Combined Exclusion Probabilities of Ten Microsatellite Markers used with Nigerian Chicken Populations

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Abstract

Ten microsatellite markers were used with 97 genomic DNA isolated from three Nigerian chicken populations, naked neck (NN = 24), frizzle feathered (FF = 32) and normal feathered (NF = 41) to determine level of inbreeding in the chicken populations, genetic divergence among populations (GD), polymorphism information content (PIC) and combined exclusion probabilities of markers (CP_E/CP_F). Polymerase chain reactions (PCRs) were carried out using each marker, chicken DNA, double distilled water and PCR Master Mix. Products generated were subjected to 12% polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. Bands on gels were scored based on size of ladder with GeneScan 3.1.2. Bands were designated as alleles and prepared into Excel. Allele frequencies were generated with Microsatellite Analyser v4.05. Inbreeding coefficient/population ranged from -0.26 to 1.00 and GD was between 1600 (NF and FF) to 2100 years (FF and NN). Sixty nine alleles were observed across populations which contained 20.29% rare and 79.71% fixed alleles. PIC/marker across populations ranged from 0.50 (MCW0078) to 0.82 (LEI0192), meaning that markers used were informative (PIC ≥ 0.50). CP_E across markers and populations was 9.99998 x 10^{-1} (exclude a parent) and CP_F was 9.99999 x 10^{-1} (exclude parents) suggesting the suitability of the markers for parentage verification in Nigerian chicken populations.

Keywords: chicken populations; genetic divergence; resolving power; routine; threshold value

1.0 Introduction

The roles of microsatellite markers in molecular studies are manifold. They are the most accurate and efficient tools for exploring genetic diversity and relationships among populations (Davila *et al.,* 2009) and genetic fitness of whole populations including paternity determination (Witzenberger and Hochkirch, 2011). Since the advent of the PCR technology, it has been very easy to amplify DNA isolated either from plants or animals. Microsatellite has short repeat units and is a good example of nuclear DNA markers (Hillel *et al.,* 2003, 2007; Olowofeso, 2005; Davila *et al.,* 2009; Rosenbom *et al.,* 2014) that has found wide application in molecular studies. The high variability and distribution gives microsatellite markers more advantages than other markers (Hillel *et al.,* 2003; FAO, 2011; Yilmaz *et al.,* 2015). Extent of diversity in livestock populations across the globe has been gained through use of microsatellite markers (Berthouly *et al.,* 2008; Granevitze *et al.,* 2009). Microsatellite markers when used with animal genetic resources reveals with ease the useful information that are essential for the formulation of long term breeding plans, designing of breeding programmes, planning conservation strategies, traceability and in parentage verifications. In recent years, selection of microsatellite markers for use with genetic resources has been based on single criterion called polymorphism information content. In determining this parameter, researchers have been using the formula suggested by Botstein *et al.* (1980) and when PIC value of a marker equals the threshold value of 0.50 or above, such marker is said to be informative.

However, selection of microsatellite markers for use must not be based on single criterion. Apart from PIC, there might be the need to consider gene diversity indices, exclusion probabilities of most of the microsatellite markers in the public domain and their combined exclusion probabilities. Exclusion probability is the estimation of power of microsatellites to genetically exclude individuals as parents and it is a function of number of microsatellite markers used in any study. It has been reported that high exclusion power of microsatellite markers makes them more valuable for parentage analysis (Zajc and Sampson, 1999; Rehout *et al.,* 2006; Davila *et al.,* 2009). Combined exclusion probabilities for microsatellite markers when estimated and exceeds 0.9995 indicate that such microsatellite markers are quite informative and suitable for parentage analysis according to Rehout *et al.* (2006), Davilla *et al.* (2009) and Souza *et al.* (2012). Exclusion probabilities are calculated using different approaches, but the general idea is to describe the power of microsatellites to genetically exclude individuals as parents (Dakin and Avise, 2004). Suitable equations for the estimation of exclusion probabilities and combined exclusion probabilities of microsatellite markers have been suggested by Jamieson and Taylor (1997).

