Genetic Diversity and Gene Flow among Three Chicken Populations in Nigeria Using Microsatellite Markers

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Summary

To understand the level of genetic diversity among and within three improved locally adapted chicken populations in Nigeria, six microsatellite markers were used with 100 genomic DNA from Shika Brown (SB = 34), FUNAAB Alpha (FA = 33), and Noiler (NL = 33). The allelic and genotypic profiles of each representative from each population were determined through polymerase chain reaction amplification of the repeat region. Genetic diversity, genetic distance, level of inbreeding, polymorphism information content, and combined exclusion probabilities of markers (CPE/CPF) were analyzed using Microsoft Excel microsatellite toolkit, GenAlex, Microsatellite Analyser, FSTAT, and Poptree2. 416 alleles with 18.99% rare and 81.01% fixed alleles were observed across populations. The mean number of alleles was 23.111 ± 0.43, mean effective number of alleles was 16.975 ± 0.75, the expected heterozygosity was 0.940 ± 0.00, observed heterozygosity was 0.396 ± 0.02, mean PIC value was 0.937, and mean gene flow rate was 10.874 ± 0.817. The mean FIS was 0.579 ± 0.037 and the global FST was 0.023 ± 0.002. Nei's genetic distance revealed that Shika Brown and the Noiler chicken populations were related (0.6985). The combined exclusion probability (CPE) across markers and populations was 0.999 (excluding a parent) and CPF was 1.000 (excluding both parents). The PIC/marker values across populations were greater than the minimum value of 0.5. High FIS and low FST value indicated a high inbreeding level within and low degree of genetic differentiation among the chicken populations. In conclusion, the microsatellite markers used are highly polymorphic and suitable for parentage analysis, control inbreeding, and could be used as baseline genetic information in conservation programs.

Key words

chicken, microsatellite markers, genetic diversity, combined probability of exclusion

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Introduction

The present and future improvement and sustainability of chicken production systems are dependent upon the availability of genetic variation (Fraga Benitez, 2002; Ige and Salako, 2014). Genetic diversity is an important asset for all breeding programs and as such, allowing breeders to improve current traits or to develop new characteristics (Talle et al., 2005; Asbjarnardottir et al., 2010). Despite its importance, genetic diversity is declining globally, which is seen as the result of market extension and economic globalization, which calls for increased use of fewer high-output breeds (Asbjarnardottir et al., 2010). With decreasing genetic diversity, the importance of characterizing individual breed increases as that can aid in the identification and conservation of valuable genetic diversity (Asbjarnardottir et al., 2010). Ramadan et al. (2011) reported that the importance of maintaining genetic diversity in domestic livestock is advocated worldwide by the Food and Agriculture Organization (FAO) of the United Nations.

The indigenous chickens constitute about 80 percent of the chicken found in the rural area of Nigeria, providing the teeming populace with a good source of animal protein and a source of income and play a vital role within the context of many social events (ceremonies) without any religious bias (FAO, 2007). Nigerian indigenous chickens are known to be good foragers, efficient mothers, self-reliant, hardy, and require minimal care to grow. Their products are preferred by Nigerians because of the taste, leanness, and suitability for special dishes (Adebambo et al., 1999; Ajayi, 2010). They manifest quite some variations which are due to their genetic make-up and environmental factors, hence, making them important for genetic studies, improvement, preservation, and conservation (Daikwo et al., 2011). As a result of many years of intensive selection and breeding, a wide range of chicken breeds exist today (Granevitze et al., 2007) and this might have led to lower genetic diversity than is observed in other domesticated species (Hillel et al., 2003; Granevitze et al., 2007). Genetically improved chicken breeds present in Nigeria are the FUNAAB Alpha, Shika Brown, Noiler among others. These chicken have just been newly developed for commercial purpose and are not being studied at the genome level (ACGG Factsheet, 2015; Ilori et al., 2017; Yakubu et al., 2019). The FUNAAB Alpha and Noiler chickens are dual-purpose lines while the Shika brown is an egg line. These birds, however, perform better in terms of production than the Nigerian indigenous chicken. Therefore, these genetic resources need special attention for their conservation and improvement because no information on their detailed genetic characterization is available.

