GENETIC EVALUATION OF HELMETED GUINEA FOWL (*Numida meleagris galeata* Pallas) IN NIGERIA USING MICROSATELLITE MARKER.

BY

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BY

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ZARIA NIGERIA

JANUARY, 2016.

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for a higher degree. All quotations are indicated and sources of information are specially acknowledged by means of references.

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Umar, Umar Aliyu Date

CERTIFICATION

This thesis entitled "Genetic Evaluation of Helmeted Guinea Fowl (*Numida meleagris galeata* Pallas) in Nigeria Using Microsatellite Marker" by Umar, Aliyu UMAR meets the regulations governing the award of the degree of Master in Animal Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my late Father and Mentor (Prof. A.M. Falaki) may Allah forgive his sins, and may his soul rest in jannatul firdaus. Ameen.

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ABSTRACT

A study was carried out to evaluate helmeted guinea fowl (*Numida meleagris galeata* pallas) in Nigeria using 31 set of microsatellite marker, a total of 200 randomly sampled indigenous guinea fowl strains namely; lavender (ash), gray breasted (white breasted), pearl, black and broiler guinea fowl was used for the study with a sample of forty (40) animals per strain. the Result revealed that the markers were lowly (PIC<0.5) polymorphic in White, Ash and highly (PIC>0.5) polymorphic in Broiler, Black and Pearl guinea fowl. The mean observed heterozygosity (0.102), (0.012), (0.042) is lower than the mean expected heterozygosity (0.591), (0.065), (0.126) in White breasted, Ash and Broiler guinea fowl respectively, with the exception of black and pearl guinea fowl with mean observed heterozygosity (0.101), (0.022) being greater than the mean expected heterozygosity (-0.149), (-0.063). All the populations were in equilibrium, with the exception of AB819301 in black guinea fowl loci that deviated ($p<0.01$) from Hardy-Weinberg Equilibrium (HWE). The shortest distance (0.4472) was observed between black and white breasted guinea fowl while the distance between Pearl-White breasted (0.6325) and Pearl-Ash (0.6325) were the same. The highest distance (1.0) was observed between broiler guinea fowl and white breasted. The AMOVA showed that the estimated variation (variance) is 6.70 and 0.00 with percentage variation of 100% among population and 0.00 within population respectively. It was also observed that in PC1, pearl (0.57) has the highest positive value followed by broiler guinea fowl (0.388) and then ash (0.057) having the lowest value of eigen vector While Black (0.241) has the highest eigen vector in PC2 followed by Broiler guinea fowl (0.205) and highest negative value of -0.220 attributed by Ash and Pearl followed by -0.007. in PC3 Black, Broiler guinea fowl and White Breasted present a zero eigen vector values of 0.00 with ash having the highest value (0.281) followed by pearl presenting the

negative (-0.220) value. These 3 PC's account for 97.715% as total variance of which 19.320% was contributed by PC1, while PC2 and PC3 contributed 32.710% and 40.485% respectively. All the eigen values contributed by all the PC'S were below 1.00 finally a distinct cluster was presented by broiler guinea fowl while black and white breasted tends to clustered together in the 1st quadrant. However, Ash and Pearl presented very closer cluster in the 3rd quadrant. Conclusively, the result revealed that the markers were lowly (PIC<0.5) polymorphic in White, Ash and highly (PIC>0.5) polymorphic in Broiler, Black and Pearl guinea fowl indicated a moderate to high gene diversity among the studied markers, the population points towards out breeding with more heterozygote in the studied population and the increase in heterozygosity in the studied population is a good indication for genetic variance at polymorphic sites, rich genetic diversity and selection potential and consistent cluster was formed between the strains which may help to quantify the degree of relationships between guinea fowl strains and provide information of closeness and evolutionary history.

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CHAPTER ONE

1.0. INTRODUCTION

Traditionally, the genetic improvement of livestock breeds has been based on phenotypic selection. The past century was characterized by the development of quantitative theory and methodology towards the accurate selection and prediction of genetic response (Walsh, 2000). This resulted in the selection of a number of economically important genetic traits in cattle, sheep, pigs and poultry. The study of genetics is about the why and how of the traits responsible for the differences in and between various organisms. It is also about all the differences in races, species, breeds, varieties, colours, textures, tastes, shapes, sizes, behaviors, productivity, state of health, and many more traits that characterize plants and animals. It is about why there is variation within variants. The dramatic size contraction of local poultry breeds due to replacement with cosmopolite improved breeds showed the need for native genetic resources conservation. Rare poultry breeds and population characterised by a limited size strictly depend on the maintenance of genetic differences (Wimmers *et al*., 2000). Conservation of genetic variability is of great importance in animal science; the analysis of breed's genetic structure can supply the basis for effective conservation programs and improvement.

It is well known that species can face great environmental changes over time, such as in climate, pollution and diseases, genetic diversity is required for populations to adapt to these changes (Frankham *et al*., 2002). Loss of genetic diversity is often associated with inbreeding and reduction in reproductive fitness (Frankham *et al*., 2002; Wasi *et al*., 2006) although there has been some disagreement regarding the importance of genetic factors in population extinctions (Frankham *et al*., 2002). Africa is blessed with a lot of indigenous poultry.

The indigenous poultry are resistant to most of the endemic poultry diseases and pests ravaging their exotic cousins (Sil *et al*., 2002). Apart from being a source of meat and eggs, they are also a source of income for many resource-poor people particularly those living in the rural areas. They are valued also for their scavenging ability, tolerance, meat quality and general hardiness (Ssewanyana *et al*., 2003). The indigenous chickens are repositories of unique genes that could be used in other parts of the world (Adebambo, 2004). Hence, the need for their conservation to keep genetic variation within and between local breeds.

Guinea fowl (*Numida meleagris*) is thought to have originated in Africa; its production has increased rapidly throughout the entire world. Guinea fowl can be successfully reared under semi-intensive conditions with less effort. That is why it has been very important for third world countries. With the help of developments in organic farming techniques in recent years the guinea fowl has been given importance as an alternative poultry (Sarıca *et al.*, 2003). While partly known in some parts of Asia and South America it is widely bred in some European countries such as France, Italy and Belgium (Champagne, 2003; Embury, 2001). Its implementation in intensive production has very little used, however, is a good chance of recovery of the animal due to nutritional and dietary outstanding of its meat and eggs (Agwunobi and Elpenyong, 1991) Guinea fowl is believed to have evolved from a francolin-like Asiatic ancestor, but it is believed that the evolution, mutation and development to modern forms occurred solely in Africa (Cracraft, 1973 and Oslon, 1974). Out of the five world *galliformes*, it is only the guinea fowl, *Numidinae*, which is entirely endemic to Africa (Delacour, 1997). Guinea fowl has been classified into four genera (*Agelastes-Phasiadus, Guttera, Acryllium* and *Numida*), six species and 16 subspecies. The major genera of guinea fowls can be distinguished

by the lateral views of the head, neck and collar region as well as by the biome they occupy. The *Numida* is the genera of all the helmeted guinea fowls found ubiquitously distributed throughout the African savanna outside desert, Mediterranean and mountain areas (Ayorinde, 2004).

It is however, the development of molecular biology during the past three decades that created an exciting new means for studying livestock genetics and animal breeding. Selection according to genotype has indeed become an important tool in the breeding of farm animals. In very recent years the molecular biological approach has also been revolutionized by the application of new technologies. Conservation of genetic variability is of great importance in animal science; the analysis of breed's genetic structure can supply the basis for effective conservation programs, genetic diversity is defined as the sum of genetic differences in multiple loci among individuals in a population, and is most readily reflected in the phenotypic variation seen in many populations. It is a valuable asset as the adaptability of a population, that is the population's ability to adjust to changes, depends on it (Woolliams *et al.*, 2005).

The application of molecular biology techniques helps in avoiding the risk of compromising genetic variability of conservation programs of small populations. Until 1997, the genotyping of the individual animals for marker assisted conservation scheme was carried out using the amplified fragment length polymorphism (AFLP) technique (Christopher *et al.*, 1997). Afterward, microsatellites have been applied because they are well dispersed in the genome and highly polymorphic (Cheng *et al.*, 1998). Their application to characterize chicken breeds is relatively recent but it has been used in many countries to study the genetic relationships among native breeds of poultry (Takahashi *et al*., 1998 and Hillel *et al*., 2003). Microsatellites were described by (Tautz, 1989) as simple sequence-stretches with a high degree of hyper-variability, and are abundant and well distributed in eukaryotic genomes (Cheng and Crittenden, 1994).

These simple sequences consist of short segments of DNA with motif repeats, usually less than six base pairs. Microsatellite markers have been shown to be appropriate tools for linkage mapping, identification of quantitative trait loci and parentage testing (Bruford and Wayne, 1993; Crooijmans *et al.*1996 and Ponsuksili *et al.* 1998) also demonstrated the usefulness of microsatellites in the estimation of genetic relatedness and diversity in chickens. Approximately 500 fluorescently labelled primers for microsatellite analysis in chickens have already been described and are commercially available in the form of mapping kits (Cheng *et al*., 1998). The microsatellites were developed and mapped by the East-Lansing group, Chickmap (Roslin Institute) and Wageningen Agricultural University, Netherland.

1.1. Justification

Information on guinea fowl production is scarce in Nigeria, which hampers rapid development of this industry probably due to long generation interval even though they are suitable for organic breeding. Performance increase through selection show that the production of these birds can be prevalent and needed in the near future.

Information from this research will be a good complementary to the conventional methods of trait selection based on performance records. Estimation of genetic parameters is primordial to the establishment of strategies used in animal breeding programs because with the study of these parameters, the evaluation of response to selection for a trait and genetic associations among traits become possible (Farahat *et al.,* 2010). Therefore, studies on genetic diversity and the structure of population within and between species may not only illustrate the evolutionary process and mechanism but also provide information useful for breeding and biological conservation of the livestock species and draws the attention of scientist universally to make

more meaningful contribution to improvement and year round production of guinea fowl and their products in Nigeria and help toward achieving National Food Security.

1.2. Hypothesis

Null hypothesis (Ho):

1. There is no polymorphism in markers from the indigenous guinea fowl strains.
2. There is no genetic diversity within and among Nigerian guinea fowl strains.
3. There are no genetic relationships among Nigerian guinea fowl strains.

1.3. Objectives

The objective of this study includes:

1) To analyze the degree of polymorphism in markers from the indigenous guinea fowl strains.
2) Determination of genetic diversity within and among some Nigerian guinea fowl strains.
3) Determination of genetic relationships among the Nigerian guinea fowl strains.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Guinea Fowl

2.1.1. Physical characteristics of guinea Fowl

The major genera of guinea fowl can be distinguished by the lateral view of the head, neck and collar region as well as the territory they occupy. NRC (1991) reported that Guinea fowl are somewhat larger than average scavenger type chicken, with adult weighing up to 2.5kg. Some have dark grey feathers with small white spots, bare heads with a bony ridge (helmet) on top, which make them look somehow like vultures. The short tail feathers usually shape downwards. The chicks known as keets resemble young quails. The sexes are indistinguishable until about 8 weeks of age. Oyedipe *et al.(*1982) indicated that the first origin of the scientifically known Guinea fowl was in West Africa. He also observed that the home of this specie is the "guinea" savanna, which explains its common English name. In Nigeria, the most widely distributed guinea fowl is the helmeted Guinea fowl *Numida meleagris galeata*. It inhabits the grassland areas spreading from the derived savanna near the forest zone in the south through the true savanna vegetation zone (Ayeni and Ayanda, 1981; Ayeni, 1979). Four varieties of *N.meleagris* which include Lavender (ash), Black, Pearl and White have been domesticated but only Pearl exists in the There are three main genera's of the Numidae family in Africa. These are: Royal guinea fowl (Figure 2.1: A to I) that has only some feathers on the nape of the neck and has Somalia, Tanzania and Zanzibar as its habitat. Crested guinea fowl (Figure 2.1: K to S) found in the African tropical forest and helmeted guinea fowl, which are those with either blue or red carouncles. It is the *N. meleagris* that has been introduced all over the world from West Africa,

which is its native origin. From it the gray (Plate I to V), white, lilac, violet, lavender (Plate I**)** and Isabelle varieties have been developed. Of the four common subspecies of Numida found in Africa, only two have been domesticated. These are the *N. meleagris*, indigenous to West Africa, and *N. ptalorhrca*, which is indigenous to East Africa. There are two main guinea fowl subspecies found in Nigeria. The helmeted guinea fowl *N. meleagris* galeata *Pallas*, occur freely throughout the grassland areas spreading from the derived savanna near the forest zone in the south to the true savanna into northern guinea savanna vegetation zones. The second, the crested guinea fowl, (figure 2.1), Guttera edouardi edouardi, is restricted in distribution to the forest and derived savanna forest edges (Ayeni, 1979). The number of free ranging semi-wild guinea fowl kept in captivity in Nigeria alone is said to be about 45 million (Akinwumi *et al*., 1979) with more millions still in the wild. It is second to the domestic fowl in terms of number and supply of poultry protein in Nigeria. Thus a huge number exists for various studies and from which to select for improvement. Nigerian guinea fowl is slow growing, weighing less than 1 kg at 8 weeks of age, come into lay at about 1 year of age, monogamous in the wild, and sex ratio of 1:4 can give good fertility. Egg production is confined to the rainy season. Sex identification presents an enormous problem for most guinea fowl farmers (Nwagu and Alawa, 1995).

