Genetic Diversity and Population Structure of Nigerian Indigenous Chicken Populations Inferred from Microsatellite Markers

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Summary

Knowledge of genetic diversity is a prerequisite for better utilization of any genetic resource. However, such information is insufficient for Nigerian indigenous chicken (NIC). Deoxyribonucleic acid (DNA) of NIC was extracted from FTA® paper and amplified with predefined microsatellite primer sets. A total of 180 chickens: northeast (NE; n=44), northwest (NW; n=25), north-central (NC; n=42) and southwest (SW; n=69) were genotyped along with 15 microsatellite markers to assess genetic diversity, demographic and population stratification. All microsatellites typed were found to be polymorphic (mean PIC = 0.53), and a total of 44 distinct alleles were detected. For all the loci, average inbreeding values (F_{IS}) were ranged from -0.01 (NW chickens) to 0.17 (SW chickens), with an average value of 0.12, thus suggesting heterozygote excess. Most of the microsatellites deviated from Hardy-Weinberg equilibrium. SW and NC chickens related more closely having a genetic distance value of 0.02. The cluster analyses using STRUCTURE program indicated there were three primary populations, which provided evidence of extensive sharing of genetic variability, revealing varying levels of admixture among the studied population. The AMOVA analysis result indicated the proportion of genetic variation due to differences among populations and within populations was 5.46% and 96.56% respectively. Our results revealed multiple waves of introduction of diverse gene pools, and high panmixia has created and maintained a unique set of Gallus biodiversity in Nigeria.

Key words

genetic differentiation; local chicken; genetic marker; population structure

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Introduction

Nigeria native chicken constitutes about 80% of the 120 million poultry type raised in the rural areas (Ajayi, 2010; RIM, 1992), which products (egg and meat) are readily available and preferred because of their pigmentation, suitability, taste, and toughness (Ohagenyi et al., 2013). They serve as an excellent source of animal protein and income to people in the rural and semi-urban areas, and also, represent a valuable animal genetic resources for the development of livestock value chain due to their broad genetic diversity which allows for poultry rearing under different environmental condition. Local chicken genetic resources are waiting to be fully exploited in developing locally adapted strains to the ever-changing production environments and breeding objectives to the benefit of poultry farmers (Ajibike et al., 2017; Sonaiya et al., 2002)

Despite the importance of Nigerian domestic chicken, there are little reports about their genetic diversity, which is a significant step towards revealing the uniqueness of these populations as well as the essential component for population survival, evolution, genetic improvement and adaptation. With the present alarming global challenges such as climate change, emerging diseases, population growth and rising consumer demands, it is likely that new genotypes (strains) will be required in the future to meet the ever-changing environmental and production conditions. Therefore, information on genetic diversity is necessary to optimize conservation and breeding program of animal genetic resources to ensure food security (Ajibike et al., 2017)

The use of molecular tools has facilitated biodiversity studies, particularly microsatellite markers because of their sufficient number, easy identification, and ubiquitous presence throughout the genome, high polymorphism and co-dominant nature (FAO, 2011). Thus, making it a marker of choice in the estimation within and between-breed genetic diversity, genetic admixture among breeds, determination of parentage, the establishment of genetic linkage maps and reconstruction of phylogenetic relationships among populations.

This study was undertaken to estimate the genetic diversity, relationship and population structure among domestic chickens based on geographical zones in Nigeria.

Materials and Methods

Ethics Statement

The Institutional Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Nigeria approved all experimental procedures. The field surveys involved no endangered or protected animal species. A veterinarian helped in the blood sample collection, and manually restrained the animals; no tranquilizers or short-acting anesthetics were used. Blood samples were collected using appropriate equipment.

Sample Collection and DNA Extraction

Blood samples (2 ml each) from the wing vein of 180 chickens from 4 geographical zones of Nigeria (northeast (NE), n=44; northwest (NW), n=25; north-central (NC), n=42; and southwest (SW), n=69) were collected using a new needle and syringe for each bird to avoid cross contamination. The blood collected was dropped on the FTA classic cards (Whatman® Bioscience, UK), allowed to dry, transferred and stored in bags containing silica gel. As described in detail previously by Ajibike et al. (2017), genomic DNA was extracted using five disks (2 mm²) punched from the FTA® Classic cards stained with the blood sample. The extract was pipetted and put in a new tube; the batch had 60 – 150 μL of DNA.