Ohwojakpor et al. (2012) reported that for proper characterization of major livestock species to conserve superior genotypes, it is advisable to use molecular markers called microsatellites. These markers are capable of revealing all the genetic information inherent in any species population and can be used to measure important genetic diversity indices. Microsatellite markers are widely used for characterizing populations and have been applied in numerous studies aimed at domestic animals, both to evaluate their genetic relationships between breeds and to estimate genetic diversity and structure within populations (Kantanen et al., 2000; Tapio et al., 2006; Asbjarnardottir et al., 2010). They can also be used in paternity testing because of their multi-allelic nature, wide genome coverage, and easy detection (Souza et al., 2012).

However, studies have been carried out with microsatellite markers in Nigeria indigenous chicken population (Olowofeso et al., 2016; Oni et al., 2017) but no research has been done on FUNAAB Alpha, Shika Brown, and Noiler chicken populations in Nigeria in terms of genetic characterization using microsatellite markers. This study is the first to investigate the genetic characteristics of these chicken populations using microsatellite and the results of this study can be used as baseline genetic information for conservation activities to control inbreeding and safeguard the genetic variability of the populations. The aim of the study therefore is to determine the genetic diversity and genetic relationship among three chicken populations (Shika Brown, FUNAAB Alpha, and Noiler) using 6 microsatellite markers.

Materials and Methods

Experimental Birds, Blood Collection and DNA Extraction

A total of 100 chickens (FUNAAB Alpha (33), Shika Brown (34), and Noiler (33)) from the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Nigeria were used for this study. Blood samples were collected from the brachial vein of individual birds using a new 1 ml disposable needle and syringe for individual birds to avoid cross-contamination. The skin was moistened and dabbed with alcohol to disinfect the area and make the vein visible. Approximately, 1 ml of blood was collected from each of the birds into a labeled ethylene diamine tetraacetic acid (EDTA) tube. Blood samples were stored at ≤ -20°C before analysis. DNA extraction was carried out using Zymo research Quick-gDNATM Miniprep Kit following the manufacturer's procedures at the Biotechnology Laboratory, Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta Nigeria. Secondary data from our three local chickens: normal feather, frizzle feather and naked neck using the same markers in our laboratory was also added to the phylogenetic reconstruction analysis to give a better presentation of the position of the new breeds in Nigeria

Microsatellite Markers Used, PCR Program and Reaction Profile

The six microsatellite markers used (Table 1) were among the 30 suggested markers by the International Society for Animal Genetics (ISAG) and FAO for chicken genetic diversity and population studies. These microsatellite markers were previously used by Ozdemir and Cassandro (2017) in local Turkish Denizli Chicken, while 3 polymorphic markers out of 6 were also previously reported by Olowofeso et al. (2016) in Nigerian indigenous chicken. The selected markers were previously tested in preliminary chicken studies in our lab to be highly polymorphic and are present in different frequencies in the different chicken populations of Nigeria. Amplification was carried out using 1 µL of each forward and reverse PCR primers as shown in Table 1, 1 µL of genomic DNA, 3.25 µL of Nuclease free water, and 6.25 µL of One Taq® Quick-Load 2X Master Mix (New England BioLabs) in a final volume of 12.5 µL. Initial denaturation was done at 94 °C for 2 mins, then 40 cycles of 94 °C for 30 s, 57.5 °C for 1 min, and 72 °C for 2 mins with a final extension of 72 °C for 5 mins. Electrophoresis was carried out at 90 V for 30 mins. The gels

Table 1. Microsatellite Markers used in this study

Markers	Primer Sequence	Chromosome number
MCW0016	F = ATGGCGCAGAAGGCAAAGCGATAT R = TGGCTTCTGAAGCAGTTGCTATGG	3
MCW0037	F = ACCGGTGCCATCAATTACCTATTA R = GAAAGCTCACATGACACTGCGAAA	3
MCW0111	F = GCTCCATGTGAAGTGGTTTA R = ATGTCCACTTGTCAATGATG	1
MCW0216	F = GGGTTTTACAGGATGGGACG R = AGTTTCACTCCCAGGGCTCG	13
ADL0268	F = CTCCACCCCTCTCAGAACTA R = CAACTTCCCATCTACCTACT	1
LEI0094	F = GATCTCACCAGTATGAGCTGC R = TCTCACACTGTAACACAGTGC	4

Note: F - forward primer; R - reverse primer

Source: Ozdemir and Cassandro (2017)

were then viewed on an imaging system to reveal the amplified fragments and their sizes, by comparison with a 100 bp DNA ladder (New England BioLabs). The alleles' fragment sizes were determined after gels had been cropped using GelAnalyzer (v. 2010a) software (Lazer and Lazer, 2010).

