



EVALUATION OF GENETIC DIVERSITY IN TWO ECOTYPES OF NIGERIAN INDIGENOUS CHICKEN (NIC) USING ISSR MARKER AND PRINCIPAL COORDINATE ANALYSIS (PCoA)

Etta, Hannah Edim*¹ and Josiah, J. G.²

¹: Department of Animal and Environmental Biology, Cross River University of Technology, Calabar, Cross River State, Nigeria.

²: Department of Biotechnology, Mewar International University, Masaka, Nasarawa State.

*Corresponding author: hannahetta76@gmail.com; 08035835981

ABSTRACT

In order to determine the genetic diversity of local chickens (Naked neck strain) from two ecotypes (Calabar and Obudu) in Cross River State, Inter Simple Sequence Repeat (ISSR) using four primers and Principal Coordinate analysis were performed. Genomic DNA extraction was carried out using Cetyltrimethyl ammonium bromide (CTAB) protocol, followed by Polymerase Chain Reaction (PCR) amplification and observation by gel electrophoresis. The power maker software (ver. 3.5) was used to analyze the genetic diversity of the local chickens. Principal Coordinate Analysis (PCoA) was also carried out. Results obtained, showed that genetic diversity ranged from 0.62 to 0.82 while polymorphic information content (PIC) ranged from 0.547 to 0.797. PCoA results revealed a total of 40.26% variation explained by axis 1, 2 and 3 based on genetic relationship. The results of the genetic evaluation can be used as baseline genetic information on the variation of both ecotypes of naked neck chicken that can be assimilated with genetic information programs, to implement further genetic studies and breeding of the naked neck strain local chicken.

Key words: Genetic diversity; naked neck chickens; Ecotypes; ISSR; PCoA

1.0 INTRODUCTION

A review of available literature from sub-saharan African countries show that local chickens on average accounts for 78% of the total poultry population (Tadelle, 1996). Despite the fact that local chickens are still widely consumed in developing countries, little research and development work has been carried out to characterize, and improve on their breeding methods (Cumming, 1992). Although, indigenous chicken have a number of adaptive traits and genes with special utility in the tropics the real value of indigenous chicken breeds, like the naked neck breed (Plate 1), is often underestimated mostly due to their poor appearance, relatively low productivity and alleged low “commercial values”(Horst,1989). Thus,

they have been neglected and little attention has been given to them by researchers, breeders, and policy makers to put them in research and development agenda.

The Nigerian indigenous chicken is a dual-purpose bird that is used for both meat and egg production in the rural and semi-urban areas of the country. They are well adapted to the adverse climatic conditions of the tropical environment and low management inputs. They contain a highly conserved genetic system with high levels of heterozygosity (Wimmers *et al.*, 2000). Better understanding of population genetic structure is crucial to develop a sustainable strategy for conservation and effective utilization of low productive livestock species.



PLATE 1: Naked-Neck Nigerian Indigenous chicken (NIC)

Poultry production in tropical countries is based on the traditional scavenging system. The unfortunate scenario in Nigeria and indeed sub-Saharan African countries is the fact that no data are there for the management and conservation of our local indigenous chicken, which has been genetically eroded. The implication is that they are endangered and tilting into extinction (Apuno *et al.*, 2010).

Several studies have been reported on the variation of NIC in different localities using traditional morphometric methods (Egahi *et al.*, 2010; Apuno *et al.*, 2011; Daikwo *et al.*, 2011). The choice of local areas in diversity studies of chicken has often been on the probability of finding genetic originality of this species (Daikwo *et al.*, 2011), as well as their abundance in these areas. Very recently DNA markers such as Restriction Fragment Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), microsatellite etc., were adopted for the characterization of livestock breeds. Inter simple sequence repeat (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellites arranged in opposite direction. They are usually 16-25bp long as primers in a single primer PCR reaction in multiple genomic loci for the amplification of different sizes of ISSR sequences. Information on the diversity of the Nigerian local chicken is scanty hence the need for this study.

