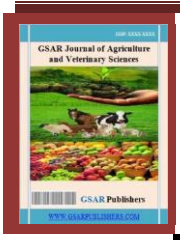

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Estimation of Genetic Diversity in Nigerian Local Chickens Using Mitochondrial Cytochrome C Oxidase Subunit 1 (COI) Gene Sequences

By

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Abstract

This study evaluated the genetic diversity of Nigerian indigenous chickens using sequences obtained from the mitochondrial cytochrome c oxidase subunit I (COI) gene. A total of fifty local chickens of five distinct phenotypic groups (Normal Feather, Naked Neck, Frizzle Feather, Crested Feather, and Ptilopody) were randomly sampled from Kwara State, Nigeria. Blood samples were collected from fifteen chickens according to feather type for genomic DNA extraction and sequencing using mitochondrial cytochrome C oxidase 1 (COI) primers.

Sequence alignment and diversity analyses were carried out using MEGA 11.0 and DnaSP V6 software, respectively. Among the 358 nucleotide sites examined, only three variable positions were identified, all of which had mutations that occurred at alignment positions 13, 14, and 15. The sequences had haplotype diversity of 0.154 and nucleotide diversity of 0.00135, which were very low, indicating limited mitochondrial genetic variation among the sampled indigenous chicken phenotypes. Neutrality analyses generated negative but statistically non-significant values for Tajima's D (−1.652), Fu and Li's D (−2.025), and Fu and Li's F* (−2.189). These findings indicated that the mitochondrial COI marker has limited discriminatory power for detecting genetic differences among Nigerian local chicken phenotypes but recommend future investigations with a larger population so as to obtain a more comprehensive understanding of genetic diversity and population structure in Nigerian local chickens.*

Keywords: *Genetic diversity, Nigerian local chicken, mitochondrial cytochrome c oxidase1, haplotype diversity.*

1. INTRODUCTION

Domestic chickens (*Gallus gallus domesticus*) are from several maternal ancestral populations that originated in Asia (Liu et al., 2006). With an estimated 166 million birds, they are the most prevalent livestock species in Nigeria. Particularly in rural areas, indigenous chickens contribute significantly to household livelihoods by providing food, income, and cultural value. They have developed a variety of unique phenotypic traits as a result of their adaptability to the nation's varied agroecological settings. These groups have historically been categorized based on obvious physical characteristics. However, these traits frequently fall short of accurately representing the underlying genetic links across various chicken populations because they are heavily influenced by environmental factors, dietary habits, and management techniques (Hanotte *et al*., 2010).

Despite the significance of native chickens to Nigeria's rural economy and agricultural sector, thorough knowledge of their genetic makeup is still lacking. Mitochondrial DNA (mtDNA) is one of the most informative markers, and molecular genetic techniques have become essential for evaluating and preserving animal genetic resources. mtDNA offers important information on population history, maternal ancestry, and evolutionary relationships because of its maternal mode of inheritance, lack of recombination, and comparatively high mutation rate. Because it can identify species and show patterns of genetic variation among populations, the cytochrome c oxidase subunit I (COI) gene is one of the mitochondrial genes that has gained widespread recognition as a useful DNA barcode (Hebert *et al*., 2003).

The COI gene has been successfully used in genetic diversity studies involving poultry in many regions of the world, but its

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application to native Nigerian chickens remains scarce. There is a large gap in knowledge based on mitochondrial DNA because the majority of studies that are currently accessible have mostly focused on morphological evaluation or microsatellite markers. This shortage of molecular evidence hampers the creation of scientifically informed breeding and conservation initiatives. Furthermore, the persistent practice of unregulated crossbreeding, along with the steady loss of indigenous genetic resources and changes in production systems, poses a severe threat to the long-term survival of locally adapted chicken populations. Addressing these difficulties demands a greater knowledge of their genetic variability at the molecular level. This study was conducted to examine the genetic diversity of native Nigerian chickens of different plumage types using mitochondrial cytochrome c oxidase subunit I.