Data Analysis

Data generated were subjected to analysis first by using Microsoft Excel microsatellite toolkit (Park, 2001) and GenAlex (Peakall and Smouse, 2012) to generate input files for Microsatellite Analyser (MSA) v4.05 developed by Dieringer and Schlotterer (2003), FSTAT 2.9.3.2 (Goudet, 2001) and Poptree2 (Tamura et al., 2010). GenAlex was used to determine the number of alleles (NA), allele frequencies, observed heterozygosity (H_o), expected heterozygosity (H_E), mean number of alleles (MNA), effective number of alleles (Ne), total heterozygosity (H_T), polymorphism information content (PIC), Nei's genetic distance (GD) and allelic richness (Ar) for the six microsatellite markers across the populations.

Table 2. Genetic parameters measured in the three chicken populations with six microsatellite markers

Locus	Population	N	Na	Ne	ASR(bp)	ROF	H_O	H_E
MCW0016	SB	34	29.000	22.667	161 – 206	0.015 - 0.088	0.294	0.956
	FA	31	21.000	15.754	161 – 191	0.016 - 0.097	0.452	0.937
	NL	33	27.000	20.547	161 – 202	0.015 - 0.091	0.515	0.951
MCW0037	SB	31	23.000	17.473	140 – 170	0.016 - 0.097	0.484	0.943
	FA	28	22.000	15.838	140 – 169	0.018 - 0.125	0.571	0.937
	NL	31	22.000	17.315	140 – 167	0.016 - 0.113	0.419	0.942
MCW0111	SB	33	25.000	18.615	93 – 130	0.015 - 0.091	0.485	0.946
	FA	31	25.000	15.626	87 – 135	0.016 - 0.129	0.419	0.936
	NL	32	25.000	19.321	98 – 129	0.016 - 0.078	0.563	0.948
MCW0216	SB	33	18.000	11.524	138 – 159	0.015 - 0.152	0.242	0.913
	FA	32	21.000	15.754	130 – 159	0.016 - 0.125	0.281	0.937
	NL	33	20.000	15.338	129 – 153	0.015 - 0.136	0.303	0.935
ADL0268	SB	33	24.000	16.754	94 – 125	0.015 - 0.106	0.364	0.940
	FA	32	20.000	15.059	93 – 126	0.016 - 0.109	0.344	0.934
	NL	31	23.000	16.427	92 – 126	0.016 - 0.113	0.355	0.939
LEI0094	SB	28	24.000	18.667	240 – 290	0.018 - 0.107	0.321	0.946
	FA	31	25.000	18.843	243 – 291	0.016 - 0.113	0.323	0.947
	NL	31	22.000	14.029	240 – 278	0.016 - 0.129	0.387	0.929
Mean			23.111	16.975			0.396	0.940

Note: N = Sample size, Na = Number of allele, ASR (bp) = Allele size range (base pair), ROF = Range of frequency, Ne = Effective number of allele ($1/H_o$), H_O = Observed Heterozygosity, H_E = Expected Heterozygosity, SB: Shika Brown, FA: FUNAAB Alpha, NL: Noiler.

MSA v4.05 was used to cross-check the genetic parameters obtained by GenAlex software. The GD values were used in the construction of a dendrogram using the neighbor-joining consensus tree method implemented with 1000 bootstraps using Poptree2 (Tamura et al., 2010). FSTAT 2.9.3.2 (Goudet, 2001) was used to determine Wright's F-statistics [i.e. inbreeding coefficient for the total population (FIT), inter-population genetic differentiation (FST) and within-population inbreeding coefficient (FIS).]