2.0 MATERIALS AND METHODS

The total number of local (naked neck strains) chicken used for this evaluation were ten (10); six (6) from Calabar ecotype and four (4) from Obudu ecotype. DNA was extracted using CTAB (Cetyltrimethyl ammonium Bromide) method, according to Allen *et al.*, 2006. To check the quality of the extracted DNA samples, 1.5% Agarose was weighed, dissolved in TBE (Trisma Boric acid EDTA) buffer before casting the gel for electrophoresis. The gel was stained and de-stained in 1.5mg/ml of ethidium bromide solution and distilled water respectively before viewing under UV trans-illuminator. Some that were contaminated with RNA were removed using RNase treatment. PCR amplification was then carried out. The PCR products were electrophoresed in a 1.5% Agarose gel containing 0.5mg/ml ethidium bromide and photographed on trans-illuminator UV light. Power marker software (version 3.5) was used to analyze the ISSR marker effects on genetic diversity of the local chickens while the Genalux software version 3.5, was used for the PCoA analysis.

3.0 RESULTS AND DISCUSSION

Results of the ISSR markers evaluation from two ecotypes - Calabar and Obudu - of naked neck NIC are presented in Table 1. The major allele frequency ranged from 0.3 to 0.5 while the number of alleles ranged from 0.3 to 0.7. Gene diversity ranged from 0.62 to 0.82 while polymorphic information contents (PIC) ranged from 0.547 to 0.797. Many marker systems have been adopted to decipher the extent of genetic diversity in

organisms, including poultry. However, local indigenous chicken, especially in Nigeria has been neglected in this scheme. Some reports have focused on morphological differences while few had delved into protein profiling. Of the few DNA markers used, ISSR has not been reported on. This result though lower than that obtained by Patel *et al.*, (2015), (0.892 -0.943) in their investigation on genetic variation in *Ocimum* spp., were high enough to show the efficiency of the selected primers as a marker discrimination power in the study of diversity in local chicken populations. The PIC values are commonly used in genetics as a measure of polymorphism for a marker locus using linkage analysis. Genetic diversity is commonly measured by genetic distance (GD) or genetic similarity (GS + 1-GD), both of which imply that there are either differences or similarities at the genetic level. The genetic diversity signifies genetic distance between the two ecotypes of local chicken. Results of the major allele frequency and number of alleles, corroborates the genetic diversity scores obtained in this study. Plate 2 (a-d) shows Agarose gel pictures representing 4 ISSR markers used for the amplification of DNA samples of the local chickens. The four primers used were 818 ISSR marker, HB- 11, HB -8 and HB – 13.

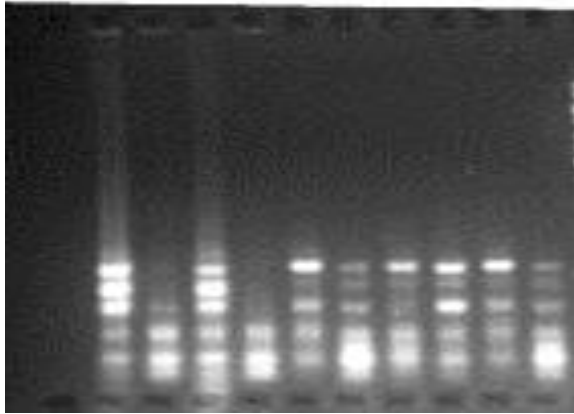
The PCoA of the two ecotypes of the NIC are on Figure 1. This showed that the total