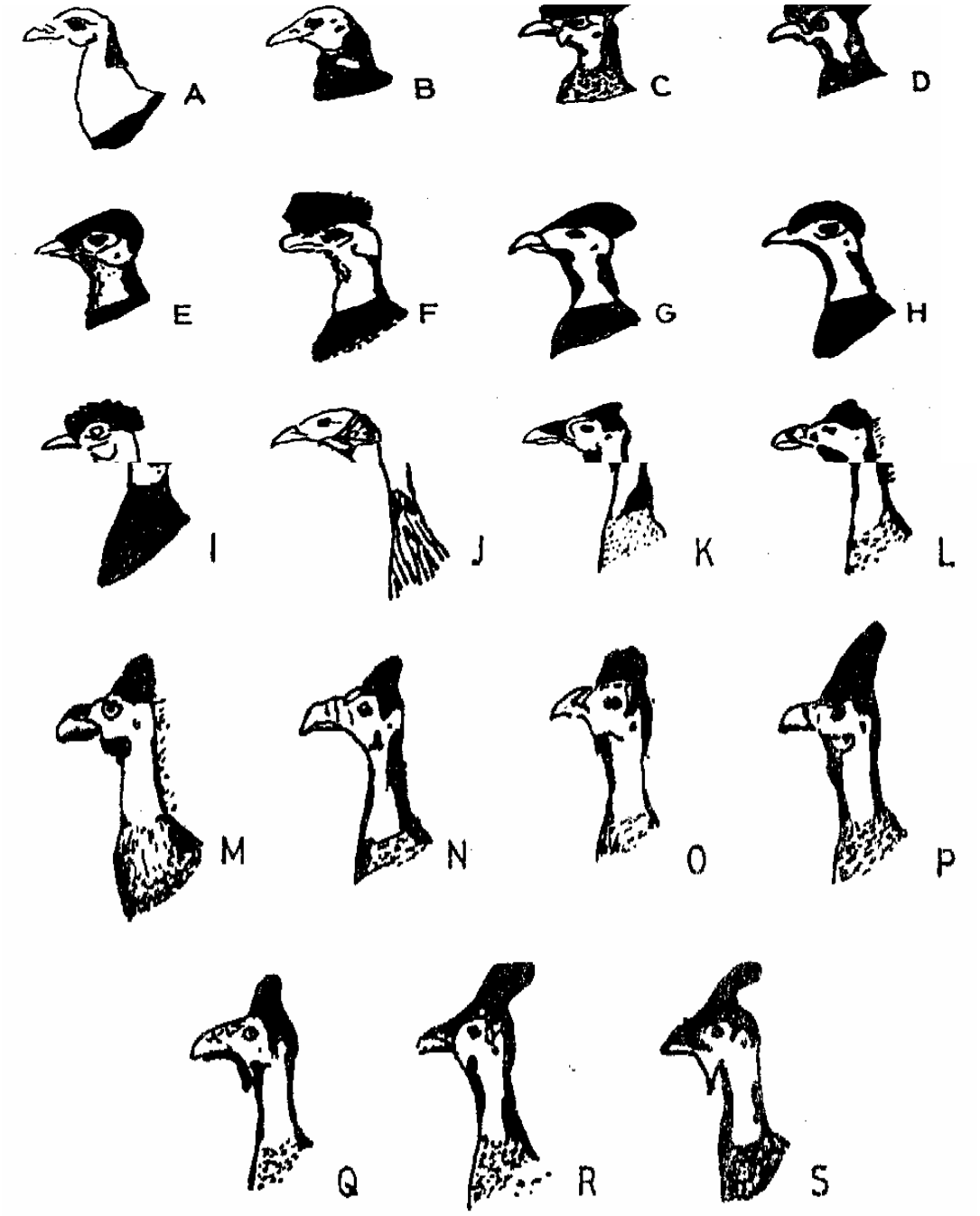

Figure 2. 1. Guinea fowl taxa in Africa
Source: Ayorinde (2004).
A, B, C, D, E, F, G, H and I: Royal guinea fowl
J, K, L, M, N, O, P, Q, R and S: Crested guinea fowl

Plate II. White breasted guinea fowl

Plate III. Lavender (Ash) guinea fowl

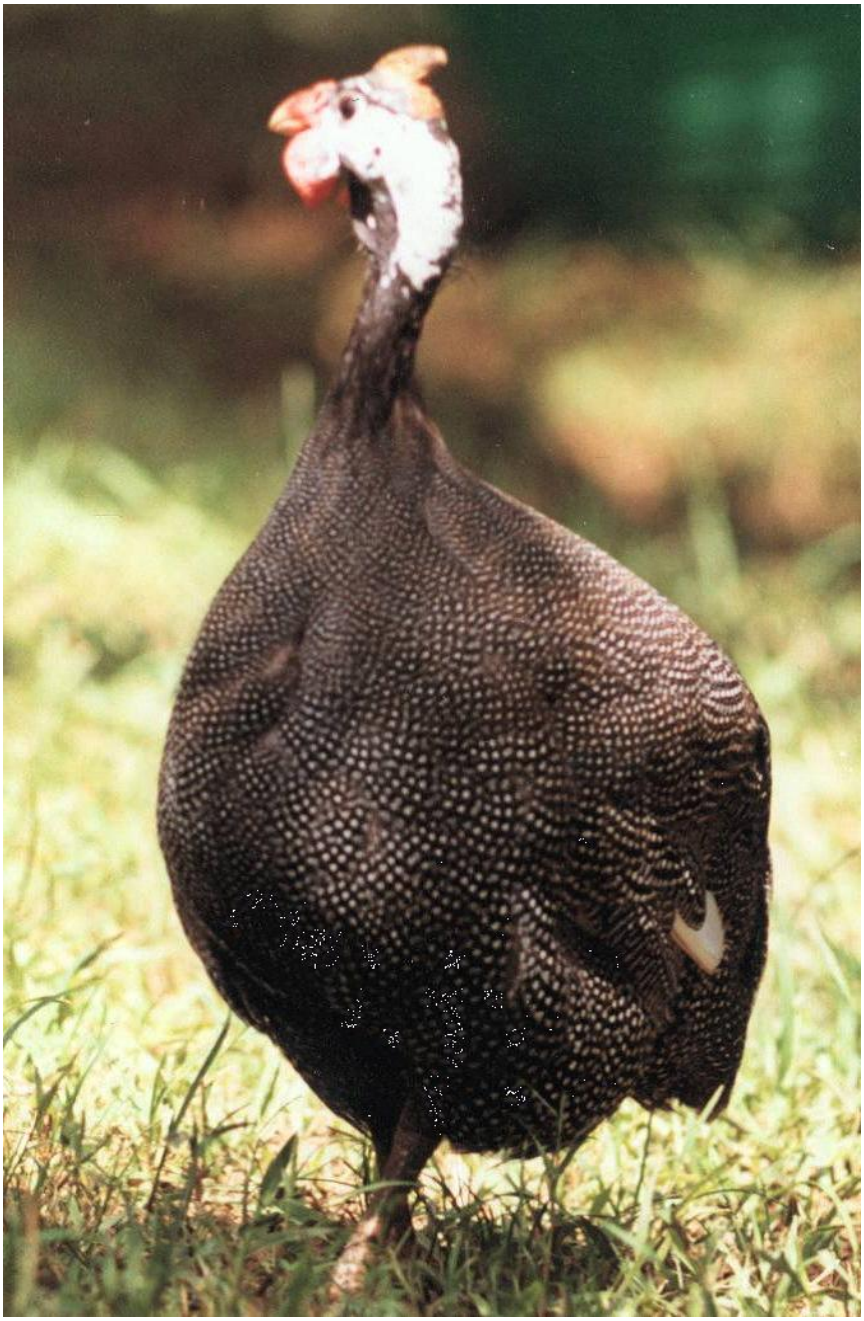

Plate IV. Pearl Guinea fowl

Plate V. Broiler guinea fowl

2.2. DNA Markers and Genotyping

Deoxyribonucleic acid known as "DNA" is the genetic material in every living organism. The genetic information in the DNA molecule is encoded in the nucleotides Guanine (G), Adenine (A), Thymine (T) and Cytosine (C). These make sequences that encode for amino acids as presented in plate 2.6. The DNA has a double stranded helix structure. The backbone of the helix is made of deoxyribose sugar and phosphate groups. Nucleotides are attached to the backbone with hydrogen bindings. DNA is tightly packed into chromosomes in the nucleus of the cell. A functional sequence of DNA is called a gene. In a gene the sequence of nucleotides vary in repeats, insertions/deletions and transitions/transversions from an individual to another. This leads to genetic variation in the same species (Eenennaam, 2009).

The genetic variation has led to the study of effective population size, population history that includes migration and recent expansion, population structure and various genetic diseases (Mburu and Hannote, 2005). Genotyping is a process of analysing the variation of an individual's genotype by examining the DNA sequence from its biological assay and also by comparing it with other individual's sequences. This term is generally used to describe a method that determines the DNA-marker alleles that an individual carries at a particular genomic locus. This makes it easy to identify the inherited properties of the individual within its family and also how they differ between other species. Many alleles come in dominant and recessive forms, but there may also be many more ways that these alleles express a specific trait which lead to hereditary variations. In other words, genotype is the molecular code of the DNA that can also lead to the study of phenotype of an organism (Eenennaam, 2009).

Several studies have been successful in identifying the DNA regions that enhance the production traits. Tests have been successfully developed to identify whether an animal carries a particular trait of interest with the help of several type of genetic markers.

2.2.1. Mitochondrial DNA (mtDNA)

Mitochondrial DNA Is a small circular molecule that comprises of approximately 37 genes coding for 22 tRNAs, 2 rRNAs and 13 mRNAs within the cytochrome "b" coding region that can be used in phylogenetic work. Phylogenetic studies can also be conducted in the non-coding region, where the displacement loop (D-loop) controls the mtDNA expression. mtDNA polymorphisms are widely used to determine the structure of population, variability among species, and evolutionary relationships (Akey *et al.*, 2002; Mburu and Hannote, 2005).

2.2.2. Restriction fragment length polymorphism (RFLP's)

RFLP is based on the patterns derived from a DNA sequence digested by a known restriction (Endonuclease) enzyme. The length of the digested fragments differs as a result of point mutation, which can be created/ destroyed at the restriction site or by insertion/deletion that can alter the length of restriction fragment. RFLPs are widely used as co-dominant markers.

Only high quality of DNA should be used. Although RFLPs are suited for phylogenetic studies, due to the tedious methodology the use of RFLP's as markers become less reliable (Nagaoka and Ogihara, 1997).

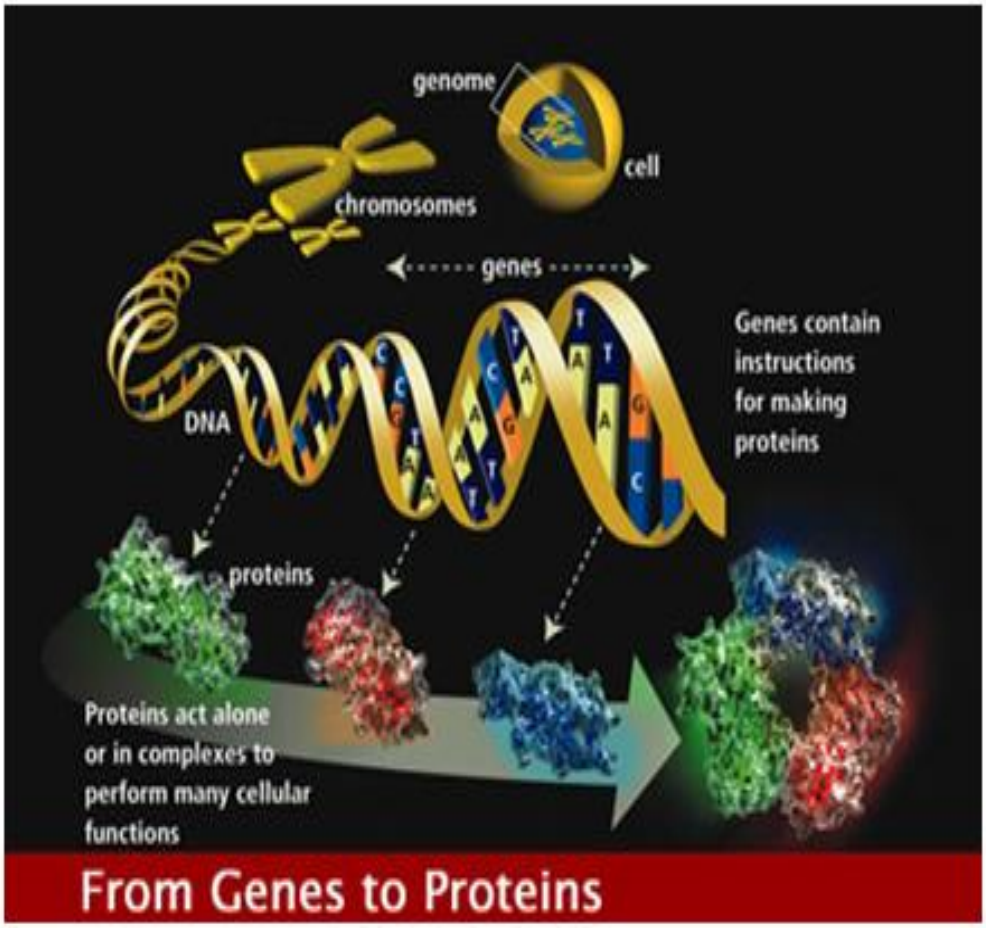

Plate VI. From genomic DNA to protein cluster. Sourced from Eenennaam (2009)

2.2.3. Amplified fragment length polymorphism (AFLP's)

AFLP Differed from RFLPs with detection of presence or absence of restriction fragments and not by the size of fragments. Normally AFLP primers are highly specific to the targeted restriction site of whole digested genome. As these markers are dominant markers they could only be used to estimate genetic variation for example in DNA finger printing and cannot be used in population genetic studies (Mueller and Wolfenbarger, 1999).