The gene flow/migrant rate was calculated using the formula suggested by Weir and Cockerham (1984).

$$N_m=(0.25(1-F_{ST}))/F_{ST} \quad (1)$$

CP_E across markers and populations were calculated using multiple products of each marker's exclusion probabilities suggested by Rehout et al. (2006) and defined as:

$$CP_E=1-(1-P_{E1})(1-P_{E2})(1-P_{E3})\ldots(1-P_{EK}) \quad (2)$$

where $P_{E1}\ldots P_{EK}$ is exclusion probabilities of k-number of markers used, by excluding one parent according to Jamieson (1994) and Rohrer et al. (2007) it is expressed as:

$$P_{E1}=1-2\sum_{i=1}^{n}p_i^2+\sum_{i=1}^{n}P_i^3+2\sum_{i=1}^{n}P_i^4-3\sum_{i=1}^{n}P_i^5-2\left(\sum_{i=1}^{n}p_i^2\right)^2+3\left(\sum_{i=1}^{n}P_i^2\right)\left(\sum_{i=1}^{n}P_i^3\right) \quad (3)$$

Similarly, when two parents are excluded (Jamieson and Taylor, 1997; Souza et al., 2012), combined exclusion probability (CPF) of across markers and chicken populations become:

$$CP_F=1-(1-P_{F1})(1-P_{F2})(1-P_{F3})\ldots(1-P_{FK}) \quad (4)$$

Where $P_{F1}\ldots P_{FK}$ is the exclusion probabilities of k-number of markers obtained as:

$$P_{F1}=1+4\sum_{i=1}^{n}p_i^4-4\sum_{i=1}^{n}P_i^5-3\sum_{i=1}^{n}P_i^6-8\left(\sum_{i=1}^{n}p_i^2\right)^2+8\left(\sum_{i=1}^{n}P_i^2\right)\left(\sum_{i=1}^{n}P_i^3\right)+2\left(\sum_{i=1}^{n}P_i^3\right)^2 \quad (5)$$

Results

The genetic variability in each population was studied in terms of the mean number of alleles (Na), allelic richness (NAR), Wright's fixation index (FIS), observed heterozygosity (HO), and expected heterozygosity (HE). The highest number of alleles observed was 29.000 in the Shika Brown chicken population at locus MCW0016, followed by the Noiler chicken population with a value of 27.000 at locus MCW0016 and the lowest of 18.000 was also observed in the Shika Brown chicken population at locus MCW0216. In total, 416 alleles were observed from the 6 loci across the three chicken populations, with an average of 23.111. As it is apparent from Table 2, locus MCW0016 had the highest mean number of alleles across populations with a value of 25.667 ± 2.40 and the lowest value of 19.667 ± 0.88 was found in locus MCW0216. The effective number of alleles ranged from 11.524 (MCW0216) to 22.667 (MCW0016) with a mean of 16.975. Allele size range observed across loci and chicken populations together with the Range of frequencies were shown in Table 2. The observed heterozygosity values were generally lower than the expected heterozygosity values in the three chicken populations and six loci considered. The mean value of heterozygosity is 0.396 while the mean value for expected heterozygosity is 0.940. Both observed and expected heterozygosity showed variations in the chicken populations and the six markers. Allele frequencies of each marker across chicken populations (Table 2) were used to compute PIC, which ranged from 0.924 (MCW0216) to 0.945 (MCW0016), and the mean PIC across markers was 0.937 whereas, the PIC per marker ranged from 0.924 (MCW0216) to 0.945 (MCW0016), and the mean PIC across markers was 0.937 (Table 3).

The fixation index of all the markers was above zero, an indication that inbreeding occurs in all three chicken populations, the reason may however be to generate inbred lines.

Table 3. Polymorphism information content (PIC), migrant rate (Nm), allelic richness (Ar), fixation indices (F_{IS}, F_{IT}, F_{ST}), gene differentiation (GST), exclusion probabilities of marker calculated in two ways (excluding one parent) and (excluding two parents) and combined exclusion probabilities (CPF) across markers and 100 samples from the three chicken populations

