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Research Article

Microsatellites Diversity Analysis of Nigerian Chicken Genetic Resources in the Rising Climatic Change

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Abstract

Microsatellite diversity analysis is crucial for Nigerian chicken genetic resources (NCGR) in the rising climatic change which can help to identify genetic diversity within the population. The study described the microsatellites diversity of NCGR. Genomic Deoxyribonucleic Acid (DNA) was isolated from chicken blood samples using Invitrogen DNA extraction kit. Twenty FAO/ISAG microsatellite markers were used in multiplex Polymerase Chain Reaction to amplify 96 Nigerian chickens' genomes. The fluorescent amplicons were analyzed through Capillary Electrophoresis using Hitachi ABI PRISM 3130 X 1 DNA Sequencer at the Laboratorio de Genetica Animal, EMBRAPA Suinos e Aves, Concordia-SC, Brazil. Genetic diversity and calculations of the variations among the NCGR were performed in GenALEx software 6.5. The microsatellites analysis of NCGR revealed selection against heterozygosity in the population with the exception of 7 markers that showed negative inbreeding levels. The mean number of alleles per locus of 4.279 with sizes ranging from 87 to 360 base pairs were detected. A relative average of 50% heterozygosity was obtained in the NCGR population. Mean fixation index (F) over all loci for the chicken strains of 0.134±0.037 indicates free interbreeding. The inbreeding co-efficient of FIS 0.145 across markers and population differentiations deviated from Hardy-Weinberg Equilibrium. The NCGR population subdivision of FST 0.132 over the loci indicated moderate differentiation. Migrant rate of 2.01 was obtained for each marker across the NCGR population. Pairwise FST by AMOVA of 0.175 indicates low to moderate differentiation among the chicken sub-populations while the NW and SB chickens are highly differentiated, FST 0.175. Developing appropriate management strategies will help to protect the microsatellite diverse but less differentiated Nigerian chicken genetic resources (NCGR) population against the rising climatic change and will further support their adaptation and productivity.

Keywords: Nigerian Chicken Genetic Resources; Climate Change; Microsatellite Markers; Within Populations, Inbreeding Reduction

Introduction

The resilience of indigenous livestock is threatened by various factors including extreme climate variability coupled with the indiscriminate cross-breeding [1]. Thus, climate change is causing a number of environmental changes in the world and these changes are having a negative impact on the livestock population [2]. The chicken population diversity and climate change in Nigeria are interconnected issues. Genetic diversity is thus important for the long-term survival of any population [3,4]; a decline in genetic diversity could also predispose the Nigerian chicken genetic resources (NCGRs) to climate change and other stressors. Inbreeding and genetic drift can occur when populations are small and isolated. It is important to note that the climate change impact on NCGRs will vary depending on the specific climate challenges faced by a particular region and adaptive features of the chicken sub-populations. The diversity of NCGRs is an important asset in the face of climate change and chickens with a wider range of microsatellite alleles are more likely able to adapt to the changing environment.

Chicken is one of the poultry species found throughout the country wherever there is human settlement and characterized by a free-range agricultural production system [5] exposing them to a wider range of diseases and environmental challenges. The chicken (*Gallus gallus domesticus*) belongs to the genus *Gallus* of a domesticated fowl and a subspecies of Red junglefowl [6]. They are very important and have been recognised as important genetic resources among all avian species; they are both preferred sources of protein and most numerous domesticated animals [7]. Chicken can be egg-laying, meat-type broilers or dual purpose which is found all over the world in different strains, lines and breeds. Nigeria has the second largest chicken population in Africa after South Africa [8]. Undeniably, there are a wide variety of chicken populations, categorized to indigenous, exotic and locally adapted chicken breeds in Nigeria [9]. The native (indigenous chickens, IC) are the Yoruba [10], Fulani [10] and Nsukka ecotypes [11,12] which often classified based on the phenotype and geographical locations. They are widely distributed in different geographical/ecological zones as classified by vegetation types such as rainforest zones of South-east, South-west and South-south; Savannah zones of North-central, North-east and North-western Nigeria [13]. The exotic counterparts are commercial chicken breeds, classified based on the productivity traits [14] and have the ability to thrive in modified conditions. ISA Brown, Novogen Brown, Ross 309, ISA Black and so on are being imported continuously while the locally adapted Shika Brown® [15;9] and improved indigenous FUNAAB Alpha® dual purpose chickens have their distinct information contained in the National Chicken Registry [9,16,17].