2. MATERIALS AND METHODS

2.1 Study Location, Experimental Birds and Sampling

The study was carried out in Kwara State, Nigeria, which is located in the North-Central geographic zone between latitudes 7°45′N and 9°30′N and longitudes 2°30′E and 6°35′E. A total of fifty native Nigerian chickens, representing five feather types in North Central Nigeria, were randomly sampled and categorized to Normal Feather, Naked Neck, Frizzle Feather, Crested Feather, and Ptilopody (feathered shank) based on their feather pattern. Blood were collected from fifteen chickens into EDTA bottle with no cross-contamination and packed in ice to ensure preservation before taken to the molecular laboratory of the Department of Animal Production, University of Ilorin, Nigeria for DNA extraction.

2.2. DNA Extraction, Intergrity Testing, Gene Sequencing and Analysis.

Genomic DNA was extracted from the blood using the Quick-DNA™ Blood/Tissue kit (Zymo Research, Catalogue No ZR D4068) Nano Spectrophotometer was used to test the quality of the DNA extracted at the Molecular and Diagnostic Research Laboratory (MDLR) of the University of Ilorin Teaching Hospital, Nigeria. The internal transcribed spacer (ITS) target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No NEB M0486S) with the forward primer COIF (5´-CCTAACGCTTCAACACTC-3´) and reverse primer COIR (5´- TTCAACTTCTTGGGCATC-3´). Polymearse Chain Reaction was carried out at the Molecular Unit of the University College Hospital, Ibadan, Nigeria using a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a standard PCR profile and the integrity of the amplified gene fragment was checked on a 1% Agarose gel ran to confirm amplification and the amplified fragments were ethanol-purified to remove the PCR reagents, after which the purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hour to confirm the presence of the purified product. The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using the manufacturer's manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. The mitochondrial COI subunit gene sequences obtained were manually edited and aligned using Clustal W implemented in Molecular Evolutionary Genetics Analysis (MEGA) software version 10.02 and Sequence Demarcation Tools (SDT) version 1 (Kumar 2018). The sequences were estimated for both haplotypes and nucleotides diversities using DNA SP Version 6.12.2 (Rozas 1999).

2.3. Ethical Considerations

The University Animal Care and Use Committee (UACUC) of Kwara State University, Malete, accepted the ethical guidelines for animal research, which were followed in the animal handling protocols. pain, stress, and the quantity of birds sampled were all kept to a minimum standard.

3.0 RESULTS AND DISCUSSION

Three hundred and forty-one (341) sites from the 13 COI sequences used for analysis from positions 1 to 358 of the alignment, were informative as shown in Table 1.0, and the results showed that 338 sites (99.1%) were invariable (monomorphic), while three sites were variable, with two of them being single mutations and obtained from a single sequence. No parsimony-informative sites were found and the total number of mutations (Eta) were three (3). These results show a remarkably high degree of sequence conservation among the 13 sampled that were eventually sequences and this is consistent with the tight functional restrictions that are typically applied to the mitochondrial COI gene within a single species as reported by Martem and Vivek (2018); Niu *et al*. (2019). Sequences obtained from two distinct phenotypes—Normal Feather and Naked Neck were completely identical from positions 51 to 350, indicating a significant purifying effect throughout the fragment. With a conservation value of 1.000 and homozygosity of 1.000 (P = 0.0000), a single large conserved region (Region 1) covering locations 16–352 was found.

Two haplotypes were found among the 13 analysed sequences; the dominant haplotype was shared by 12 of the 13 sequences, while the remaining sequence formed a unique single haplotype. Nucleotide diversity (π) was 0.00135 and haplotype diversity (Hd) was 0.154 ± 0.126 of two sequences in the sample differed, on average, at fewer than one nucleotide position, as indicated by the average number of pairwise nucleotide differences (k) of 0.462. Tajima's D value was −1.652, Fu and Li's D* was −2.025, and Fu and Li's F* was −2.189, the Neutrality tests yielded negative but statistically non-significant values and fell within the range of 0.10 > P > 0.05. Fu's Fs statistic was likewise non-significant and somewhat positive (0.976) as shown in Table 2.0. Even though these statistics are not statistically significant, their consistently negative direction is consistent with a recent population expansion, a historical selective sweep, or a strong purifying effect acting on the COI focus, with the small sample size limiting the statistical power to detect significance.