percentage contribution to the variability was 40.26%. This value is lower than most others largely due to the fact that variability within breeds is more genetic than environmental. The genetic distances between both breeds was not highly significant ($P < 0.05$) seemingly for the same reason as the variability. Kuhnlein *et al.* (1989) determined genetic distance (broverbreed, random bred and inbred lines of Whitenleghorn) using DNA finger-printing (DNA-Finger printing). Genetic diversity between different breeds (Cameroon naked neck and normal feathered) and Dahlem Red breed) using DNA-FP'S had been carried out (Mafeni 1995) with results reported similar to ours. The estimation of genetic variation within and between different lines (two white leghorn lines, 3 commercial broiler lines, Rhoda island Red and 8 other breeds) using DNA-FP'S was done by Ponsuksili *et al.*, (1996) while genetic characterization of different lines (ten lines of white leghorn, two Fayomi and one Spanish breed) using DNA-FP's and RAPD was investigated by Campo and Orozco 1986. Genetic variability among layers and their correlation with performance (nine different lines bred from commercial strains imported from Romania and USA) using DNA-FP's had been reported (Meng *et al.*, 1996). Most of these reports are similar to our findings that diversity in poultry populations is more of a product of origin.

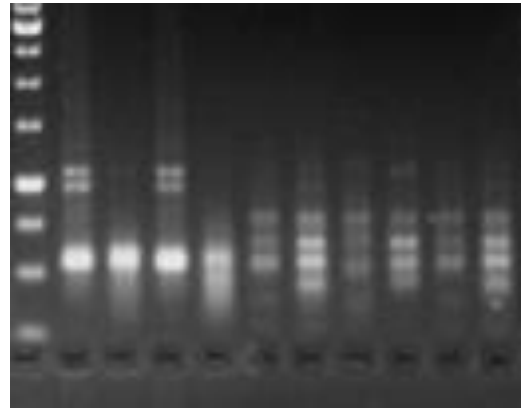
Table 1: Genetic parameters of the four ISSR markers for Genetic Diversity studies of local chickens from Calabar and Obudu Ecotypes

Marker	Major Allele Frequency	Sample Size	No. of obs.	Allele No.	Availability	Gene Diversity	PIC
818	0.3000	10.0000	10.0000	7.0000	1.0000	0.8200	0.7978
HB11	0.4000	10.0000	10.0000	6.0000	1.0000	0.7600	0.7300
HB13	0.5000	10.0000	10.0000	3.0000	1.0000	0.6200	0.5478
HB8	0.5000	10.0000	10.0000	4.0000	1.0000	0.6600	0.6102

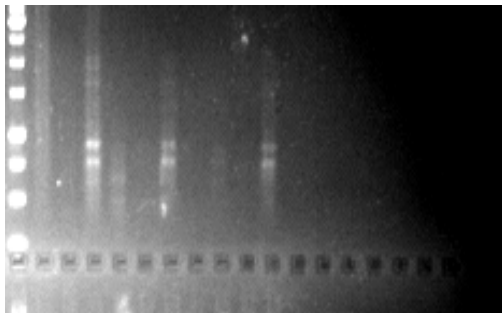
Source: Field Work (2019)



