Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2023.20536.1854

Evaluation of Genetic Variability in Four Nigerian Locally-Adapted Chicken Populations Using Major Histocompatibility Complex-Linked LEI0258 Microsatellite Marker

Opeyemi Adetola Oladejo[1,2], Saidu Oyarekhua Oseni[2], Martina Kyallo[3], Jean-Baka Domelevo Entfellner[3], Noah Edson Tor[4], Christian Keambou Tiambo[3,5] & Roger Pelle[3]

[1] Animal Science and Fisheries Management, Bowen University, Iwo, Osun State, Nigeria
[2] Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
[3] Biosciences eastern and central Africa-International Livestock Research Institute (BecA-ILRI) Hub, Nairobi, Kenya
[4] Department of Animal Breeding and Physiology, Federal University of Agriculture, Makurdi, Benue State, Nigeria
[5] Centre for Tropical Livestock Genetics and Health (CTLGH) – ILRI, Nairobi Kenya

Poultry Science Journal 2023, 11(2): 189-201

Keywords
Characterization
Genetic diversity
Immune response
LEI0258 marker
Nigerian locally-adapted chicken

Corresponding author
Opeyemi Adetola Oladejo
opeaoladejo@gmail.com

Article history
Received: August 22, 2022
Revised: February 14, 2023
Accepted: April 07, 2023

Abstract

Major Histocompatibility Complex (MHC) is a group of genes that generally influence immune response in vertebrates, and it has been explored among different animal species in various countries. However, there is a paucity of information on its application in Nigerian locally-adapted chickens (NLAC). This research investigated genetic polymorphism, allele variability, and genetic relationships using LEI0258 major histocompatibility complex-linked microsatellite marker among four NLAC populations: Fulani × Yoruba ecotypes, FUNNAB Alpha × Noiler breeds. Blood samples were randomly collected from 50 mature birds in each population and DNA was extracted and subsequently subjected to PCR, Sanger sequencing, and bioinformatic analysis. There were two variable numbers of tandem repeats (VNTRs), with 90% of the alleles containing only one R13 and varying numbers of the R12 motifs that ranged from 1 to 19. Additional polymorphism was revealed by the presence of five SNPs and three indels in the upstream and downstream regions of LEI0258. A total of 48 alleles were observed with sizes ranging from 188 to 530 base pairs while the allele frequencies within the populations ranged from 1.9 to 29.2%. However, only 17 out of the 48 alleles had corresponding MHC-B haplotypes. Haplotypes B2, B12, and B21 found in this study had been reported to confer resistance to infectious poultry diseases especially avian influenza in locally adapted chickens. There were high allelic variability and genetic polymorphisms observed via the atypical LEI0258 microsatellite in describing the MHC-B region.

Introduction

Nigerian locally adapted chickens (NLACs) show high diversity in body sizes, plumage colors, feather distribution, and even their adaptation within the same flock (Ajayi, 2010). There have been studies elucidating the genetic diversity of Nigerian chickens using morphological and biochemical descriptors (Apuno *et al*., 2011; Ige *et al*., 2013), and molecular studies on the genetic diversity of Nigerian chickens (Adebambo *et al*., 2010; Ajibike *et al*., 2017; Tor *et al*., 2021). However, limited molecular studies have been done on these chicken populations to illustrate the genetic mechanism of their adaptation using the microsatellite markers in the major histocompatibility complex (MHC) region (Olufowobi *et al*., 2020).

Most vertebrates possess the MHC genetic region, which is essential for recognizing foreign antigens and triggering the immune system's defense against infections (Piertney and Oliver, 2006). In chickens, the genetic area primarily known for influencing disease resistance and immunological responses is the MHC, commonly known as the B-complex (Miller and Taylor, 2016). Additionally, the avian MHC genes play several non-immune roles in the production process and the success of reproduction (Nikbakht and Esmailnejad, 2015) and other important economic

