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GENETIC FINGER PRINT OF HELMETED GUINEA FOWL *(NUMIDA MELEAGRIS GALEATA* PALLAS) USING MICROSATELLITE MARKER IN NIGERIA

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ABSTRACT

A study was carried out to genetically evaluate helmeted guinea fowl (*Numida meleagris galeata* pallas) using 31 set of microsatellite marker. A total of 160 randomly sampled indigenous guinea fowl strains namely; Lavender (ash), Pearl, Black and Broiler guinea fowl was used for the study with a maximum of forty (40) animals per strain. A template DNA for PCR reaction was isolated from whole blood of the selected samples and PCR was done using 31 set of microsatellite marker and electrophoresed on agarose gel stained with ethidium bromide. The bands on the electropherogramme were analysed with image Lab^{TM} Software of BIO-RAD to score the bands while GenAlEx was used to calculate mean number of allele (Na), Polymorphism Information Content (PIC), observed and expected heterozygosity (H_O and He), Hardy Weinberg equilibrium (HWE) and Analysis of Molecular Variance (AMOVA) while phylogenetic tree and bi-dimensional graph showing the distribution of the guinea fowls studied were constructed using R-package. The Result revealed that the markers were lowly (PIC<0.25) polymorphic in Ash while normally polymorphic in pearl (PIC<0.5) and highly (PIC>0.5) polymorphic in Broiler and Black guinea fowl. With the exception of broiler and ash guinea fowl with mean observed heterozygosity (0.042), (0.012) being lower than the mean expected heterozygosity (0.126), (0.065) the observed heterozygosity (0.022), (0.101) in pearl and black guinea fowl is greater than expected heterozygosity (-0.063), (-0.149) respectively. Shortest distance (0.6325) was observed between Pearl and Ash guinea fowl while the distance between Pearl and Black (0.7746), Pearl and Ash (0.7746), and also Broiler guinea fowl and Ash (0.7746) were the same. The highest distance (0.8944) was observed between Broiler guinea fowl and black. 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. A distinct cluster was form by all the groups studied with black formed a clustered in the 1st quadrant while broiler guinea fowl formed a distinct cluster at the 2nd quadrant. However, Ash and Pearl form very closer cluster in the 3rd quadrant. Conclusively, the result revealed that the markers were lowly (PIC<0.25) polymorphic in Ash while normally polymorphic in pearl (PIC<0.5) and highly (PIC>0.5) polymorphic in Broiler and Black guinea fowl, the population has more heterozygote in the studied population and Consistent cluster was formed between the strains which help to quantify the degree of relationships between guinea fowl strains and provide information of closeness and evolutionary history.

Key words: Microsatellite, Helmeted Guinea fowl, Nigeria.

INTRODUCTION

Guinea fowl is believed to have evolved from a francolin-like Asiatic ancestor, but it is believed that the evolution, and development to modern forms occurred solely in Africa (Cracraft, 1973; Oslon, 1974). Of the five world *galliformes*, it is only the guinea fowl, *Numidinae*, which is entirely endemic to Africa (Delacour, 1997). 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).There are three main genera's of the *Numidae* family in Africa. These are Royal guinea fowl that has only some feathers on the nape of the neck and has Somalia, Tanganyika and Zanzibar as its habitat, Crested guinea fowl found in the African tropical forest and Helmeted guinea fowl, which are those with either blue or red carouncles. It is this *Numida meleagris* that has been introduced all over the world from West Africa, which is its native origin and is the genus from which the gray, white, lilac, violet, lavender and Isabelle varieties have been developed. Of the four common subspecies of *Numida* found in Africa, only two have been domesticated. These are the *Numida meleagris*, indigenous to West Africa, and *Numida ptalorhrca*, indigenous to East Africa. There are two main guinea fowl subspecies found in Nigeria (Ayorinde, 2004). The helmeted guinea fowl *Numida meleagris galeata* Pallas, which occurs 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 sub specie is the crested guinea fowl, *Guttera edouardi edouardi*, is restricted in distribution to the forest and derived savanna forest edges (Ayeni, 1979).

