GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIP OF INDIGENOUS CHICKEN (*Gallus domesticus)* POPULATIONS IN KANO STATE

I.U. Zango*, I.O. Suleiman and F. Bibi-Farouk
Department of Animal Science, Bayero University, Kano-Nigeria.
**Corresponding author: ibrahimubaz@gmail.com: +2348185528989*

ABSTRACT
Genetic diversity and phylogenetic analysis of three genotypes of indigenous chicken in Kano state, Nigeria was carried out. Twenty-four (24) matured free-ranging chickens between the ages of 6 months and above were sampled. The chickens were observed individually and classified into the genotypes based on the expression of feather distribution and structure phenotypically. Two (2 ml) of blood samples from each genotype were collected and genomic DNA was extracted individually using Quick DNA Kits. Five microsatellite markers were used for genotyping, and the data generated were subjected to analysis in GenAlex software (version 6.5). Nei's genetic distances among all the populations were: 0.078 (normal feathered versus naked neck chickens), 0.084 (normal feathered versus frizzle feathered chickens) and 0.174 (naked neck versus frizzle feathered chickens). Phylogenetic tree showed that two clusters were identified; Normal and Frizzle feathered, joined by the Naked neck genotype which was separated from the others. Relatively high genetic distance was discovered and phylogenetic tree showed two hierarchical clusters between the populations. It is recommended that conservation and improvement strategies be adopted to curb further genetic dilution and erosion.
Keyword: Genetic diversity, Phylogenetic tree, DNA Extraction, Indigenous Chicken

INTRODUCTION

Domesticated chicken is the most abundant species of domestic bird in the world which belongs to the genus Gallus (Garrigus, 2007). The evolutionary history of domestic fowl development was characterized into three most known phases. The first phase started with the evolution of *Gallus gallus*, followed by the emergence of the domesticated chicken from its progenitors and thirdly is the introduction of the large number of the new breeds, varieties, strains and lines (Mogesse, 2007). Domestic chicken survives food and water scarcity and have developed resistance to parasitic infection (Ibeagha-Awemu, Peters & Bemji, 2019).

The indigenous chicken ecotypes of Africa represent a reservoir of chicken genomes and useful genes which are desirable for their productive adaptability (FAO, 2012), vary widely in body size, body conformation and many other phenotypic characteristics (Cabarles *et al*., 2012). They have genes that are adaptive to their tropical conditions and diseases tolerance (Aklilu *et al*.,2013).Genetic diversity is undertaken to classify an individual or population compared to other individuals or populations based on morphological, biochemical and molecular information (Goncalves *et al*.,2009). It quantifies the magnitude of genetic variability within a population which is a fundamental source of biodiversity (Ebegbulem and Asanga, 2018). Major forces creating genetic differences are mutation and recombination, genetic drift, natural and artificial selection and migration (Lush, 1994).

MATERIALS AND METHODS

Study Area and Experimental Animals

The study was conducted in Kano State, Nigeria, which located between latitudes 10˚30'N and 12˚38'N and longitudes 7˚45'E and 9˚29'E. *Twenty-four (24) matured free-ranging chickens between the ages of 6 months and above were sampled.*The birds were properly restrained to avoid injury. Eight blood samples were randomly selected from each genotype for molecular analysis. 2mL sterile syringe was used to collected two millilitres (2mL) of blood and stored in Ethylene Di-amine Tetra Acetic Acid (EDTA) tube. And then taken to laboratory for DNA extraction, purification and amplification. All experimental procedures were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC).

DNA Extraction and Polymerase Chain Reaction (PCR)

Zymo-Spin research kit (ZR kit/DNA MiniPrep) was used to the extraction of genomic DNA from the sampled blood according to the manufacturer's procedure. Five microsatellite loci markers were used for genotyping. PCR primers, tandem repeat units of microsatellites detected using PCR with primer corresponding to the unique-sequence. The primers used for the research included ADL0268, LEI0166,

MCW0183, MCW0067 and MCW0222. PCR primers, tandem repeat units of microsatellites detected using PCR with primer corresponding to the unique-sequence. Gel electrophoresis was conducted on 1% Agarose gel to separate DNA according to size.

Statistical Analysis

Nei's genetic diversity per population was computed using GenAlex software (version 6.5) to compare the relationship between different populations. The result was compared with Nei's (1978) unbiased genetic distance and identity calculated to assess their genetic diversity. Differentiation in populations fixation indices of genetic variation within sub population (F_{IS}), between subpopulations (F_{ST}) and total population (F_{IT}) per locus across the populations were determined using the GeneAlex 6.5 program (Peakall and Smouse, 2012). Hierarchical clustering was constructed to determine the phylogenetic genetic relationship among populations. GenAlex 6.5 software program was used.

RESULTS AND DISCUSSION

Grand means and standard errors over loci are presented in Table 1. Total number of sample size was 7.07 with a range of 6 to 8 across loci. Total observed heterozygosity was 0.41 indicating that the markers were sufficiently polymorphic to show the genetic diversity of the chicken populations. The overall observed and expected heterozygosity for population 0.41±0.05 and 0.42±0.02 respectively as estimated within the breeds were based on a set of markers showing substantial number of detected alleles 0.42. So, the grand mean of observed heterozygosity (H_O) was lower than the expected mean heterozygosity (H_E), indicating that the indigenous population is not following the assumption of Hardy-Weinberg principles. Number of alleles produced by these markers and their heterozygosity were in line with the report of Hillel *et al.* (2003), but lower than what have been reported by Olowofeso *et al*. (2005), A similar result (0.45-0.67) has been reported. for African, Asian and South American indigenous chickens. Alipanah *et al*. (2011) had reported 5.90 for three indigenous chicken populations in Iran. The variation in heterozygosities may be adduced to differences in breeds, location, different sample sizes, number and sources of microsatellite markers (Olowofeso *et al.,* 2005), crossbreeding, gene flow, low level of inbreeding and low selection pressure.