In Nigeria, different chicken breeds have been developed through purebreeding and crossbreeding and because of ease of trade between multiplier herds/flocks and end users, possibility of ascertaining the exact parents contributing genes to certain individual have been very difficult. Tracing the main gene source of some genotypes so as know which deserve proper selection, conservation and improvement has been a source of worry to poultry breeders. Added to this difficulty is the indiscriminate mating methods being adopted by individuals as a result of recent interest in poultry production enterprise in Nigeria. However, these challenges may be minimised with the availability of microsatellite markers; as these markers are potential tools that can be used to properly assign individuals to the appropriate population as well as tracing their gene sources (Olowofeso, 2011). With these markers, it is very possible to know the gene contribution of a sire and dam to an offspring, or the possibility of excluding either sire or dam or both parents of an individual.

Considering the large number of microsatellite markers that are available, it is therefore suffice to ascertain their efficacies before validating them for multiple uses. Though, use of microsatellite markers with some animal genetic resources have been reported (La Manna *et al.,* 2015; Levy *et al.,* 2015 and Yilmaz *et al.,* 2015), but utilization of microsatellite markers with some Nigerian chicken populations for

routine parentage analysis with the key consideration of calculating exclusion probabilities and combined exclusion probabilities in naked neck, frizzle and normal feathered chickens in Nigeria have not been reported which is the crux of this investigation. In fact, Ohwojakpor *et al.* (2012) reported that these chicken populations were important genetic resources and that concerted efforts be made to guide against genetic erosion of these valuable resources and that genetic information about the chicken populations must be obtained using molecular approach to get vital information to complement the phenotypic attributes of these chicken populations long reported by Nwosu *et al.* (1985) and Adebambo *et al.* (1999), respectively. The objectives of this study were to probe further the applicability of more microsatellite markers with Nigerian chicken populations to know the level of inbreeding in the populations, genetic divergence among populations and to determine PIC, exclusion probabilities and combined exclusion probabilities of the ten microsatellite markers used with the chicken populations.

2. 0 Materials and Methods

2.1. Experimental birds, blood collection and DNA extraction

Three Nigerian chicken populations, namely naked neck, frizzle and normal feathered chickens were sampled for this study. Blood samples from which DNA were extracted were collected from unrelated birds in seven different states. Five of the states (Akwa Ibom, Bayelsa, Delta, Edo and Rivers States) were in the South-South region of Nigeria and two states (Ogun and Ondo) are parts of the South-West region of the country. These seven states were considered because of the availability of these chicken populations. The veins in the wings of the birds were searched for and by puncturing of the brachial vein with 13 mm, 27 gauge syringe; 1 ml of blood was collected from individual bird into 1.5 ml haematocrit tube containing ethylene diamine tetra acetic acid as anticoagulant. Samples collected from the states and transferred to the Biotechnology Laboratory, Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria where further analyses were carried out comprised (NN = 24, FF = 32 and NF = 41). Blood samples were stabilised in Genomic Lysis Buffer (GLB) with addition of four volumes of GLB to 1 volume of whole blood for a day before DNA extraction. Genomic DNA was extracted using Zymobead^{TM} Genomic DNA extraction kit. This method was employed because it is an easy purification method of high quality DNA from whole blood within a very short time using innovate Zymobead^{TM} silica-bead method. The supernatant obtained using this procedure contained purified DNA which were stored at -20^{0}C. Detailed description of this rapid procedure for DNA extraction is available at www.zymoresearch.com.

