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Original Article

Phylogenetic Polymorphisms of Gambian and Togolese Rural Chickens Assessed with Mitochondrial DNA

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Abstract: *Gambia and Togo are geographically, two of the smallest of the sixteen-member block of Economic Community of West African States (ECOWAS). Like the rest of Africa, not much is known about the phylogenetic variations, evolution and molecular (DNA) signatures of the indigenous chicken populations of the two countries. To achieve this, genomic DNA was extracted from a total of 40 village chickens of both countries using FTA cards, following standard protocols. Sanger sequencing of the D-loop amplicons of the mitochondrial DNA was outsourced to StabVida Laboratory, Portugal, using specific primers. Multiple alignments of a final length of 920bp of the sequences with a reference sequence reveals 8 SNPs. Reconstruction of evolutionary origins using MEGA 7 and NETWORK 4.6 matrix suggests that Gambian and Togolese chickens are most likely of south Asian descent. Molecular indices revealed that the chickens in the two populations are of comparatively lower genetic diversity, nonetheless, the Gambian population was slightly more diverse than the Togolese. This finding may be employed in the genetic conservation strategies of chickens in the two countries.*

Keywords*: Chicken, Gambia, Mitochondrial DNA, Phylogenetics, Togo.*

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). These village chickens have been observed to be hardy and well adapted to the local environment with excellent ability to convert wastes and available feed around the houses and village into highly nutritious products (Mtileni *et al.,* 2009). However, there is a dearth of molecular information on the animal genetic resource in the region (FAO, 2007). This study aimed at reconstructing the possible evolutionary origins of Gambian and Togolese chickens out of Asia and evaluating their genetic diversities at the highly polymorphic section of D-loop region of the mt DNA.

II. MATERIALS AND METHODS

The birds used for this study were village chickens from rural areas of The Gambia and Togo, two countries on the West coast of Africa (Figures 1 and 2). Sampling was done from village settlements that were at least 20km apart. Blood was collected from the wing veins of the chickens onto FTA classic cards, using needles and syringes

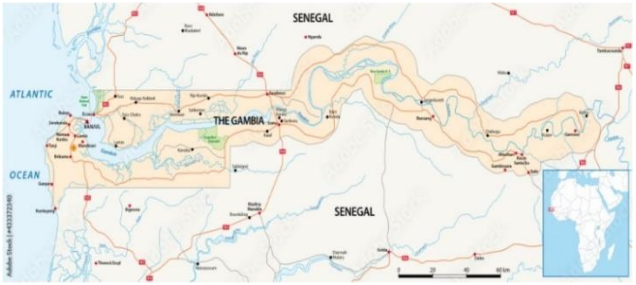

Figure 1: Map of the Gambia

Source: https://as2.ftcdn.net/v2/jpg/

Figure 2: Map of Togo

Source: https://stock.adobe.com/ng/images/togo

A. Genomic DNA isolation and Polymerisation

DNA was isolated from 40 dried FTA classic papers containing genomic DNA samples from 24 Gambian and 14 Togolese chickens, following standard procedures (www.whatman.com). Cloning of the target D-loop hypervariable segment was achieved with the primer AV1F2:F- 5′-AGGACTACGGCTTGAAAAGC-3′ and R-3′-TGCTTAAGGTTAATTACTGCTG-5′ (Nishibori,*et al.,* 2001), after the following PCR (Polymerase Chain Reaction) conditions: a final volume of 30µl containing 1µl of genomic DNA, 2.5mM of each dNTPs, 14pmol of primer, 1.5mM $Mgcl_2$ 1 x PCR buffer containing 10mM Tris-HC1 (PH 8.3) and 50mM KCL and 1.26U Taq DNA polymerase (Roche Applied Sciences, Germany). With thermo-cycling conditions at initial denaturation of 94^{o}C for 2 minutes, followed by 10 cycles at 94^{o}C for 15 seconds, annealing at 58^{o}C for 30 seconds and extension at 72^{o}C for 40 seconds. Final extension step was at 72^{o}C for 10 minutes. DNA isolation and PCR were carried out at ACUTIG genetics Laboratory, Abeokuta, Nigeria. Resultant PCR products were sequenced at Stab Vida Laboratory, Portugal, based on Sanger's di-deoxy chain termination method.

B. Phylogenetic Analysis

Multiple alignments of all sequences to detect nucleotide variations and reconstruct evolutionary relationship with Asiatic and other African chickens were conducted with ClustalW in MEGA 7 application. Similarly, results from median-joining network using NETWORK 4.6 were used to confirm the dendrograms. DnaSP V.5.10 software was used to evaluate the number of haplotypes and their diversities. Mismatch neutrality tests were determined with Arlequin 3.5.1.3.