Please cite this article as Opeyemi Adetola Oladejo, Saidu Oyarekhua Oseni, Martina Kyallo, Jean-Baka Domelevo Entfellner, Noah Edson Tor, Christian Keambou Tiambo & Roger Pelle. 2023. Evaluation of Genetic Variability in Four Nigerian Locally-Adapted Chicken Populations Using Major Histocompatibility Complex-Linked LEI0258 Microsatellite Marker. Poult. Sci. J. 11(2): 189-201.

traits in these birds. In size, the MHC of the chicken is minimal, compact, and 20-fold smaller than that of mammals (Davison, 2003) and hence is called minimal essential MHC (Kaufman *et al.,* 1999). The LEI0258 locus which is located within the MHC B-complex is highly polymorphic and complex with a variable number of tandem repeats (VNTRs) that have been optimally used for identifying MHC-B haplotypes and studying the genetic diversity of chicken populations (Fulton, 2020).

Numerous studies have utilized LEI0258 microsatellite marker among chicken populations from several countries including Ethiopia (Addisu *et al.,* 2020), Tanzania (Mwambene *et al.,* 2019), Kenya (Ngeno *et al.,* 2015), Cameroon (Touko *et al*., 2015) and South Africa (Ncube *et al.,* 2014). High levels of MHC diversity generally in the non-serologically defined random mating population, indigenous and commercial breeds have been reported previously (Izadi *et al.,* 2011). Novel alleles have also been identified in chickens from different extensively reared populations from different countries (Chazara *et al.,* 2013; Ncube *et al.,* 2014). However, there is a paucity of information on MHC applications among Nigerian chickens. Thus, this research used the MHC-linked LEI0258 marker typing to identify the novel and unique alleles to aid in understanding the genetic variability within Nigerian locally-adapted chickens. This research investigated genetic polymorphism, allelic variability, and genetic relationships using LEI0258 major histocompatibility complex-linked microsatellite marker among four NLAC populations Genetic information of these chickens is important for deciding on conservation and improvement in their various production settings for sustainable use.

Materials and Methods

Sample collection and DNA extraction

Blood samples (2.5 ml) were randomly collected from 200 adult chickens (6 months or older) from four NLAC populations (50 chickens each) of Fulani ecotype chickens (FEC), Yoruba Ecotype chickens (YEC), FUNNAB Alpha (FAC) chickens and Noiler chickens (NC) as shown in Table 1.

Blood samples were drawn by venipuncture from the wing vein and stored in EDTA blood collection tubes. The genomic DNA extraction was carried out at the FUNAAB Biotechnology and Acutigenetics Laboratory, both in Abeokuta, Ogun State, Nigeria. Genomic DNA was extracted from whole blood following the Zymo® Quick DNA Miniprep plus kit for biological fluids and cells protocol (Zymo Research Corp; www.zymoresearch.com). The DNA quality and quantity were assessed using NanoDrop 2000c Spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). The integrity of the extracted DNA was assessed using 0.8% agarose gel electrophoresis in the presence of 0.25X GelRed nucleic acid gel stain (Biotium, USA) and visualized using GelDoc-It®2310 Imager (Ultra-violet products - UVP Bioimaging System Ltd, Cambridge, UK.

Polymerase Chain Reaction amplification of LEI0258 locus

The primer pair LEI0258-F: 5'-CACGCAGCAGAACTTGGTAAGG-3' and LEI0258-R: 5'-AGCTGTGCTCAGTCCTCAGTGC-3' (Fulton *et al.,* 2006) were used for PCR amplification of the MHC-linked microsatellite. The 5' end of the forward primer was tagged with the T7 promoter sequence (underlined): T7-LEI0258F 5'-TAATACGACTCACTATAGGGCACGCAGAACT TGGTAAGG-3' and the reverse primer with the SP6 promoter sequence (underlined): 5'-ATTTAGGTGACACTATAAGCTGTGCTCAGTCC TCAGTGC-3'. T7 and SP6 primers were used for Sanger sequencing.