2.2.4. Random amplified polymorphic DNA (RAPD)

RAPD Are short arbitrary primers that can bind to any place in a sequence without any prior knowledge of the DNA. The target product is amplified and studied. In case of mutations, mismatches between the target and primer may not result in PCR product. Moreover these are dominant markers and detection of polymorphisms is limited. In general, the use of dominant markers like RAPD for identifying heterozygotes will require twice the amount of co-dominant markers (Williamson *et al*., 2002). In birds, RAPD has been employed for several purposes such as evaluating genetic variation within and among populations (Smith *et al*., 1996; Horn *et al*., 1996; Sharma *et al*., 1998; Padilla *et al*., 2000), identification of geographic populations of migratory species (Haig *et al*., 1997), in distinguishing segregation of alleles in crosses between inbred lines (Wei *et al*., 1997) and in sex determination (Lessells and Mateman, 1998). RAPD Markers have also been recently employed to evaluate Genetic Diversity (Pawel *et al*., 2009; Mehlenbacher *et al*., 2006) and genomic polymorphisms **(**Zhang *et al*., 2010). Thus, this technique samples the genome more randomly than other methods based on the coding region variability (Lynch, 1991). The RAPD analysis requires minimum sample material, no prior sequence information, no radioactive probes, and enhances the feasibility of molecular studies when access to specimens is restricted (Kozol *et al*., 1994).

2.2.5. Microsatellites

Microsatellites Are stretches of DNA consisting of short tandem repeats of nucleotides (usually 1 to 5bp). The tandem repeats varies among every individual such that no individual can have the same number of repeats. Microsatellites have been used commonly in forensics, disease diagnosis, population genetic studies, in conservation biology and linkage analysis due to their length polymorphism and abundance in all genomes as reported by (Rodrigues and Kumar, 2006). Thirty one (31) set of microsatellite marker was also used to genotype West African and guinea fowl from japan (Botchway *et al*., 2013), For the 31 polymorphic loci, the observed number of alleles ranged from 2 to 9 (mean 3.39) with allele sizes ranging from 94 bp to 286 bp, while the effective number of alleles ranged from 1.03 to 4.97 (mean 2.04). The observed (HO) and expected heterozygosities (He) ranged from 0.033 to 1.000 (mean 0.396) and 0.033 to 0.799 (mean 0.419), respectively. A large number of highly polymorphic microsatellites have been characterized and mapped in poultry, including chicken, quail and other phasianidae family (Inoue-Murayama *et al*., 2001; Pang *et al*., 1999; Kayang *et al*., 2000, 2002, 2003, 2004; Kikuchi, 2005), facilitating the use of these markers in parentage testing. Acceptance of this kind of test must provide a high degree of certainty in the assignment of parentage, which can be evaluated with the Exclusion Probability (Weir and Bruce, 1996). Wild and improved quails were also genotype to characterize them and their genetic distance was determined by (Ayanga *et al.*, 2002).

2.3. Application of Microsatellite Markers

2.3.1. Genetic studies

Ben *et al*, (2014) studied the first detailed analysis of genetic diversity of the Tunisian rabbit populations using 36 microsatellite markers, out of which 294 were genotyped. The genetic

differentiation among the population implies that 98.9 % of the total genetic variation was explained by individual variability with heterozygosity ranging from 0.3 to 0.53. The re-colonization process was studied by using the population genetics approach in wolves from Alps and Apennines were genotyped using 12 microsatellite loci and the study concluded that the wolves from Alps showed lower genetic diversity compared to those from the Apennines (Fabbri *et al*., 2007 and Cantú-iris Mariana *et al.,* 2012). Another study was conducted on dogs to validate the efficiency of the microsatellite markers on Dogs and showed thet dogs were normally differentiated by their phenotypic traits such as size, shape, coat colour and behavior. Apart from these characteristics, 28 breeds of dogs were analysed to assess the genetic variation using 100 microsatellite markers. The resulting breed-specific allele frequencies were then used to interpret the genetic distances between the breeds. These results also concluded that the heterozygosis tended to decrease as the population sizes decreased (Irion *et al*., 2003). As microsatellites markers are present on the sex chromosomes, they are also utilised for sex determination. The consistencies of the markers to differentiate X and Y-chromosomes even in the immature individuals lead to the reliability of microsatellite markers in sex differentiation (Takahito *et al*., 2011). Thirty one (31) set of microsatellite marker was also used to genotype West African and guinea fowl from japan (Botchway *et al*., 2013), For the 31 polymorphic loci, the observed number of alleles ranged from 2 to 9 (mean 3.39) with allele sizes ranging from 94 bp to 286 bp, while the effective number of alleles ranged from 1.03 to 4.97 (mean 2.04). The observed (HO) and expected heterozygosities (He) ranged from 0.033 to 1.000 (mean 0.396) and 0.033 to 0.799 (mean 0.419), respectively.

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2.3.2. Microsatellite marker/ primer design

With the help of PRIMER3 software (Marcelle-Moore, 2010) redesigned 31 microsatellite markers and a sex marker from the existing flanking sequences in order to genotype pets in California and Sweden. They also used the MULTIPLEX program to group the primers into 5 multiplexes. Similarly, (Shen *et al*., 2010) added that the main goal in designing good primers is to produce efficient and sufficient amplification. Quality of the primers is proportional to the success rate of amplification. The length of the primer is linked with the specificity of the amplification. Primers with reasonable GC content are designed. Care is taken so that primers are not complimentary especially at 3' prime end (Dieffenbach *et al.*, 2010).

2.3.3. Multiplex PCR

Is a very powerful and widely used genotyping technique that enables amplification of two or more products in a single reaction. It simultaneously amplifies multiple regions of a DNA template or multiple DNA templates using more than one primer set comprising of forward and reverse primer in single tube (Shen *et al*., 2010). It is also used for qualitative and semi – quantitative gene expression analysis, DNA testing in research, forensic and diagnostic laboratories on both eukaryotes as well as prokaryotic sources (Qiagen Multiplex PCR Handbook, October 2010). A fluorescent primer based PCR has the ability to genotype animal population carrying normal or mutant alleles.

2.3.3.1. *Applications of multiplex PCR :* In Animals/human: Analysis of satellite DNA, genotyping of transgenic animals, lineage analysis, detection of pathogens, diet analysis, sex determination, mutation detection and qualitative and semi quantitative analysis of gene expression. In Plants: GMO analysis, analysis of satellite DNA, lineage analysis, pathogen detection, qualitative and semi quantitative analysis of gene expression and genotyping of transgenic plants. In Bacteria/viruses: Hygiene analysis, diagnostics/pathogen detection and qualitative and semi quantitative analysis of gene expression.

2.3.3.2. *PCR inhibitors:* Many scientists have faced PCR inhibitors as an obstacle for successful PCR amplification. These inhibitors either directly interact with DNA or with DNA polymerase thereby preventing the amplification completely or reduced product yield. The magnesium that acts as a critical cofactor for the DNA polymerase is found to be the major target for the inhibitors to act on (Bessetti, 2007). These inhibitors normally bind to or reduce the magnesium, and thus inhibit the PCR. Commonly these inhibitors are found in blood, fabrics, tissues and soil sources as a result of excess of potassium chloride, sodium chloride and other salts (Manivannan, 2013). They also occur in the presence of iso-detergents, phenol, and ethanol and other alcohols (Katcher and Schwartz, 1994).

2.3.3.3. *Methods to overcome inhibition:* The best way to avoid PCR inhibition is by preventing it from being processed along with the samples. Prevention is almost impossible while handling samples like blood or other tissue material. Scientists have approached prevention of PCR inhibition or failure using the following steps:

- Swab-transfer method for sample collection has been proved to avoid inhibitor containing materials rather than processing spliced materials. DNA purification methods were performed with specialised kits like the QIAamp DNA Stool Mini kit. This kit contains Inhibitex tablets, which has the property to absorb the inhibitors in the purification process while extracting the DNA from the stool. When the tablet adsorbs the inhibitors, ASL buffer is used to remove them. Bacteria and other pathogens are lysed or killed by incubating the stool homogenate at 70°C (QIAamp® DNA Stool Handbook).

- Increasing the DNA polymerase amount in the reaction and also by the use of additives like Bovine Serum Albumin (BSA). BSA is found to provide resistance against inhibitors in blood (Bessetti, 2007).

Use of Internal Positive Control (IPC) in the multiplex real-time PCR helps to detect the inhibitors by analysing target amplification efficiency (Bessetti, 2007). From the above references, genotyping was carried out on the DNAs isolated from the muscle tissues, hair and scat of the organisms using multiplex PCR. As the hair and scat can be collected without capturing the animals, these methods show great promise in estimating the population data (Scott and Elias, 2013). The genotyping data were used to analyze and study genome mapping, population genetics. Genotyping data with neutral markers led to the determination of underlying mutation, which is proportional to the polymorphism of neutral markers. The rate of mutation helps in estimating the genetic distance and is also observed that it leads to direct transmission of alleles from the parent to offspring. The data is further analyzed to determine the linkage and lineage among the individuals. Genotyping data involving microsatellite markers are also used in identification of paternity, forensic studies and linkage disequilibrium mapping studies (Ellegren, 2004).

2.4. Standard Diversity Indices

2.4.1. Genotypic frequency:

Estimates genotypic frequencies observed at each locus from raw data only for co-dominant markers.

2.4.2. Hardy-Weinberg equilibrium test (HWE-Test):

Hardy-Weinberg Equilibrium Test explains that the both gene and genotype frequencies will be constant from generation to subsequent next generations (Labate, 2000) HWE assumption is under following consideration: Random mating, no selection, no mutation and no migration (Mariette and Kremer 1999). Deviation from HWE indicates that one or some of mentioned factors make disequilibrium from this test. Chi-square is the usual tool for determination of whether the allelic frequencies are in HWE (Levene, 1949) at each locus only for co-dominant markers. Botchway *et al*. (2013) reported that out of the 31 polymorphic loci analyzed only Nine loci deviated significantly from Hardy–Weinberg Equilibrium ($p<0.05$) following Bonferroni correction. NAF was less than 0.2 in all loci except locus Nmg22 (0.3).

$HWE= \sum(Oi-Ei)/ Ei$

When

HWE= statistical test

Oi = Observed frequency

Ei = Expected frequency

Df= Degree of freedom

If $X^2cal \leq X^2$ tab then Ho is accepted, it means that allele frequencies for loci in a given population are in HWE, if $X^2cal \geq X^2$ tab then H0 is rejected (Hartle and clark 1989).

2.4.3. Allele frequency:

Estimates gene frequencies at each locus from raw data. Missing values are excluded from such estimation.

2.4.4. Allele number:

Counts the number of alleles with nonzero frequency.

2.4.5. Effective allele number:

Estimates the reciprocal of homozygosity (Hartl and Clark, 1989).

2.4.6. Polymorphic loci:

Percentage of all loci those were polymorphic regardless of allele Frequencies.

2.4.7. Observe homozygosity:

Estimates proportion of observed homozygotes at a given locus.

2.4.8. Expected heterozygosity (Gene diversity):

Estimates proportion of expected heterozygotes under random mating only for co-dominant markers. Two estimates are given. The first is (Saitou and Nei, 1987) heterozygosity. The second the expected heterozygosity estimated using the algorithm of Levene (1949), which is the same as (Nei, 1978) unbiased heterozygosity. It is defined as the probability that two randomly chosen haplotypes are different in the sample. Gene diversity and its sampling variance are estimated as

$$H = \frac{\mathrm{n}}{\mathrm{n}-1}\left(1 - \sum_{i=1}^{n} P_i^2\right)$$

$$V(H) = \frac{2}{n(n-1)}\left\{2(n-2)\left[\sum_{i=1}^{k} P_i^3 - \sum_{i=1}^{k} P_i^2)2\right] + \sum_{i=1}^{k} P_i^3 - \sum_{i=1}^{k} P_i^2)2\right\},$$

Where *n* is the number of gene copies in the sample, *k* is the number of haplotypes, and is the sample frequency of the *i*-th haplotype. *Ip*

(Saitou and Nei, 1987). the standard deviation of the Heterozygosity computed as

$$s.d.(H) = \sqrt{V(H)}.$$

2.4.9. Expected heterozygosity per locus

For each locus, an estimation of the expected heterozygosity simply as

$$H = \frac{\mathrm{n}}{\mathrm{n}-1}(1 - \sum_{i=1}^{n} P_i^2)$$

2.4.10. Homogeneity test:

Constructs two-way contingency tables and carries out chi-square (C2) and likelihood ratio (G2) tests for homogeneity of gene frequencies across populations. The tests are carried out for Groups or Multiple Populations.