2.2. Microsatellite markers used, PCR programme and reaction profile

Ten microsatellite markers (Table 1) out of those recommended for biodiversity studies in chickens and according to International Society for Animal Genetics/Food and Agriculture Organisation of the United Nations-Measurement of Domestic Animal Diversity Project (FAO, 2004) were used. Reasons for selecting the markers were four-fold: availability in the public domain, high polymorphisms, ability to amplify DNA from chicken populations (Hillel *et al.,* 2003) and based on the fact that they have not been used with Nigerian chicken populations. PCR programme carried out in TC 4000 series thermal cycler was at 94^{0}C, 300 s of initial denaturation, followed by 35 cycles of denaturation at 94^{0}C, 60 s, annealing temperatures of the primers ranged from 57^{0}C to 60^{0}C (Table 1) and initial extension at 72^{0}C, 60 s, followed by final extension at 72^{0}C, 600 s. The microsatellite markers, DNA, double distilled water and other PCR reaction mixture were amplified in a total volume of 25 µl, which contained 1 µl DNA (approximate concentration of 50 ng/ml), forward and reverse primer (2 µl), double distilled water (16.10 µl) and PCR Master Mix (5.90 µl) which included (*Taq* DNA polymerase, dNTP, 10 x PCR buffer and cation (Mg^{2+}). PCR products were

electrophoresed on an ABI 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Allele lengths (fragment sizes) were determined with reference to the ladder (PCRRanger 100 bp DNA Ladder (50 bp to1000 bp) (NORGEN) using GeneScan 3.1.2 software.

Table 1. Sequence of microsatellite markers and annealing temperature used in this study*

Marker	Forward form	Reverse form	Annealing temperature (^{0}C)
LEI0192	TGCCAGAGCTTCAGTCTGT	GTCATTACTGTTATGTTTATTGC	59
MCW0016	ATGGCGCAGAAGGCAAAGCGATAT	TGGCTTCTGAAGCAGTTGCTATGG	60
MCW0069	GCACTCGAGAAAACTTCCTGCG	ATTGCTTCAGCAAGCATGGGAGGA	58
MCW0078	CCACACGGAGAGGAGAAGGTCT	TAGCATATGAGTGTACTGAGCTTC	58
MCW0098	GGCTGCTTTGTGCTCTTCTCG	CGATGGTCGTAATTCTCACGT	60
MCW0103	AACTGCGTTGAGAGTGAATGC	TTTCCTAACTGGATGCTTCTG	60
MCW0111	GCTCCATGTGAAGTGGTTTA	ATGTCCACTTGTCAATGATG	60
MCW0216	GGGTTTTACAGGATGGGACG	AGTTTCACTCCCAGGGCTCG	58
MCW0222	GCAGTTACATTGAAATGATTCC	TTCTCAAAACACCTAGAAGAC	60
MCW0248	GTTGTTCAAAAGAAGATGCATG	TTGCATTAACTGGGCACTTTC	57

*Allele size range (in basepair) of the marker in each and across chicken populations are shown in Table 3.

2.3. Data analysis

For each chicken population per marker, number of alleles (N_A), allele frequencies, observed (H_O) and expected heterozygosities (H_E), rare alleles or alleles present in one population but completely absent in the other populations (R_A) and inbreeding coefficient/population (F_{IS}/pop) were obtained using the program package, Microsatellite Analyser v4.05 developed by Dieringer and Schlotterer (2003). Chi square test was conducted to test if differences in allele numbers/marker among chicken populations were significant. Number of alleles across populations, global fixation indices (i.e. individual within subpopulation F_{IS}, individual within total population F_{IT}, subpopulation within total population F_{ST}), gene differentiation (G_{ST}) for each microsatellite marker using the total allele frequencies observed across populations and Nei's standard genetic distances (D_s) among populations were generated with the same software package. Effective number of alleles (EN_A), gene flow (N_m) and gene diversity (G_D) which equals total heterozygosity (H_T) for each marker across chicken populations were calculated using relations:

$$EN_A = \frac{1}{\sum_{i=1}^{n} P_i^2} \quad \text{.....(1)}$$

$$N_m = \frac{0.25(1 - F_{ST})}{F_{ST}} \quad \text{.....(2)}$$

$$H_T = 1 - \sum_{i=1}^{n} P_i^2 \quad \text{.....(3)}$$

PIC for each marker was obtained using Botstein *et al.* (1980) equation depicted as:

$$PIC = H_T - 2(\sum_{i=1}^{n} \sum_{j=i+1}^{n} P_i^2 P_j^2) \quad \text{.....(4)}$$