The PCR reaction mixture consisted of 40 ng DNA, 0.1 μM each primer, 1X Bioneer AccuPower PCR PreMix (Bioneer, Korea), and 3.4 μL of MilliQ water added to a final volume of 10 μl. Amplification was performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA) using the following PCR program: 94 ℃ for 3 minutes, followed by 30 cycles of 94 ℃ for 45 seconds, 63 ℃ for 1 minute, 72 ℃ for 2 minutes, and a final extension at 72 ℃ for 20 minutes. The PCR products were analyzed in a 2% agarose gel stained with 0.025X GelRed (Biotium, USA). Before Sanger sequencing, gel purification was done for heterozygote samples to purify single bands (Figure 1) according to the Qiagen QIAquick Gel Extraction Kit (Hilden, Germany) protocol. Both the PCR and the gel extraction/purification were carried out at the BecA-ILRI Hub. The purified amplicons were Sanger-sequenced at Macrogen, Netherlands. The sequenced DNA fragments were subsequently used for Bioinformatics analyses.

Sequence Data Analysis

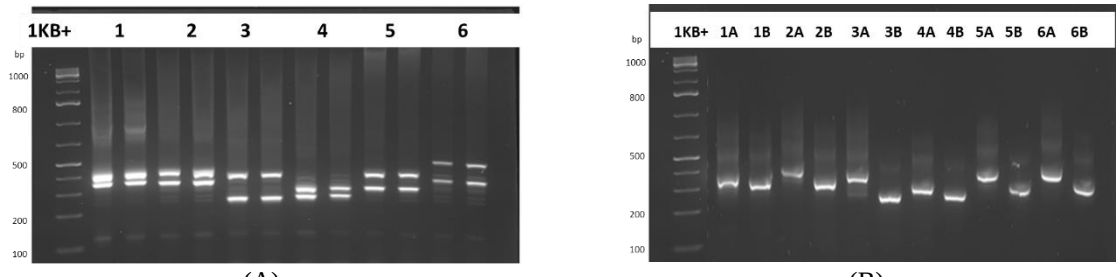
After sequencing, the forward (F) and reverse (R) sequences of each of the chicken samples were assembled to form a single consensus sequence using the CLC Genomics Workbench 8 (http://www.clcbio.com/download) software. Basic local alignment search tool (BLAST) was used to check the percentage (%) sequence similarity between the query (obtained consensus sequences) and the target LEI0258 sequence retrieved from the GenBank database of the National Centre of Biotechnology Information (NCBI). In addition, pairwise and multiple sequences alignments were performed using the Clustal W function of the MEGA version 6.0 software (http://megasoftware.net) and consensus sequences blasted in the NCBI GenBank database (Tamura *et al.,* 2013). The identification of the genetic

polymorphism and conserved sequence motifs from the aligned sequence was done using DnaSP version 6.0 (http://www.ub.edu/dnasp/) and a summary table used to reveal polymorphisms at microsatellite repeats (R13 / R12) and flanking regions of all sequenced alleles based on conserved regions (Librado and Rozas, 2009) and other were downloaded using the appropriate links. The population structure and the haplotype plots of the NLAC sequences were accomplished using the population and evolutionary genetics analysis system PEGAS and Arlequin software (Excoffier and Lischer, 2011).

Table 1. The physical attributes and locations of the sampled Nigerian locally-adapted chickens

Local ecotypes	Attributes	Improved breed	Attributes
Fulani 	Heavy local ecotypes, found majorly in the northern part of Nigeria and among the Fulani tribes. • Average matured body weight (BW) of 1.46 kg (males) and 1.07 kg (females). • GPS: -9.0765ºN 7.3986ºE (FCT), • Elevation: 1,180 feet.	**FUNAAB-Alpha** 	Crossbred of local ecotypes (naked-neck and frizzled) with exotic breeds. • Average matured BW of 2.06 kg (males) and 1.47 kg (females). • GPS: -7.49' N, 4.55' E (Ile-Ife) • Elevation: 873 feet.
Yoruba 	Small-bodied, light ecotype found majorly in southwestern Nigeria. • Average matured BW of 0.87 kg (females) and 0.94 kg (males). • White eggshell. • GPS: 7.49' N, 4.55' E • Elevation: 873 feet.	**Noiler**  	Heavy breeds, a crossbreed of layers, broilers, and local chickens. • Average matured BW of 2.53 kg (males) and 2.23 kg (females). • 150-200 eggs / year. • GPS: 7.49' N, 4.55' E • Elevation: 873 feet.

