GENETIC DIVERSITY AND PHYLETIC EVOLUTION OF NORTHEAST NIGERIAN INDIGENOUS CHICKEN POPULATION BY MTDNA SEQUENCE

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ABSTRACT

Nigerian indigenous chicken (NIC) breeds are geographically widespread, with more than 12 breeds listed as national genetic resources. However, NIC breeds are facing declining population and germplasm degeneration as a result of lots of commercial chicken strains been introduced. In this study, the genetic diversity and phylogenetic structure of Northeast (NE) Nigerian chicken were investigated based on mitochondrial DNA D-loop sequences of 397 base-pair in length. A total of 38 individuals were examined, and 8 haplotypes were observed in 9 polymorphic sites (5 singleton and 4 parsimony-informative) with high A+T content (63.46%) compared to G+C content (36.54%). The haplotype and nucleotide diversity indices were 0.588 and 0.002 respectively indicating relatively low genetic variability. Phylogenetic analysis and a star-like pattern median joining (MJ) network revealed that these individuals were grouped into one distinct genetic clade (cluster A). A non-statistically significant Tajima (*D's*) and Fu (*F's*) values of -1.718 and -3.964 respectively, and sum of squared deviation (ssd: 0.007) and Harpending's raggedness index (rag: 0.105) suggested no rapid expansion event that occurred in NE Nigerian. This study concluded that Northeast Nigerian chicken has relatively high genetic variability, but, low genetic diversity which could be exploited for efficient breeding selection and genetic resources conservation.

Keywords: Genetic variation, indigenous chicken, diversity, phylogney

INTRODUCTION

Nigeria has a rich and unexploited chicken genetic resources due to diversified agro-geographical condition and long history of animal husbandry. There are more than 12 breeds of chicken listed as national genetic resources (FAO, 2007a) which are yet to be fully utilized for national breeding and genetic improvement programme towards better production and reproductive performance. Although most Nigerian chicken ecotypes are not viable economically, yet, they form part of the national genetic resource because of their unselective long breeding history.

The widespread diffusion of commercial chicken strains is threatening the survival of well-adapted Nigerian local chickens (Rege and Gibson, 2003), which is evident as many local husbandry practices are being abandoned (Köhler-Rollefson *et al.*, 2009), thus, causing erosion of indigenous chicken genetic diversity (FAO, 2007b) with attendant negative consequences such as loss of viability, fertility and disease resistance and inbreeding depression (FAO, 2007a; Taberlet *et al.*, 2008; Ajibike *et al.*, 2016).

This study, therefore, aims to determine the genetic variation of Northeast Nigerian chicken as a population as well as provide a genetically based tool for effective conservation programme based on the D-loop mitochondrial DNA (mtDNA) gene.

MATERIALS AND METHODS

Sample collection and DNA extraction

Genomic DNA was extracted from air-dried blood preserved on FTA classic cards (Whatman Biosciences), using the recommended manufacturer protocol, from 37 unrelated individual indigenous chicken from five towns (Kano, n=14; Kari, n=9; Potiskum, n=7; Maiduguri, n=8) in the North-east (NE) region of Nigeria. The DNA concentration and purity, A260/A280 ratio between 1.8 and 2.0, were assessed using a NanoDrop® 1000 Spectrophotometer. Potential DNA degradation was visualized on 1.5% agarose gel.

PCR amplification and sequencing of the mtDNA

Using the primers: L16750 (5' – AGGACTACGGCTTGAAAAGC - 3') as the forward primer (Fu *et al*., 2001) and H547 (5' – ATGTGCCTGACCGAGGAACCAG - 3') as a reverse primer (Liu *et al*., 2006), PCR amplification was carried out as previously described by (9).

Two internal primers: CR-for (5' - TCTATATTCCACATTTCTC - 3') and CR-rev (5' – GCGAGCATAACCAAATGG - 3') were used in a 20 µL comprising approximate 20 ng of purified PCR product as template DNA, 3.2 pmol of primer and 8 µL of Big Dye Terminator Ready Reaction Mix, 8 µL of deionized water, 2 µL of primer and 2 µL template DNA, using a ABI 3730 XL Capillary DNA Analyzer (Applied Biosystems, USA) programmed as: 25 cycles at 96 ^{o}C for 10 seconds, 50 ^{o}C for 5 seconds and 60 ^{o}C for 4 minutes. After the last cycle, there was a rapid thermal ramp to 4 ^{o}C and holding until the purification of the sequencing product.