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 breeding; 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 year 2005 the genotyping of the individual animals for marker assisted conservation scheme was carried out using the amplified fragment length polymorphism (AFLP) technique (Botchway, *et al*., 2013). 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 (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 & Crittenden, 1994).

MATERIALS AND METHODS

Study Location

This study was carried out using guinea fowl populations in three states of Nigeria namely (Figure 1); Katsina State ($12^0$15'N and $7^0$30'E), Kano State ($12^0$00'N and $9^0$45'E), and Kaduna State ($10^0$15'N and $8^0$13'E) (Google, 2012).

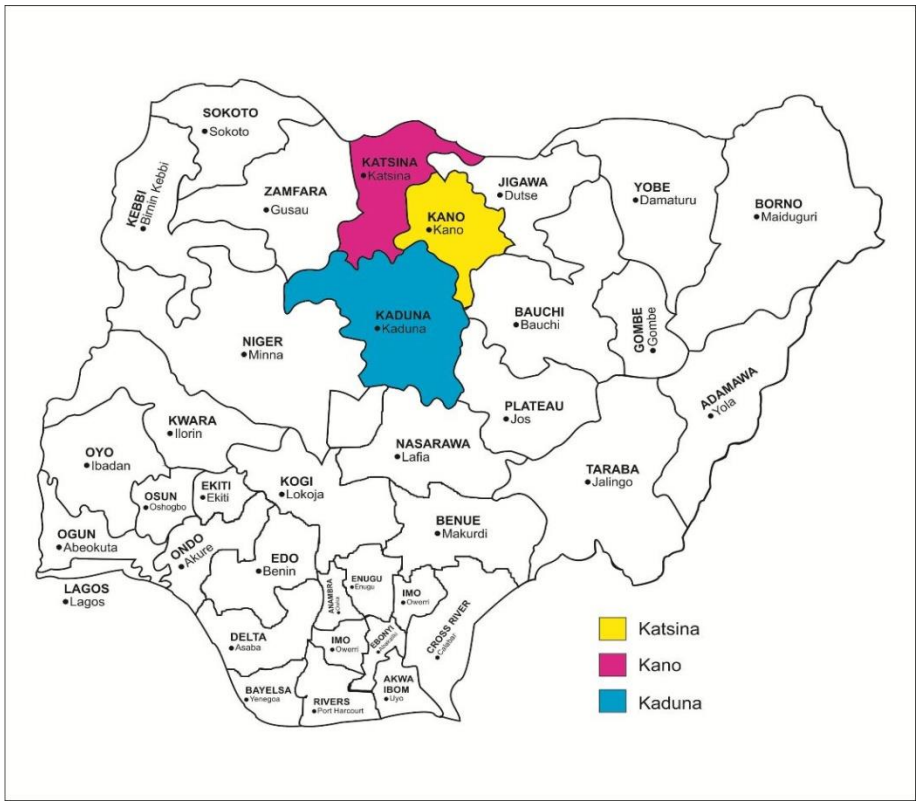


Figure 1: *Map of Nigeria showing locations where samples were collected*

Experimental Birds

A total of 160 randomly sampled (Table 1) indigenous Guinea fowl strains namely; Lavender (Ash), Pearl, Black and Broiler Guinea fowl was used for the study. For each strain, a maximum of forty (40) birds ware sampled and blood (2ml) was collected from the wing vain of each bird using needle and syringe, and immediately transferred into a well labeled EDTA bottles and stored at –20°C until analysis. Thirty-one sets (31) of micro-satellite markers were used to genotype the sampled animals.

The blood samples were analysed at Centre for Biotechnology Research and Training (CBRT/ABU), and Molecular Diagnosis (Avian influenza) Laboratory of Veterinary Teaching Hospital, Ahmadu Bello University (ABU) Zaria.

