EVOLUTIONARY AND HAPLOTYPIC DIVERSITY STUDIES AMONG GAMBIAN AND TOGOLESE RURAL CHICKENS USING MTDNA

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ABSTRACT

Gambia and Togo are among the smallest in terms of geographic size, of the sixteen-member block of Economic Community Of West African States (ECOWAS). Like the rest of Africa, not much is known about the evolution and molecular genetic 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 mtDNA 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 for the chickens in the two countries.

Keywords: Gambia, Togo, Chicken, diversity, mtDNA

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 mtDNA.

MATERIALS AND METHODS:

DNA was isolated from 40 dried FTA classic papers containing gDNA 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 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°C for 2 minutes, followed by 10 cycles at 94°C for 15 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 40 seconds. Final extension step was at 72°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. 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.

RESULTS AND DISCUSSION

Table 1: Nucleotide Polymorphisms observed in the sampled populations

	222233888 034924899 211574901			N
		Gambia	Togo	
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

The result revealed seven haplotypes and eight polymorphic sites. 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). The mean haplotype diversity also follows a similar patern, suggesting low genetic diversity among Gambian and Togolese chickens, indicative that the Gambian and Togolese chickens may be products of relatively more recent evolution. The relatively higher 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.

Results of the neighbour joining phylogenetic analysis based on the reference sequences of Miao *et al.,* 2013 (Figure 1) suggests an Indian maternal 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 2), also shows all Gambian and Togolese chickens (blue) in the same node with *Gallus g. murghi* (red), indicative of an Indian matrilineage.