where P_i is allele frequencies produced by each marker/population, P_j is the other allele frequencies when the first is assumed to be P_i, and H_T is total heterozygosity. With exception of genetic divergence, mean of other parameters aforementioned were calculated for each marker and across chicken populations. Genetic divergence (in years) was calculated as product of genetic distances among populations and generation interval for chickens over two times microsatellite mutation rate. Combined exclusion probability (CP_E) across markers and populations was calculated using multiple products of each marker exclusion probabilities defined as:

$$CP_E = 1-(1-P_{EI})(1-P_{E2})(1-P_{E3})----(1-P_{EK}) \quad \text{.....(5)}$$

by Rehout *et al.* (2006), where $P_{E1}\ldots P_{EK}$ is exclusion probabilities of k-number of markers used, expressed as:

$$P_{EI} = 1-2\sum_{i=1}^{n} P_i^2 + \sum_{i=1}^{n} P_i^3 + 2\sum_{i=1}^{n} P_i^4 - 3\sum_{i=1}^{n} P_i^5 - 2(\sum_{i=1}^{n} P_i^2)^2 + 3(\sum_{i=1}^{n} P_i^2)(\sum_{i=1}^{n} P_i^3) \quad \text{.....(6)}$$

by excluding one parent according to Jamieson (1994) and Rohrer *et al.* (2007). Similarly, when two parents are excluded (Jamieson and Taylor, 1997; Souza *et al.,* 2012), combined exclusion probability (CP_F) across markers and chicken populations becomes:

$$CP_F = 1-(1-P_{F1})(1-P_{F2})(1-P_{F3})------(1-P_{FK}) \quad \text{.....(7)}$$

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

$$P_{FI} = 1+4\sum_{i=1}^{n} P_i^4 - 4\sum_{i=1}^{n} P_i^5 - 3\sum_{i=1}^{n} P_i^6 - 8(\sum_{i=1}^{n} P_i^2)^2 + 8(\sum_{i=1}^{n} P_i^2)(\sum_{i=1}^{n} P_i^3) + 2(\sum_{i=1}^{n} P_i^3)^2 \quad \text{.....(8)}$$

3.0 Results and Discussion

In frizzle feathered, naked neck and normal feathered chicken populations, the markers detected 49, 50 and 59 alleles. To ascertain if there are significant differences in the number of alleles produced by markers in each chicken population, number of alleles was subjected to chi square analysis, our result was $\chi_c^2 = 5.15$, which was lower than $\chi^2_{(0.05,18)} = 28.90$ (See Table 2). Based on this result, our hypothesis was accepted and concluded that there were no significant differences in number of alleles detected by the markers in three chicken populations. Summary of these results are presented in Table 2.

With exception of MCW0098 in which observed heterozygosity was greater than expected heterozygosity, it was vice versa in the remaining nine microsatellite markers used. Both observed and expected heterozygosities showed variations in the chicken populations and in the ten markers. Inbreeding coefficient in the chicken populations ranged from less inbreeding level (-0.26) seen in both normal and naked neck chicken populations detected by MCW0098 to high inbreeding level (1.00) in naked neck revealed by MCW0078 (Table 3). MCW0098, unlike other markers produced negative inbreeding values within and across chicken populations, but fixation index (F_{IS}) of other markers were above zero, an indication that inbreeding occur in the three Nigerian chicken populations, reason may however being to generate inbred lines. Allelic frequencies distribution observed in each chicken population and among populations together with number of rare alleles identified by each microsatellite marker were summarised in Table 3.

Table 4 shows results of the ten microsatellite markers among chicken populations. A total of 69 alleles were detected by the markers among populations with mean number of alleles across markers and populations equals 6.90. Out of the total alleles detected by markers, 14 (20.29%) were rare alleles and 55 (79.71%) were fixed alleles (i.e. alleles present in two or more populations). LEI0192 and MCW0248 produced four rare alleles each (highest across populations), followed by 3 produced by MCW0222, while MCW0016, MCW0069 and MCW0103 produced 1 rare allele each. Total number of rare allele produced by the markers were 2, 5 and 7 in frizzle feathered, naked neck and normal feathered chicken populations (Table 3). Across populations/marker, number of alleles ranged from 4 (MCW0078) to 11 (MCW0222), effective number of alleles oscillate between 2.33 (MCW0098) and 7.14 (LEI0192), gene diversity range limits were 0.57 (MCW0098) to 0.86 (LEI0192). Migrant rate and coefficient of gene differentiation across markers and populations were 1.04 and 0.08, respectively. Global fixation indices of nine markers across populations shows that $F_{IT} > F_{IS} > F_{ST}$, except for MCW0098 where $F_{ST} > F_{IT} > F_{IS}$ across chicken populations.