Table 1: List of sampling locations and states

LOCATION	STATE	STRAIN
Sabongari Market	Kano	Non specific
Dakastale gada	Kano	Non specific
Kwanar dangora	Kano	Non specific
Samaru College of Agric DAC/ABU Zaria	Kaduna	Broiler guinea fowl
Galadimawa	Kaduna	Non specific
Dumbi	Kaduna	Non specific
Giwa	Kaduna	Non specific
Funtua	Katsina	Non specific

Genomic DNA Extraction

Thirty-one (31) sets of primers included in the lists of recommended primers for guinea fowl analysis suggested by (Botchway *et al*., 2013), fowl genomic DNA used as a template for PCR reaction was isolated from blood using a modified DNA purification kit (Thermo Scientific®) according to the procedures described by (Sambrook *et al*., 1998). The purified DNA was checked by running it on a 1.0 % Agarose gel stained with G-green.

PCR Amplification

A multiplex PCR was done using the 31 microsatellites (Table 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™) 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).

Table 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
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

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 45m to 1 h depending on the voltage for minimum 10 cm distance (3/4 gel slab).

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

Data Analysis

The bands in the electropherogramme were analysed and scored using the image Lab™. Observed allele frequencies were computed and 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 were calculated using GenAlEx version 6.5 (Dieringer & Schlötterer, 2003).

In this study, deviations of the locus/population from Hardy Weinberg equilibrium (HWE) were determined using the programme GenAlEx version 6.5 (Raymond, 2004).

Analysis of molecular variance, AMOVA (Excoffier, 1992) was carried out using the GenAlEx 6.5(Peakall & Smouse, 2012) and was performed to quantify further the extent of population and the distribution of genetic variation in the sample populations. A genetic distance measure was computed using the GenAlEx 6.5. A phylogenetic tree was constructed from (Nei, 1973) standard genetic distance Cavalli-Sforz generated using the Unpaired Group with Arithmetic Mean (UPGMA) methods in R-Studio version 3.4. (R-Studio, 2015). The reliability of the constructed trees was examined according to (Felsenstein, 1985).

Ethical clearance

Ethical clearance for samples collection was approved by animal use and handling committee of Ahmadu Bello University, Zaria.

RESULTS

Mean Number Allele (Na), Observed (Ho) and Expected Heterozygosity (He), Polymorphism Information Content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Guinea Fowl Population Studied

Table 3, showed mean number allele (Na), observed (Ho) and Expected Heterozygosity (He), polymorphism information content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Pearl guinea fowl. The mean number of allele ranged from 0.226 to 3.129 (Ash and Black). The value of PIC ranged from 0.207 (Ash) to 0.715 (Black) with mean of 0.47 showed low (PIC<0.25) polymorphism in Ash while normally polymorphic in Pearl (PIC<0.5) and highly (PIC>0.5) polymorphic in Broiler and Black guinea fowl. The observed (Ho) and expected (He) heterozygosity ranged from 0.012 to 0.101 and -0.1490 to 0.126 with mean of 0.044 and -0.01, respectively. None of the population deviated from Hardy-Weinberg Equilibrium (HWE) and the values ranged between -2.4014 (Pearl) to 0.070171 (Broiler) with mean of -0.57.

Table 3: Number of allele (Na), Observed (Ho) and Expected (He) Heterozygosity, Polymorphism Information Content (PIC) and Hardy-Weinberg Equilibrium (HWE) of Guinea fowl population studied

Strain	MNa	PIC	Ho	He	HWE	P<0.01
Pearl	0.710	0.310	0.022	-0.063	-2.4014	NS
Black	3.129	0.715	0.101	-0.1490	0.034708	NS
Broiler	1.290	0.642	0.042	0.126	0.070171	NS
Ash	0.226	0.207	0.012	0.065	0.036078	NS
Mean	0.340	0.470	0.044	-0.010	-0.570	

Genetic Distance

Table 4 Showed the pair-wise population matrix of Nei's genetic distance estimates of Nigerian Guinea Fowl strains. The result showed that, the shortest distance (0.6325) was observed between pearl and ash guinea fowl while the distance between Pearl and Black (0.7746), Pearl and Ash (0.7746), and also Broiler guinea fowl and Ash (0.7746) were the same. The highest distance (0.8944) was observed between Broiler guinea fowl and black.