Avian genome is small and domestic chicken (*Gallus gallus*) specifically is estimated to contain one-third of the number of base pairs of that in the human genome [18]. Microsatellite markers are short, repetitive sequences of DNA that are found throughout the genome [4,19] and mostly occur in the non-coding region of the genome. Detecting microsatellite repeats on fluorescently-labelled PCR primers [20] has improved resolution and lends itself to automation. The marker runs in multiplexes for efficient and rapid genotyping. In Nigeria, microsatellite diversity is particularly important for the chicken population because of the challenging environmental conditions. Chicken breeds with a high level of microsatellite diversity are more likely to be productive, healthy and resist diseases and parasites. [21] concluded, native breed chickens are reservoirs of genomes and possess major genes for tropical adaptability and disease resistance.

The Nigerian indigenous chickens found in each of the geographical zones are believed to constitute different genetic populations with limited inter-population gene flow, which could be attributed to long distances separating them [13]. Over time, microsatellite markers have been widely utilized to describe the genetic diversity and population structure of different domestic species. [22] and [23] described microsatellite markers as the efficient tools for exploring genetic diversity and relationships among populations; likewise, effective markers in poultry species [24,25]. Consequently, microsatellite diversity can help to reduce the risk of inbreeding by making it less likely that individuals will mate with close relatives. The microsatellite markers have been extensively utilized in evaluating heterozygosity and genetic relatedness in chickens [26,25] and many microsatellite loci are available in chickens [27]. Microsatellite diversity analysis is thus necessary for Nigerian chickens in the face of rising global change, the study investigated the microsatellites diversity within and among the NCGRs.

Materials and Methods

Chicken blood sampling, storage and DNA extraction

A total of one hundred (100) individual chicken blood samples were sampled across the six agro/geo-political zones of Nigeria representing six sub-populations and Shika Brown (SB-98) chicken. A total of 1ml of blood was collected from brachial vein of each Nigeria indigenous chicken using needles and syringes, preserved in a tube containing Ethylene diamine tetra acetate (EDTA) and

then stored in a cooler containing ice during exploration. Part of the blood samples were also dropped on the sample area of the Whatman FTA classic card. The samples were allowed to dry at room temperature and then stored inside the desiccator with silica gel until further analysis. The EDTA bottles containing the chicken blood samples were stored at -20°C at the Molecular Biology Laboratory of National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State, Nigeria where Deoxyribonucleic Acid (DNA) extraction was done. The genomic DNA was isolated from thawed blood samples using Invitrogen DNA extraction kit following the manufacturer's protocol. The extracted gDNA samples were lyophilized and preserved in a bag for other molecular analyses abroad.

Markers name	Primer sequence (5' - 3') Forward Reverse	Chromosome number	Annealing Temperature (°C)	Allele range base pair (bp)
ADL0112	GGCTTAAGCTGACCCATTAT ATCTCAAATGTAATGCGTGC	10	58	120-134
ADL0268	CTCCACCCCTCTCAGAACTA CAACTTCCCATCTACCTACT	1	60	102-116
ADL0278	CCAGCAGTCTACCTTCCTAT TGTCATCCAAGAACAGTGTG	8	60	114-126
LEI0166	CTCCTGCCCTTAGCTACGCA TATCCCCTGGCTGGGAGTTT	3	60	354-370
LEI0192	TGCCAGAGCTTCAGTCTGT GTCATTACTGTTATGTTTATTGC	6	60	244-370
LEI0234	ATGCATCAGATTGGTATTCAA CGTGGCTGTGAACAAATATG	2	60	216-364
MCW0014	TAGCACAACTCAAGCTGTGAG AGACTTGCACAGCTGTGTACC	6	58	164-182
MCW0016	ATGGCGCAGAAGGCAAAGCGATAT TGGCTTCTGAAGCAGTTGCTATGG	3	60	162-206
MCW0034	TGCACGCACTTACATACTTAGAGA TGTCCTTCCAATTACATTCATGGG	2	60	212-246
MCW0037	ACCGGTGCCATCAATTACCTATTA GAAAGCTCACATGACACTGCGAAA	3	64	154-160
MCW0067	GCACTACTGTGTGCTGCAGTTT GAGATGTAGTTGCCACATTCCGAC	10	60	176-186
MCW0081	GTTGCTGAGAGCCTGGTGCAG CCTGTATGTGGAATTACTTCTC	5	60	112-135
MCW0104	TAGCACAACTCAAGCTGTGAG AGACTTGCACAGCTGTGTACC	13	60	190-234
MCW0111	GCTCCATGTGAAGTGGTTTA ATGTCCACTTGTCAATGATG	1	60	96-120
MCW0183	ATCCCAGTGTCGAGTATCCGA TGAGATTTACTGGAGCCTGCC	7	58	296-326
MCW0206	CTTGACAGTGATGCATTAAATG ACATCTAGAATTGACTGTTCAC	2	60	221-241