C. Haplotype Polymorphisms:

The results for haplotype polymorphisms of Gambian and Togolese chickens and their geographic distributions are shown in Figures 1 and 2. The results show that seven haplotypes (WAC1-7) were detected, of which WAC1 was the most frequent and widely distributed. The result revealed seven haplotypes (WAC1-7). The number of haplotypes was at par with that reported by Eltanany and Hemeda (2016) (7) but generally lower than other reports across Africa (Adebambo *et al.,* 2010; Mwacharo *et al.,* 2011 and Hassaballah *et al.,*2015), an indication of relatively low molecular genetic diversity among the studied chicken populations. The mean haplotype diversity also follows a similar pattern, suggesting low genetic diversity among Gambian and Togolese chickens, indicative that the Gambian and Togolese chickens may be products of relatively more recent evolution.

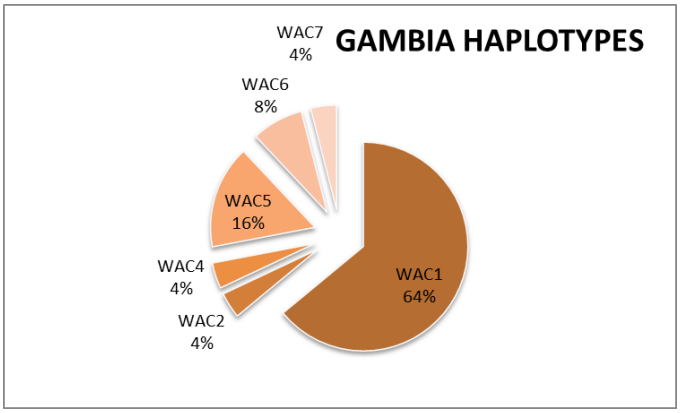

Figure 3: Percentage Distribution of Gambian Village Chicken Haplotypes

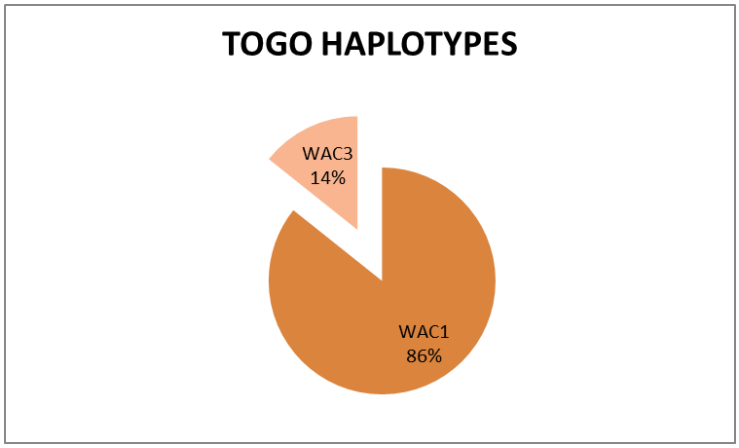

Figure 4: Percentage Distribution Togolese Village Chicken Haplotypes

D. Nucleotide Polymorphisms:

Eight polymorphic sites were observed among the studied village chicken populations (Table 1). Seven of these segregating sites were among the Gambian chickens while only one was seen in the Togolese population (Table 2). Seven and one substitutions were detected in Gambia and Togo respectively. No insertions nor deletions were observed. The low number of polymorphic sites recorded in this study is a further indication of low genetic variability of the Gambian and Togolese chickens. The relatively higher mean nucleotide diversity (0.025±0.02) compared to others; Wani *et al.* 2014 (The Sudans, 0.00282), Adebambo *et al.* 2010 (Nigeria, 0.00157±0.0137), could be evidence of purifying selection.

Table 1: **Nucleotide Polymorphisms Observed in the Sampled Populations**

	222233888 034924899 211574901	Gambia	Togo	N
Ref	CCATACTCA			
GT1	..C.....	16	12	28
GT2	.TC.....	1	-	1
GT3	..C.G....	-	2	2
GT4	..C..TATC	1	-	1
GT5	..CC.....	4	-	4
GT6	..C..T...	2	-	2
GT7	T.C.....	1	-	1

The first column signifies identification number of sampled chickens. Vertically oriented numbers indicate the variable sites position. Dots (.) indicate identity with the reference (Ref) sequence (GenBank accession number AB829474) (Osman et al., 2016) sequences shown are only the variable sites.