Table 4: Genetic distance estimates of Nigerian Guinea Fowl strains

PEARL	BLACK	BG	ASH	
				PEARL
0.7746				BLACK
0.7746	0.8944			BG
0.6325	0.7746	0.7746		ASH

BG: broiler guinea fowl

Analysis of Molecular Variance (AMOVA)

The AMOVA estimated showed that (Table 5) 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) was only attributable to variation among population.

Table 5: 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: Populations

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

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

The result based on the allele frequency obtained is shown in figure 2. A distinct cluster was form by all the groups studied with black formed a clustered in the 1st quadrant while broiler guinea fowl formed a distinct cluster at the 2nd quadrant. However, Ash and Pearl form very closer cluster in the 3rd quadrant.

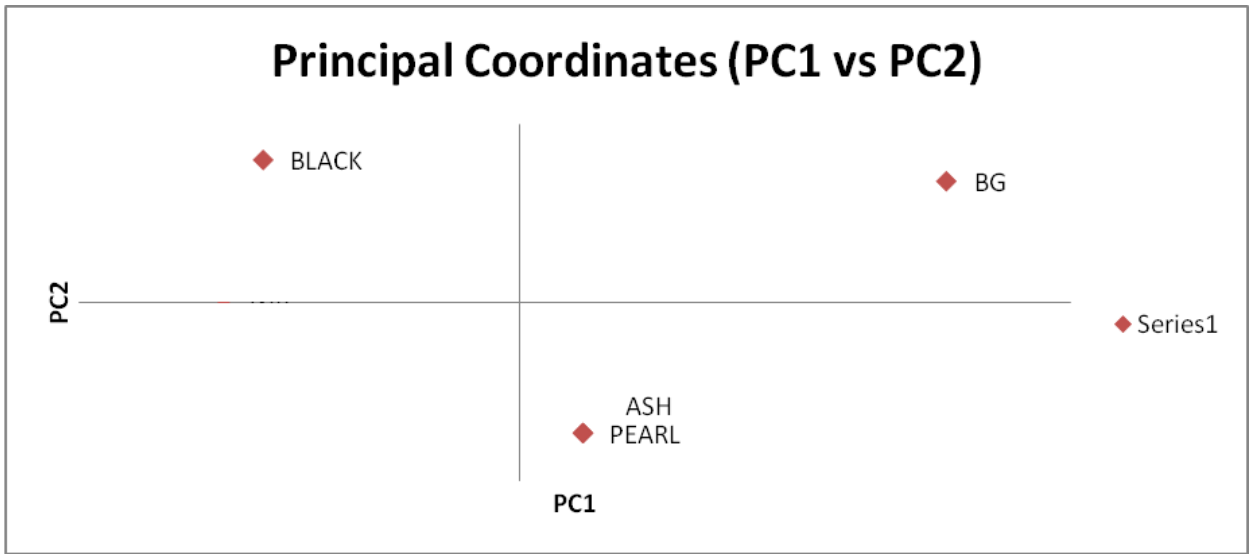
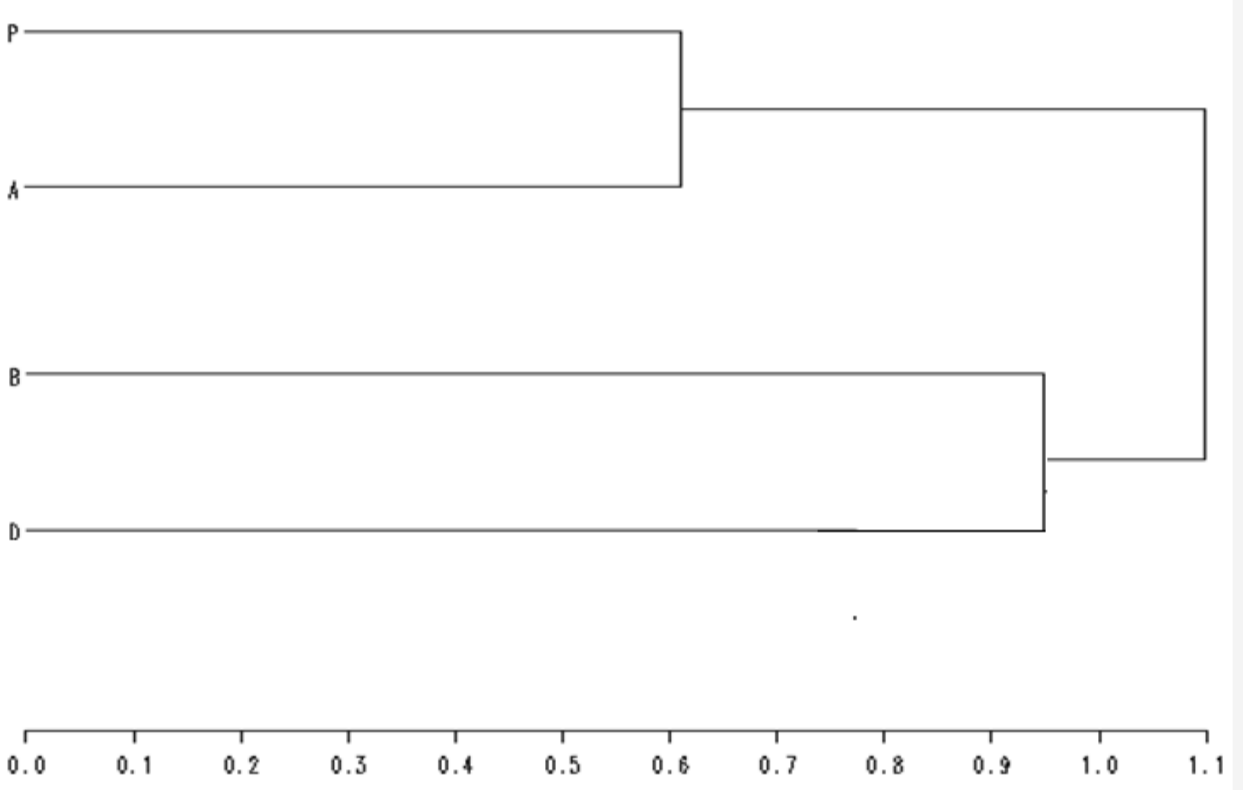


