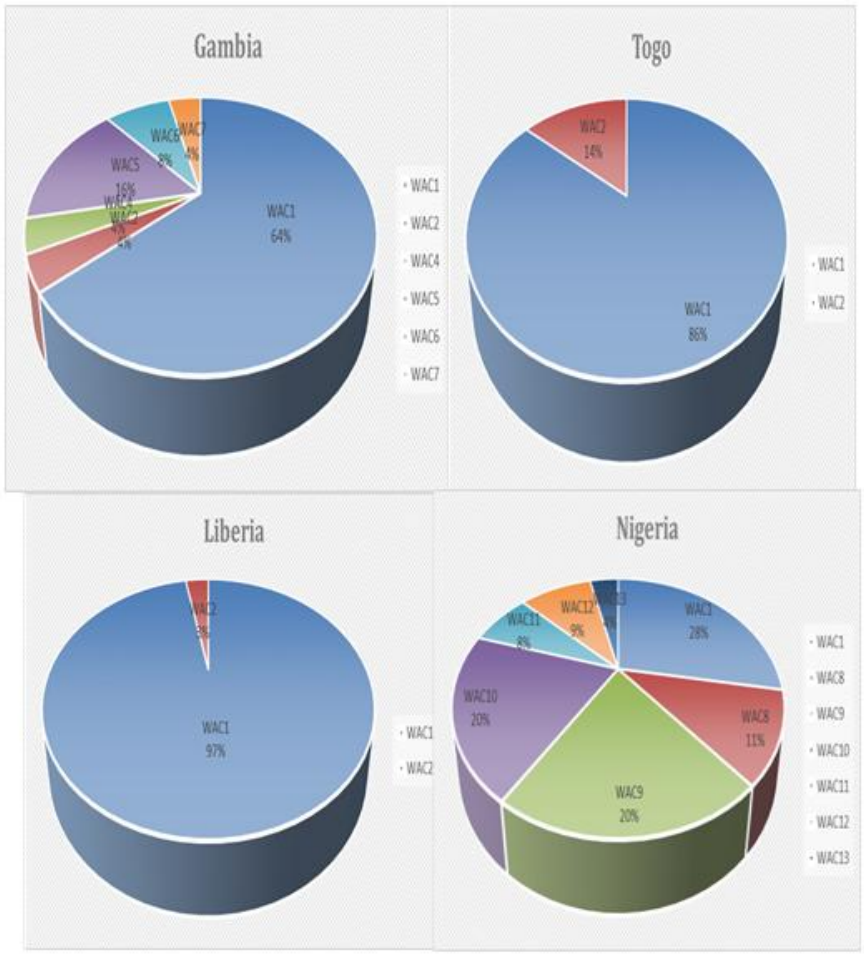

Figure 3: Geographic distribution of the 13 haplotypes observed in the mtDNA D-loop of the sampled West African chicken populations

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

	1111222222222233333346888 5899001233446912444378 99 2457270814166557034119 01	Population				
		Gambia	Togo	Liberia	Nigeria	N
Ref	TTCGCACCCAATATCATACTGTCA					
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
θ_H	1.02±0.45	0.27±0.19	0.05±0.05	3.86±0.67	1.30±1.5
θ_k	2.18±0.85	0.37±0.08	0.27±0.06	1.92±0.83	1.18±0.87
θ_s	1.85±0.89	0.31±0.31	0.24±0.24	4.17±1.45	1.65±1.60
θ_π	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

θ_H: Theta value based on expected homozygosity; θ_k: Theta value based on number of alleles; θ_s: Theta value based on number of segregating sites; θ_π: 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
θ_0	0.00	0.00	0.00	0.00	0.00
θ_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 ≥ 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. ≥ 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 *Fs*	-2.23	0.19	-1.32	7.25	0.97
P (Sim. Fs < Obs. Fs)	0.037	0.32	0.05	0.99	0.35

