GENETIC DIVERSITY OF MITOCHONDRIAL DNA (MTDNA) D-LOOP SEQUENCES IN SIX IMPROVED TROPICALLY ADAPTED CHICKEN BREEDS (iTABS)

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Abstract
Improved tropically adapted chicken breeds (iTABs) are characterized as dual purpose, low-input high-output birds suitable for smallholder poultry (SHP). A 592-bp of mitochondrial DNA (mtDNA) D-loop region of 77 iTABs from six populations was amplified to assess the genetic differentiation of six iTABs introduced to smallholder poultry farmers in Imo State, Nigeria by the African Chicken Genetic Gains (ACGG) project. The specific objectives were to determine the level of polymorphism among and within the breeds, origin, phylogenetic relationship and evolutionary distance between and among these breeds. The haplotype diversity and nucleotide diversity of the iTABs were 0.80±0.025 and 0.391±0.013, respectively. 14 haplotypes were identified from 62 polymorphic sites. These haplotypes clustered into three clades with 99.7 % of the total maternal genetic variations occurring within population. Sasso and Shika Brown had the least (0.385) genetic distance. The results indicate the existence of three distinct maternal lineages from Southeast Asia, Indian subcontinent and Southwest China evenly distributed among the iTABs. The high genetic diversity observed within population can be utilized for the long term genetic improvement and stabilization of the breeds.

Keywords: mtDNA, smallholder-poultry, Phylogenetics, Polymorphism.

Introduction
The improved tropically adapted chicken breeds (iTABs) are low-input high-output chickens suitable for smallholder poultry (SHP). The establishment of an effective long-term genetic improvement, multiplication and delivery system is essential for the adoption and continuous supply of the iTABs to SHP farmers. Understanding the genetic diversity and phylogenetic architecture of the iTABs using mitochondrial DNA (mtDNA) will contribute to the development of the long-term genetic improvement program. MtDNA is an extremely variable genome which has unique characteristics that make it an ideal genetic marker (Avise, 2004).Therefore, this study was conducted to determine the level of mitochondrial DNA polymorphism, and establish phylogenetic relationships among the iTABs introduced to SHP farmers in Imo State, Nigeria.

Materials and methods
A total of 77 blood samples were collected from six populations of iTABs (Noiler (No), FUNAAB

Alpha (Fu), ShikaBrown (Sb), Kuroiler (Ku), Sasso (Sa) and Fulani (Fi)) from three African Chicken Genetic Gains (ACGG) project sites in Imo State, Nigeria. Genomic DNA was extracted using standard phenol-chloroform method.

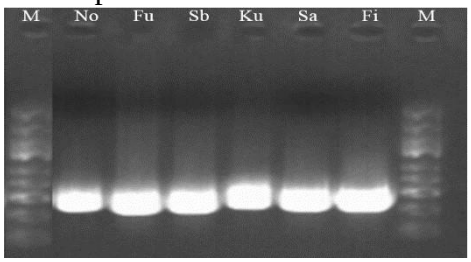

Figure 1**:** PCR Gel electrophoresis result of mtDNA D-loop region showing a 592-bp amplicon size for the iTABS: Noiler (No), FUNAAB Alpha (Fu), Shika Brown (Sb), Kuroiler (Ku), Sasso (Sa), and Fulani (Fi), M= 200-bp molecular –weight size marker.

PCR reactions were conducted at 96℃ for 15 mins, followed by 35 cycles consisting of 30 sec denaturation at 95℃, 30 sec annealing at 56℃ and 30 mins extension at 70℃, with final extension at 70℃ for 5 mins using a Gene Amp PCR System 9700 (USA). The PCR products were electrophoresed (120 V, 20 min) on 1.5% agarose gels, and purified before sequencing. A 592-bp of the mtDNA D-loop region was amplified, and sequenced (3730XL DNA Analyzer sequencer, Applied Biosystems) using primers from Mobegi *et al*. (2005): L16750 (5′AGGACTACGGCTTGAAAAGC-3′, accession No.: NC_001323, Desjardins and Morais, 1990) as the forward primers and H547 (5′-ATGTGCCTGACCGAGGAACCAG-3′, Accession No.: AB098668, Komiyama *et al*., 2003) as the reverse primer.