Locus	PIC	Nm	Ar	F_{IS}	F_{IT}	F_{ST}	G_{ST}	P_E	P_F
MCW0016	0.945	13.130	25.214	0.557	0.565	0.019	0.019	0.723	0.825
MCW0037	0.937	12.982	21.95	0.477	0.487	0.019	0.019	0.685	0.795
MCW0111	0.941	10.610	24.759	0.482	0.494	0.023	0.023	0.698	0.791
MCW0216	0.924	9.350	19.605	0.703	0.711	0.026	0.026	0.625	0.672
ADL0268	0.934	11.137	22.152	0.622	0.631	0.022	0.022	0.671	0.747
LEI0094	0.938	8.037	23.174	0.635	0.646	0.030	0.03	0.692	0.763
Mean	0.937	10.874	22.809	0.579	0.589	0.023	0.023	0.682	0.766
C_{PE} and C_{PF}								0.999	1.000

Table 4. Number of private and shared alleles across loci in the three chicken populations

Locus	No of private alleles	No of shared alleles among populations	No of shared alleles between populations		
			SB x FA	SB x NL	FA x NL
MCW0016	18	11	3	5	1
MCW0037	8	16	2	2	3
MCW0111	11	8	4	10	5
MCW0216	9	10	4	2	4
ADL0268	10	7	5	8	5
LEI0094	23	6	3	8	4
Total	79	58	21	35	22

Note: SB: Shika Brown, FA: FUNAAB Alpha, NL: Noiler

Global fixation indices of the six markers across populations show that FIT > FIS > FST. The inbreeding coefficient within the populations (F_{IS}) across loci ranges from 0.477 (MCW0037) to 0.703 (MCW0216), with a mean of 0.579, while the overall inbreeding coefficient (F_{IT}) is between the range of 0.487 (MCW0037) and 0.711 (MCW0216) overall loci across the populations (Table 3). Exclusion probabilities of marker across populations when one parent is excluded (P_E) ranged from 0.625 (MCW0216) to 0.723 (MCW0016). Similarly, exclusion probability when both parents are excluded (P_F) ranged from 0.672 (MCW0216) to 0.825 (MCW0016). Combined exclusion probabilities across markers and chicken populations were 9.999 x 10^{-1} when one parent was excluded and 10.000 x 10^{-1} for both parents excluded (Table 3). The mean migrant rate, allelic richness, and coefficient of gene differentiation across markers and populations were 10.874, 22.809, and 0.023, respectively.

As it is apparent from Table 4, out of the total alleles detected by markers, 18.99% were rare alleles and 81.01% were fixed alleles (i.e. alleles present in two or more populations). Locus LEI0094 produced the highest number of rare alleles across populations (23), followed by locus MCW0016 (18), while locus MCW0037 had the lowest value of 8 rare alleles. Locus MCW0037 had the highest value (16) of the number of shared alleles across the population while locus LEI0094 had the lowest value (6) of the number of shared alleles across populations. The number of shared alleles between populations was observed to have the highest value between Shika Brown and Noiler chicken population with a value of 35, followed by FUNAAB Alpha and Noiler chicken population with a value of 22 while Shika Brown and FUNAAB Alpha had the lowest value of 21 (Table 4). Table 5 shows the genetic distances between the chicken population which were calculated using Nei's genetic distance (DA).

Table 5. Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Population	Shika Brown	FUNAAB Alpha	Noiler
Shika Brown	****	0.4267	0.4973
FUNAAB Alpha	0.8516	****	0.4149
Noiler	0.6985	0.8796	****

Nei's genetic distance shows a range from 0.6985 (between Shika Brown and Noiler) to 0.8516 (between Shika Brown and FUNAAB Alpha). Fig. 1 shows a dendrogram revealing the relationship among the three chicken populations. The dendrogram is based on Nei's genetic distances in Table 5. It was revealed that Shika Brown and Noiler are closely related but distant to FUNAAB Alpha chicken. However, the dendrogram showed that the Shika Brown and Noiler populations formed a cluster revealing some level of genetic relatedness between these two populations than the FUNAAB Alpha population.