2.4.11. Genetic distance:

Estimates Nei (1972) genetic identity and genetic distance and Nei's (1978) unbiased genetic identity and genetic distance. The estimation is made for Groups or Multiple Populations.

2.4.12. Number of usable loci

Number of loci that show less than a specified amount of missing data.

2.4.13. Number of polymorphic sites *(S)*

Number of usable loci that show more than one allele per locus.

2.4.14. PIC (Polymorphic Information Content)

An important index of polymorphism of microsatellite DNA sites ware first used to estimate the polymorphism of gene markers in linkage analysis. The value of PIC indicates the degree of polymorphism. A site is highly polymorphic when PIC>0.5, normally polymorphic when

PIC<0.5, and lowly polymorphic when PIC<0.25. Kong *et al. (*2006) assessed the genetic variation and established the relationship amongst breeds and strains using 15 chicken specific microsatellite markers, and founded that PIC of UMA1019 was the highest (0.872) and that of ADL0234 was the lowest (0.562). Chang *et al. (*2007) studied genetic diversity of quail breeds in China based on microsatellite markers, and indicated the highest PIC was 0.573. In contrast to that of chicken and other fowls, quail's microsatellite DNA has not received much attention from researchers.

2.4.15. Principal component analysis (PCA)

The construction of phylogeny and calculation of genetics distances have the disadvantage of not being able to detect the effects of past bottleneck effects on population and any admixture that has taken place between population (Cavalli-Sforza *et al*., 1994).

An alternative method taking in to account the admixture is multivariate analysis, for example, principal component analysis (PCA). PCA can be used to reduce a large number of variables to a smaller number of variables without losing too much information (Cavalli-Sforza *et al*., 1994). PCA involves a mathematical procedure that transforms a number of (possibly) correlated variable into a (smaller) number of uncorrelated variable called principal components. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible.PCA simply describes the variation of the multivariate data in term of some linear combinations of the variables, in this case the observed microsatellite allele frequencies, which are resolved into smaller reference vectors. The new variables are uncorrelated with each other. The coefficients defining the linear transformation are chosen so as to minimize the variation of the transformed data measure along each new co-ordinate axis (Cavalla-Sforza *et al*., 1994). Any component with

Eigen values less than 1.00 are not considered to be stable. They account for less variability than does a single variable (Girden, 2001). When a factor has an Eigen value less than 1.00, in a sense it has more than one variable contributing to the value.

2.4.16. Construction of phylogeny

The Neighbor-Joining (NJ) methodology (Saitou and Nei, 1987) is used to construct the phylogenetic tree of population relationships. The NJ is known to be more efficient than many other methods in obtaining the correct tree (Rzhetsky and Nei, 1992).The neighbor joining method does not assume an equal rate of evolution between lineages unlike the un-weighted pair group-method with arithmetic mean (UPGMA), another method commonly used to construct phylogenetic trees (Nei *et al*.,1983).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study Location

This study was carried out using guinea fowl populations in three states of the country namely; Katsina state ($12^0$15'N and $7^0$30'E), Kano State ($12^0$00'N and $9^0$45'), and Kaduna State figure 3.1 and table 3.1 (Google, 2012).

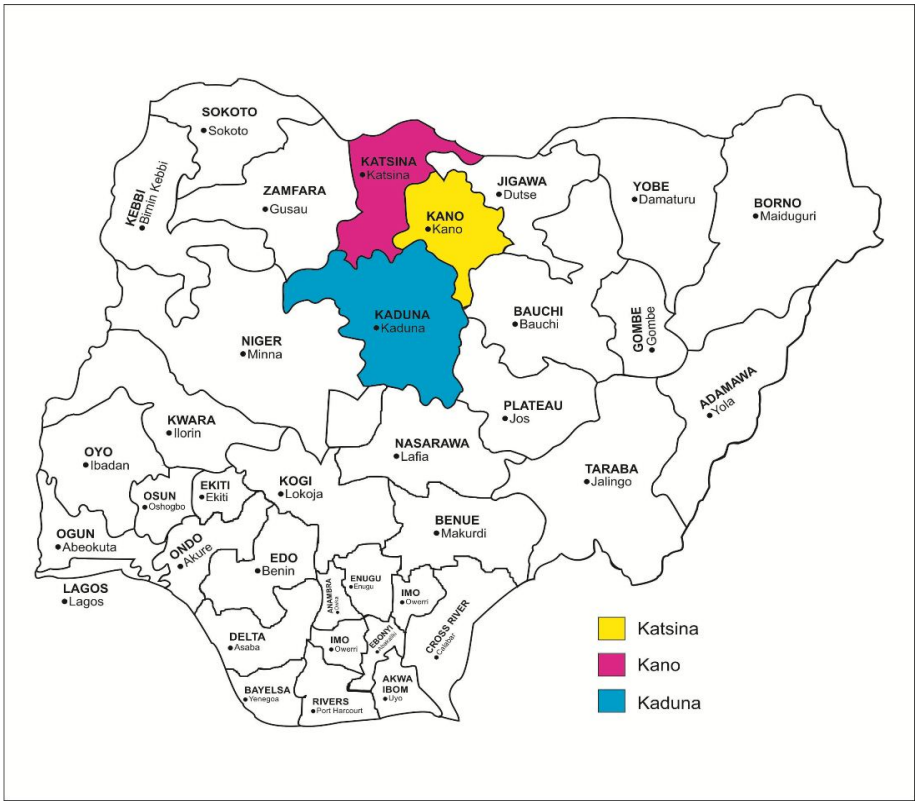


Figure 3. 1 Map of Nigeria showing locations where samples were collected

Table 3.1. List of sampling locations and states

LOCATION	STATE	STRAIN
Sabongari Market	Kano	No specific
Dakastale gada	Kano	No specific
Kwanar dangora	Kano	No specific
Samaru College of Agric DAC/ABU Zaria	Kaduna	Broiler guinea fowl
Galadimawa	Kaduna	No specific
Dumbi	Kaduna	No specific
Giwa	Kaduna	No specific
Funtua	Katsina	No specific

3.2. Experimental Birds

A total of 200 indigenous strains of Guinea fowl convenient/randomly collected from eight (8) different location Table 3.1 in three states namely; Lavender (Ash), Gray breasted (white breasted), Pearl, Black and Broiler Guinea fowl was used for the study. For each strain, forty (40) animals ware sampled and blood (2ml) was collected in EDTA bottles from the selected birds and stored at –20°C until analysis.

3.3. Laboratory Analysis

The laboratory analysis was done at Centre for Biotechnology Research and Training Ahmadu Bello University Zaria (CBRT/ABU), and Molecular Diagnosis (Avian influenza) Laboratory of Veterinary Teaching Hospital, Ahmadu Bello University (ABU) Zaria.

3.4. Genomic DNA Extraction

Fowl genomic DNA used as a template for PCR reaction was isolated from blood using a modified DNA purification kit (Thermo Scientific® Plate VII.) according to the procedures described by (Sambrook, Fritch, and Maniartis, 1998) figure 3.2.

3.5. DNA Quantification

The purified DNA was checked by running it on a 1.0 % Agarose gel stained with G-green.

Plate VIII. Modified Genomic DNA Extraction and purification Kit

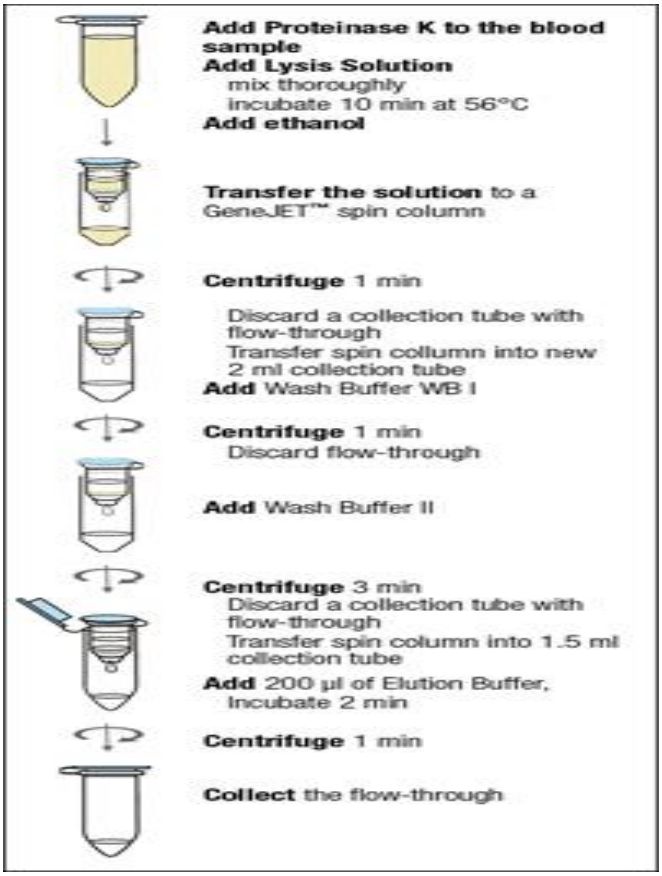


Figure 3. 2 Protenase K gDNA Extraction procedure

3.6. PCR amplification

A multiplex PCR was done using the 31 microsatellites (Table 3.2) by a PX2 Thermo hybaid thermal cycler at the following conditions. A working dilution of 5ng/µL of genomic DNA was used. A 25µL PCR reaction was performed using 9.0µL of Taq 2x Master Mix (Tqman of Thermo fisher scientific^{TM}) which includes 0.4mM dNTPs, 50U/ml Taq polymerase, 1.5mM $MgCl_2$, 1.5µL Standard Taq Reaction Buffer and stabilizers and 8µL of primers. The PCR amplification was done on a PX2 Thermal Cycler (Thermo Electron Corporation) using the following setup: an initial denaturation at 96°C (4 min), annealing at 53°C (45 sec), and extension at 72°C (1.5 min) for 35 cycles according to procedure of Sanger *et al.* (1977).

3.7. Electrophoresis

PCR products were subjected to electrophoresis on Bio-Rad gel electrophoresis unit on 1.5% Agarose gel prepared in 0.5 × Tris borate EDTA (TBE) buffer. Ethidium bromide (2 µL / 200 mL gel) was added to the gel for visualizing DNA bands. One lane was run for 100 bp ladder by loading 10 µL (Gei Nei, Meark) in that well as a size standard. The running buffer comprised of 0.5 X TBE. PCR product (25 µL) was loaded in each well. The Gel was run at 70 mA Current for 45minutes to 1 hour depending upon the voltage for minimum 10 cm distance (3/4 gel slab).

After electrophoresis the product was visualized by gel documentation and analysis system plate 3. 1 (Molecular Imager® Gel Doc^{TM} XR+ system with image Lab^{TM} Software of BIO-RAD) calibrated to high sensitive level (75%) (Jean-Louis, 2000).

Plate IX. Gel DocTM XR+ of BIO RAD

3.8. Data Analysis

The bands in the electropherogramme (Plate 4.1 to 4.5) were analysed and scored using the image Lab^{TM} . Observed allele frequencies were computed using the excel macros and the allele frequencies was used in the determination of both within and between-population genetic variation. Genetic variation was determined as the Average observed and expected heterozygosity and was calculated using GenAlEx version 6.5 (Dieringer and Schlötterer, 2003). Deviations of the locus/population from Hardy Weinberg equilibrium (HWE) were determined using GenAlEx version 6.5 programme (Raymond and Rousset, 1955). Analysis of molecular variance, AMOVA (Excoffier *et al*., 1992) was carried out using the GenAlEx 6.5(Peakall and Smouse, 2012) and was performed to quantify further the extent of population differentiation and the distribution of genetic variation in the sample populations. A genetic distance measure was computed using the GenAlEx 6.5. Phylogenetic trees was constructed from (Nei, 1973) standard genetic distance (DS) and Cavalli- Sforz and Edwards genetic distance measure (DA) generated using the Unpaired Group with Arithmetic Mean (UPGMA) methods in the GENEPOP version 3.4 (Raymond and Rousset, 1955). The reliability of the constructed trees was examined according to (Felsenstein, 1985). Principal component analysis, which simply describes the variation of the multivariate data in terms of some linear combinations of the variables, was performed by Eigen analysis of covariance matrix calculated from allele frequencies data using the GenAlEx 6.5.