PCR Amplification and Genotyping

The genomic DNA was amplified using 15 microsatellite markers (shown in Table 2) recommended by ISAG/FAO for chicken biodiversity study (FAO, 2011) following the published protocol for each marker. Each 25 μL PCR reaction containing 20 – 40 ng extracted genomic DNA as a template, 2.5 mM $MgCl_2$, 200 μM each of dNTPs, 1 unit *Taq* DNA polymerase, 0.05 μM of each primer, and 1× Magnesium free PCR buffer (Promega, Madison, USA). PCR was carried out on a thermal cycler (GeneAmp PCR System 9700) as described in detail previously by Lioi and Piergiovanni (2013). Multiplexing and Allele size calling was performed as previously described by Adebambo et al. (2011) using an ABI 3730XL automated capillary sequencer (Applied Biosystems, USA).

Statistical Analysis

POPGENE (Yeh et al., 1999), CERVUS ver 3 (Kalinowski et al., 2007) and GenAlex (Peakall and Smouse, 2012) were used to determine the number of alleles, an effective number of alleles, observed and expected heterozygosity, and test for Hardy–Weinberg equilibrium. Allelic frequencies were utilized for assessing polymorphic information content (PIC) using Bostein et al. (1980) formula. Population genetic differentiation was evaluated using Wright (1978) fixation indices for each locus across populations based on Weir and Cockerham (1984) method using FSTAT 2.9.3 software (Goudet, 2002). The extent of inbreeding within each population was estimated using GENEPOP software (Rousset, 2008).

Gene flow between populations was determined as F_{ST} = $1/(4Nm+1)$. A nuclear AMOVA implemented in Arlequin ver.3.5.2.2 (Excoffier and Lischer, 2010) was used to estimate and partition genetic variation within and between sampled populations. The population genetic structure and the degree of admixture of Nigerian chicken populations were investigated using STRUCTURE program ver.2.3 (Pritchard et al., 2000) based on Bayesian clustering procedure. 50 independent runs for each K value ranging from 1 to 6 were carried out based on admixture model of 100,000 iterations after a burn-in period of 20,000 iterations for all runs. To identify the most likely group (*K*) that best fit the data, we used the Evanno method (Evanno et al., 2005) in web-based STRUCTURE Harvester (Earl and vonHoldt, 2012). The program CLUMPP ver. 1.1 (Jakobsson and Rosenberg, 2007) was used to align the 50 repetitions of each K, and the out files were visualized using DISTRUCT ver. 1.1 (Rosenberg, 2004).

Results and Discussion

Allelic and Genetic Diversity

Measures of allelic and genetic diversity computed across the 15 loci for each population as shown in Table 1 revealed the mean number of alleles (MNA) per population with an average value of 5.92±0.31, ranging from 5.00±0.05 (NW chickens) to 6.73±0.74 alleles (SW chickens). The NE and NW chickens had less than the average number of alleles, while NC and SW chickens had the higher mean number of alleles. These suggested that the observed MNA over a range of loci across the different population could be considered as a good indicator of genetic variation within a given animal population (Hassen et al., 2016). It might also suggest the level of inbreeding being experienced at the different geographical location or differences in effective population size.

The number of the effective alleles (NEA) contributing to the population ranges from 2.02±0.16 (NE chickens) to 2.86±0.25 (SW chickens), with an overall mean of 2.42±0.10. The number of loci with particular alleles was the highest in SW chicken (67 loci) and the lowest in NE chickens (27 loci) while the proportion of loci not in HWE was the highest in NW chickens (11 loci) and the lowest in NE chickens (5 loci). The mean PIC per population had an average value of 0.53±0.04, ranging from a minimum of 0.42±0.04 alleles (NE chickens) to a maximum of 0.57±0.04 (SW chickens), which suggested that all loci used in this study are moderately informative for analyzing genetic diversity in chickens (Mateescu et al., 2005).