The study's findings show that the mitochondrial COI gene region is highly conserved among the five phenotypes of

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Nigerian Local hens sampled, with only three single polymorphic sites found among 341 relevant places. This conservation limits the COI gene's ability to detect fine-scale genetic differences across phenotypes within a single species, even while it makes it a reliable barcode for species-specific identification as earlier reported by Nui *et al*. (2019). Together with the identification of only two haplotypes among 13 sequences, the low haplotype diversity (Hd = 0.154) and nucleotide diversity (π = 0.00135) found in this study imply that the sampled Nigerian local chicken phenotypes share a recent and similar maternal lineage as earlier reported by Liu *et al*. (2006) for Asian Jungle fowl. This trend may reflect a founder effect during the establishment of these characteristics and a historically smaller effective population size, or a series of domestication bottlenecks and human-mediated translocations as known to affect mitochondrial lineage diversity in *Gallus gallus* domesticus more generally (Ballard and Whitlock, 2004). Notably, the Ptilopody phenotype, which has historically been linked to introduced stock as stated by Ajayi (2010) shared the dominant haplotype with other indigenous phenotypes and this suggests that indigenous and introduced populations shared similar values at this genomic region. Thus, highlighting the wider mitochondrial homogeneity of domestic chickens across a variety of management histories.

The lack of statistical significance is most likely due to the small sample size (n = 13) rather than underlying evolutionary processes. However, the negative, though non-significant, values obtained for Tajima's D and the Fu and Li tests are directionally consistent with a demographic history of population expansion or a selective sweep (Mifta *et al*. 2024), which suggest the use of more general use of single-locus, low-variability markers like COI for determining population structure and demographic history in genetically homogeneous domestic populations.

These results support the idea that additional, more variable markers are needed to characterize genetic structure among Nigerian Local chicken phenotypes adequately. These findings align with earlier mitochondrial studies of Nigerian chicken populations that have similarly reported limited phylogeographic structure using D-Loop and cytochrome b markers, as reported by Adebambo *et al*. (2010), Abdulhakeem *et al*. (2017). Adeleke *et al*. (2011). Nuclear SNPs from genome-wide association studies, insertion-deletion polymorphisms, and complete mitochondrial genome sequencing, which includes the more variable D-loop control region and additional protein-coding regions, might provide higher resolution for intraspecific differentiation and would significantly strengthen future population genetic inference in this system.

4.0. Conclusions.

The minimal polymorphism obtained is limited to three single sites, and only two haplotypes found among 13 sequences also confirms that the mitochondrial COI gene region is highly conserved among Nigerian local chicken phenotypes. While the COI barcode is not informative for resolving fine-scale genetic differentiation among local Nigerian chicken phenotypes, the findings nonetheless provide valuable baseline molecular data for the conservation and management of these genetic resources, and molecularly confirm the close maternal relatedness of the sampled phenotypes.

Future research should increase sample sizes, expand the molecular marker panel to include microsatellites, nuclear SNPs, and complete mitochondrial genome sequencing, and combine molecular data with morphometric and production trait information to develop a more holistic characterization of Local chicken phenotypes and to provide information that will aid the protection of Nigeria's indigenous chicken populations from genetic erosion caused by incessant breeding and unchecked crossbreeding.

Table 1.0. Mt COI sequence values and genetic diversity of Nigerian local chickens Sampled

Parameter	Obtained Values
Number of sequences analysed	13
Total nucleotide sites	358
Informative sites	341
Monomorphic (invariable) sites	338
Polymorphic (variable) sites	3 (all singleton)
Number of haplotypes	2
Haplotype diversity (Hd)	0.154 ± 0.126
Nucleotide diversity (π)	0.00135
Average pairwise differences (k)	0.462

Table 2.0. Neutrality tests and sequence conservation of Nigerian Local chickens sampled

Test Statistic	Value	Significance

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Tajima's D	-1.65231	Not Significant (0.10 > P> 0.05)
Fu & Ii's D*	-2.02547	Not Significant (0.10 > P> 0.05)
Fu & Ii's F*	-2.18908	Not Significant (0.10 > P> 0.05)
Fu's Fs	+0.976	Not Significant
Haplotype prob. ≤ 2	0.392	Moderately probable under neutrality

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