Allele frequencies of each marker across chicken populations (Table 3) were used to compute PIC, which ranged from 0.50 (MCW0078) to 0.82 (LEI0192) and mean PIC across markers was 0.70. Exclusion probabilities of marker across populations (P_{EI}) ranged from 0.66 (MCW0078) to 0.83 (MCW0098). Similarly, P_{FI} ranged from 0.40 (MCW0078) to 0.89 (LEI0192). Combined exclusion probabilities across markers and chicken populations were 9.99998×10^{-1} when one parent was excluded and 9.99999×10^{-1} for both parents excluded. Genetic divergence between normal and frizzle feathered chickens occurred 1600 years ago, normal feathered and naked neck genetically diverged 1700 years ago and divergence between frizzle feathered and naked neck occurred 2100 years ago (Table 5).

The ten microsatellite markers used with three Nigerian chicken populations showed that inbreeding occurred in the populations. Nine of the markers used were able to detect this with the exception of one marker that revealed negative inbreeding level. Negative fixation index when obtained in a population by a marker is an indication that inbreeding has been minimised. Using microsatellite markers, Manatrinon *et al*. (2008) has reported negative F_{IS} values, this therefore mean that negative fixation index is a common occurrence when microsatellite markers are used with animal genetic resources as seen by MCW0098 in this study. Mean number of alleles/marker, effective number of alleles, gene diversity, migrant rate, coefficient of gene differentiation, PIC observed in three Nigerian chicken populations by the markers used were similar to those reported in literature (Olowofeso, 2005; Berthouly *et al.,* 2008; Davila *et al.,* 2009; Ohwojakpor *et al.,* 2012 and Mwacharo *et al.,* 2013). Mean number of alleles across markers in this study was 6.9, lower than 12-26 and 9.6 reported by Zhang *et al.* (2002) and Hillel *et al.* (2003), respectively. However, our value was within 2-21 alleles observed by Romanov and Weigend (2001). Gene diversity range limits across markers in this study (0.57-0.86) were similar to 0.63-0.86 reported by Zhang *et al.* (2002), but higher than 0.47 obtained by Hillel *et al.* (2003). Considering number of alleles/marker, high expected heterozygosity and inbreeding level within chicken population, it means that the markers were useful in revealing genetic information abound in these Nigerian chicken populations. The high values of these parameters observed in this study were consistent with values reported by Rikimaru and Takahashi (2007); Berthouly *et al.* (2008) and Davila *et al.* (2009). Gene flow occurred among chicken populations because the calculated migrant rate for each marker across populations was more than zero. The occurrence of negative inbreeding coefficient seen in the chicken population(s) is as a result of gene flow among populations. Mean gene diversity or total heterozygosity as high as 0.74 observed may be due to inbreeding level in the population and gene flow among populations. High value of fixation indices (F_{IT} and F_{IS}) observed indicates that inbreeding occurs within these Nigerian chicken populations.

Genetic divergence is an extension of genetic distances among populations and it is a reflection of the genetic distances among populations. Genetic divergence among populations in this study ranged from 1600 to 2100 years and genetic distances among chicken populations observed further supports the genetic distances of these chicken populations reported by Ohwojakpor *et al.* (2012). In a chicken biodiversity study reported by Hillel *et al.* (2003), proportion of rare alleles was greater than 10% and this study also detected 20.29% rare alleles in Nigerian chicken populations, thus confirmed the suitability of microsatellite markers in detecting rare alleles in chicken populations. Normal feathered chicken has the highest number of rare alleles and can be selected for increased production ahead of the other two chicken populations examined. The ten microsatellite markers used produced PIC ≥ 0.50, meaning that the markers were informative (Bostein *et al.,* 1980).