The observed mean heterozygosity (H_o) was lower than the expected mean heterozygosity (H_e) for all studied chicken populations. The H_o had an average value of 0.49±0.05 with the lowest value observed in NE chickens (0.39±0.06) and the highest values in NW and SW chickens (0.53±0.05) while the H_e ranged from 0.47±0.04 (NE chickens) to 0.61±0.04 (SW chickens) with an overall mean value of 0.55±0.04. These values are comparable with studies carried out on other native chicken breeds or populations (Ohwojakpor et al., 2012; Olowofeso et al., 2005; Quain et al, 2006; Wei et al 2008; Ye et al, 2006; Zhen-Hue et al, 2010). The presence of long term of natural selection for adaptation and existence of interbreeding as a result of free movement of animals within the country in search of a better vegetation and more marketing of the

stocks (Ajibike, 2016), are believed to significantly contribute to the observed genetic diversity in Nigerian chickens.

A significant inbreeding coefficient (F_{IS}) ($P < 0.01$) was observed in all the populations studied ranging from 0.01 (NW) to 0.17 (NE), with a mean value across populations of 0.12. These represented an average decrease in the number of homozygous loci of 1% in NW chickens and a reduction of 17% in NE chickens. The within-population heterozygosity varied among the sampled populations, but, estimated variability did not differ from the report of Mwacharo et al. (2007). Despite the attempt to avoid sampling closely related individuals, a significant low positive mean F_{IS} value (range = 13 to 17%) was detected indicating heterozygote deficiency which confirmed that the populations are not entirely panmictic. This significant positive mean F_{IS} could be due to having similar ancestral origins or inbreeding effect as dominant cocks get to mate more females especially if the chickens share common overnight roosting ground (Adebambo et al., 2000)

Genetic Differentiation across Populations

Population genetic differentiation was evaluated across the populations using 15 microsatellite markers (Table 2). A total number of alleles per locus for all sampled population ranged from 4.00 (MCW0020 and MCW0165 loci) to 15.00 (MCW0104 locus) with an overall mean value of 8.20. The observed allele size ranged from 6.00 (MCW0020 and MCW0165) to 44.00 (MCW0081) with a mean value of 18.67 while the allelic richness ranged from 3.23 (MCW0248) to 9.12 (MCW0104) with mean value of 5.37. MNA and the number of effective alleles across populations had mean values of 5.92 and 2.42, with values ranging from 3.25 to 10.75 and 1.28 to 3.51 respectively.

The observed expected heterozygosity value ranged from 0.22 (MCW0248) to 0.73 (MCW0104) with an overall mean of 0.57, whereas the observed heterozygosity value ranged from 0.14 (MCW0165) to 0.94 (MCW0081) with an overall mean of 0.49. All used loci except MCW0165, MCW0014, and MCW0248 were highly polymorphic having PIC value higher than the threshold of 0.50 assumed for a maker to be informative (Chatterjee et al., 2008; Olasunkanmi, 2010). The PIC value ranged from 0.21 (MCW0248) to 0.70 (MCW0104), with an overall mean value

Table 1. Population level allelic and genetic diversity

Pop	N	Allelic diversity				Genetic diversity				HWE
		MNA	NEA	PIC	PA	H_o	H_e	I	F_{IS}	
NE	44	5.67±0.54	2.02±0.16	0.42±0.04	0.27±0.12	0.39±0.06	0.47±0.04	0.92±0.08	0.169**	5
NW	25	5.00±0.50	2.16±0.12	0.47±0.03	0.33±0.13	0.53±0.05	0.52±0.03	0.99±0.07	-0.009**	11
NC	42	6.27±0.64	2.65±0.20	0.54±0.04	0.40±0.16	0.51±0.05	0.59±0.04	1.19±0.09	0.135**	6
SW	69	6.73±0.74	2.86±0.25	0.57±0.04	0.67±0.21	0.53±0.05	0.61±0.04	1.25±0.10	0.128**	7
Mean		**5.92±0.31**	**2.42±0.10**	**0.53±0.04**	**0.42±0.15**	**0.49±0.05**	**0.55±0.04**	**1.10±0.05**	**0.12****	