Table 2: **Standard, Molecular and Mismatch Diversity Indices from mtDNA of Studied Chicken Populations**

	Gambia	**Togo**	**Mean**
Number of Sequences	25	14	19.50
Number of Haplotypes	6	2	4
Haplotypes diversity (Hd)	0.58±0.11	0.26±0.14	0.42±0.12
Nucleotide diversity	0.04±0.03	0.01±0.01	0.025±0.02
Mean number of pairwise differences	0.90±0.64	0.26±0.31	0.58±0.47
Sum of square frequency	0.45	0.76	0.61
Number of observed transitions	5	1	3
Number of observed transversions	2	0	1
Number of substitutions	7	1	2
Number of observed indels	0	0	0
Number of polymorphic sites	7	1	4
Tajima's *D*	-1.590	-0.341	-0.97
Fu's Fs	-2.23	0.19	-1.02

E. Evolutionary Origins

Results of the neighbour joining phylogenetic analysis based on the reference sequences of Miao *et al.,* 2013 (Figure 3) suggests an Indian maternal evolutionary descent for the chickens of Gambia and Togo, as all the sequences cluster with that *Gallus gallus murghi* which is the wild Indian Red Jungle Fowl, in clear distinction from the other Gallus sub species and clades. In the same vein, result of the median joining network analysis (Figure 4), also shows all Gambian and Togolese chickens (blue) in the same node with *Gallus g. murghi* (red), indicative of an Indian matrilineage.