MCW0222	GCAGTTACATTGAAATGATTCC TTCTCAAAACACCTAGAAGAC	3	60	220-226
MCW0248	GTTGTTCAAAAGAAGATGCATG TTGCATTAACTGGGCACTTTC	1	60	205-225
MCW0284	GCCTTAGGAAAAACTCCTAAGG CAGAGCTGGATTGGTGTCAAG	4	60	235-243
MCW0295	ATCACTACAGAACACCCTCTC TATGTATGCACGCAGATATCC	4	60	88-106

Table 1: The microsatellites Primers details used to genotype the Nigerian chicken genetic resources.

Source: [28].

Microsatellites primers' details utilized for the study

Twenty recommended ISAG/FAO pairs of primers corresponding to microsatellite regions of chicken autosomes 1, 2, 3, 4, 5, 6, 7, 8, 10 and 13 [28] are shown on table 1 in conjunction with the markers name, forward and reverse sequences, chromosome locations, annealing temperature conditions and the allele sizes.

DNA quantification

The lyophilized chicken gDNA samples were re-solubilized in 50 µL milliQ water placed in a Thermo mixer (dry bath); subsequently quantified for concentration and purity using Nanodrop Spectrophotometre [29] at the Laboratorio de Genetica Animal, EMBRAPA Suinos e Aves, Concordia-SC, Brazil (Figure 1). The ratios OD_{260}/OD_{280} and OD_{260}/OD_{230} were compared to assess purity of the extracted DNA. The DNA concentration was adjusted to 25 ng/µL.

Figure 1: Chicken DNA quantification at the Laboratorio de Genetica Animal, EMBRAPA Suinos e Aves, Concordia-SC, Brazil.

Polymerase Chain Reaction (PCR) conditions and amplification of the chicken DNA using 20 Microsatellite markers

Twenty panels of FAO/ISAG recommended microsatellite markers [28] were used in multiplex PCR (A - D) to amplify the Nigerian chickens' genome which were fluorescently labeled FAM (blue) and HEX (green) respectively. The mix A and C were programmed on TD58 while the mix B and D were executed on TD56 with the PCR conditions of 2 minutes initial denaturation at 95°C, followed by 30 cycles of final denaturation at 95°C for 30 seconds, annealing at 62°C for 30seconds decrease 1° for cycle, 72°C for 1 minute; repeated 5x from step 2, 95°C for 30seconds, 58°C for 30 seconds, 72°C for 1 minute; 27x from step 6 was repeated and a final extension at 4°C. The Polymerase Chain Reaction was performed in a total volume of 10 µL containing 25 ng of genomic DNA, Master Amp, 10x Taq buffer, 0.2 mM of each forward and reverse primer, 0.1 mM Deoxynucleosides triphosphate (dNTP), 0.5 mM of $MgCl_2$, and 5 U/uL Taq polymerase. Thermal cycling was performed by GeneAmp PCR 9700 Thermal Cycler at Laboratorio de Genetica Animal EMBRAPA Suinos e Aves, Concordia-SC, Brazil.

Chicken DNA samples used for PCR reactions and microsatellite genotyping

Each bird was genotyped for 20 nuclear microsatellite loci and for the purpose of a plate genotyping of 96 wells, a total of 96 chicken DNA samples were selected and used for the PCR reactions and microsatellite genotyping. The chicken DNA samples included different strains of NCGR from the agro/geo-political zones of Nigeria and Shika Brown® (SB-98) served as an outgroup for the study (Table 2).

	Sampled chicken populations							
Locations	**SS**	**SW**	**SE**	**NE**	**NC**	**NW**	**SB**	**Total**
	9	33	15	10	11	13	5	**96**

Table 2: Chicken DNA samples used for PCR reactions and genotyping.

Note: SS: South-south, SW: South-west, NW: North-west, NC: North-central, NE: North-east, SE - South-east, SB: Shika Brown®.

Capillary electrophoresis (CE) and genotyping of chicken DNA amplicons

The microsatellite loci were amplified using fluorescently labeled primers; GENESCAN™-500 was used as internal size standard and the fluorescent PCR products were analyzed through the Capillary Electrophoresis (CE) using Hitachi ABI PRISM 3130 X 1 DNA Sequencer [30] following the manufacturer's recommendations to separate the allele by size. The genotyping reaction was prepared as shown on figure 2. The GeneMapper version 4.1 [30] was used to determine the fragment sizes in base pairs and the analysis was performed by GeneScan 3.1.2 and Genotyper 2.5 software [30].