Combined exclusion probability is a function of markers used and need to be considered before employing sets of microsatellite markers for parentage verifications. In this study, both exclusion probability and combined exclusion probabilities of markers were calculated using two-fold method. In the first method, mean exclusion probability for markers was 0.73 and with second approach, it was 0.71 across markers. The ten markers revealed combined exclusion probabilities of 9.99998×10^{-1} and 9.99999×10^{-1} across chicken populations when one parent and both parents were excluded, respectively. These values are similar to combined exclusion probabilities obtained by Rikimaru and Takahashi (2007), and Davila *et al.* (2009) that used microsatellite markers with chickens. Our results were also similar to values reported for same parameter by Primmer *et al.* (1995); Bowling *et al.* (1997); Zajc and Sampson (1999); Rohrer *et al.* (2007) and Souza *et al.* (2012) when microsatellite markers were used with some animal genetic resources. The power of microsatellite markers increased when more markers were used. The ten markers were used in our computation because of the non-existence of null alleles which might complicate results.

Table 2. Chi square analysis for number of alleles in Nigerian chicken populations/marker*

Marker	Chicken population			Total	Chi square calculated (χ^2_c)	Chi square at 95% confidence level) ($\chi^2_t = \chi^2_{(0.05,18)}$)
	Normal feathered n = 41	Frizzle feathered n = 32	Naked neck n = 24		5.15	28.90
LEI0192	7 (6.35)	4 (5.27)	6 (5.38)	17		
MCW0016	7 (5.23)	4 (4.34)	3(4.43)	14		
MCW0069	6(4.86)	4 (4.03)	3 (4.11)	13		
MCW0078	4 (3.73)	4 (3.10)	2 (3.17)	10		
MCW0098	5 (5.23)	5 (4.34)	4 (4.43)	14		
MCW0103	4 (5.60)	5 (4.65)	6 (4.75)	15		
MCW0111	6(6.35)	5 (5.27)	6 (5.38)	17		
MCW0216	6.(6.35)	6(5.27)	5 (5.38)	17		
MCW0222	9 (9.34)	8 (7.75)	8 (7.91)	25		
MCW0248	5 (5.98)	4 (4.96)	7 (5.06)	16		
Total	59	49	50	158		

*Numbers in parentheses represent calculated expected number of alleles and 'n' = Sample size/chicken population.

Table 3. Observed and expected heterozygosities, allele size range, rare alleles, inbreeding coefficient, alleles and their frequencies/marker in each and across chicken populations*