NE – North-east chicken; NW – North-west chicken; NC – North-central chicken; SW – South-west chicken; N – Sample size; MNA – Mean number of alleles; NEA – number of effective alleles; PIC – Polymorphic information content; PA – Number of private alleles; H_o – Mean observed heterozygosity; H_e – Mean expected heterozygosity; I – Shannon information index; F_{IS} – inbreeding coefficient; $P < 0.01$; HWE – Number of loci deviating from HWE

of 0.53. Thus, those markers had real advantages for detecting DNA identity and diversity in these populations and are therefore suitable for use in the characterization of natural populations and determination of genetic differentiation in Nigerian chicken.

The mean values of F-statistics obtained over loci were F_{IS}= 0.12, F_{IT} = 0.15, and F_{ST} = 0.03. The inbreeding coefficients (F_{IS}), assessed within population deficit or excess in heterozygosity value, ranged from -0.37 (MCW0081) to 0.64 (MCW0165) with an average of 0.12 across all loci. The degree of differentiation within the population (F_{IT}) and the extent of differentiation among subpopulation (F_{ST}) value were 0.15 and 0.03, respectively. All loci with positive F_{IS} values indicated heterozygotes deficiencies while ADL0268, MCW0123, MCW0248 and MCW0081 loci with negative F_{IS} values indicated heterozygotes excess. The heterozygotes deficiencies suggested that random mating was being practiced between the sampled chicken populations, resulting in more common alleles which did not significantly differ regarding frequency across sampled populations (Rosenberg et al., 2001).

The highest value of F_{ST} (0.10) was observed for MCW0165, while MCW0020 and MCW0248 had the lowest value of 0.0102. The mean F_{ST} value (0.03) indicated the absence of genetic divergence within the sampled populations, and most of the total genetic variation corresponded to differences among individuals within populations. Both high F_{IS} and F_{ST} values imply a considerable degree of inbreeding and genetic differentiation among sampled chicken populations respectively. Values of G_{ST} ranging from 0.00 (MCW0020 and LEI0094) to 0.08 (MCW0165), with a mean of 0.02, suggest that genetic variation among the studied chicken populations was relatively low (2%). The estimated number of migrants by locus ranged from 2.37 (MCW0165) to 17.79 (MCW0248), with a mean of 7.20.

Genetic Distances and Relationship among Populations

The Nei's D_A genetic distance is a correlation among the allele frequencies between sampled populations (Nei, 1972). Table 3 shows the genetic distance between each pair for the four populations based on the selected 15 microsatellite loci. The Nei's genetic distance ranged from 0.012 (NC and SW chickens) to 0.088 (NE and SW chickens). The genetic identity among four chicken populations was observed to be quite high (0.916 to 0.989). The unweighted pair group method with arithmetic mean (UPGMA) dendrogram constructed for the four chicken populations from D_A genetic distances (Fig. 1) revealed the clustering of the four chicken populations into three distinct separate groups, with NC and SW chicken population forming a single cluster, while NW and NE chicken were individually divided into an apparent cluster.