Figure 2: Preparing genotyping reaction outside and under the Laminar flow Hood at the Laboratorio de Genetica Animal, EMBRAPA Suinos e Aves, Concordia-SC, Brazil.

Chicken microsatellite datasets analysis

The Excel in GenALEx software 6.5 [31] was used to estimate the genetic diversity and calculate the variations among the NIC, including the mean number of alleles (N_A), effective number of alleles (N_E), observed (H_O) and expected (H_E) heterozygosity, gene flow (N_M) and statistics (F_{IS} and F_{ST}) across all populations for each locus. The analysis of molecular variance (AMOVA) included variations within and between the chicken strains were also implemented by the program, GenALEx 6.5 [31]. Pairwise F_{ST} (proportion of genetic variability due to population sub-structuring) values and gene flow estimates between sub-population were computed using the GenALEx 6.5 [31]. Nei's standard genetic distances and identity [32] were estimated among the pairs of population using the same program.

Results and Discussion

The heterozygosity information over all 20 microsatellite loci utilized for each Nigerian chicken population

The microsatellite diversity of indigenous and locally adapted chicken breeds of Nigeria is important for better understanding of breeds variability and differentiation against several factors responsible for their genetic dilution and erosion. The number of alleles, N_A, is an indicator of genetic variability necessary for chicken populations' evolution and adaptation to different environmental changes. The N_A average value (4.279) obtained for the NIC populations in the present study (Table 3) was similar to those reported by [22] in the Nigerian chicken breeds (4 - 12 alleles), [33] in native chicken lines from India and Egypt (2 and 11 alleles), [34] in the locally adapted Southern African chickens (4 - 12 alleles) under the Fowl for Africa program and [23] in three Brazilian chickens obtained 4.96 to 5.04 alleles. The productive potential of any animal is relative to its genetic make-up which interacts with the environmental factors to determine the extent to which the potential is realized.

The more diverse genes a species has, the better its chances of resisting diseases, prevailing over other stresses and adapting to changing conditions. The genetic variability measured by the heterozygosity Ho and He for the Nigerian chicken population showed some variations (Ho < He) which indicates there is no equilibrium between Ho and He and this could be pointing to the fact that the studied chicken population were free-range stocks from Nigeria where their variability is not controlled. The birds interbreed freely with innate mate choice selection and free flow of alleles in

Populations	N	Na	Ne	I	Ho	He	uHe	F
SS	5.850	4.450	3.400	1.253	0.625	0.652	0.716	0.055
	0.254	0.380	0.327	0.091	0.065	0.032	0.036	0.085
SW	19.850	5.950	3.190	1.291	0.518	0.632	0.649	0.185
	0.921	0.550	0.305	0.095	0.055	0.033	0.034	0.081
NW	6.900	4.000	2.551	1.033	0.386	0.551	0.602	0.315
	0.497	0.332	0.213	0.088	0.055	0.041	0.046	0.091
NC	7.900	4.150	2.658	1.090	0.510	0.577	0.617	0.125
	0.307	0.365	0.210	0.083	0.065	0.034	0.036	0.104
NE	6.400	4.000	2.913	1.105	0.529	0.584	0.640	0.053
	0.387	0.370	0.246	0.107	0.069	0.050	0.055	0.104
SE	9.250	4.400	2.953	1.107	0.528	0.569	0.603	0.074
	0.428	0.499	0.333	0.117	0.066	0.049	0.051	0.093
SB	3.400	3.000	2.414	0.894	0.433	0.519	0.612	0.137
	0.210	0.262	0.217	0.090	0.070	0.043	0.051	0.117
Grand Mean ±SE	8.507	4.279	2.868	1.111	0.504	0.583	0.634	0.134
	0.454	0.165	0.103	0.037	0.024	0.016	0.017	0.037

Table 3: Mean and SE of Heterozygosity information over all 20 microsatellite loci utilized for each Nigerian chicken sub-populations.

Note: Sample Size N, Number of Alleles Na, Number of Effective Alleles Ne, Information Index I, Observed Heterozygosity Ho, Expected He and Unbiased Expected Heterozygosity uHe, and Fixation, Index F, SS: South-south, SW: South-west, NW: North-west, NC: North-central, NE: North-east, SE: South-east, SB: Shika Brown®, ±SE - Standard error

the population leading to genotypic frequencies occurrence. The average heterozygosity over all loci of 50% observed in the Nigerian chicken populations (Table 3) including the outgroup Shika Brown® (SB) chickens showed the genetic potential and variability of the stocks which must be preserved in the rising climatic conditions. Different variations values may be adduced to differences in the geographical locations, sample sizes and birds. The obtained values are similar to the reports of [22,34- 37]. Species with high heterozygosity are thus required for maintaining diversity and evolutionary process and NCGR possesses such for adaptation and productivity. However, [23] obtained 62 - 65% heterozygosity for three Brazilian chickens in their study; likewise, [38] reported high genetic variability for Brazilian Caipira chickens. [39] reported higher heterozygosity values of 64 - 66% (Zimbabwe), 60.7% (Malawi) and 56.1% (Sudan) chickens respectively. Much lower genetic variability ranging from 28 – 44% for commercial broiler and layer lines was reported by [40].

