ISSN:
Print - 2277 - 0755
Online - 2315 - 7453

Journal of Agricultural Science and Environment

A PRELIMINARY COMPARISON OF MITOCHONDRIAL D-LOOP REGION OF FUNAAB ALPHA AND NIGERIAN INDIGENOUS CHICKENS

***[1]S.O. DUROSARO, [1]B.T. OSHINOWO, [1]A.C. AKPOJO, [1]L.T. OLUYOMBO, [2]I.C. NWOSU, , [3]O.S. IYASERE, [1]B.M. ILORI, , [4]E.V. IKPEME, AND [1]OZOJE, M.O.**

[1]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria
[2]Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria
[3]Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Nigeria
[4]Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross River State, Nigeria
***Corresponding Author**:durosaroso@funaab.edu.ng, **Tel**: +2347069464530

ABSTRACT

Nigerian indigenous chickens possess immunity from endemic diseases and have a better survival rate than commercial hybrid strains under local production conditions. FUNAAB Alpha chicken was developed by improving Nigerian indigenous chickens through crossbreeding and selection. This study compared the mitochondrial d-loop of FUNAAB Alpha and Nigerian indigenous chickens to check likely genetic erosion and loss of diversity in development of FUNAAB Alpha breed. Blood samples were collected from Nigerian indigenous (n=23) and FUNAAB Alpha (n=20) chickens sampled from farms and houses in Ogun state, Nigeria. The Hypervariable 1 (HV1) of the mitochondrial d-loop region was amplified and sequenced. Single nucleotide polymorphisms present in HV1 of chickens were identified using Clustal W. Genetic diversity of the region was determined using DnaSp v5 while selective forces acting on the chickens were predicted using HyPhy software implemented inside MEGA 6 software. Phylogenetic relationship among FUNAAB Alpha, Nigerian indigenous and other chicken breeds was determined using MEGA 6 software. Five polymorphisms were identified in FUNAAB Alpha chickens while twelve were identified in Nigerian indigenous chickens. All the polymorphisms identified in FUNAAB Alpha chickens were also observed in Nigerian indigenous chickens while seven polymorphisms were unique to Nigerian indigenous chickens. Higher diversity indices were observed in Nigerian indigenous chickens (number of haplotype: 4; haplotype diversity: 0.743±0.012; nucleotide diversity: 0.014±0.0013 and average number of nucleotide differences: 4.332) compared with FUNAAB Alpha chickens (number of haplotype: 2; haplotype diversity: 0.485±0.001; nucleotide diversity: 0.008±0.0001 and average number of nucleotide differences: 2.424). Positive selective forces were acting on FUNAAB Alpha chickens while negative selective forces were acting on Nigerian indigenous chickens. Phylogenetic analysis revealed that FUNAAB Alpha chickens clustered with Nigerian indigenous and South American chickens. It can be concluded that there was likely genetic erosion and loss of diversity in development of FUNAAB Alpha breed. Breeding programmes aimed at improvement of genetic diversity and reduction of genetic erosion should be applied in subsequent improvement of FUNAAB Alpha chickens.

Keywords: chickens, diversity, genetic erosion, phylogeny, polymorphism, selection

INTRODUCTION

Nigerian indigenous chicken is a dual purpose bird raised for meat and egg production in the rural and peri-urban communities in Nigeria (Sonaiya and Olori, 1990). These indigenous chickens play major roles in Nigerian rural economies and contribute significantly to Gross National Product of Nigeria (Momoh *et al*., 2007; Omondi, 2018). They have a better meat flavour and are self-reliant with the capacity to withstand harsh weather condition. The birds possess the ability to hatch their own eggs, brood and scavenge for major parts of their feeds. Their products are preferred by many Nigerians because of the pigmentation, taste, leanness and suitability for special dishes (Horst, 1989). They also possess immunity from endemic diseases and have a better survival rate than commercial hybrid strains under local production conditions (Minga *et al*., 2004).

FUNAAB Alpha chicken, which was developed by improving Nigerian indigenous chickens, was officially registered as a new breed in 2018. The selection process for the traits of economic importance (meat and egg) in this chicken breed started in 1997 with over 10 generations of selections for improved meat and egg production. There are two types of FUNAAB Alpha chickens: the meat line and the dual purpose line. The feather colour of the meat line is usually white. The average body weight at hatch of meat line FUNAAB Alpha chickens ranges from 35-42 g while average body weight at 8 weeks varies between 1.2-1.5 kg. The dual purpose FUNAAB Alpha chickens are phenotypically the same in term of feather colours with Nigerian indigenous chickens. The average chick weight at hatch is between 30-35 g while body weight at maturity can reach 1600 g (Ilori *et al*., 2017).