3.9. Bands Pattern of Microsatellite DNA Maker of Guinea Fowl

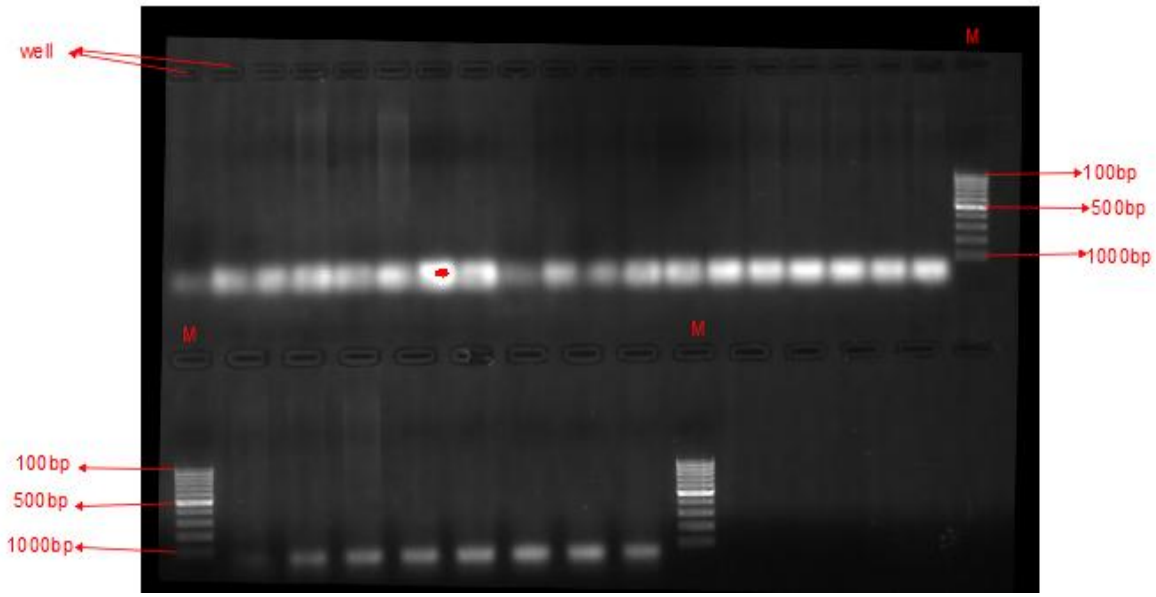


Plate X. Pattern of microsatellite DNA marker of pearl Guinea fowl.

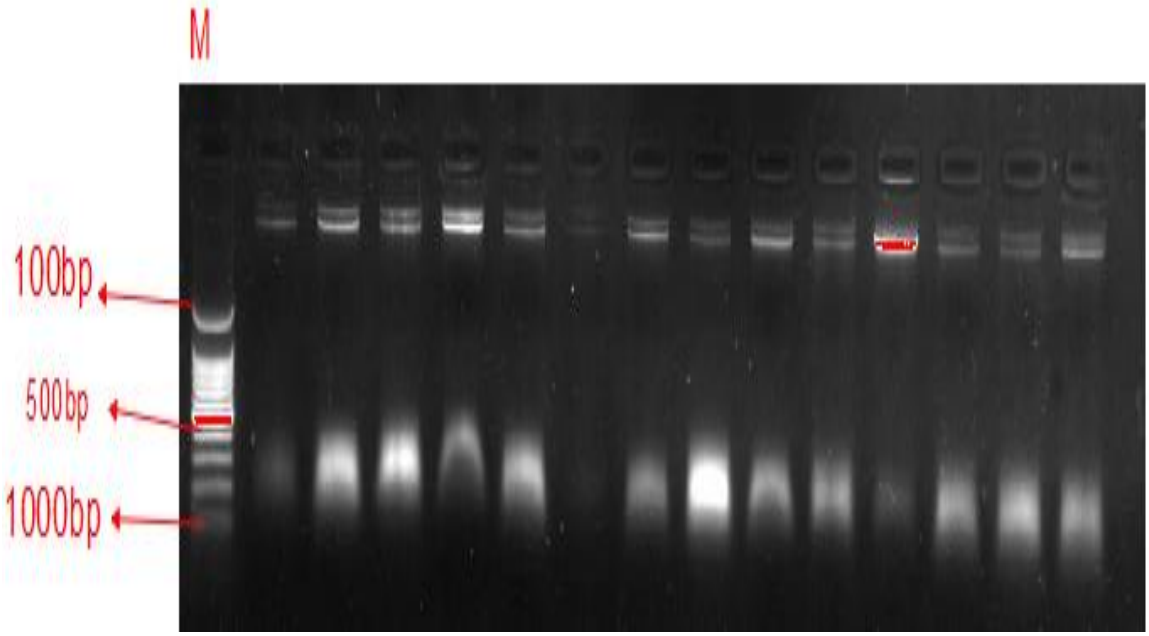


Plate XI. Pattern of microsatellite DNA marker of Black Guinea fowl

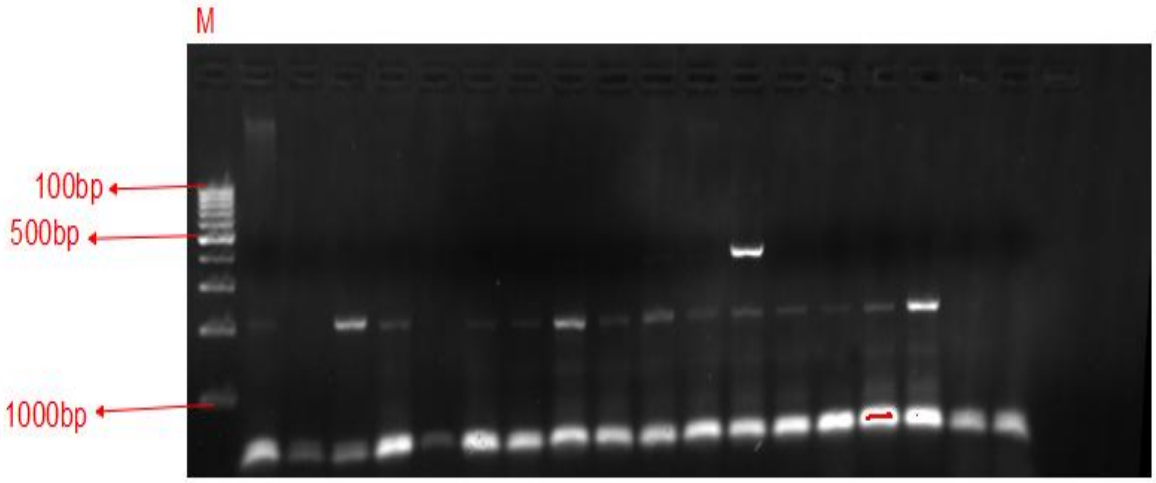


Plate XII. Pattern of microsatellite DNA marker of Broiler Guinea fowl

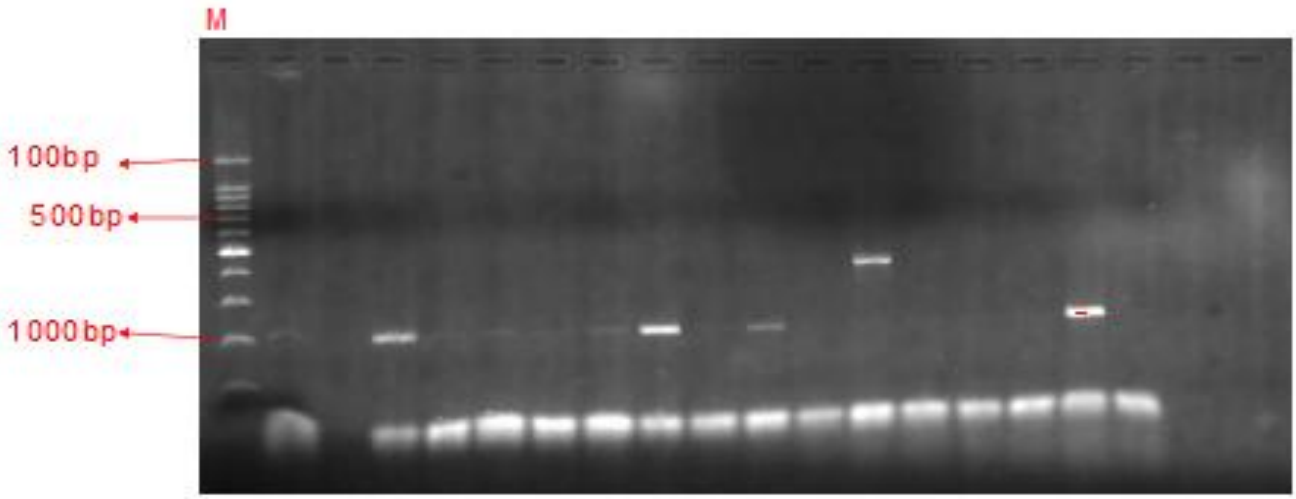


Plate XIII. Pattern of microsatellite DNA marker of Ash (lavender) Guinea fowl.

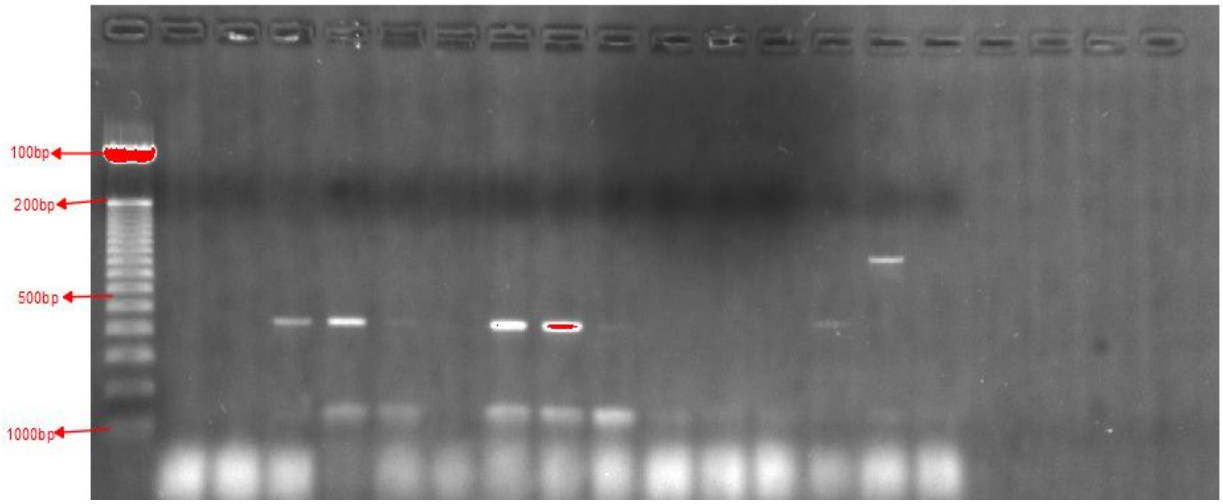


Plate XIV. Pattern of microsatellite DNA marker of white Breasted Guinea fowl.

Table 3. 2. Guinea fowl primers (directly labeled) for genetic diversity assessment

LOCUS	ACCESSION NUMBER	PRIMER SEQUENCE	NUMBER OF BASES	SIZE RANGE (bp)
Nmg01_F	AB819290	CATCCAATACCCTGAACCTA	20	192-196
Nmg01_R		TTAAACCACAAGACCATTCC	20	
Nmg02_F	AB819291	TGAACACGGGCTTAGATAGT	20	123-213
Nmg02_R		GCTTTAGATGGCAATAGTGG	20	
Nmg03_F	AB819292	GTCTTCTCTGACTTTTGGAAAT	22	164-168
Nmg03_R		TACCCACACTGGTACTCTCC	20	
Nmg04_F	AB819293	GCACTAATAGTAGAGTACGCAGAA	24	94-110
Nmg04_R		TGCTAACTCCAAATGACACA	20	
Nmg05_F	AB819294	TGTACATGGTGCGTGTTTAT	20	116-152
Nmg05_R		CGTTTTTGTCCGTACTCAAC	20	
Nmg06_F	AB819295	TGCAAATCATCTTTTTCCTT	20	164-172
Nmg06_R		TCCTCTGACTTATACCAGTTGA	22	
Nmg07_F	AB819296	TGAGAGTGAAATACCTGCAA	20	169-172
Nmg07_R		GATCTGTTAGGGCTGCTAGA	20	
Nmg08_F	AB819297	GATGGCTATTGGGAAATACA	20	206-214
Nmg08_R		CTGGCTTACATATCCTTCCA	20	
Nmg09_F	AB819298	GTCTCCGAGATGTTGGTTT	19	142-147
Nmg09_R		AATCTTTCGCCTCTTACACA	19	
Nmg10_F	AB819299	TCTTGTTCCAGTTGTCATCA	19	108-116
Nmg10_R		ATGCCTCTGCAAATTAGTGT	20	
Nmg11_F	AB819300	AAGTTTTCAGCAAAATCCAG	20	242-246
Nmg11_R		CACATACAGATCATGGGACA	20	
Nmg12_F	AB819301	CAACTAAGTTCCTTGATTTCTCA	23	145-210
Nmg12_R		TGCAGAGTTTCTCTCTTTGAC	21	
Nmg13_F	AB819302	AACAAAGGATGTTTTGTGCT	21	214-233
Nmg13_R		TAAACCAATTTCCAGCATTT	20	
Nmg14_F	AB819303	GGCTGTGTGAAAGGAGAGTA	22	149-163
Nmg14_R		GCCAACATGCCTAACTGTAT	20	
Nmg15_F	AB819304	TCTTGTTCTCCATCAGCTCT	20	118-120
Nmg15_R		CACAAAACACCTGCTCACTA	20	
Nmg16_F	AB819306	CAACATTTGTCTGGTGTCAG	20	252-257
Nmg16_R		AGTCAAATGGTTGTGGATGT	20	
Nmg17_F	AB819307	TCTTCCTTCAGAGGTACCAA	20	223-232
Nmg17_R		TGAAGACCATAGAAGCCTGT	20	
Nmg18_F	AB819308	ACTTCATGAGGTTCAAATGG	20	264-266