Average heterozygosity values over all 20 microsatellite loci

The Wright's Fixation index which is increased homozygosity values close to zero are expected under random mating and positive values indicate inbreeding while the negative values indicate excess of heterozygosity due to selection for heterozygotes [31]. The obtained mean fixation index over all loci for the chicken strains (0.134) as shown in Table 4 indicates the NCGR population interbreed freely where the beneficial alleles are fixed and the deleterious alleles are lost due to natural selection, likewise the assortative mating pattern exhibited among them leads to homozygous population, slowly though. This result substantiates the deviation from the Hardy - Weinberg Equilibrium observed in the seven chicken sub-populations studied. The recent study by [41] corroborated this result. The 20 microsatellites analysis of the seven NCGR population revealed selection against heterozygosity/inbreeding occurrence in the populations with the exception of 7 markers that showed negative inbreeding levels. [22] stated when negative fixation index value is obtained in a population by a marker, it is an indication that inbreeding has been minimized.

The negative F_{IS} frequencies obtained for the seven (7) markers indicate relatively better allele/heterozygosity fixation in the loci when compared to others studied and this suggested that these markers can be further utilized in the controlled-inbreeding study. Assortative mating reduces the intraspecific geneflow which results in phenotypic divergence and may end up in a reproductive isolated population [42].

Locus	N	Na	Ne	I	Ho	He	Uhe	F
ADL0112	7.143	3.143	2.359	0.913	0.465	0.533	0.583	0.111
	1.519	0.261	0.291	0.111	0.072	0.061	0.065	0.103
ADL0268	9.571	4.143	2.820	1.165	0.449	0.628	0.672	0.263
	2.057	0.340	0.246	0.080	0.045	0.035	0.041	0.106
ADL0278	9.143	3.571	2.432	0.972	0.252	0.551	0.591	0.471
	1.981	0.571	0.262	0.130	0.024	0.062	0.066	0.112
LEI0166	5.286	3.000	2.407	0.902	0.692	0.530	0.602	-0.308
	1.475	0.378	0.249	0.155	0.134	0.089	0.103	0.121
LEI0192	8.143	3.286	2.410	0.955	0.806	0.579	0.628	-0.410
	1.933	0.474	0.177	0.063	0.097	0.021	0.021	0.186
LEI0234	7.000	6.714	5.205	1.733	0.645	0.797	0.879	0.200
	1.662	0.714	0.483	0.095	0.094	0.020	0.024	0.104
MCW0014	7.143	2.857	2.259	0.861	0.181	0.521	0.573	0.659
	1.519	0.340	0.250	0.116	0.058	0.058	0.065	0.119
MCW0016	11.000	3.143	1.486	0.562	0.317	0.295	0.314	-0.095
	2.837	0.670	0.132	0.132	0.065	0.063	0.066	0.068
MCW0034	8.714	6.429	4.290	1.609	0.552	0.761	0.817	0.280
	1.809	0.685	0.259	0.071	0.64	0.017	0.015	0.075
MCW0037	10.429	4.429	3.482	1.296	0.457	0.679	0.722	0.286
	2.608	0.429	0.409	0.119	0.031	0.050	0.049	0.299
MCW0067	9.143	3.286	2.384	0.953	0.380	0.551	0.589	0.101
	1.981	0.360	0.238	0.102	0.070	0.050	0.050	0.139
MCW0081	10.286	5.143	3.031	1.307	0.786	0.660	0.705	-0.189
	2.589	0.404	0.223	0.064	0.072	0.024	0.025	0.096
MCW0104	8.857	7.286	4.002	1.601	0.649	0.718	0.774	0.073
	2.176	0.944	0.503	0.133	0.044	0.044	0.049	0.085
MCW0111	10.000	5.429	3.430	1.407	0.878	0.701	0.755	-0.256
	2.837	0.429	0.227	0.069	0.061	0.019	0.017	0.092
MCW0183	8.000	6.143	4.413	1.581	0.659	0.745	0.807	0.095
	1.690	0.705	0.517	0.134	0.056	0.043	0.039	0.097
MCW0206	7.000	2.857	1.968	0.757	0.162	0.445	0.505	0.581
	1.988	0.340	0.227	0.126	0.046	0.071	0.083	0.177
MCW0222	7.571	4.571	3.081	1.242	0.522	0.646	0.709	0.140
	1.863	0.612	0.377	0.111	0.095	0.041	0.045	0.149
MCW0248	9.000	3.000	1.597	0.585	0.311	0.309	0.334	-0.020
	2.042	0.617	0.213	0.160	0.091	0.083	0.093	0.082