Genetic analysis: A 315-bp sequence fragment was subsequently analysed. FinchTV version 1.4.0 (www.geospiza.com/finchtv) was used to assemble, view and edit the sequences. The MEGA 7.0 (Tamura *et al*., 2015) was used to align the D-loop sequence to the *Gallus gallus* RefSeq (Accession No:KX987152.1), following 1000 bootstrap replicates, a maximum Likelihood (ML) tree was generated. 3 D-loop sequences of *Galliformes*; *Meleagris gallopavo*, *Cortunix japonica* and *Anas platyrhynchos* (Accession No: EF153719, AP003195 and MF069250, respectively), were the out-groups. Eight RefSeq of *Gallus sp.* and two sub-species from the most common haplotypes of different clades found in Asian domestic chickens were included in the analysis. Genetic distances and diversities were analysed using Mega 7.0 and DnaSP 6.11.01 (Librado *et al*., 2017).

Results and Discussion

The 77 sequences from 6 iTABs populations generated 14 haplotypes from 62 polymorphic sites (Figure 2). All variable sites were due to substitution mutations, and 94.6% of these mutations were transitions.

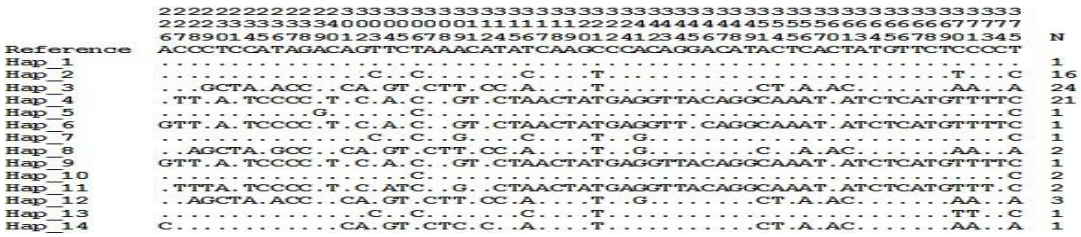

Figure 2**:** Nucleotide polymorphism and their frequencies (N) based on the haplotypes. Vertically oriented numbers indicate the RefSeq position, dots (.) indicate identity with the RefSeq, Hap 114are haplotype numbers.

Results in Table 1 showed that the highest (H=10) and the lowest (H=5) number of haplotypes were found within FUNAAB Alpha, and ShikaBrown/Noiler, respectively. The haplotype diversity (Hd) and nucleotide diversity (Pi) within population ranged from 0.731 (Sasso) - 0.970 (FUNAAB Alpha), and 0.383 (Sasso) – 0.471 (FUNAAB Alpha), respectively while the Hd and Pi among population were 0.796±0.025 and 0.386±0.013, respectively.

Table 1: Genetic diversity indices of iTABs in Imo State

Diversity indices	Among pop.	Within pop.					
		No	Fu	Sb	Ku	Sa	Fi
Number of sequences	77	12	12	14	13	13	13
Number of sites	618	618	618	618	618	618	618
Polymorphic sites	62	180	188	184	174	189	183
Number of Haplotype	14	5	10	5	6	7	8
Haplotype diversity	0.80 (0.025)	0.82 (0.025)	0.97 (0.070)	0.81 (0.0454)	0.85 (0.065)	0.73 (0.113)	0.90 (0.067)
Nucleotide diversity	0.39 (0.013)	0.46 (0.045)	0.47 (0.045)	0.43 (0.053)	0.45 (0.040)	0.38 (0.070)	0.44 (0.045)
Sequence conservation	0.22 (22.3%)	0.23 (23.3%)	0.23 (23.1%)	0.21 (20.8%)	0.23 (23.1%)	0.29 (29.9%)	0.24 (23.8%)

Pop=Populations, No=Noiler, Fu=FUNAAB Alpha, Sb=ShikaBrown, Ku=Kuroiler, Sa=Sasso, Fi=Fulani, Number in parenthesis () are standard error of the mean.

In this study, Hd were higher than the values previously reported for African chicken populations by Adebambo *et al*. (2010) (Nigeria: 0.421), and Nwacharo *et al.* (2011) (Ethiopia: 0.374, Sudan: 0.413, Uganda: 0.322). Among the populations under study, Sasso showed the highest sequence conservation (29.2%) whereas FUNAAB-Alpha and Kuroiler both had the lowest sequence conservation (23.1%). Diversity indices Table 1 further revealed high mtDNA polymorphism within populations and low among populations. Table 2 shows that the highest genetic distance (0.457) was between Sasso and FUNAAB Alpha, and lowest (0.385) between Sasso and
ShikaBrown. It was observed that all the breeds shared a common ancestry, with Sasso and ShikaBrown being more closely related. Furthermore, Analysis of Molecular variance shows that 99.67% of the maternal genetic variation occurred within populations while the remaining variation (0.38%) was found among populations.