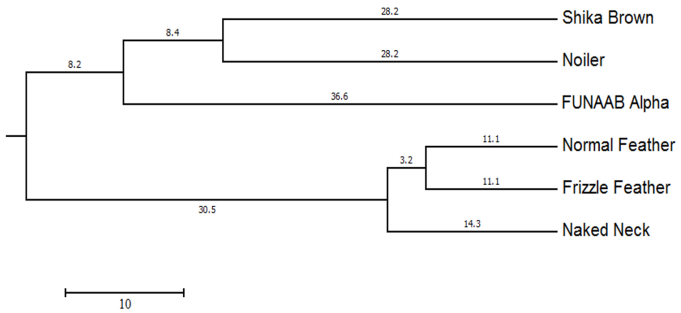

Figure 1. Dendogram constructed based on Nei's genetic distance using a neighbor-joining method showing the genetic relationship among the three improved chicken and our local chicken populations

Discussion

Six (6) microsatellite loci recommended by ISAG and FAO for chicken genetic diversity analysis were used for this study. The high mean number of alleles observed over the six microsatellite loci for the three studied chicken populations is an indication of great allelic diversity which could have been influenced by crossbreeding or admixture among the populations. The number of alleles in this study was similar to what was reported by Hillel et al. (2003) (16 - 23) but differed widely compared to 4 - 14 reported by Ozdemir and Cassandro (2017) in Local Turkish Denizli Chicken and 6 - 11 by Olowofeso et al. (2016) in Nigerian indigenous chicken. The sample size and differences in the chicken populations used might be the reasons for the varying values observed. The number of alleles observed across the loci is greater than the effective number of alleles, which is as expected and also

as reported by Liu et al. (2008). However, the markers used in this study are appropriate, since their polymorphisms are higher than the minimum of 4 alleles required for microsatellite markers for estimation of genetic diversity as suggested by Baker (1994). The author opined that the microsatellite markers investigated should display a minimum of 4 alleles per locus for proficient judgment of genetic differentiation. The mean effective number of alleles (Ne) in this study confirms the diversity of Shika Brown over FUNAAB Alpha and Noiler chicken populations and also confirms the usefulness of locus MCW0016 over other loci used. Moreover, the mean Ne obtained in the current study is quite different from those documented by Olowofeso et al. (2016) in Nigerian indigenous chicken (4.45) and Ohwojakpor et al. (2012) in chicken populations in the South South region of Nigeria (6.16). The discrepancies in the observed values might be attributed to sample size and variation in the studied population.

The mean observed heterozygosity (the percentage of loci heterozygous per individual) for all populations across loci was less than the expected heterozygosity (gene diversity) in this study, and this could be as a result of selection against heterozygosity. This conformed to the earlier reports by Mogesse (2007) on indigenous chicken populations in Ethiopia and Ohwojakpor et al. (2012) in chicken populations in the South South region of Nigeria. The above authors opined that selection against heterozygosity would cause the observed heterozygosity to be lower than the expected heterozygosity. More so, the chicken populations used in this study had been selected over years for a particular production purpose. For instance, FUNAAB Alpha and Noiler chicken populations were selected over years for the dual purpose of both meat and egg productions while the Shika Brown chicken population was selected for the production of eggs. The observed heterozygosity obtained in this study falls within the range (0.399 – 0.562) reported by Ozdemir and Cassandro (2017). Also, the expected heterozygosity was quite similar to what was reported by Ohwojakpor et al. (2012) (0.8072 – 0.8622) and Zhang et al. (2002) (0.63–0.86) in Chinese native chickens but differed from those reported by Hillel et al. (2003) (0.47), Olowofeso et al. (2005) (0.6486 ± 0.06 to 0.7017 ± 0.03) and Wimmers et al. (2000) (0.45–0.67) in African, Asian, and South American local chickens. The variation in the expected heterozygosity may be adduced to differences in location, sample size and population structure (Olowofeso et al., 2005; Kaya and Yildiz, 2008).