MCW0284	6.286	2.286	1.842	0.669	0.107	0.434	0.494	0.724
	1.174	0.184	0.155	0.077	0.054	0.438	0.060	0.168
MCW0295	10.429	4.857	2.468	1.142	0.810	0.586	0.631	-0.379
	2.759	0.459	0.151	0.064	0.053	0.023	0.030	0.051
Grand Mean/±SE	8.507	4.279	2.868	1.111	0.504	0.583	0.634	0.134
	0.454	0.165	0.103	0.037	0.024	0.016	0.017	0.037

Table 4: Mean and SE of Heterozygosity information over all 20 microsatellite loci.

Note: Sample Size N, Number of Alleles Na, Number of Effective Alleles Ne, Information Index I, Observed Heterozygosity Ho, Expected He and Unbiased Expected Heterozygosity uHe, and Fixation Index F, Standard error ± SE.

F-Statistics and estimates of gene flow over the Nigerian chicken populations for each microsatellite locus

Wright's fixation indices further give an idea about the population structure in respect of inbreeding coefficient and population differentiation due to non-random mating [43]. Supposedly all the subpopulations are in Hardy-Weinberg Equilibrium with the allele frequencies, the F_{ST} will be zero [31]. As shown on Table 5, the obtained inbreeding co-efficient F_{IS}, (0.145) across markers and population differentiations indicates the existence of genetic variability within breeds, controlled percentage of homozygosity and deviation from Hardy-Weinberg Equilibrium. [41] obtained inbreeding co-efficient F_{IS} 0.12 in 180 chickens sampled from four regions of Nigeria. F_{IS} values of 0.16-0.35 were obtained for Black Australorp and Ovambo chickens [34]. The negative inbreeding levels within individuals relative to the subpopulation (F_{IS}) by the seven microsatellite markers substantiates the fixation index value earlier reported.

The NCGRs population subdivision (F_{ST}) over all loci indicated moderate differentiation occurrence, thereby minimizing the risk of diversity loss. Similar trend of F_{ST} values to this result was reported by [34], 0.11 in the Naked neck and New Hampshire, 0.12 in Ovambo and Naked neck, and 0.14 in Naked neck and Lebowa-Venda chickens. Lower F_{ST} values of 0.039 were obtained by [39] for African chicken lines. The higher the inbreeding coefficient within and among the individual population, the higher the risk of diversity loss leading to genetic drift in a population.

Gene flow is the migration of genes between the populations' homozygous allele frequencies and determines the relative effects of selection and genetic drift [27]. The obtained migrant rate for each marker across the population was more than zero (Nm = 2.01) indicating there is gene migration to negate the effects of genetic drift in the NCGR population. The Nigerian local chickens found in each of the geographical zones are believed to constitute different genetic populations with limited inter-population gene flow which could be attributed to long distances separating them [13]. [44] attributed the existence of genetic homogeneity of NICs resulting from intermixes of germplasms in the country as a result of the free flow of human and animal traffic. [22] obtained a migrant rate of 1.04 across 10 markers in the NCGR population which is however lower to the obtained value in this study. The occurrence of negative inbreeding coefficient in the NCGR populations was a result of gene flow among the populations; usually mediated by reproduction and vertical gene transfer from parents to chicks.

The relationship and structure of NCGR populations: Pairwise population relatedness of Nei's Genetic Distance and Identity in Nigerian chickens

Due to the fact that indigenous chickens are often grouped together and described according to their phenotypic characteristics, geographical locations; the structure of population was analyzed for genetic relationships as shown on Table 6; North-west NW (Fulani ecotype chicken) and outgroup Shika Brown® SB chickens had higher genetic distance and least genetic identity when compared to other five populations of Nigerian chickens studied, the genetic distance of such magnitude was predictable for the outgroup Shika Brown® (SB) because it was selected for different production system, egg production [15] and the North-west (Fulani) chickens are known for confined production system. Indian Ramanagara and Chamrajnagara chickens were most distant (0.22) and highly similar (0.802) among the 15 ecotypes studied by [27]. [45] recorded a high genetic distance and lower genetic identity for commercial White Leghorn and Rhode Island Red chickens when compared with the Ethiopian and South African chickens. [44] found no sig-