T: time of expansion; θ_0 and θ_1: mutation parameters

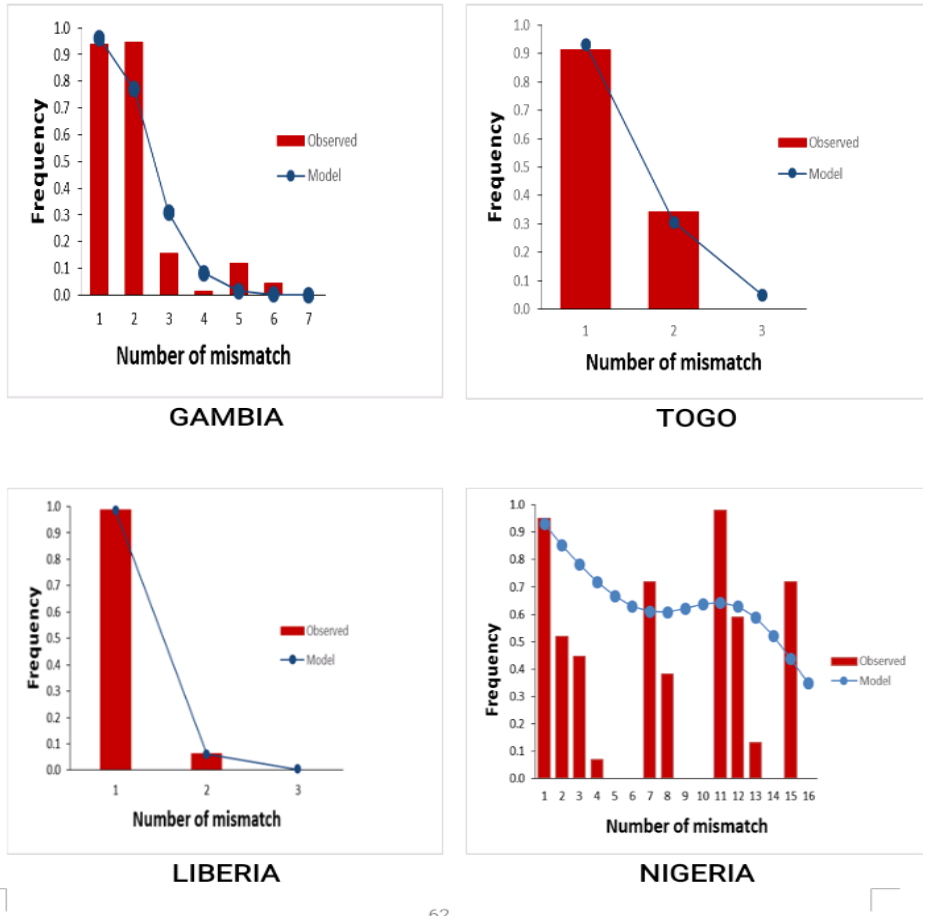

Figure 4: Mismatch distributions of West African village chicken populations

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	
Total	126	230.913	1.95713		

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 FST 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±0.02) and Gambia (0.58± 0.11) in this study were higher than previous reports on Nigerian chickens (0.4217±0.0419, Adebambo *et al.,* 2010; 0.39±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±0.03, Osman *et al.,* 2016; 0.608±0.09, Eltanany and Hemeda, 2016); East Africa, 0.638±0.024(Mwacharo *et al.,* 2011); Sudan and South Sudan, 0.577 (Wani *et al.,* 2014); Chad, 0.541±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±0.05) and Togolese (0.26±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 value0 (0.083±0.02) from this study was higher than the observations of Osman *et al.* (2016) and Eltanany and Hemeda (2016) (Egypt, 0.0045±0.0013 and 0.004±0.001 respectively); Mwacharo *et al.* (2011) (East Africa, 0.00745±0.00042); Wani *et al.* (2014) (The Sudans, 0.00282); Hassaballah *et al.* (2015) (Chad, 0.00312±0.00049), Adebambo *et al.* (2010) (Nigeria, 0.00157±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±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 *Fs* 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 *Fs* 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.

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