Marker	Pop. Name	H_O	H_E	Allele size range (bp)/Pop.	R_A	Fis /pop.	Allele observed (bp) and their frequencies/marker in each and across chicken populations										
							1	2	3	4	5	6	7	8	9	10	11
LEI 0192							236	238	240	242	244	246	248	250	252	254	
	NF	0.15	0.83	236-248	3	0.82	0.11	0.27	0.11	0.03	0.16	0.17	0.15	0.00	0.00	0.00	
	FF	0.03	0.63	238-254	1	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.08	0.31	0.53	
	NN	0.13	0.78	238-254	0	0.84	0.00	0.22	0.00	0.00	0.26	0.07	0.04	0.00	0.33	0.09	
	Across			236-254	4		0.05	0.20	0.05	0.01	0.13	0.09	0.07	0.03	0.18	0.20	
MCW 0016							132	134	136	156	158	180	182				
	NF	0.10	0.78	132-182	1	0.88	0.07	0.30	0.18	0.29	0.14	0.01	0.01				
	FF	0.34	0.63	132-182	0	0.00	0.08	0.05	0.00	0.00	0.00	0.44	0.43				
	NN	0.04	0.66	134-156	0	0.94	0.00	0.44	0.33	0.22	0.00	0.00	0.00				
	Across			132-182	1		0.06	0.25	0.16	0.18	0.06	0.15	0.15				
MCW 0069							263	265	267	269	271	273					
	NF	0.29	0.70	263-273	1	0.58	0.12	0.05	0.17	0.49	0.15	0.02					
	FF	0.06	0.75	263-269	0	0.00	0.30	0.13	0.29	0.29	0.00	0.00					
	NN	0.21	0.43	267-271	0	0.51	0.00	0.00	0.21	0.73	0.06	0.00					
	Across			263-273	1		0.15	0.06	0.22	0.48	0.08	0.01					
MCW 0078							133	135	137	139							
	NF	0.07	0.59	133-139	0	0.88	0.01	0.48	0.44	0.08							
	FF	0.16	0.64	133-139	0	0.00	0.02	0.51	0.29	0.19							
	NN	0.00	0.50	135-137	0	1.00	0.00	0.44	0.57	0.00							
	Across			133-139	0		0.01	0.48	0.42	0.10							
MCW 0098							251	253	255	257	259						
	NF	0.78	0.63	251-259	0	-0.26	0.17	0.57	0.07	0.06	0.12						
	FF	0.72	0.62	251-259	0	0.00	0.11	0.59	0.09	0.06	0.14						
	NN	0.50	0.40	251-257	0	-0.26	0.21	0.75	0.02	0.02	0.00						
	Across			251-259	0		0.16	0.62	0.07	0.05	0.10						
MCW 0103							260	262	264	266	268	270					
	NF	0.24	0.63	262-268	0	0.61	0.00	0.15	0.16	0.56	0.13	0.00					
	FF	0.19	0.55	260-268	0	0.00	0.03	0.06	0.11	0.66	0.14	0.00					
	NN	0.83	0.75	260-270	1	-0.12	0.02	0.08	0.11	0.42	0.21	0.17					
	Across			260-270	1		0.02	0.10	0.13	0.56	0.16	0.04					
MCW 0111							98	100	102	104	106	110					
	NF	0.59	0.82	98-110	0	0.29	0.24	0.22	0.21	0.11	0.12	0.10					
	FF	0.66	0.78	100-110	0	0.00	0.00	0.25	0.36	0.13	0.16	0.11					
	NN	0.21	0.75	98-110	0	0.72	0.04	0.17	0.13	0.13	0.11	0.44					
	Across			98-110	0		0.11	0.22	0.24	0.12	0.13	0.19					

MCW 0216							121	123	125	127	129	131					
	NF	0.68	0.82	121-131	0	0.16	0.20	0.15	0.15	0.13	0.29	0.09					
	FF	0.69	0.79	121-131	0	0.00	0.14	0.33	0.30	0.11	0.09	0.03					
	NN	0.42	0.70	121-131	0	0.40	0.15	0.08	0.13	0.15	0.00	0.50					
	Across			121-131	0		0.17	0.19	0.19	0.13	0.15	0.17					
MCW 0222							216	218	220	222	236	238	240	242	244	246	248
	NF	0.08	0.83	216-248	1	0.91	0.03	0.03	0.00	0.01	0.11	0.25	0.00	0.05	0.25	0.13	0.15
	FF	0.53	0.77	218-248	1	0.00	0.00	0.03	0.00	0.05	0.00	0.35	0.02	0.05	0.33	0.11	0.06
	NN	0.79	0.84	216-248	1	0.04	0.04	0.00	0.09	0.09	0.00	0.26	0.00	0.04	0.28	0.11	0.11
	Across			216-248	3		0.02	0.02	0.02	0.04	0.05	0.28	0.01	0.05	0.28	0.12	0.11
MCW 0248							199	201	207	209	211	213	215	217			
	NF	0.73	0.75	199-211	1	0.01	0.38	0.25	0.14	0.05	0.19	0.00	0.00	0.00			
	FF	0.67	0.74	199-211	0	0.00	0.38	0.27	0.14	0.00	0.22	0.00	0.00	0.00			
	NN	0.71	0.76	199-217	3	0.06	0.40	0.25	0.08	0.00	0.02	0.15	0.02	0.08			
	Across			199-217	4		0.38	0.25	0.12	0.02	0.16	0.04	0.01	0.02			

*Naked neck (NN = 24), frizzle feathered (FF = 32), normal feathered (NF = 41), R_A = rare alleles, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS}/pop. = inbreeding coefficient per population.