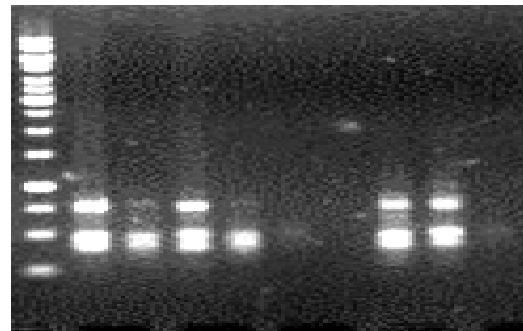
a. 818



b. HB-11

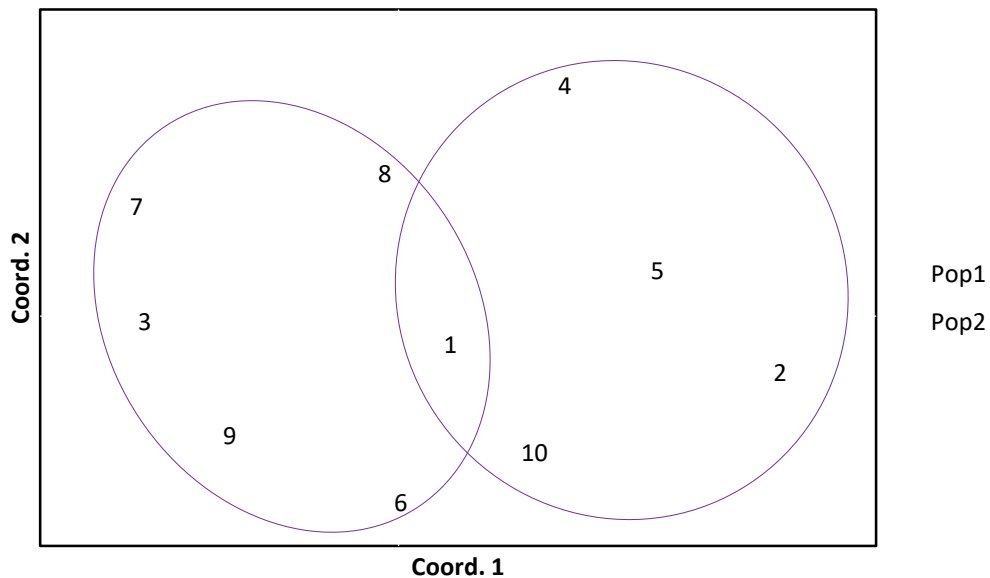


C. HB-8



d. HB - 13

Plate 2 (a - d): DNA amplification of naked neck local Chicken using four primers.
Source: Field work, 2019



Key:

Population 1 - Calabar
Population 2 - Obudu

Axis	1	2	3
%	14.52	13.58	12.16

Fig. 1: Principal coordinate analysis (PCoA) of genetic variation based on the ISSR dataset for naked neck local chicken obtained from Calabar and Obudu Ecotypes. 40. 26% variation explained by axis 1, 2 and 3.

Clustering on the chart also seemed to be population dependent. Although indigenous birds have a number of adaptive traits and genes with economic importance in the tropics (Horst, 1989), regrettably, their real value is often under-estimated probably due to their poor appearance and relatively low productivity. The assumptions concerning the indigenous chicken also stemmed from the fact that detailed genetic diversity analysis have not been carried out in terms of morphology, biochemistry as well as molecularly (either nuclear or mitochondria). Various genetic diversity studies will help in selecting superior genotype(s) for breeding and improvement purposes. From Figure 1, two major clusters were generated based on genetic relationship. Cluster-1 is made up of 5 accessions while cluster-2 had 4 accessions. Mollah *et al.*, (2009) reports using RAPD markers to analyze genetic diversity in Bangladeshi chicken between indigenous and exotic chicken populations. Their report showed

sufficient variability for studied populations. This could be as a result of the variability introduced by the exotic chicken population. From the PCoA plot, it is also evident that genotypes are fairly dispersed, which reflects a good genetic base of the local chickens (Patel *et al.*, 2015). Differences observed in our results as compared to other genetic studies could be as a result of the mono-specie population of naked-neck chicken involved in this investigation.

4.0 CONCLUSION

Genetic variability has been measured directly with different genetic markers in chicken, for example with restricted fragment length polymorphism (RFLP). PIC analysis can be used to evaluate markers so that the most appropriate marker can be selected for genetic mapping and phylogenetic analysis (Anderson *et al.*, 1993; Powell *et al.*, 1996). Result of ISSR makers showed that genetic diversity ranged from 0.62 to 0.82 while polymorphic information contents (PIC)

ranged from 0.547 to 0.797. The major allele frequency ranged from 0.3 to 0.5 while the number of alleles ranged from 0.3 to 0.7. Results on the principal coordinate analysis (PCoA) showed 40.26% variation in the two ecotypes of local chickens on the basis of three axes with the first axis accounting for 14.52% variations, while the second axis accounted for 13.58 % variation and the third 12.16%. From figure 1, two major clusters were generated based on genetic relationship.

5.0 RECOMMENDATIONS

From the results obtained, it is recommended that genetic diversity studies be carried out on other species of local chickens, like the frizzled feather, muffled feather and the normal local chicken. Other molecular markers apart from the ISSR marker can also be used for these investigations. These studies will come in handy as baseline information in breed improvement programs.

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