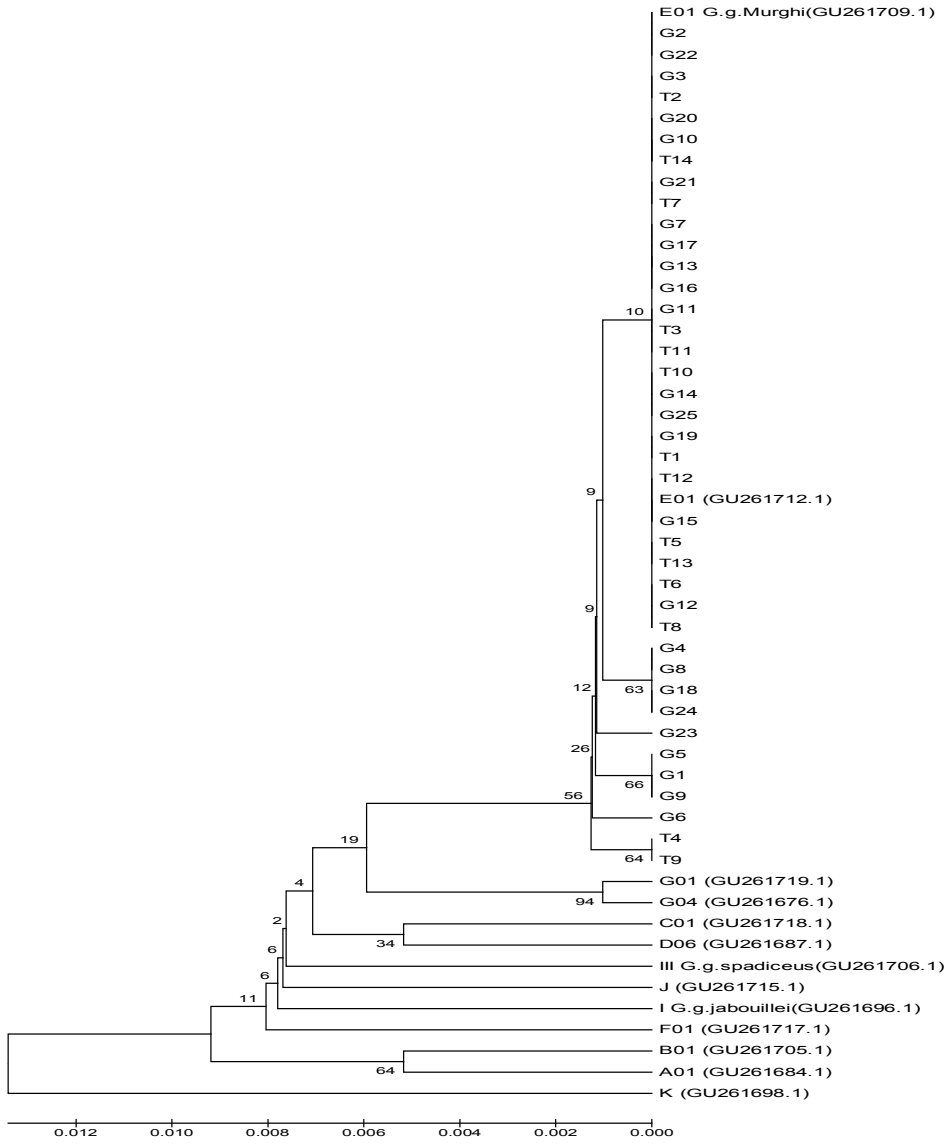

Figure 1: Neighbour-joining tree showing the relationship of Gambian and Togolese chickens with some the major chicken clades of Miao *et al.* (2013).

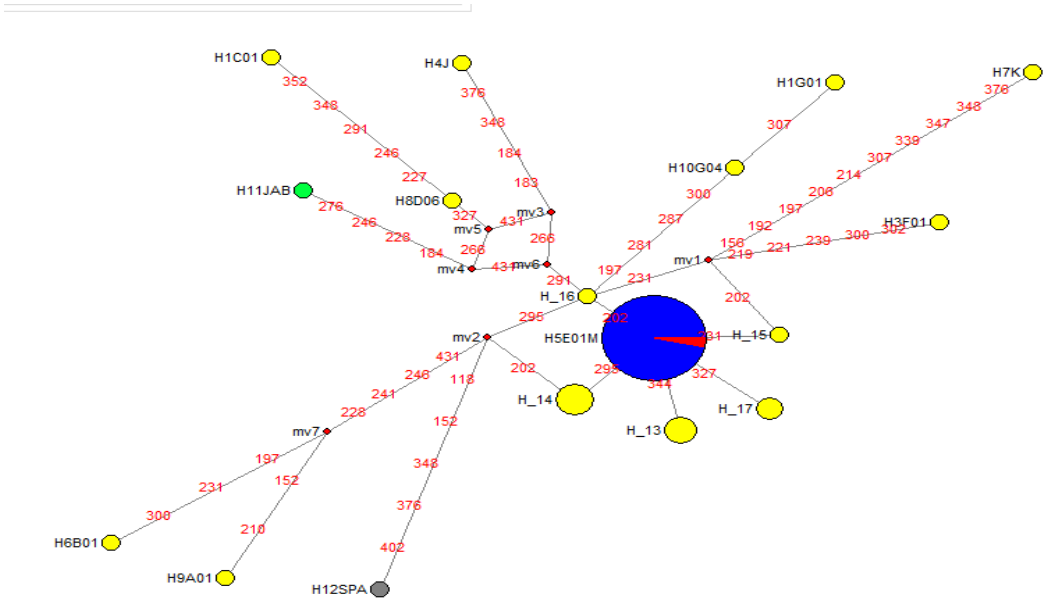

Figure 2: Median-joining network result for the relationship between the studied chicken haplotypes and the international chicken haplotypes defined by Miao *et al*. (2013). Area of each circle is proportional to the frequency of the corresponding haplotype(s). Different populations are distinguished 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.

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 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.

REFERENCES

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.

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.

Food and Agriculture Organisation (FAO) 2007. Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. FOA, Rome, Italy.

Miao,Y.W., Peng, M.S., Wu,G.S., Ouyang,Y.N., Yang,Z.Y.,Yu, N., Liang,J. P., Pianchou,G., Beja-Pereira,A., Mitra,B., Palanichamy,M.G., Baig,M., Chaudhuri, T. K., Shen,Y. Y.,Kong, Q. P., Murphy,R. W., Yao,Y. G. and Zhang,Y. P. 2013. Chicken domestication: an updated perspective based on mitochondrial genomes. *Heredity.* 110:277–282.

Mtileni, B. J., Muchadeyi, F.C., Maiwashe, A., Phitsane, T. E., Halimani, M., Chimonyo, G. and Dzama,
G. 2009. Characterisation of production systems for indigenous chicken genetic resources ofSouth Africa. *Appl. Anim. Husb. Rural Dev*. 2:18–22.

Mwacharo, J. M., Bjornstad, G., Mobegi, V., Nomura, K., Hanada, H., Amano, T., Jianlin, H and Hanotte, O. 2011. Mitochondrial DNAreveals multiple introductions of domestic chickens in East Africa.*Mol. Phylogenet. Evol*. 58:374–382.

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.

Rikaterere, D. and Luseba, D. 2010.*Ethnoveterinary Botanical Medicine: Herbal Medicines for Animal Health,* CRC Press, 450pp.