Figure 2: *Bi-dimensional graph representing the relationship between PC1 and PC2 using microsatellite makers derived from 5 guinea fowl strains in Nigeria*

The Cluster Analysis

The dendrogramme (figure 3) from the result of 31 SSR used based on Nei's genetic distance indicated that the studied population forms three (3) clusters at various degree of similarities.

At 0.6 pearl (P) and Ash (A) forms cluster, indicating a high level of genetic similarity. At 0.9 co-efficient D and B Finally, the first (1st) and second (2nd) group joined form the third (3th) cluster at 0.95 showing genetic similarity among all the strains.



Figue 3: *Dendrogramme representing Phylogenic relationship of guinea fowl strains in Nigeria (p:pearl, A:ash, B:black, D:broiler guinea fowl)*

DISCUSSION

The result of the studied guinea fowl population using 31 set of microsatellite markers revealed that they(markers) were lowly (PIC<0.25) polymorphic in Ash while normally polymorphic in pearl (PIC<0.5) and highly (PIC>0.5) polymorphic in Broiler and Black guinea fowl, A locus is said to be highly polymorphic if PIC is greater than 0.5, medium(moderate) polymorphic if it is between 0.25 and 0.5 and low polymorphic if it is less than 0.25 (Botstein *et al*., 1980). The PIC level observed is an indication for a moderate to high gene diversity among the studied markers which is also reported by Chang *et al*.(2007) and they can be used for molecular genetic studies. The mean observed heterozygosity (0.012), (0.042) was lower than the mean expected heterozygosity (0.065), (0.126) for Ash and Broiler, respectively which(follows the same trend) agrees with the report of Botchway *et al*.(2013)who reported that, observed (Ho) and expected heterozygosity (He) ranged from 0.033 to 1.000 with a mean of 0.396, and 0.033 to 0.799 with a mean of 0.419, respectively from guinea fowl population of West Africa using the same markers as applied in this research, meaning that the population points towards out breeding with more heterozygote in the studied population. The increase in heterozygosity in the studied population is a good indication of genetic variance at polymorphic sites, rich genetic diversity and selection potential (Liu *et al*, 2006). With the exception of broiler and ash guinea fowl with mean observed heterozygosity (0.042), (0.012) being lower than the mean expected heterozygosity (0.126), (0.065) the observed heterozygosity (0.022), (0.101) in pearl and black guinea fowl is greater than expected heterozygosity (-0.063), (-0.149)