Population genetics studies focus on finding genetic variations in mitochondrial DNA because mutations in this region are higher than other genomic regions (Mannen *et al*., 2004). Animal mitochondrial DNA follows maternal inheritance strictly and is highly variable within a species, making it important for genetic diversity analysis and phylogenetic inference (Wolf *et al*., 1999). Also, analysis of the mitochondrial DNA can be used for tracing the origins of animals as well as identifying individual animals (Anderson *et al*., 1981; Teinlek et al., 2018).

The present study compared the genetic diversity between FUNAAB Alpha and Nigerian indigenous chickens in order to check the likely genetic erosion and loss of diversity involved in the development of FUNAAB Alpha chickens. The type of selection force acting on the two chicken breeds was also predicted. The relatedness of the two chicken breeds with other chicken breeds was also determined.

MATERIALS AND METHODS

Sampling location and experimental animals

Twenty-three (23) Nigerian indigenous and 20 FUNAAB Alpha chickens were sampled from various farms and households in Ogun State Nigeria. Each bird was sampled from farms and houses separated by 10km to avoid sampling of birds that are closely related. The birds were sampled from Ilaro, Ota, Ijebu-Igbo, Ifo, Odeda, Sagamu, Ipokia and Ijebu-Ode. Ogun State has a landmass of 16,980 Km^2, altitude of 66 m above sea level, temperature range of 21-34 ˚C, relative humidity of 85% and located on 7˚00'N 3˚ 35'E coordinates. The capital of the state is Abeokuta and the major river in the State is Ogun River.

Blood collection

About 1 ml of blood was collected from brachial vein of each chicken using needle and syringe. Individual blood sample was deposited in heparinised bottle.

DNA extraction

Genomic DNA (gDNA) was extracted from the blood of the birds at Biotechnology Laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta from the chickens using Zymo research quick-gDNA™ miniprep kit (catalogue number: D3024)

Amplification and sequencing of mitochondrial d-loop region

Amplification was carried out using

L16750_Fwd 5'-AGGACTACGGCTTGAAAAGC-3' (Akishinonomiya *et al*., 1996) and H547_Rev 5'-ATGTGCCTGACCGAGGAACCAG-3' (Liu *et al*., 2006)

primers to amplify 600 bp region covering the whole of Hypervariable 1 (HV1) region and other parts of chicken mitochondrial d-loop. For amplification, 5 µl of genomic DNA (~10-20 ng) was added to a reaction mixture containing 12.8 µl of nuclease free water, 2.5 µl of 1× PCR buffer, 1.5 µl of 1.5mM $MgCl_2$, 1 µl of 0.2mM dNTP, 1 µl of 0.4UM forward primer, 1 µl of 0.4UM reverse primer and 0.2 µl of 2U/ µl surf Hot Taq. The PCR conditions included initial denaturation at 96^{o}C for 15 minutes, 35 cycles of final denaturation at 95^{o}C for 30 seconds, annealing at 56^{o}C for 30 seconds, extension at 70^{o}C for 1 minute and final extension at 70^{o}C for 5 minutes. The amplicon was purified with Magnetic Beads Carboxylate (MCLab, USA). Sequencing of amplicon was done with BigDye Terminator v. 3.1 using the instrument 3730 XL following the supplier's protocol at STAB Vida Genetics Laboratory, Campus FCT UNL Edificio Departmental de Quimica, Laboratorio 009, 2829-516 Caparica, Portugal.

Analysis of mitochondrial d-loop sequences

The Hypervariable 1 (HV1) sequences (546 bp) of mitochondrial d-loop region of FUNAAB Alpha and Nigerian indigenous chickens were trimmed and edited using Bioedit (Hall, 1999) and MEGA v6 (Tamura *et al*., 2013). The single nucleotide polymorphisms (SNPs) present in the HV1 region were identified by alignment with reference d-loop sequence (AB829490) using Clustal W (Thompson *et al*., 1994).

Genetic diversity indices such as singleton variable site, parsimony informative site, number of haplotype, haplotype diversity, average number of nucleotide differences and nucleotide diversity were estimated using DnaSp v5 (Librado and Rozas, 2009).