Locus	Ht	mHe	mHo	Fis	Fit	Fst	Nm
ADL0112	0.682	0.533	0.465	0.127	0.318	0.219	0.894
ADL0268	0.710	0.628	0.449	0.284	0.367	0.116	1.906
ADL0278	0.630	0.551	0.252	0.542	0.600	0.126	1.728
LEI0166	0.675	0.530	0.692	-0.305	-0.025	0.214	0.916
LEI0192	0.635	0.579	0.806	-0.392	-0.269	0.088	2.586
LEI0234	0.879	0.797	0.645	0.190	0.266	0.093	2.432
MCW0014	0.620	0.521	0.181	0.653	0.708	0.160	1.313
MCW0016	0.315	0.295	0.317	-0.075	-0.006	0.063	3.694
MCW0034	0.849	0.761	0.552	0.275	0.350	0.103	2.170
MCW0037	0.764	0.679	0.457	0.327	0.402	0.111	1.994
MCW0067	0.640	0.551	0.380	0.311	0.407	0.140	1.542
MCW0081	0.730	0.660	0.786	-0.191	-0.077	0.096	2.358
MCW0104	0.799	0.718	0.649	0.095	0.187	0.102	2.203
MCW0111	0.754	0.701	0.878	-0.253	-0.165	0.070	3.304
MCW0183	0.825	0.745	0.659	0.115	0.201	0.097	2.325
MCW0206	0.595	0.445	0.162	0.636	0.728	0.252	0.743
MCW0222	0.749	0.646	0.522	0.193	0.303	0.136	1.582
MCW0248	0.353	0.309	0.311	-0.005	0.120	0.125	1.752
MCW0284	0.600	0.434	0.107	0.753	0.822	0.278	0.650
MCW0295	0.622	0.586	0.810	-0.381	-0.302	0.057	4.099
Mean SE				0.145 0.077	0.247 0.072	0.132 0.014	2.010 0.209

Table 5: F-Statistics and Estimates of gene flow over the Nigerian chicken populations for each microsatellite locus.

Na = No. of different alleles

Ne = No. of Effective Alleles = 1/(Sum pi^2)

I = Shannon's Information Index = -1* Sum (pi * Ln (pi))

Ho = Observed Heterozygosity = No. of Hets/N

He = Expected Heterozygosity = 1 - Sum pi^2

uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He

F = Fixation Index= (He - Ho)/He = 1 - (Ho/He)

Where pi is the frequency of the ith allele for the population and Sum pi^2 is the sum of the squared population allele frequencies.

Fis = (Mean He - Mean Ho)/Mean He

Fit = (Ht – MeanHo)/Ht

Fst = (Ht – MeanHe)/Ht

Nm = [(1/Fst)1]/4

Mean He = Average He across the populations

Mean Ho = Average Ho across the populations

Ht = Total Expected Heterozygosity = 1 – Sum tpi ˆ2 where tpi is the frequency of the ith allele for the total and Sum tpi ˆ2 is the sum of the squared total allele frequencies.

nificant difference in the genetic distance of Southwest, Northwest and Northeast indigenous chickens.

The genetic distance between populations provides a relative estimate of the time elapsed since the sub-divisions existed as a single population and helps in defining the breeds. However, the least distance and genetic similarity of the South-west (SW) and North-central NC could be attributed to the boundaries sharing of the regions where the chickens were sampled from. The South-west and North-central stocks can be said to be transboundary chickens. This result corresponds with that of [41]. [45] recorded a similar trend of least genetic distance (0.073) and highest genetic similarity (0.929) for the Ethiopian D/Elias and Mecha chicken populations. The chickens of Bangalore rural and Mysore were least distant (0.056) and highly similar (0.946) as reported by [27].

	SS	SW	NW	NC	NE	SE	SB
SS	1.000	0.884	0.801	0.786	0.764	0.795	0.576
SW	0.123	1.000	0.898	0.923	0.841	0.862	0.648
NW	0.222	0.108	1.000	0.898	0.753	0.805	0.500
NC	0.241	0.080	0.108	1.000	0.752	0.847	0.613
NE	0.270	0.173	0.284	0.285	1.000	0.746	0.613
SE	0.229	0.148	0.217	0.167	0.293	1.000	0.699
SB (Outgroup)	0.552	0.434	0.693	0.537	0.489	0.359	1.000

Table 6: Pairwise Population Matrix of Nei's Genetic Distance (below diagonal) and Identity (above diagonal).