Table 2. Genetic variability indices by locus across population

Locus	N	TNA	AS	AR	MNA	Ne	Ho	He	PIC	F_{IT}	F_{ST}	F_{IS}	G_{ST}	I	Nm	F(null)	HWE
ADL0112	173	5.00	8.00	4.24	4.75	2.74	0.58	0.64	0.58	0.11	0.02	0.09	0.01	1.15	12.72	0.05	NS
ADL0268	173	6.00	11.00	5.06	4.75	2.24	0.52	0.56	0.52	0.04	0.05	-0.02	0.04	1.01	4.47	0.03	NS
MCW0014	175	8.00	20.00	3.78	4.25	1.57	0.28	0.39	0.35	0.26	0.03	0.24	0.02	0.66	8.88	0.15	*
MCW0020	162	4.00	6.00	3.99	4.00	2.58	0.59	0.63	0.58	0.04	0.01	0.03	0.00	1.13	18.70	0.01	NS
MCW0123	172	10.00	20.00	6.84	7.00	2.47	0.58	0.60	0.57	0.03	0.04	-0.01	0.03	1.22	5.68	0.03	NS
MCW0248	175	5.00	8.00	3.23	3.25	1.28	0.23	0.22	0.21	-0.05	0.01	-0.06	0.01	0.43	17.79	-0.02	ND
MCW0216	179	7.00	12.00	4.96	5.50	2.50	0.44	0.63	0.56	0.28	0.03	0.26	0.02	1.10	8.42	0.16	*
ADL0278	177	6.00	12.00	4.27	4.75	2.53	0.45	0.63	0.57	0.26	0.03	0.24	0.02	1.09	8.34	0.18	***
LEI0094	179	14.00	37.00	7.04	8.50	2.14	0.42	0.54	0.52	0.17	0.02	0.15	0.00	1.18	16.51	0.15	***
MCW0034	176	12.00	24.00	7.50	8.75	2.98	0.62	0.68	0.66	0.08	0.02	0.06	0.01	1.46	11.60	0.04	NS
MCW0081	180	10.00	44.00	6.84	7.50	3.26	0.94	0.72	0.68	-0.34	0.02	-0.37	0.02	1.44	10.61	-0.17	***
MCW0069	178	10.00	20.00	5.55	6.50	2.65	0.58	0.64	0.57	0.08	0.02	0.06	0.01	1.18	12.80	0.04	NS
MCW0104	173	15.00	42.00	9.12	10.75	3.51	0.65	0.73	0.70	0.07	0.03	0.04	0.02	1.57	7.08	0.06	NS
MCW0165	177	4.00	6.00	3.51	3.50	1.71	0.14	0.42	0.39	0.67	0.10	0.64	0.08	0.69	2.37	0.49	***
MCW0222	174	7.00	10.00	4.55	5.00	2.20	0.36	0.59	0.53	0.38	0.08	0.33	0.07	0.97	2.90	0.23	***
Mean		8.20	18.67	5.37	5.92	2.42	0.49	0.57	0.53	0.15	0.03	0.12	0.02	1.08	7.20		

N: Sample size; TNA: Total number of alleles; AS: Observed allele size; AR: Allelic richness; MNA: Mean number of alleles; Ne: Effective number of alleles; Ho: observed heterozygosity; He: expected heterozygosity; PIC: Polymorphic information content; F_{IS}: Inbreeding coefficient F_{IT}: Total inbreeding estimates; F_{ST}: fixation index; G_{ST}: Observed genetic differentiation; I: Shannon diversity index Nm: Estimated gene flow; F(null): null allele frequency; HWE: Hardy-Weinberg Equilibrium; NS – Not significant; * - $p<0.1$; ** - $p<0.01$; *** - $p<0.001$

The neighbour-joining tree further revealed the genetic homogeneity and lack of genetic sub-structuring of the studied chicken populations, even though small values of F_{ST} showed a significant differentiation. The phylogenetic analysis showed the same trend with a little bootstrap value of 3.17. A similar low bootstrap value was reported by Wimmers et al. (2000), though the bootstrap value is not enough to identify the chickens as a separate population, but could be a result of having a common ancestor or intermixing of germplasm being driven by human population traffic.