The results obtained in this study highlight that all of the studied populations have higher levels of allelic richness than those reported by Maretto et al. (2013) (2.12) in local Italian and Polish chicken breeds and Tadano et al. (2012) in closely related Japanese native Nagoya chicken breeds. Greenbaum et al. (2014) reported that a decrease in the allelic richness could lead to a reduction in the population's potential to adapt to future environmental changes, probably because this diversity index (allelic richness) is the raw material for evolution, especially by natural selection. All the microsatellite markers used in this study are useful for population identification since they all detect at least one rare allele across the chicken populations. However, the high value of rare alleles observed across the chicken population at locus LEI0094 indicates that the locus is reliable for detecting rare alleles inherent in these chicken populations. Hence, the locus will be more useful than other microsatellite loci for population identification in the chicken populations considered in the current study. Rare alleles in this study had an overall frequency of 18.99% which is lower than 20.29% and 33.33% reported by Olowofeso et al. (2016) and Oni et al. (2017) respectively using different microsatellite markers. Even though locus MCW0037 had the lowest value of private alleles, it had the highest number of shared alleles across the three chicken populations. Hence, it suggests that this locus may be useful for detecting shared alleles inherent among the three chicken populations. Also, since there is a high value of shared alleles across the three chicken populations considered in the current study, it then follows that these alleles are important for the survival of these chickens. This implies that the alleles could be responsible for the adaptation of these chicken populations to their environment. Interestingly, Shika Brown and Noiler chicken populations had the highest value of shared alleles. This may be attributed to the fact that the two populations were selected for the same purpose of egg production. Also, they are tropically adapted strains of chicken in Nigeria and therefore might have descended from a common ancestor as they were selected and developed for a common trait. In the same vein, FUNAAB Alpha and Noiler chicken populations had a reasonable number of alleles shared between them, probably because they were selected for common production traits (egg and meat). This may account for similar phenotypic conformations in terms of body structure and plumage color between the two chicken populations. Shika Brown and FUNAAB Alpha had the lowest number of shared alleles between them and this might be adduced to the fact that the alleles they shared might just be responsible for egg production and adaptation to their environment.

The informativeness of microsatellite markers is better determined by calculating the polymorphism information content (PIC) (Suwabe et al., 2004). Locus MCW0016 had the highest mean value of PIC indicating that this locus is the most informative locus among the set of loci used in this study. However, the values of the PIC recorded for this study showed that all microsatellite markers used for the analysis are highly polymorphic and informative for genetic diversity studies. Also, all loci PIC values in this study were greater than the threshold value of 0.5. According to Botstein et al. (1980) and Chatterjee et al. (2008), a marker is said to be highly informative when the PIC value is above 0.50. The overall mean PIC value calculated based on the number and frequency of alleles per marker at a specific locus across populations obtained in this study was higher than values reported by Ohwojakpor et al. (2012) in chicken populations in the South South region of Nigeria (0.8010). The variation observed in the reported study might be a result of variation in the source and type of microsatellite markers used. Loci MCW0016 and MCW0037 had the lowest values of genetic differentiation (G_{ST}) among the loci within and across the chicken populations. This might be because the two loci had a high number of shared alleles across the loci in the studied chicken populations. Locus LEI0094 had the highest value of G_{ST} among the microsatellite markers used in the three chicken populations and this might also be because this locus had the highest value of private alleles. The mean value of G_{ST} observed in this study reveals a low population differentiation between the studied populations. This suggests that each population of chickens considered in this study had been selected over time to develop a new breed to suit a specific production purpose. This result disagreed with earlier reports by Ohwojakpor et al. (2012) (0.0721 ± 0.01) and Oni et al. (2017) (0.1778 ± 0.03).

Population differentiation was examined by Wright's F-Statistics (F_{IS}, F_{IT}, and F_{ST}) which provide important insights into the evolutionary processes that influence the structure of genetic variation within and among populations and they are among the most widely used descriptive statistics in population and evolutionary genetics (Holsinger and Weir, 2009). In the current study, the global F_{ST} over all loci and across the populations indicates a low degree of genetic differentiation among populations and this justifies the values of G_{ST} obtained for the three populations of chickens. The values of F_{ST} obtained in the current study were low and agreed with the findings reported by Ozdemir and Cassandro (2017) in Local Turkish Denizli Chicken (0.03), Berima et al. (2013) in native Sudanese chicken breeds (0.026), Lyimo et al. (2013) in five Tanzanian chicken ecotypes (0.048) and Touko et al. (2015) in Cameroon chickens (0.040). The inbreeding coefficient (F_{IS}) is often used to show the potential reduction in heterozygosity due to non-random mating which serves as an indication of inbreeding within the populations. In this study, the observed mean F_{IS} indicates the existence of inbreeding (heterozygosity deficit) within the three chicken populations which could be partly because the chicken populations in this study have been subjected to selection over time to improve the breed-specific characteristics, such as feather patterns, number of eggs, egg size and body weight. The high F_{IS} results in this study are comparable to those reported by Ozdemir and Cassandro (2017) (0.167) and Kaya and Yıldız (2008) (0.301) in Denizli fowl. In summary, the global fixation indices of the six markers across the three chicken populations show that $F_{IT} > F_{IS} > F_{ST}$.