respectively implies that the decrease in heterozygosity in the studied population may be due to low population size which is a driving factor for inbreeding and shifted the population towards reduction in heterozygosity. All the populations were at Hardy Weinberg Equilibrium, suggesting that selection has not been done in the population studied likewise mating is random, and no migration (gene flow). The observed shortest genetic distance confirms the fact that the guinea fowl studied are living in the same geographical location, having common origin and evolutionary progress, time of speciation and bottleneck effect at the beginning of domestication this agree with the report of Kayang *et al*. (2010) who reported that the distances between west African guinea fowl population is 0.079 and 0.169 likewise the findings of (Sharma *et al.*, 1998) using RAPD to describe the population of Indian guinea fowl have found high genetic similarity. The Ash and White breasted were considered as variants of Pearl (Moreki, 2009) which expressed the shortest distance between ash- pearl (0.6325) Guinea fowl and Broiler guinea fowl was introduced into Nigeria from Belgium/France after many years of breeding activities even though they have the same origin of evolution. The individuals studied were found to exhibits high degree of homozygosity (0% within) within group, which was probably contributed due to the fact that guinea fowl is monogamous in nature (Nwagu & Alawa, 1995).The high estimated percentage variation (100%) between population, indicate that they are separated from each other to maintain the strain characteristics which was also reported by Umar *et al*. (2015), Bawej *et al*. (2012) reported that The higher genetic similarity between than within the analysed guinea fowl varieties could result from the fact that the birds of the analysed varieties originated from the same founder flock. The lower genetic similarity within variety was probably due to the higher variation of the analysed animals and the fact that the results obtained using the primers were averaged. Broiler guinea fowl was distantly related to other strains studied probably due to long term selection and breeding activities that led to its development. Ash and Pearl presented very closer cluster. Consistent cluster was formed between the strains which may help to quantify the degree of relationships between different species and provide information on closeness within the same species where variant occur.

CONCLUSIONS

The result revealed that the markers were lowly (PIC<0.5) polymorphic in Ash while normally polymorphic in pearl (PIC<0.5) and highly (PIC>0.5) polymorphic in Broiler and Black guinea fowl indicated a moderate to high gene diversity among the studied markers which is a good indication of high polymorphism.

The increase in heterozygosity in the studied population is a good indication of genetic variance at polymorphic sites, rich genetic diversity and selection potential in broiler and ash with mean observed heterozygosity (0.042), (0.012) being lower than the mean expected heterozygosity (0.126), (0.065). The increase in heterozygosity is a good indication for genetic variance at polymorphic sites, rich genetic diversity and good selection potential.

Consistent cluster formed between the strains which may help to quantify the degree of relationships between different species and provide information on closeness within the same species where variant occur with Broiler guinea fowl being distantly related to other strains studied.

RECOMMENDATION

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 especially for Ash. 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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Conflict of interest: No conflict of interest as far as this research work is concerned and other researcher's findings were considered and respected in form of citation and referencing.

Author's contribution:

Umar, U.A.: Student and corresponding author of the research and the manuscript is from my M.Sc thesis.

Dr. Kabir, M: My major supervisor and advisor of the research who help in correcting and packaging of the research for award and publication.

Prof. Nwagu, B.I: Research professor, second supervisor and advisor of the research.

M, Abdurrahman and A, Suleiman: A breeder /staffs in training at the National Animal Production Research Institute that support in the sample collection and transportation.

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