Table 3.2. continue

Nmg18_R		TGGAATCTAGCTTGTTGGTT	20	
Nmg19_F	AB819309	TCTGAAGTATCTGCCCTGAG	20	135-139
Nmg19_R		TTATCAAGTGAGCGATCAGA	20	
Nmg20_F	AB819310	ATTCTCTGGAATGGGAATTT	20	200-204
Nmg20_R		ATAGTGGCATGGTTCTCTTC	20	
Nmg21_F	AB819311	TGCTAAATTATGTGCAGCAG	20	164-173
Nmg21_R		CCAACCTAAGCCATACTAGG	20	
Nmg22_F	AB819312	TTCCAGCTTGAAAACTGACT	20	203-234
Nmg22_R		CACAGACACAGACCATTGAG	20	
Nmg23_F	AB819313	AACCTGCAGAAACACATTTT	20	230-236
Nmg23_R		CTGCAATACTTCATTTGTGG	20	
Nmg24_F	AB819314	TCTCTCCTGACTTCCAAAAA	20	226-236
Nmg24_R		AGGCTTGAACTCATGGACTA	20	
Nmg25_F	AB819315	CACAAGTGTGAAGCAATGTC	20	207-211
Nmg25_R		ACATCTATGGCCTCAGACAC	20	
Nmg26_F	AB819316	AAACAGAAGGTGAATGCTGT	20	261-286
Nmg26_R		GTAGCTGTGCACCTCACC	18	
Nmg27_F	AB819317	GGCCCTATCCTCAAATAGTCTCC	23	236-252
Nmg27_R		TCAAAGCCTGTAAGAAGTGCTC	22	
Nmg28_F	AB819318	GAATCCAGTGACACACCTTT	20	209-235
Nmg28_R		TATTTAATGAGGGCTGCTTT	20	
Nmg29_F	AB819319	CCTCTTCCTCCTCGTTTAAT	20	241-278
Nmg29_R		TCCTTTTGCTAGAACACACA	20	
Nmg30_F	AB819320	TGAGATCAGGTGGAAAAACT	20	203-209
Nmg30_R		TACATTTCCTGCTCTCCAGT	20	
Nmg31_F	AB819321	CCTTGTTCCAGAGCTGTAGT	20	175-181
Nmg31_R		GGAAGGACAAATAAAGCAAA	20	

F: forward sequence **(5'➔3')**, **R:** reverse sequence **(3'➔5'), bp**: Base pair

CHAPTER FOUR

4.0. RESULTS

4.1. Number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Pearl guinea fowl

Number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Pearl guinea fowl was showed in Table 4.1. The number of allele ranges from 1-8 which is polymorphic and informative even though only nine (9) markers (AB819290, AB819291, AB819293, AB819297, AB819301, AB8192312, AB819315, AB819316, AB819318) gives positive result. The value of PIC ranges from 0.65(AB819312)-0.96(rest) with mean of 0.310. The Ho and He range from 0.032 to 0.258 and -3 to 0.008 with mean of 0.022 and -0.063 respectively. None of the loci deviated from Hardy-Weinberg Equilibrium (HWE) and the value range between -3.06434(AB819291) to 0.169923 (AB819301) with mean of -2.4014.

4.2. Number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Black guinea fowl

Number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Black guinea fowl was presented on Table 4.2. The number of allele ranges from 1-19 with average of 3.129 which is polymorphic and informative even though only twenty four (24) markers give positive result. The value of PIC ranges from 0.65-0.96 with mean of 0.715. The H_o and He range from 0.290 to 0.032 and 0.260 to 2.900 with mean of 0.101 and -0.149 respectively. The result showed that only 6.050234(AB819301) loci deviated ($p<0.01$) from Hardy-Weinberg Equilibrium (HWE)

and the rest does not and the values range between -3.06486(AB819311) to 6.050234(AB819301) with mean of 0.034708.

4.3. Number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Broiler guinea fowl

Number of allele ranges from 1-9 which is polymorphic and informative even though only twenty (20) markers gives positive result. The value of PIC ranges from 0.985 to 1 with mean of 0.642. The Ho and He range from 0.29 to 0.032 and 0.09 to 0.322 with mean of 0.042 and 0.126 respectively. None of the loci deviated from Hardy-Weinberg Equilibrium (HWE) and the value range between 0.00182323(AB819301) to 0.26071561(AB819321) with mean of 0.070171 as shown in table 4.3.

Table 4. 1. Microsatellite markers, number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Pearl guinea fowl

LOCUS	Na	PIC	Ho	He	HWE	LS(P<0.01)
AB819290	3	0.96	0.097	0.185	0.041859	NS
AB819291	1	0.96	0.032	-3.000	-3.06434	NS
AB819292	0	-	-	-	-	
AB819293	5	0.96	0.161	0.168	0.000292	NS
AB819294	0	-	-	-	-	
AB819295	0	-	-	-	-	
AB819296	0	-	-	-	-	
AB819297	1	0.96	0.0322	0.050	0.006337	NS
AB819298	0	-	-	-	-	
AB819299	0	-	-	-	-	
AB819300	0	-	-	-	-	
AB819301	8	0.96	0.258	0.117	0.169923	NS
AB819302	0	-	-	-	-	
AB819303	0	-	-	-	-	
AB819304	0	-	-	-	-	
AB819306	0	-	-	-	-	
AB819307	0	-	-	-	-	
AB819308	0	-	-	-	-	
AB819309	0	-	-	-	-	
AB819310	0	-	-	-	-	
AB819311	0	-	-	-	-	
AB819312	1	0.65	0.0322	0.030	0.000161	NS
AB819313	0	-	-	-	-	
AB819314	0	-	-	-	-	
AB819315	1	0.96	0.0322	0.133	0.076396	NS
AB819316	1	0.96	0.0322	0.008	0.073205	NS
AB819317	0	-	-	-	-	
AB819318	1	0.96	0.0322	0.168	0.109772	NS
AB819319	0	-	-	-	-	
AB819320	0	-	-	-	-	
AB819321	0	-	-	-	-	
Mean	0.71	0.31	0.022	-0.063	-2.4014	

Number Allele: (Na), Observed Heterozygosity : (Ho), Expected Heterozygosity: (He), Polymorphism Information Content: (PIC), Not Significant: (NS), Level of significance: (LS), ***: (P<0.01).

Table 4. 2. Microsatellite markers, Number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Black guinea fowl

LOCUS	Na	PIC	Ho	He	HWE	LS(P<0.01)
AB819290	4	0.785	0.129032	0.1875	0.018232	NS
AB819291	8	0.785	0.258065	0.1172	0.169308	NS
AB819292	0	-	-	-	-	
AB819293	2	0.806	0.064516	0.0000	-	
AB819294	6	0.857	0.193548	0.1481	0.013947	NS
AB819295	1	0.857	0.032258	0.0800	0.028491	NS
AB819296	1	0.857	0.032258	0.0420	0.00226	NS
AB819297	1	0.857	0.032258	0.1300	0.073488	NS
AB819298	2	0.860	0.064516	0.1000	0.012591	NS
AB819299	1	0.860	0.032258	0.0910	0.037919	NS
AB819300	1	0.860	0.032258	0.0210	0.006035	NS
AB819301	19	0.860	0.612903	0.0520	6.050234	***
AB819302	6	0.992	0.193548	0.1480	0.014018	NS
AB819303	1	0.992	0.032258	0.0210	0.006035	NS
AB819304	0	-	-	-	-	
AB819306	1	0.992	0.032258	-2.9100	-2.97487	NS
AB819307	2	0.992	0.064516	0.0000	-	-
AB819308	0	-	-	-	-	-
AB819309	0	-	-	-	-	-
AB819310	0	-	-	-	-	-
AB819311	1	0.992	0.032258	-3.0000	-3.06486	NS
AB819312	7	0.992	0.225806	0.1312	0.068219	NS
AB819313	6	0.994	0.193548	0.1481	0.013947	NS
AB819314	6	0.994	0.193548	0.1875	0.000195	NS
AB819315	1	0.994	0.032258	0.1172	0.061563	NS
AB819316	4	0.996	0.129032	0.0890	0.018006	NS
AB819317	0	-	-	-	-	
AB819318	9	0.998	0.290323	0.1480	0.136864	NS
AB819319	3	1	0.096774	0.1240	0.005978	NS
AB819320	0	-	-	-	-	-
AB819321	4	1	0.129032	0.2600	0.065972	NS
Mean	3.129	0.715	0.101	-0.1490	0.034708	

Number Allele: (Na), Observed Heterozygosity : (Ho), Expected Heterozygosity: (He), Polymorphism Information Content: (PIC), Not Significant: (NS), Level of significance: (LS), ***: (P<0.01).

Table 4. 3. Microsatellite markers, Number allele (Na), Observed (Ho) and Expected Heterozygosity (He), and polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) for Broiler guinea fowl

ACCESSION NUMBER	Na	PIC	Ho	He	HWE	LS(P<0.01)
AB819290	1	0.985	0.032258	0.1000	0.04588979	NS
AB819291	9	0.985	0.290323	0.1312	0.19298879	NS
AB819292	0	-	-	-	-	-
AB819293	1	0.993	0.032258	0.1481	0.09061019	NS
AB819294	3	0.994	0.096774	0.1852	0.04222007	NS
AB819295	0	-	-	-	-	
AB819296	0	-	-	-	-	
AB819297	0	-	-	-	-	
AB819298	1	0.994	0.032258	0.081	0.02933065	NS
AB819299	0	-	-	-	-	
AB819300	1	0.995	0.032258	0.009	0.06010384	NS
AB819301	3	0.995	0.096774	0.111	0.00182323	NS
AB819302	3	0.996	0.096774	0.168	0.03019728	NS
AB819303	2	0.997	0.064516	0.200	0.09177957	NS
AB819304	0	-	-	-	-	
AB819306	1	0.997	0.032258	0.045	0.00360797	NS
AB819307	3	0.997	0.096774	0.185	0.04207474	NS
AB819308	0	-	-	-	-	-
AB819309	0	-	-	-	-	-
AB819310	1	0.998	0.032258	0.090	0.03704598	NS
AB819311	0	-	-	-	-	-
AB819312	1	0.998	0.032258	0.148	0.09051494	NS
AB819313	1	0.998	0.032258	0.000	-	-
AB819314	2	0.998	0.064516	0.132	0.03450068	NS
AB819315	0	-	-	-	-	-
AB819316	0	-	-	-	-	-
AB819317	1	0.999	0.032258	0.148	0.09051494	NS
AB819318	2	0.999	0.064516	0.185	0.078467	NS
AB819319	2	0.999	0.064516	0.140	0.04069882	NS
AB819320	1	1.000	0.032258	0.000	-	
AB819321	1	1.000	0.032258	0.322	0.26071561	NS
MEAN	1.29	0.642	0.042	0.126	0.070171	

Number Allele: (Na), Observed Heterozygosity : (Ho), Expected Heterozygosity: (He), Polymorphism Information Content: (PIC), Not Significant: (NS), Level of significance: (LS), ***: (P<0.01).

4.4. Number allele (Na), Observed (Ho) and Expected Heterozygosity (He), and polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Ash guinea fowl

Table 4.4 presented the number allele, observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Ash guinea fowl. The number of allele ranges from 0-1 which is lowly polymorphic and informative even though only seven (7) markers gives positive result. The value of PIC ranged from 0.857 to 0.985 with mean of 0.207. The Ho and He range from 0.031 to 0.032 and 0.0023 to 0.15 with mean of 0.012 and 0.065 respectively. None of the loci deviated from Hardy-Weinberg Equilibrium (HWE) and the value range between 0.0016 (AB819301) to 0.092827 (AB819318) with mean of 0.036078.

4.5. Number allele (Na), Observed (Ho) and Expected Heterozygosity (He), and polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Ash guinea fowl

The number of allele ranged from 0-1 (Table 4.5) which is lowly polymorphic and informative even though only seven (7) markers gives positive result. The value of PIC ranged from 0.857 to 0.985 with mean of 0.207. The Ho and He ranged from 0.031 to 0.032 and 0.0023 to 0.15 with mean of 0.012 and 0.065 respectively. None of the loci deviated from Hardy-Weinberg Equilibrium (HWE) and the value ranged between 0.0016 (AB819301) to 0.092827 (AB819318) with mean of 0.036078.