Table 4. Number of alleles, effective number of alleles, gene diversity, migrant rate, fixation indices, gene differentiation, polymorphism information content, exclusion probabilities of marker calculated in two ways and combined exclusion probabilities across markers and 97 samples from three Nigerian chicken populations

Marker	N_A	EN_A	G_D	N_m	F_{IS}	F_{IT}	F_{ST}	H_S	H_T	G_{ST}	PIC	P_{EI}	P_{FI}
LEI0192	10	7.14	0.86	1.22	0.86	0.89	0.17	0.73	0.86	0.15	0.82	0.78	0.89
MCW0016	7	5.88	0.83	0.89	0.77	0.82	0.22	0.68	0.83	0.18	0.78	0.72	0.84
MCW0069	6	3.23	0.69	2.88	0.70	0.73	0.08	0.62	0.69	0.10	0.65	0.73	0.68
MCW0078	4	2.44	0.59	12.25	0.85	0.86	0.02	0.57	0.59	0.03	0.50	0.66	0.40
MCW0098	5	2.33	0.57	12.25	-0.22	-0.20	0.02	0.54	0.57	0.05	0.54	0.83	0.52
MCW0103	6	2.78	0.64	12.25	0.42	0.43	0.02	0.63	0.64	0.02	0.60	0.75	0.57
MCW0111	6	5.56	0.82	3.92	0.35	0.39	0.06	0.77	0.82	0.06	0.79	0.68	0.81
MCW0216	6	5.88	0.83	2.25	0.21	0.29	0.10	0.75	0.83	0.10	0.81	0.72	0.83
MCW0222	11	5.26	0.81	-62.75	0.49	0.50	-0.004	0.79	0.81	0.03	0.78	0.73	0.81
MCW0248	8	4.00	0.75	24.75	0.06	0.07	0.01	0.73	0.75	0.03	0.71	0.70	0.75
Mean	**6.90**	**4.45**	**0.74**	**1.04**	**0.45**	**0.48**	**0.07**	**0.68**	**0.74**	**0.08**	**0.70**	**0.73**	**0.71**
CP_E and CP_F												0.999998	0.999999

N_A= Number of alleles observed, EN_A = effective number of allele, G_D = gene diversity, N_m = gene flow, F_{IS}, F_{IT} and F_{ST} are fixation indices, H_S = sub-population heterozygosity, H_T = total heterozygosity, G_{ST} = genetic differentiation, PIC = polymorphism information content, P_{E1} and P_{FI} are exclusion probabilities of k-number of markers for one parent and both parents excluded and CP_E and CP_F are combined exclusion probabilities for one and both parents excluded.

Table 5. Genetic divergence in years (below diagonal) and Nei's standard genetic distances among chicken populations (above diagonal)

Chicken population	Normal feathered	Frizzle feathered	Naked neck
Normal feathered	0.00	0.32	0.34
Frizzle feathered	1600.00	0.00	0.42
Naked neck	1700.00	2100.00	0.00

4.0 Conclusion

The 69 alleles and their frequencies observed will provide repository information with regards to these Nigerian chicken populations. Novel information of this study are the level of inbreeding revealed by the microsatellite markers, proportion of rare alleles (20.29%) and fixed alleles (79.71%) present in the chicken populations. Normal feathered chicken has the highest number of rare alleles and can be selected for increased production. LEI0192, MCW0248, MCW0222, MCW0016, MCW0069 and MCW0103 were more effective in detecting rare alleles, with MCW0222 appearing more promising in detecting rare alleles. Genetic divergence among populations ranged from 1600 to 2100 years. Polymorphism information content of the markers met the minimum threshold value of 0.50, thus confirmed that the markers were informative. Combined exclusion probabilities of the markers were within adequate plateau of 0.9995 to 0.9999 recommended for microsatellite markers to be used for parentage analysis. The possible implications of the achieved results are that Nigerian 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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