The gene flow rate (Nm) is the number of migrants per generation. Locus MCW0016 had the highest value of Nm across the three chicken populations, which might be the result of its high number of alleles and the high number of shared alleles among the chicken populations while locus LEI0094 had the lowest value of Nm across the chicken population because it had the highest value of private alleles and the lowest number of shared alleles among the chicken populations. The mean Nm value derived in these populations is above zero, thereby revealing a significant level of gene flow among the chicken populations. This result agreed with the findings of Olowofeso et al. (2016) and Oni et al. (2017), who reported a mean Nm value above zero.

In the current study, Nei's genetic distance was used to ascertain the degree of relationship among the studied chicken populations. The Shika Brown and the Noiler chicken populations had the closest relationship while the farthest relationship was observed between the FUNAAB Alpha and Noiler chicken populations. The close relatedness between the Shika Brown and the Noiler chicken population is an indication of the level of intermixing between both breeds. Also, there might have been introgression along the line in the parent stock of the two breeds or during the selection process in their development. The distant related FUNAAB Alpha population is an indication of possible isolation from other breeds. The consensus neighbor-joining tree constructed from genetic distance data including data from our three local chicken populations depicts a phylogenetic relationship that corroborates the genetic distance information. Two distinct clusters were observed. The first cluster revealed the Shika Brown and Noiler chicken population clustered together enhancing the reliability of their relationship, while the FUNAAB Alpha chicken population diverged from all other populations and cluster close to our local chickens. The tree also revealed that our local chickens are the ancestors of these three improved chicken populations which have been developed over 10 generations of the selection process from the genetic resource of our local chicken (Ilori et al., 2017; Yakubu et al., 2019). The FUNAAB Alpha chicken is, however, closer to the local populations than the other two improved chickens. Combined exclusion probability (CP_E) is the function of markers used and it needs to be considered before employing sets of microsatellite markers for parentage verifications. In this study, both exclusion probability (P_E) and combined exclusion probabilities of markers were calculated using the two-fold method. Locus MCW0016 had the highest value of P_E across all the loci in the two scenarios and this might be adduced to the high number of alleles observed at this locus. Therefore, this locus is more reliable than the other markers used in this study for parentage testing. Conversely, locus MCW0216 had the lowest value of PE across all the loci in the two scenarios and this might be a result of the low number of alleles at this locus compared to other loci. Therefore, this locus is not as reliable as other loci in this study for parentage testing. The mean C_{PE} and C_{PF} in this study fall within the required threshold (≥ 0.9999) for parentage analysis. These values are similar to combined exclusion probabilities obtained by Olowofeso et al. (2016), Rikimaru and Takahashi (2007), and Davila et al. (2009) that used microsatellite markers with chickens. Therefore, microsatellite markers used in this study are effective in parentage testing and pedigree verification beyond a reasonable doubt because they are highly informative.

Conclusion

Until now, a limited number of markers have been used to examine the genetic background of these three chicken populations in Nigeria. Novel information of this study is the level of inbreeding revealed by the microsatellite markers, the proportion of rare alleles (18.99%), and fixed alleles (81.01%) present in the chicken populations. Polymorphism information content of the markers met the minimum threshold value of 0.50, thus confirming that the markers were informative. Combined exclusion probabilities of the markers were within adequate plateau recommended for microsatellite markers to be used for parentage analysis. In this study, microsatellite markers have been used with three chicken populations and the results of the markers have revealed that Shika Brown and Noiler chicken populations are closely related. Also, FUNAAB Alpha was genetically diverse from the other chicken populations. With the results of this study and the limited information in literature, it can be confirmed succinctly that these chicken populations are valuable genetic resources that need to be conserved and microsatellite markers used were not only informative but equally suitable for routine parentage verifications of the chicken populations.

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