Table 4. 4. Microsatellite markers, number of allele, polymorphism information content, observed Heterozygosity, expected Heterozygosity, mean number of allele and HWE for Ash guinea fowl

LOCUS	Na	PIC	Ho	He	HWE	LS(P<0.01)
AB819290	0	-	-	-	-	-
AB819291	1	0.857	0.032	0.023	0.003522	NS
AB819292	0	-	-	-	-	-
AB819293	1	0.878	0.032	0.008	0.072000	NS
AB819294	0	-	-	-	-	-
AB819295	0	-	-	-	-	-
AB819296	0	-	-	-	-	-
AB819297	1	0.898	0.032	0.09	0.037378	NS
AB819298	0	-	-	-	-	-
AB819299	1	0.918	0.031	0.051	0.007843	NS
AB819300	0	-	-	-	-	-
AB819301	1	0.939	0.032	0.04	0.0016	NS
AB819302	0	-	-	-	-	-
AB819303	0	-		-		
AB819304	0	-	-	-	-	-
AB819306	0	-	-	-	-	-
AB819307	0	-	-	-	-	-
AB819308	0	-	-	-	-	-
AB819309	0	-	-	-	-	-
AB819310	0	-	-	-	-	-
AB819311	0	-	-	-	-	-
AB819312	0	-	-	-	-	-
AB819313	0	-	-	-	-	-
AB819314	0	-	-	-	-	-
AB819315	1	0.959	0.032	0.09	0.037378	NS
AB819316	0	-	-	-	-	-
AB819317	0	-	-	-	-	-
AB819318	1	0.980	0.032	0.15	0.092827	NS
AB819319	0	-	-	-	-	-
AB819320	0	-	-	-	-	-
AB819321	0	-	-	-	-	-
MEAN	0.226	0.207	0.012	0.065	0.036078	

Number Allele: (Na), Observed Heterozygosity : (Ho), Expected Heterozygosity: (He), Polymorphism Information Content: (PIC), Not Significant: (NS), Level of significance: (LS), ***: (P<0.01).

Table 4. 5. Microsatellite markers, Number of allele (Na), observed (Ho) and Expected Heterozygosity (He), and polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of white breasted guinea fowl

LOCUS	Na	PIC	Ho	He	HWE	LS(P<0.01)
AB819290	0	-	-	-	-	-
AB819291	7	0.929	0.225806	0.107	0.131915	NS
AB819292	0	-	-	-	-	-
AB819293	6	0.943	0.193548	0.21	0.001289	NS
AB819294	3	0.953	0.096774	0.1852	0.04222	NS
AB819295	0	-	-	-	-	
AB819296	0	-	-	-	-	
AB819297	1	0.955	0.032258	0.06	0.012827	NS
AB819298	2	0.955	0.064516	0.111	0.019466	NS
AB819299	2	0.956	0.064516	0.112	0.020132	NS
AB819300	2	0.958	0.064516	0.132	0.034501	NS
AB819301	1	0.959	0.032258	0.1952	0.136015	NS
AB819302	5	0.959	0.16129	0.18	0.001945	NS
AB819303	2	0.966	0.064516	0.09	0.007216	NS
AB819304	0	-	-	-	-	
AB819306	2	0.967	0.064516	0.062	0.000102	NS
AB819307	3	0.968	0.096774	0.185	0.042075	NS
AB819308	0	-	-	-	-	-
AB819309	1	0.970	0.032258	0.086	0.033584	NS
AB819310	1	0.969	0.032258	0	-	-
AB819311	0	-	-	-	-	-
AB819312	6	0.974	0.193548	0.76	0.422195	NS
AB819313	0	-	-	-	-	-
AB819314	3	0.982	0.096774	0.078	0.004519	NS
AB819315	0	-	-	-	-	-
AB819316	0	-	-	-	-	-
AB819317	3	0.984	0.096774	0.133	0.009867	NS
AB819318	5	0.986	0.16129	0.1852	0.003087	NS
AB819319	5	0.993	0.16129	0.103	0.032988	NS
AB819320	0	-	-	-	-	-
AB819321	0	-	-	-	-	-
Mean	0.591	0.062	0.102	0.591	0.053108	

Number Allele: (Na), Observed Heterozygosity : (Ho), Expected Heterozygosity: (He), Polymorphism Information Content: (PIC), Not Significant: (NS), Level of significance: (LS), ***: (P<0.01).

4.6. Genetic distance

Pair-wise population matrix of Nei's genetic distance estimates of Nigerian Guinea Fowl strains Table 4.6. The result showed that, the shortest distance (0.4472) was observed between black and white breasted guinea fowl while the distance between Pearl-White breasted (0.6325) and Pearl-Ash (0.6325) were the same. The highest distance (1.0) was observed between broiler guinea fowl and white breasted.

4.7. Analysis of Molecular Variance (AMOVA)

The AMOVA estimated showed that (Table 4.7) the estimated variation (variance) is 6.70 and 0.00 for within population and the estimated percentage variation is 100% among population and 0.00 within population and the total estimated variation (6.7) is only attributed by var. among population.

Table 4. 6. Genetic distance estimates of Nigerian Guinea Fowl strains

PEARL	BLACK	BG	WB	ASH	
					PEARL
0.7746					BLACK
0.7746	0.8944				BG
0.6325	0.4472	1.000			WB
0.6325	0.7746	0.7746	0.6325		ASH

BG: broiler guinea fowl, WB: white breasted

Table 4. 7. Analysis of Molecular Variance (AMOVA) of Guinea Fowl strains based on Microsatellite DNA Variation

Source	Df	SS	MS	Est. Var.	%
Among Pops	4	80.400	20.100	6.700	100
Within Pops	24	0.000	0.000	0.000	0
Total	28	80.400		6.700	100

Df : degree of freedom, SS: sum of squares, MS: mean square
Est. Var. : estimated variance, pops: population

4.8. Population structure of the guinea fowl strains in Nigeria studied using 31 set of microsatellite marker

4.8.1. Principal component Analysis (PCA)

The factor analysis to study the population structure of the guinea fowl strains in three (3) principal components (PC's) are shown in table 4.8, it was observed that in PC1 pearl (0.57) has the highest positive (0.057) value followed by Broiler guinea fowl (0.388) and then ash (0.057) having the lowest value of eigen vector while Black (0.241) has the highest eigen vector in PC2 followed by Broiler guinea fowl (0.205) and highest negative of value -0.220 attributed by Ash and Pearl followed by -0.007. in PC3 Black, Broiler guinea fowl and White Breasted present a zero eigen vector values of 0.00 with ash having the highest Value (0.281) followed by pearl presenting the negative (-0.281) value. These 3 PC's account for 97.715% as total variance of which 19.320% was contributed by PC1, while PC2 and PC3 contributed 32.710% and 40.485% respectively. All the Eigen Values contributed by all the PC'S were below 1.00 (0.158, 0.197, 0.284 for, PC3, PC2, and PC1, respectively).

Table 4. 8. Principal Component (PC), Sample Eigen Vectors, Eigen Values and %Variation of five guinea fowl strains

STRAIN	PC1	PC2	PC3
PEARL	0.057	-0.220	-0.281
BLACK	-0.233	0.241	0.000
BG	0.388	0.205	0.000
WB	-0.269	-0.007	0.000
ASH	0.057	-0.220	0.281
% variance	19.32	32.71	40.485
EigenValue	0.284	0.197	0.158

BG: broiler guinea fowl, WB: white breasted

4.8.2. Bi-dimensional graph representing the relationship between PC1 and PC2 using microsatellite makers derived from 5 guinea fowl strains in Nigeria

The result based on the allele frequency obtained was shown in the figure 4.1 which show that a distinct cluster was presented by broiler guinea fowl while black and white breasted tends to clustered together in the 1st quadrant. However, Ash and Pearl presented very closer cluster in the 3rd quadrant.

4.8.3. The Cluster Analysis

The dendrogramme (figure 4.2) from the result of 31 SSR used based on Nei's genetic distance indicated that the studied population forms four (4) clusters at various degree of similarities.

At 0.6 pearl (P) and Ash (A) forms cluster, indicating a high level of genetic similarity. At 0.78 co-efficient D and W grouped to form a cluster and then grouped with B at 0.9 to form a third (3rd) cluster then. Finally the first (1st) and second (2nd) group that join the third, form the forth (4th) at 0.95 showing genetic similarity among all the strains studied.

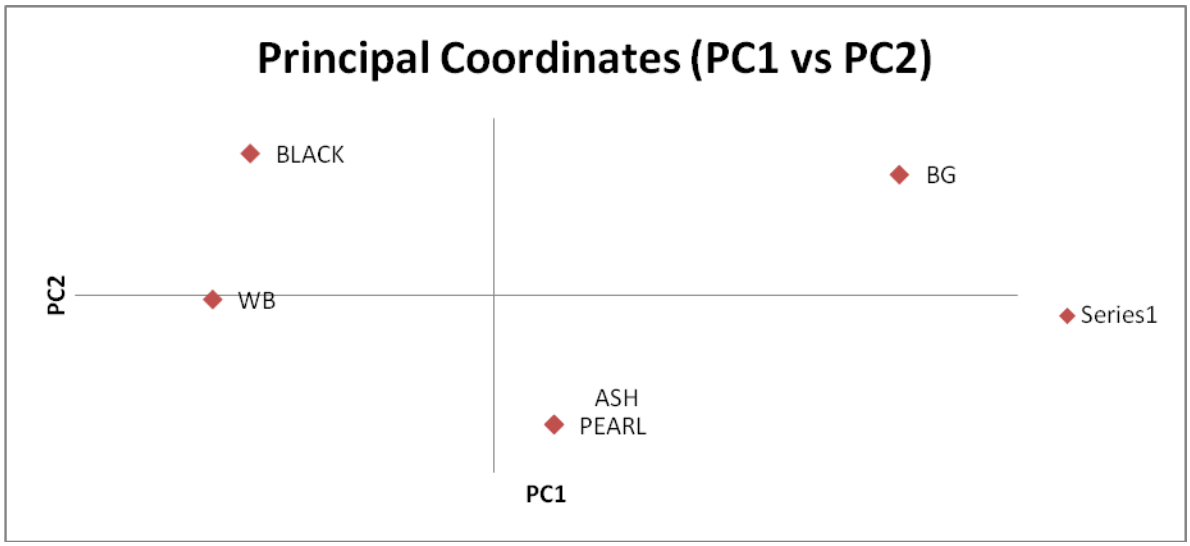
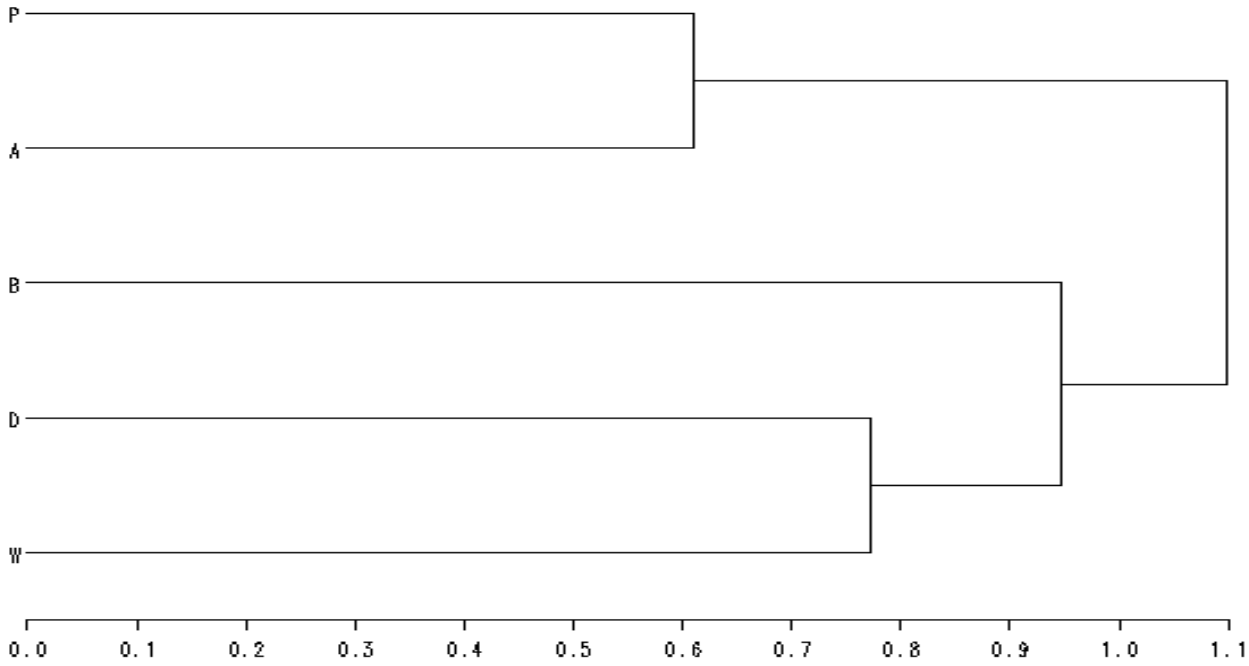


Figure 4. 1. Bi-dimensional graph representing the relationship between PC1 and PC2 using microsatellite makers derived from 5 guinea fowl strains in Nigeria



Figue 4. 2: Dendrogramme representing Phylogenic relationship of guinea fowl strains in Nigeria (p: Pearl, A: Ash, B: Black, D: Broiler guinea fowl, W: White breasted)

CHAPTER FIVE

5.0. DISCUSSION

5.1. Number allele (Na), observed (Ho) and Expected Heterozygosity (He), and polymorphism information content and Hardy-Weinberg Equilibrium (HWE) of pearl guinea fowl

From the result of the studied pearl guinea fowl using 31 set of microsatellite markers revealed that they were normally (PIC<0.5) polymorphic (mean PIC =0.31), which indicated a moderately average gene diversity among the studied markers (Chang *et al*., 2007) and they can be used for molecular genetic studies This affirmed the report of Botchway *et al. (*2013) who developed 31 polymorphic microsatellite markers for guinea fowl using next –generation sequencing. The mean Ho (0.022) is greater than the mean expected (-0.063) which is lower than the values reported by Botchway *et al. (*2013) who reported a mean Ho and He of 0.396 and 0.419 respectively, meaning that there is great decrease in heterozygosity in the studied population and agrees with the report of (Agha *et al*., 2008; Lacy, 1987; Lande and Barrowclaugh, 1987) that the decrease in heterozygosity in the studied population may be due to low population size which is driving factor for inbreeding and shifted the population towards homozygosity. The population does not deviate from Hardy-Weinberg Equilibrium (HWE) meaning that no external forces acting on it **(**is in equilibrium**)** i.e The population is not affected by genetic drift, Mating is random ,Migration (gene flow) has no affect on allele frequency, Mutation has no effect on allele frequency, All alleles are equally viable (no natural selection).