Table 3. Nei's genetic distance (below diagonal) and identity (above diagonal) between Nigerian chickens

	NE	NW	NC	SW
NE	*****	0.963	0.937	0.916
NW	0.038	*****	0.981	0.965
NC	0.065	0.020	*****	0.989
SW	0.088	0.036	0.012	*****

NE: Northeast chicken; NW: Northwest chicken; NC: Northcentral chicken; SW: Southwest chicken
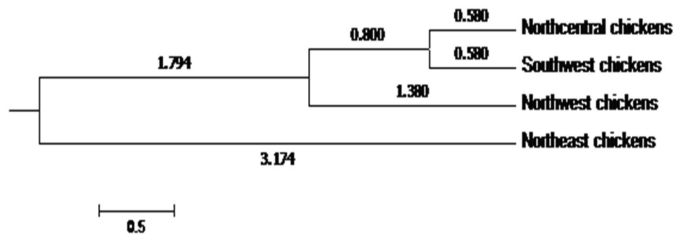

Figure 1. Genetic distance dendrogram of sampled Nigerian indigenous chicken populations

Population Genetic Structure Analyses

The AMOVA revealed that 5.46% of the total variation was present among the chicken populations, and most of the genetic variation (96.59%) was found within individual (Table 4). The possible ancestral gene pools underlying the observed genetic diversity were assessed with STRUCTURE program (Pritchard et al., 2000). The analysis revealed that K=3 had the highest maximum likelihood value of Ln Pr $(X|K)$ of -6363.466 with the lowest value (-6222.572) observed at K=5 (Fig. 2).

As inferred using Evanno et al, 2005) method, the most likely number of gene pools that contributed to the observed genetic variability in the four populations studied was K=3 having the highest ΔK value of 3.693 (Fig. 3). The contribution of the detected gene pools to the studied populations is graphically shown (Fig. 4). The admixture plot of all chicken population reveals three distinct population patterns, which shows strong support for subpopulation structure. The actual population substructure could be hindered by the sample sizes considered. These confirmed that the studied chicken populations were identical with dependent clusters of the potential ancestral gene pool as genetically distinct and there was clear evidence of genetic admixture, which is the result of variable contributions from one ancestral gene pool.
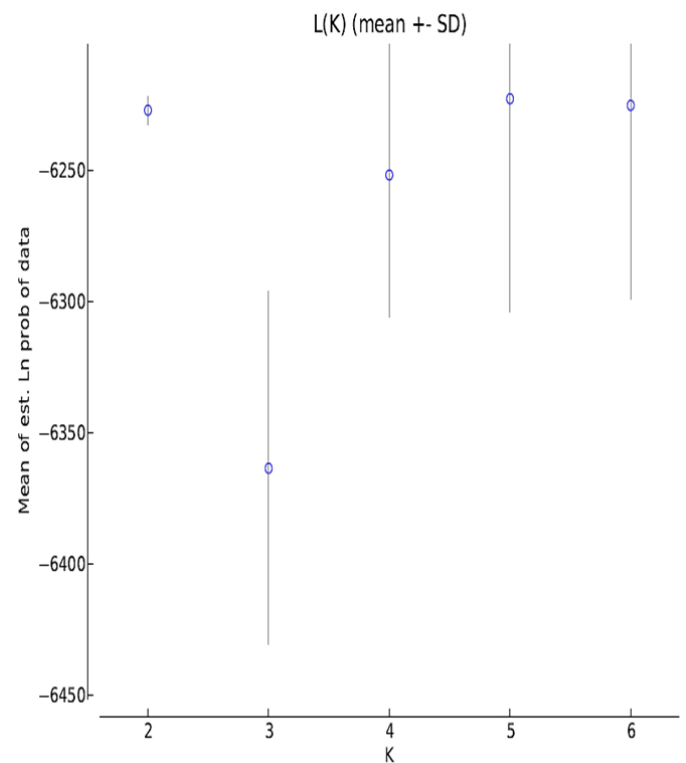