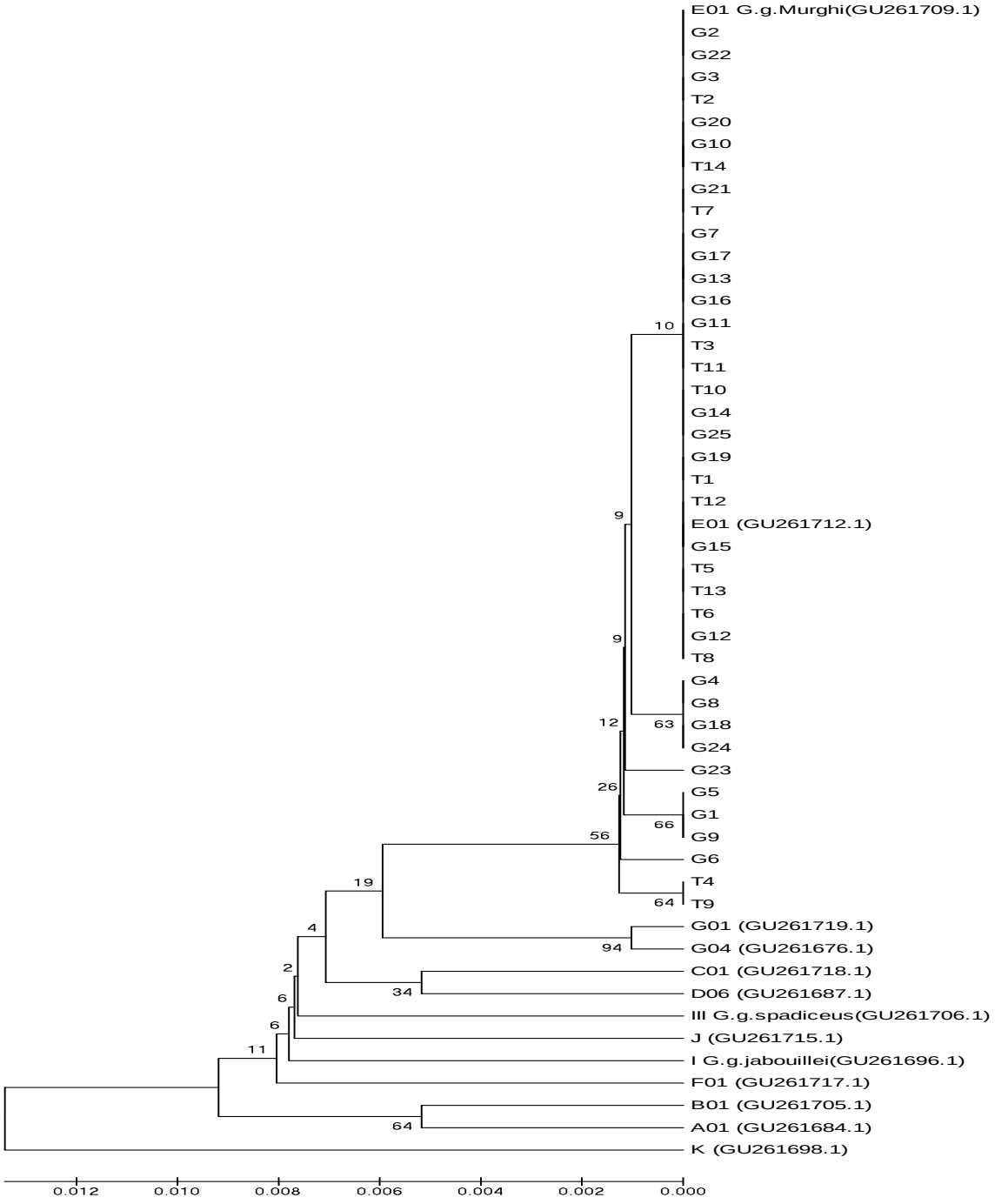

Figure 5: Phylogenetic Results

F. Tests of Selection Neutrality

The tests of selection neutrality are also contained on Table 2. The negative Tajima's D for the two populations is suggestive of purifying selection leading to sudden population expansion in the recent past (Tajima, 1996) and excess rare alleles in the populations implying that the populations may not be at equilibrium or neutrality of selection (Hahn *et al.,*2002).

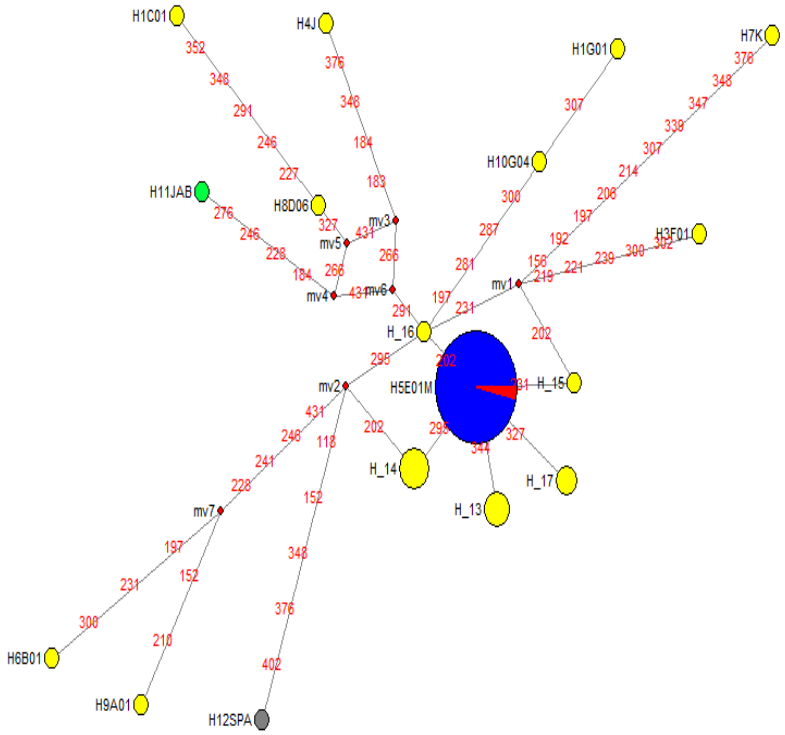

Figure 6: Network Results

III. CONCLUSION

The mtDNA marker indicated that both Gambian and Togolese chickens are of low genetic diversity, at population disequilibrium and descendants of the Indian red Jungle fowl, which might have been introduced into the two countries most likely by land through the Middle East via the Sinai Peninsula following trans-Saharan trade routes. The findings from this study could be valuable for their conservation and genetic enhancement through crossbreeding.

Figure 3: Phylogenetic results- Neighbour-joining phylogenetic tree showing the relationship of Gambian and Togolese chickens with some the major chicken clades defined by Miao *et al.* (2013), alongside other sub-species of gallus. The Genbank accession numbers of the sequences are given in parentheses.

Figure 4: Network Results- Median-joining network result for the relationship between the studied Gambian and Togolese chicken haplotypes and the international chicken haplotypes of Miao *et al.* (2013). Area of each circle is proportional to the frequency of the corresponding haplotype(s). Different populations are distinguished by use of colour codes (blue = Gambian and Togolese, red = Clade E (*G.g. murgha*), green = *G.g. jaboullei*, gray = *G.g. spadiceus*, Yellow = East Asia.

Interest Conflicts:

The authors declare that there is no conflict of interest concerning the publishing of this paper.

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