Table 2: Genetic distance of the six iTABs in Imo State

	No	Fu	Sb	Ku	Sa	Fi
No		0.014	0.015	0.014	0.015	0.015
Fu	0.426		0.015	0.014	0.015	0.015
Sb	0.443	0.444		0.015	0.013	0.014
Ku	0.425	0.426	0.430		0.015	0.015
Sa	0.456	0.457	0.385	0.439		0.015
Fi	0.447	0.449	0.422	0.446	0.405	

Below diagonal represents distance estimate (d); above diagonal represents Standard Error of Mean (SEM). Analysis was computed in Bootstrap model. (No=Noiler, Fu=FUNAAB Alpha, Sb=ShikaBrown, Ku=Kuroiler, Sa=Sasso and Fi=Fulani).

The phylogenetic analysis (Figure 3) grouped the iTABs into three main clades (clade II, III and IV) out of the seven clades identified in Asian domestic chicken (Bjornstad *et al*., 2003). Mobegi *et al*. (2006) observed that the majority of haplotypes in West African village chicken populations cluster in clade IV,

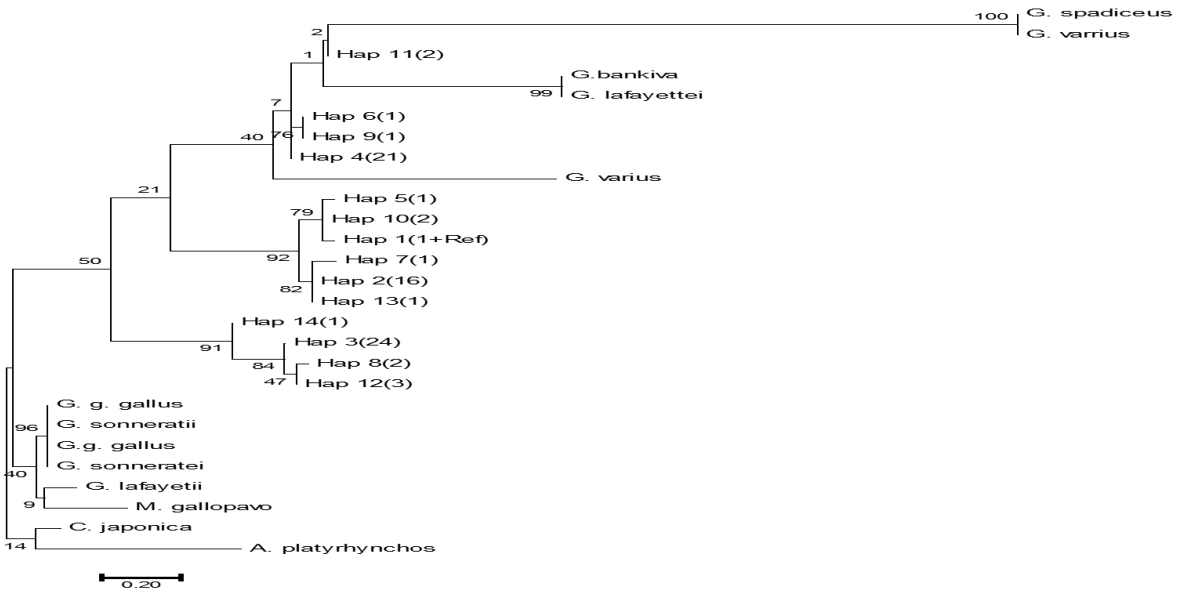

Figure 3.Phylogenetic relationship of the iTABS constructed using the haplotypes.

Muchadeyi *et al*. (2008) observed two distinct clades amongst both Zimbabwe and Malagasy chickens, while Adebambo *et al*. (2010) observed a single clade among Nigerian chicken populations, indicating a closer history of domestic chicken between West African village chicken and iTABs, but difference between Nigerian chicken and iTAB which suggests the absence of admixture. The phylogram revealed a shared ancient lineage between iTABs and the individuals of *Gallus species*. A recent common ancestor was observed between *G. varius* and iTABs haplotypes found in clade IV. Individuals in hap 11 (Sasso and FUNAAB Alpha) were found to have an intermixed lineage with *G. bankiva* and *G. lafayetii.* This distinct distribution patterns suggests that the divergent clades originated from divergent regions of Japan, Serbia and the Indian subcontinent, which supports the theory of multiple origins in South and Southeast Asia (Liu *et al.*, 2006; Kanginakudru *et al.*,2008).

Conclusion and Recommendation

The high genetic diversity within the population can be utilized for further genetic improvement of the breeds. This result can also guide in the conservation of the local germplasm, and evaluate the rate of admixture within the iTABs.

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