Tajima's D; Fu and Li's D*; and Fu's Fs tests were performed to test the mitochondrial d-loop HV1 region of the chickens for deviation from neutrality using DnaSP v5 (Librado and Rozas, 2009).

Mean synonymous substitution per synonymous site (dS) and mean non-synonymous substitution per non-synonymous site (dN) were estimated for the region to predict the type of selection acting on the birds using HyPhy software implemented inside MEGA v6 software (Tamura *et al*., 2013). Positive dN-dS suggested positive selection while negative dN-dS suggested negative selection. MEGA v6 software (Tamura *et al*., 2013) was used to determine the phylogenetic relation-

ship among FUNAAB Alpha, Nigerian indigenous and other chicken breeds. The phylogenetic tree was inferred using neighbor joining method. The reliability of the inferred tree was evaluated using bootstrap analysis of 1000 replications.

RESULTS

Polymorphisms identified in mitochondrial d-loop HV1 region of FUNAAB Alpha and Nigerian indigenous chickens

The polymorphisms identified in the mitochondrial d-loop HV1 region of FUNAAB Alpha and Nigerian indigenous chickens are shown in Table 1. Five polymorphisms were identified in FUNAAB Alpha chickens while twelve were identified in Nigerian indigenous chickens. All the polymorphisms identified in FUNAAB Alpha chickens were also observed in Nigerian indigenous chickens while seven polymorphisms were unique to Nigerian indigenous chickens.

Table 1: Polymorphisms identified in mitochondrial d-loop HV1 region of FUNAAB Alpha and Nigerian indigenous chickens

Breed	*Polymorphism	Reference allele
FUNAAB Alpha	222A>G	G
	249A>G	G
	281A>G	A
	342A>G	A
	355T>C	C
Nigerian indigenous	222A>G	G
	225C>T	C
	243C>T	C
	249A>G	G
	256C>T	C
	261T>C	T
	281A>G	A
	310C>T	T
	342A>G	A
	355T>C	C
	358A>G	A
	446T>C	T

*The polymorphisms were named based on complete mitochondrial d-loop sequence of chicken

Genetic diversity of FUNAAB Alpha and Nigerian indigenous chickens

Genetic diversity indices of FUNAAB Alpha and Nigerian indigenous chickens are presented in Table 2. Higher diversity indices were observed in Nigerian indigenous chickens compared with FUNAAB Alpha chickens.

Table 2: Genetic diversity indices of FUNAAB Alpha and Nigerian indigenous chickens

Diversity indices	FUNAAB Alpha	Nigerian indigenous
Number of sequences analysed	20	23
Singleton variable site	0	0
Parsimony informative site	5	12
Number of haplotype	2	4
Haplotype diversity	0.485±0.001	0.743±0.012
Nucleotide diversity	0.008±0.0001	0.014±0.0013
Average number of nucleotide differences	2.424	4.332

Deviation of mitochondrial d-loop HV1 region of FUNAAB Alpha and Nigerian indigenous chickens from neutrality

Test of deviation of mitochondrial d-loop HV1 region of FUNAAB Alpha and Nigerian indigenous chickens from neutrality is shown in Table 3. All the test of deviation from neutrality indices estimated for the two chicken breeds were positive and greater than 1.

Table 3: Test of deviation of mitochondrial d-loop HV1 region of FUNAAB Alpha and Nigerian indigenous chickens from neutrality

Deviation from neutrality test	FUNAAB Alpha	Nigerian indigenous
Tajima's D	1.71	1.15
Fu and Li's D	1.26	1.46
Fu's Fs	5.04	5.32

Type of selection acting on FUNAAB Alpha and Nigerian indigenous chickens

The likely selective forces acting on FUNAAB Alpha and Nigerian indigenous chickens are as presented in Table 4. Positive selective forces were acting on FUNAAB Alpha chickens while Negative Selective forces were acting on Nigerian indigenous chickens.

Table 4: Selective forces acting on FUNAAB Alpha and Nigerian indigenous chickens

Breed	dN	dS	dN-dS	Type of selection
FUNAAB Alpha	1.80	1.27	0.53	Positive selection
Nigerian indigenous	3.44	5.70	-2.26	Negative selection

Phylogenetic relationship among FUNAAB Alpha, Nigerian indigenous and other chicken breeds

The phylogenetic relationship among FUNAAB Alpha, Nigerian indigenous and other chicken breeds is presented in Figure 1. The phylogenetic analysis revealed that the FUNAAB Alpha chickens, South American and Nigerian indigenous chickens clustered together. All the Asian chickens clustered together with the Indian red jungle chicken forming their ancestor.