5.2. Number allele (Na), observed (Ho) and Expected Heterozygosity (He), and polymorphism information (PIC) content and Hardy-Weinberg Equilibrium (HWE) of Black guinea fowl

From the result of the studied black guinea fowl using 31 set of microsatellite markers revealed that the markers mean number of allele (3.129) and mean PIC (0.715) were highly polymorphic moreover the PIC range from 0.65-0.96, revealed that the studied markers were highly polymorphic (PIC>0.5), which indicated high average gene diversity among the studied markers (Chang *et al*., 2007), This affirm the report of Botchway *et al. (*2013) who developed 31 polymorphic microsatellite markers for guinea fowl using Next –Generation Sequencing. The mean Ho (0.101) is greater than the mean expected (-0.149) which is lower than the values reported by Botchway *et al. (*2013) whom reported a mean Ho and He of 0.396 and 0.419 respectively, meaning that there is great decrease in heterozygosity in the studied population and agrees with the report of (Lacy, 1987; Lande and Barrow-Claugh, 1987 and Agha *et al*., 2008) that the decrease in heterozygosity in the studied population may be due to low population size which is driving factor for inbreeding and shifted the population towards homozygosity. The deviation of AB819301 loci (P<0.01) from Hardy-Weinberg Equilibrium (HWE) of black guinea fowl agree with the decrease in heterozygosity in the population and might be as a result of the non random union of gametes in the population i.e inbreeding, migration ,mutation and selection. Deviations from HWE at microsatellite loci have been reported by different studies (Botchway *et al*., 2013; Chang *et al*., 2007; Kayang *et al*., 2010; Sigh and Sharma, 2002).

5.3. Number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Broiler guinea fowl

The result of the studied Broiler guinea fowl using 31 set of microsatellite markers revealed that they were highly (PIC<0.5) polymorphic, (Chang *et al*., 2007) and they can be used for molecular genetic studies, This affirm the report of Botchway *et al. (*2013). The mean Ho (0.042) is lower than the mean expected (0.126) which indicated a high average gene diversity among the studied markers however the result is lower than the values reported by Botchway *et al. (*2013) who used the same markers on guinea fowl, meaning that the population of studied broiler guinea fowl points towards out breeding with more heterozygots in the studied population and agrees with the report of (Agha *et al*., 2008; Lacy, 1987; Lande and Barrowclaugh, 1987) this affirm with the fact that broiler guinea fowl had gone a long term selection programme in Europe. No deviation from Hardy-Weinberg Equilibrium (HWE) was observed i.e in this case we conclude that any differences between the observed and expected values are likely due to chance alone.

5.4. Number allele, observed (Ho) and Expected Heterozygosity (He), and polymorphism information content and Hardy-Weinberg Equilibrium of Ash guinea fowl

The mean value of PIC (0.207) implying that the markers were lowly (PIC<0.25) as reported by (Chang *et al*., 2007) even though the bands were monomorphic. The mean Ho (0.012) is lower than the He (0.065), meaning that the population points towards out breeding with more heterozygote in the studied population. The population does not deviate from Hardy-Weinberg Equilibrium (HWE) meaning that no external forces acting on it **(**is in equilibrium**)** i.e The population is large so it is not affected by genetic drift, Mating is random ,Migration (gene flow) has no affect on allele frequency, Mutation has no effect on allele frequency,

All alleles are equally viable (no natural selection).

5.5. Number allele, observed (Ho) and Expected Heterozygosity (He), and polymorphism information content and Hardy-Weinberg Equilibrium (HWE) of white breasted guinea fowl

From the result of the studied pearl guinea fowl using 31 set of microsatellite markers revealed that they were lowly (PIC>0.5) polymorphic (mean PIC =0.062) even though all the individual markers presented a highly polymorphism (PIC>0.5), which indicated a moderate average gene diversity among the studied markers (Chang *et al*., 2007), they can be used for molecular genetic studies and this agrees with the report of (Botchway *et al.*, 2013). The mean Ho (0.102) is lower than the mean He (0.591) with the value of He greater than the value reported by Botchway *et al. (*2013) who reported a mean Ho and He of 0.396 and 0.419 respectively, meaning that there is great increase in heterozygosity in the studied population and agrees with the report of (Agha *et al*., 2008, Lacy, 1987 and Lande and Barrow-Claugh, 1987), that the increase in heterozygosity in the studied population is a good indication for genetic variance at polymorphic sites, rich genetic diversity and selection potential as also reported by (Liu, *et al.*, 2006). The population does not deviate from Hardy-Weinberg Equilibrium (HWE) meaning that no external forces acting on it **(**is in equilibrium**)** i.e The population is large so it is not affected by genetic drift, Mating is random ,Migration (gene flow) has no affect on allele frequency, Mutation has no effect on allele frequency, All alleles are equally viable (no natural selection).

5.6. Genetic Distance

The result showed that, the shortest distance (0.4472) observed between Black and White breasted guinea fowl was probably due to the fact that they are living in the same geographical location, common origin and evolutionary progress, time of speciation and bottleneck effect at

the beginning of domestication. The equality of genetic distance value (0.6325) of Pearl-White breasted (0.6325) and Pearl-Ash (0.6325) were the same which is due to the fact that the phenotypic variation is just by chance also White breasted and Ash were considered as variants of Pearl (Moreki, 2009) Guinea fowl. The highest distance (1.0) observed between Broiler guinea fowl and White breasted is due to the fact that the Broiler guinea fowl was introduce in to Nigeria from Belgium after many years of breeding activities even though they have the same origin of evolution also based on RAPD markers, this agrees with the report of (Kayang *et al*., 2010) that the indigenous West African populations of guinea fowl are more genetically diverse but less differentiated compared to the non-indigenous populations, Sharma, *et al.* (1998) observed a low level of genetic variation within and among lavender(ash), pearl (wild type) and white helmeted guinea fowl varieties in India and attributed this to a small founder population and many years of multiplication without selective breeding.

5.7. Analysis of Molecular Variance (AMOVA)

The Molecular Variation estimated showed that the total estimated variation of (0.00) within population showed that there is no genetic variation within the studied population which means they are either completely homozygotes (0% within), and agrees with the report of (Agha *et al*., 2008) that low genetic variation within population may be due to low population size which is driving factor for genetic bottlenecks, random genetic drift, inbreeding and human activities (Lacy, 1987; Lande and Barrowclaugh, 1987) or due to method and facilities used. And the high estimated percentage variation (100%) among population, mean that they are separated from each other and agree with the findings of Ayanga *et al. (*2002) and Sharma *et al. (*1998).

5.8. Population Structure of the Guinea Fowl Strains Studied Using 31 Set of Microsatellite marker.

5.8.1. Principal component analysis (PCA)

The factor analysis to study the population structure of the guinea fowl strains in three (3) principal components (PC's) implies that all the Eigen Values contributed by the PC'S were below 1.00(0.158, 0.197, 0.284 for PC3, PC2, and PC1, respectively) meaning that they account for less variability than does a single variable (Girden, 2001). When a factor has an Eigen value less than 1.00, in a sense it has more than one variable contributing to the value. The contributing variables could be due to experimental materials that can contribute to quality and number of bands obtained.

5.8.2. Bi-dimensional graph representing the relationship between PC1 and PC2 using microsatellite makers derived from 5 guinea fowl strains in Nigeria

Based on the allele frequency obtained, broiler guinea fowl was distantly related to other strains probably due to long term selection and breeding activities while black and white breasted tends to clustered together. However, Ash and Pearl presented very close cluster, This agrees with the report of (Moreki, 2009, Sharrma *et al*., 1998) who reported that White breasted and Ash were considered as variants of Pearl Guinea fowl.

5.8.3. The cluster analysis

The four (4) clusters (in the dendrogramme) at various degrees of similarities based on Nei's genetic distance in studied population. Pearl (P) and Ash (A) forms cluster, indicating a high level of genetic similarity. Broiler (D) and White breasted (W) grouped to form a cluster and then grouped with Black (B) to form a third (3^{rd}) cluster then. Finally the first (1^{st}) and second (2^{nd}) group that join the third, form the forth (4^{th}) at 0.98 showing genetic similarity among all

the strains studied. Which agrees with report of (Ayanga *et al*., 2002; Lacy, 1987; Lande and Barrowclaugh, 1987; Singh *et al*., 2010) that all the guinea fowl studied have the same origin of evolution. These important measurements may help to quantify the degree of relationships between different species and provide information on closeness within the same species where variation occur (Mojekwu, 2014; Palti *et al*., 1997).

CHAPTER SIX

6.0. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary

From the result of the studied guinea fowl using 31 set of microsatellite markers revealed that they were lowly (PIC>0.5) polymorphic in white, Ash and highly (PIC>0.5) polymorphic in Broiler, Black and Pearl guinea fowl which indicated a moderate to high gene diversity among the studied markers (Chang *et al*., 2007) and they can be used for molecular genetic studies. The mean Ho (0.102), (ll0.012), (0.042) is lower than the mean He (0.591), (0.065), (0.126) in White breasted, Ash, Broiler respectively, meaning that the population points towards out breeding with more heterozygote in the studied population) and the increase in heterozygosity in the studied population is a good indication for genetic variance at polymorphic sites, rich genetic diversity and selection potential as also reported by Liu *et al.* (2006). With the exception of black and pearl guinea fowl with mean Ho (0.101), (0.022) being greater than the mean He (-0.149), (-0.063) and implies that the decrease in heterozygosity in the studied population may be due to low population size which is driving factor for inbreeding and shifted the population towards homozygosity. All the populations are in equilibrium meaning The population is large, Mating is random, no migration (gene flow), no mutation, All alleles are equally viable (no natural selection), with the exception of AB819301 in black guinea fowl loci deviated ($p<0.01$) from Hardy-Weinberg Equilibrium (HWE) which indicate decrease in heterozygosity in the population which might be resulted from the non random union of gametes in the population i.e inbreeding, migration, mutation and selection. It was shown that Black and White breasted guinea fowl are living in the same geographical location, having common origin and evolutionary progress, time of speciation and bottleneck effect at the beginning of domestication

due to observed shortest genetic distance. The White breasted and Ash were considered as variants of Pearl (Moreki, 2009) Guinea fowl and Broiler guinea fowl was introduce in to Nigeria from Belgium/France after many years of breeding activities even though they have the same origin of evolution. The populations studied were either completely homozygotes (0% within), or due to method and facilities used. And the high estimated percentage variation (100%) among population, mean that they are separated from each other. The factor analysis (PCA) to study the population structure of the guinea fowl strains showed that eigen value (EV<1), implied that many factors account for less variability than does a single variable (Girden, 2001) and broiler guinea fowl was distantly related to other strains probably due to long term selection and breeding activities while black and white breasted tends to clustered together. However, Ash and Pearl presented very closer cluster. Consistent cluster was form between the strains which may help to quantify the degree of relationships between different species and provide information of closeness within the same species where variant occur.

6.2. Conclusions

- The result revealed that the markers were lowly (PIC<0.25) polymorphic in White(0.062), Ash(0.207) and highly (PIC>0.5) polymorphic in Broiler,(0.642) Black(0.715) and Pearl(0.31) guinea fowl indicating a moderate to high gene diversity among the studied markers which were a good indication of high polymorphism.

- The population points towards out breeding with more heterozygotes in the studied population (0.591, 0.065 and 0.126 in white breasted, Ash and Broiler guinea fowl respectively.

- Consistent cluster formed between the strains quantified the degree of relationships between the strains and provide information of closeness within the same strain where variation occur and a common ancestry was proved for Nigerian Guinea fowl and broiler Guinea fowl.

6.3. Recommendations

- The Markers can be used for molecular genetic studies and breeding in Nigerian guinea fowl.
- The Nigerian guinea fowl should be selected for egg and meat production to exploit its production potentials.
- Controlled breeding measure should be employed to avoid the effect of inbreeding in the Nigerian population of guinea fowl.
- A plan conservation measures should be employed to preserve the unique phenotype of the guinea fowl in Nigeria.
- Automatic sequencing technologies should be employed to study the polymorphism, genetic variation within and between Nigerian Guinea fowl populations and their structure.

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