Data analysis

A 397bp long fragment was subsequently used for analysis. Viewing and editing of the sequences were done using ChromasPro software. All sequences were aligned with the *Gallus gallus* sequence available in GenBank (X52392) using ClustalW in MEGA 10.2.2 (Kumar *et al*., 2018). The number of haplotypes, nucleotide variable site, haplotype diversity and nucleotide diversity were calculated using the software DnaSP version 6.12.03 (Rozas *et al*., 2017). The phylogenetic trees of the identified haplotypes, and *Meleagris gallopavo* mtDNA D-loop sequence (AY398672) as an outgroup, were constructed with the neighbor-joining (NJ) method implemented in MEGA 10.2.2 while the robustness of phylogenetic hypotheses was calculated based on 1000 replications (Felsenstein, 1985). In addition, median-joining (MJ) network analysis was constructed for the identified haplotypes using NETWORK 10.2.0.0 (Bandelt *et al*., 1999).

RESULTS AND DISCUSSION

mtDNA D-loop sequence variability and nucleotide composition

A total 950 base-pair (bp) fragment was amplified and sequenced, of which the 397-bp sequences of HV-I were used in subsequent analyses (from nt 1 to nt397 of X52392 sequence). In the 38 sample NE chicken, the average nucleotide composition was 21.35% A, 1.17% G, 35.38% C and 42.11% T. The A+T (63.46%) contents was higher than that of C+G (36.54%) showing that A and T were richer in the chicken mtDNA-loop region which corroborated the report of Yu *et al*. (2019). There were 9 polymorphic sites with 5 singleton polymorphic sites and 4 parsimony-informative polymorphic sites. The variable types were transitions and transversions. The low observed haplotype (Table 1) may be due to low sample size and differences in sampling location used in this study.

Table 1: Polymorphism information of 8 haplotypes identified in 38 sample birds

	012222222223333 260112456791469 975075836107047 6	N
Ref	CCTTTTCTTCTACATT	
NE33	.T.CCC.CCT..T...	24
NE74	.T.CCC.CCT..TC..	1
NE73	.T.CCC.CCT..TC.C	1
NE72	.T.CCC.CCT..T..C	3
NE69	GTGCCC.CGTG.T...	1
NE68	.T.CCC.CCT..T.C.	5
NE48	.T.CCCTCCT..T...	2
NE35	.T.CCC.CCT.TT...	1

The first column signifies identification number of sampled chickens (NExx). Vertically oriented numbers indicate the variable site position. Dots (.) indicate identity with Red Jungle Fowl mitochondrial genome reference sequence (GenBank accession number: X52392*) while different base letters denote substitution. N: Number of individuals in each haplotype*

Molecular diversity estimation and population dynamics

The observed haplotype was 8 with identified haplotypic (gene) and nucleotide diversity of 0.588 and 0.002 respectively (Table 2). The observed haplotypic and nucleotide diversity were lower than what was reported by Eltanany and Hemeda (2016) for Egyptian chicken and Yu *et al*. (2019) for Chinese

chicken respectively. Despite the small sample size, the observed *Hd* and π suggest a relatively high genetic diversity in the sampled North-east Nigerian chicken.

The observed negative Tajima's *D* (-1.718) and Fu's *F* (-3.964) indicated either balancing or purifying selection occurred in NE Nigerian chicken. The positive and non-significant values of the sum of squared deviation (SSd: 0.007) and Harpending's raggedness index (Rag: 0.105) suggested there was no rapid expansion event that occurred in NE Nigerian chicken

Table 2: Estimated diversity parameters and population dynamics in NE Nigerian chicken

Population	*N*	*H*	*Hd*	π	*K*	*D*	*F*	*Ssd*	*Rag*
NE chicken	**38**	**8**	**0.588**	**0.002**	**0.896**	**-1.718^{NS}**	**-3.964**	**0.007**	**0.105**

N: Sample size; H: Haplotype; Hd: Haplotype diversity; π: Nucleotide diversity; K: Average number of nucleotide difference; D: Tajima's neutrality Test; F: Fu's neutrality test; Ssd: Sum of squared deviation; Rag: Harpending's raggedness index; NS: non-significant

Phylogenetic analysis and median-joining network of haplotypes

As shown in Figure 1, the 8 NE Nigerian chicken haplotypes were found to be in one cluster (A) as revealed by the Neighbour-joining (NJ) tree constructed. NE33 was the dominant haplotype, present in 63.16% in the sampled birds (24/38). Similarity to the NJ tree, the median-joining network showed that the haplotypes were grouped into one cluster (A), thus, revealing how less divergence that NE chicken are by sharing the most common haplotype (NE33).

Figure 1*: The Neighbour-joining tree based on 9 D loop haplotypes identified in the 38 NE chicke sequences. The tree was constructed based on kimur two-parameter distance. The numbers at the majo nodes represent the percentage bootstrap values fo interior branches after 1000 replications.*

Figure 2: *Median-joining network (ε = 0) of NE chicken haplotypes based on the polymorphic sites of the mitochondrial D-loop HV1 region. Area of each circle is proportional to the frequency of the corresponding haplotype. The red colour between the haplotype nodes refer to the positions of median vector.*

diversity and belong a single maternal lineage within substructure. This could be exploited for efficient breeding selection and genetic resources conservation.

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