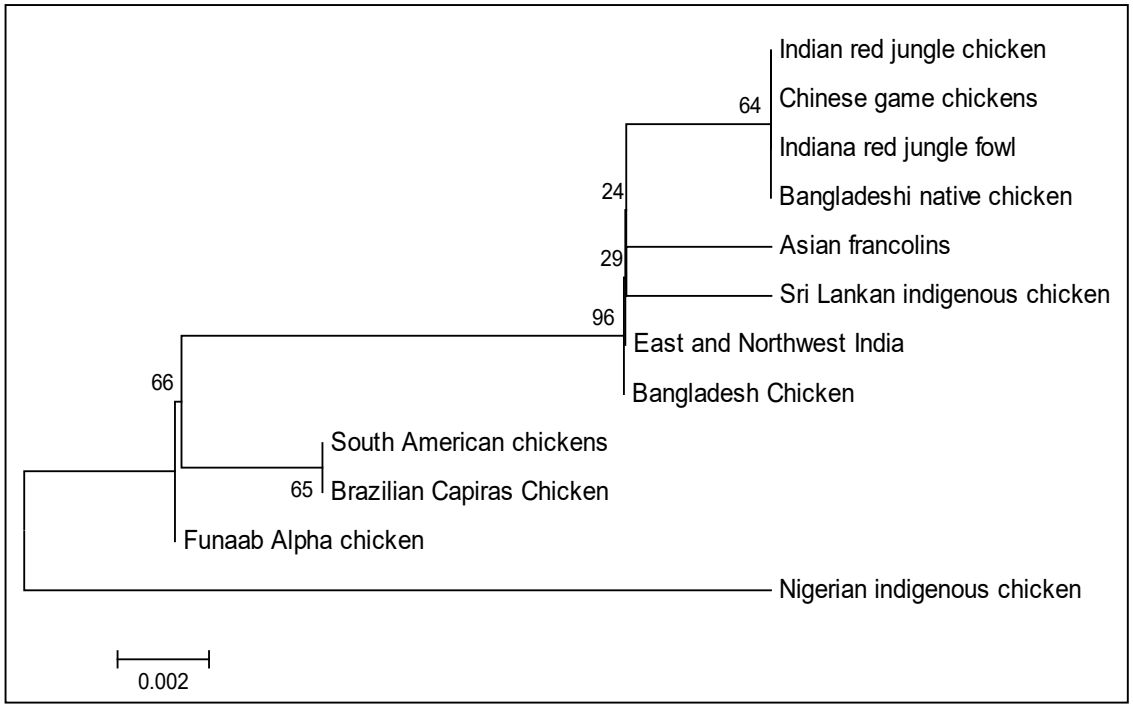

Figure 1: Phylogenetic relationship among FUNAAB Alpha, Nigerian indigenous and other chicken breeds

DISCUSSION

Comparison of mitochondrial d-loop HV1 region of FUNAAB Alpha and Nigerian indigenous chickens presented the molecular genetics evidences of likely genetic erosion and loss of diversity in development of FUNAAB Alpha chickens.

The alignment of the HV1 region of the mitochondrial d-loop region of the two breeds revealed high variability at regions 222 to 446 bp. Regions from 167 to 397 bp were found to have higher variation in Sudanese domestic chickens by Wani *et al.* (2014). The presence of more polymorphisms in Nigerian indigenous chickens compared with FUNAAB Alpha chickens was not surprising as the indigenous chickens used in this study were from rural farms and houses, with the birds not subjected to artificial selection like FUNAAB Alpha chickens. Some polymorphisms were present in Nigerian indigenous chickens but absent in FUNAAB Alpha chickens. Absence of some polymorphisms in FUNAAB Alpha chickens was an indication that some variations have been lost during development of FUNAAB Alpha chickens from Nigerian indigenous chickens.