Table 1: Grand Mean and Standard Error for Sample Size, Number of Alleles, Number of Effective Alleles, Information Index, Observed Heterozygosity, Expected and unbiased expected Heterozygosity, and Fixation Index for the Indigenous Chicken Populations

Locus	N	N_A	N_E	I	H_O	H_E	uH_E	F
Grand mean	7.07	2.00	1.76	0.61	0.41	0.42	0.46	0.02
SE±	0.18	0.00	0.05	0.02	0.05	0.02	0.02	0.11

N=Sample Size, N_A= No. of Alleles, N_E = No. of Effective Alleles, I= Infor. Index, Ho=Observed Heterozygosity, H_E = Expected, uH_E= unbiased expected Heterozygosity, F = Fixation Index

The genetic distance of the chicken populations studied is presented in Table 2. The unbiased genetic distance values (in bracket) give the genetic distance when all sources of error are removed from the analysis presented in the table. The genetic distance between normal feathered and naked neck populations (0.078) was smaller than the distance between normal feathered and frizzle feathered (0.084). This is lower than report of Adeleke *et al.,* (2011) who found that the genetic distance between normal feathered and naked neck populations is 0.5585 and the distance between normal feathered and frizzle feathered is 0.6368.

Table 2: Nei's (Unbiased) Genetic Distance among Population of Indigenous Chicken

	NF	NN	FF
NF	0.000 (0.000)		
NN	0.078 (0.016)	0.000 (0.00)	
FF	0.084 (0.026)	0.174 (0.119)	0.000 (0.000)

NF = Normal feathered chicken, NN = Naked neck chicken; FF = Frizzle feathered chicken

Table 3 is the pairwise genetic differentiation values (F_{ST}) that estimate population mutation rate and standard measure of variation. The table showed genetic differentiation between each of the populations. The pairwise F_{ST} distance between the populations ranges 0.044 to 0.099 which indicates

that some of the populations share amount of genetic diversity and the populations are differentiated. And there was free interbreeding among the population in different magnitude. High F_{ST} implies a considerable degree of differentiation among population. Highest value was observed between pop2 and pop3. The phylogenetic relationships among the three indigenous chicken populations studied were summarized in Figure 1. The Dendrogram showed the hierarchical relationship between the populations. The figure shows that the three genotypes of the indigenous chickens were clearly separated from one another. Two clusters were identified; Normal and Frizzle feathered, joined by the Naked neck genotypes which was separated from others.

Table 3: Population Pairwise F_{ST} Based on Microsatellite Loci (Gene Differentiation)

	NF	NN	FF
NF	0.000 (0.000)		
NN	0.044 (0.016)	0.000 (0.000)	
FF	0.050 (0.026)	0.099 (0.119)	0.000 (0.000)

NF = Normal feathered chicken, NN = Naked neck chicken; FF = Frizzle feathered chicken

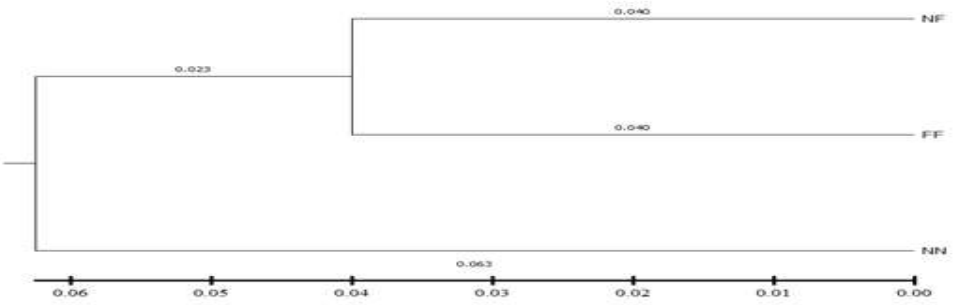

Figure 1. Phylogenetic relationships among the three indigenous chicken populations

The phylogenetic relationships among the three indigenous chicken populations were summarized in Figure 1. This showed the hierarchical relationship between the populations. The figure showed that the three genotypes of the indigenous chickens were clearly separated from one another. Two clusters were identified; Normal and Frizzle feathered, joined by the Naked neck genotypes which was separated from the others. This is in line with the report of Adekoya *et al.* (2013) and Adeleke *et al.*(2011) that showed variation between indigenous chicken populations in Nigeria. However, it is expected to have an overlap of naked neck with frizzle feathered than with the normal feathered chickens. This is due to the presence of gene for feather reduction and coverage on the naked neck and frizzle feathered chickens which allow internal heat loss and heat tolerance compared to the normal feathered chicken.

CONCLUSION

The study showed relatively high genetic distance while phylogenetic tree showed two hierarchical clusters between the three chicken populations. Therefore, conservation and improvement strategies need to be adopted to curb further genetic dilution and erosion.

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