Note: Nei Genetic Distance = -1 * Ln (Nei Identity). Nei Unbiased Genetic Distance = -1 * Ln (Nei Unbiased Identity), SS: South-south, SW: South-west, NW: North-west, NC: North-central, NE: North-east, SE: South-east, SB: Shika Brown®

Analysis of molecular variance (AMOVA) for seven Nigerian chickens' population

Table 7 and figure 3 showed the analysis of molecular variance (AMOVA) which indicated the proportion of genetic variations due to differences within-population and among-populations [46] and the knowledge is important for conservation management. The AMOVA result indicates moderate genetic variability (46% of total genetic variations) is distributed within-population (individuals) of NCGRs, which indicates genetic variation is lost to natural selection against alleles with lower reproductive fitness in the environment or by genetic drift. The percentage (54% among-populations) variations distributed in the seven chicken sub-populations indicates similarity and since they belong to the same subspecies (*Gallus gallus domesticus*). [41] obtained 5.46% and 96.56% for among-populations and within-population for Nigerian chickens. Also, [34] and [47] obtained larger proportions of within-population variations in South African and Sinai chickens. [48] attributed the huge diversity of genetic variants within and among indigenous chickens to domestication, selection and breeding.

Estimation of Nigerian chicken population structures by pair-wise F_{ST} through AMOVA

Furthermore on the sub-division of chicken strains (F_{ST}) as shown in Table 8, the indigenous chickens from South-west (SW) and North-central (NC) regions of Nigeria are diverse but less differentiated (F_{ST} = 0.025 – 0.032). This result is similar to [39] who reported 0.039 for some African chicken lines. Still, the obtained values for the latter are similar to the guidelines by [49] and values obtained by [34]. On the other hand, the North-west (NW) and outgroup Shika Brown® chickens were highly differentiated from other chicken sub-populations (F_{ST} = 0.175). F_{ST} value indicates low from 0 – 0.05, moderate between 0.05 – 0.15 and high from 0.15 to 0.25 and very high when it is above 0.25 genetic differentiation between populations [50] equated the genetic differentiation in Ghana and Benin indigenous chickens (F_{ST} = 0.162) to Nigeria's North-west (NW) and Shika Brown® chickens (F_{ST} = 0.175) respectively.

Source	Df	SS	MS	Estimated Variance	%
Among populations	6	67.555	11.259	0.012	0
Among individual	90	975.185	10.957	3.864	54
Within individual	96	310.000	3.229	3.229	46
Total	192	1352.740		7.105	100

Table 7: Analysis of molecular variance (AMOVA) for the Nigerian chicken population.

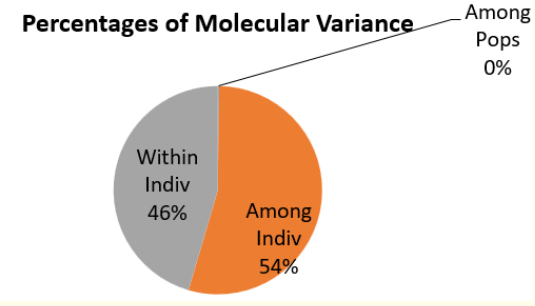

Figure 3: Analysis of molecular variance (AMOVA) for the Nigerian chicken populations.

Conclusions

The NCGR population is microsatellite diverse which is important for their adaptation and productivity in the climatic changing conditions, they are however less differentiated. The 20 microsatellites markers used were sufficient to differentiate within and among populations of seven chicken sub-populations in Nigeria. The North-west (NW) and outgroup Shika Brown® chickens are highly differentiated and can be included in the germplasm exchange program. South-west (SW) and North-central (NC) chickens are genetically similar. The microsatellite diverse NCGR population interbreed freely, exhibiting assortative mating pattern where the beneficial alleles are fixed and the deleterious alleles are lost due to natural selection. The inbreeding coefficient across markers and population differentiations deviated from Hardy-Weinberg Equilibrium and showed possible indication of inbreeding within the NCGR populations. 7 out of 20 recommended FAO/ISAG microsatellite markers been LEI0166, LEI0192, MCW0016, MCW0081, MCW0111, MCW0248 and MCW0295 revealed that inbreeding has been minimized in the NCGR population.

Recommendations

Microsatellite markers including the LEI0166, LEI0192, MCW0016, MCW0081, MCW0111, MCW0248 and MCW0295 can further be used to investigate the level of inbreeding reduction in the Nigerian chicken populations leading to information that can be used to develop management strategies that can help to maintain or increase genetic diversity and subsequently will help to protect the Nigerian chicken genetic resources against the rising climate change.

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