Haplotype diversity range of 0.485 to 0.743 observed in our study was in the range of 0.39 to 0.88 reported for African chickens by Adebambo *et al.* (2010), Mtileni *et al.* (2011), Osman *et al.* (2016) and Ajibike *et al.* (2017). The lower haplotype and nucleotide diversity observed in FUNAAB Alpha chickens was an indication that the breed is less heterozygote and younger on the evolutionary time scale of domestic chickens. Ancient populations are more likely to be genetically more diverse compared to their derived or recent counterparts (Savolainen *et al.*, 2002). Haplotype diversity is affected by breeding histories of chickens and selection (Joshi *et al.*, 2013). Generally, the low genetic diversity observed in FUNAAB Alpha chickens was an indication of loss of genetic diversity in this breed. Livestock species are most times bred under strong selection that concentrates on few traits of economic importance with this type of breeding resulting in loss of genetic diversity (Taberlet *et al.*, 2011). The genetic diversity is fundamental for sustainable genetic improvement, facilitating the rapid adaptation to necessary and unpredicted change to the development of production system (Mariante and Egito, 2002).

Positive tests of deviation from neutrality indices obtained for FUNAAB Alpha and Nigerian indigenous chickens was an indication of presence of both low and high frequency mutations which can be linked to positive selection and decrease in population size (Hahn *et al.*, 2002). Since negative selection was observed in Nigerian indigenous chickens, positive selection can be excluded as the cause of these positive tests of deviation from neutrality indices and we can confidently assume decrease in population size to be the cause. The surprising result is the observation of positive tests of deviation from neutrality indices in FUNAAB Alpha chickens as positive tests of deviation from neutrality indices result from many haplotypes (Simmonsen *et al.*, 1995) but only two haplotypes were found in the FUNAAB ALPHA breed of chicken used in our study.

Negative selection forces predicted in Nigerian indigenous chickens are likely used by the breed for selection against newly arising deleterious mutations for preservation of biological functions. Negative selection acts on all natural populations and it is mainly for genomic sequence conservation across evo-

lutionary timescales (Elyashiv *et al*., 2016). Also, negative selection is the most prevalent form of selection as it constantly sweeps away deleterious mutations that are produced in each generation and keeps the animal fit. It is a natural selection through which alleles with reduced fitness or viability are lost in the population. Since most keepers of Nigerian indigenous chickens don't provide drugs, vaccines and sometimes feeds for their birds, this type of selection aids in survival and fitness of the birds. Another importance of negative selection is to ensure that new mutations are either lost or fixed rapidly within individuals and thus exposed to selection at the population level (Bergstrom and Pritchard, 1998).

Positive selection forces observed in FUNAAB Alpha chickens might be linked to fixation of alleles. Many alleles that are involved in adaptation, growth and egg production are being fixed in FUNAAB Alpha chickens as it is a new breed that needs to be productive and adapted to tropical climate of Nigeria. Positive selection forces are involved in fixation of alleles that are involved in fitness. Identification of regions that are subjected to positive selection can help answer whether genetic differences between population have adaptive significance (Andolfatto, 2005).

Phylogenetic analysis revealed that FUNAAB Alpha, South American and Nigerian Indigenous were closely related. Close relationship among these chicken breeds implied high comparability and evolution from a common ancestor. Clustering together of FUNAAB Alpha and Nigerian indigenous chickens was not surprising as FUNAAB Alpha chickens were developed from Nigerian indigenous chickens. Also there are two lines of FUNAAB Alpha chickens, the meat and dual purpose lines. The meat line was developed by crossing Nigerian indigenous chickens with exotic broilers while the dual purpose line was developed through selection of Nigerian indigenous chickens for many generations. The exotic breed used in the development of meat line could probably be from South America as FUNAAB Alpha chicken clustered with them on the phylogenetic tree. There was a duplication event in the evolution tree with all the Asian chickens clustering together and Indian red jungle chicken formed the ancestral lineage of all chickens. Formation of ancestral lineage by Indian red jungle chicken supported the hypothesis that domestic chickens originated from the tropical jungle fowl of the genus Gallus (Crawford, 1990).

CONCLUSIONS

There was likely genetic erosion and loss of diversity in development of FUNAAB Alpha breed. Positive selective forces are acting on FUNAAB Alpha chickens while Nigerian indigenous chicken is undergoing negative selection. Phylogenetic analysis revealed likely crossing of South American chickens with Nigerian indigenous chickens during the development of meat type FUNAAB Alpha chickens

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(Manuscript received: 3rd February, 2020; accepted: 4th June, 2020).