Figure 2. The maximum likelihood value of Ln Pr $(X|K)$
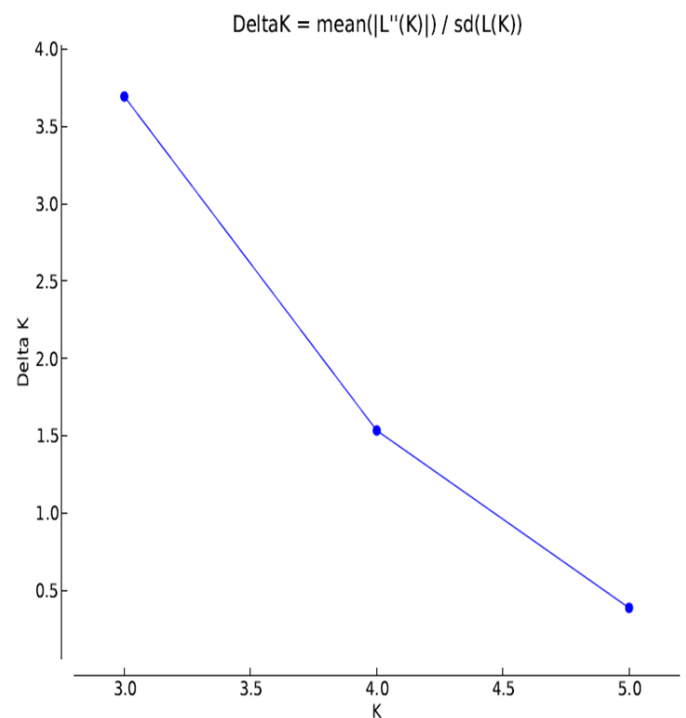

Figure 3. The optimum number of clusters as derived from structure harvester analysis

At K=3, NC and SW chickens were each grouped into a distinct cluster having an estimated membership value of 0.3783 and 0.4387 respectively. Genetic composition of the NE and NW chickens was defined by the variable frequencies of one ancestral gene pool with a contribution of 67.06% and 43.78%, respectively (Table 5).

This result indicated a sub-regional variation in the allelic composition and a high degree of genetic admixture with no significant gene pool differences, which might be due to the free movement of the chickens and exchange of genetic materials through social and agriculture interrelationships (Ajibike et al., 2017).

Table 4. Nigerian chicken population microsatellite AMOVA result

SOV	df	SS	Variance components	% variation
Among Populations	3	7904.46	25.51	5.46
Among Individuals	176	76107.36	-9.57	-2.05
Within Individuals	180	81282.50	451.57	96.59
Total	359	165294.32	467.51	

SOV: Source of variation; df: degree of freedom; SS: Sum Square

Table 5. Membership proportion of the inferred clusters

Pop	Inferred clusters			Number of individuals
	1	2	3	
NC	0.3783	0.3771	0.2446	42
NE	0.1915	0.1379	0.6706	44
NW	0.2917	0.2706	0.4378	25
SW	0.3702	0.4387	0.1912	69

NE: Northeast chicken; NW: Northwest chicken; NC: Northcentral chicken; SW: Southwest chicken
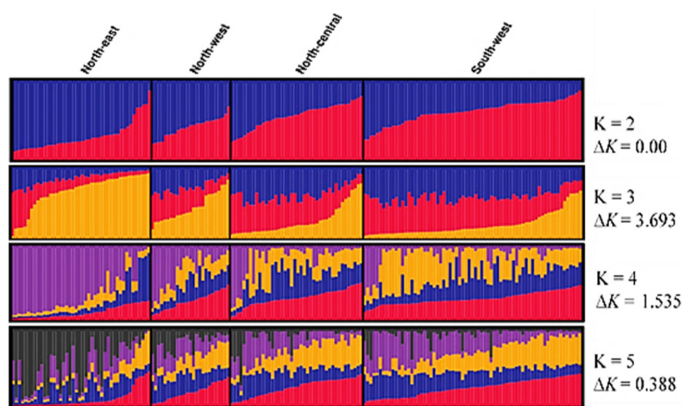

Figure 4. STRUCTURE clustering of sampled Nigerian chicken populations

Conclusion

The Nigerian chicken populations were well differentiated into three distinct groups by genetic distance and population structure values. Also, most of the markers used were highly polymorphic, informative, and suitably applicable in the genetic characterization of Nigerian chicken populations. A high degree of admixture/interbreeding and differences in the gene pool calls for an urgent further cataloguing and characterization of Nigerian chickens to have a national picture of their genetic diversity as well as putting in place of a genetic improvement and breeding program to avoid erosion of their genetic potentials.

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