Source: Oladejo *et al.*, 2021b



(A) (B)

Figure 1. Agarose gels showing heterozygous LEI0258 alleles before purification (A) and after gel purification (B).

Results

The frequencies of the LEI0258 alleles of the four chicken populations and their corresponding MHC-B haplotypes (Table 2). In total, there were 48 alleles found among the four NLAC populations. The sizes of these alleles ranged from 193 to 530 base pairs (bp) while the allele frequencies ranged from 1-18 across the populations. Seven alleles occurring at a low

frequency were found only in the NC namely 196, 220, 241, 297, 320, 383, and 444 bp. The highest occurring allele sizes were 309, 217, and 261 with a total allele frequency of 18, 12, and 7, respectively. Although none of these allele sizes were individually found in all four populations, allele 309 bp was found in FEC, NC, and YEC while allele size 217 bp was found in FA, NC, and YEC populations. Moreover, allele size 261 bp was found in FA and NC populations. Furthermore, 32 of the 48 alleles observed were unique or private to only one of the populations. For example, 12 alleles were unique to YEC namely: 193, 195, 206, 227, 342, 347, 420, 421, 422, 474, 513, and 530 bp. Additionally, ten allele sizes were private only to the NC population in this case; 196, 220, 241, 297, 311, 320, 358, 383, 443, and 444 bp. Five allele sizes 249, 262, 264, 306, and 418 bp were uniquely found in the FA population. Five allele sizes 259, 263, 319, 349, and 393 bp were also unique to the FEC population, respectively. However, out of the 48 alleles present in the four chicken populations studied, only 17 (35.4%) alleles had corresponding MHC-B haplotypes.

Table 2. The LEI0258 allele frequencies and their corresponding MHC-B haplotypes

S/N	Allele (bp)	Populations				Total allele	Serology
		FA	FEC	NC	YEC	Frequency	GenBank B-haplotype
1	193	-	-	-	2	2	B15.1, B11, B61, B27
2	194	1	-	1	1	3	BW3
3	195	-	-	-	1	1	
4	196	-	-	1	-	1	
5	206	-	-	-	1	1	B13.2, B13, B17, BW11
6	217	7	-	4	1	12	
7	218	2	-	2	-	4	
8	219	-	-	1	1	2	
9	220	-	-	1	-	1	
10	221	-	-	1	1	2	
11	227	-	-	-	1	1	
12	241	-	-	1	-	1	
13	249	1	-	-	-	1	B15.2, B22, B73
14	259	-	3	-	-	3	
15	261	5	-	2	-	7	B15, B2, B29
16	262	2	-	-	-	2	
17	263	-	1	-	-	1	
18	264	1	-	-	-	1	
19	273	1	-	1	-	2	
20	297	-	-	1	-	1	
21	306	1	-	-	-	1	
22	307	-	-	4	1	5	B72, B78
23	309	-	4	11	3	18	B10, B24, B26, B76
24	310	-	1	3	-	4	
25	311	-	-	3	-	3	
26	319	-	1	-	-	1	
27	320	-	-	1	-	1	B74
28	333	-	-	1	2	3	BW4
29	342	-	-	-	1	1	
30	345	-	1	-	4	5	B14
31	346	-	-	1	2	3	
32	347	-	-	-	1	1	
33	349	-	2	-	-	2	
34	357	-	1	3	-	4	B21
35	358	-	-	3	-	3	
36	381	-	1	-	4	5	B13.1
37	382	2	-	-	1	3	
38	383	-	-	1	-	1	
39	393	-	1	-	-	1	B1
40	418	1	-	-	-	1	
41	420	-	-	-	1	1	B62
42	421	-	-	-	1	1	
43	422	-	-	-	1	1	
44	443	-	-	4	-	4	B6
45	444	-	-	1	-	1	
46	474	-	-	-	1	1	B12.2, B71
47	513	-	-	-	1	1	B12.3
48	530	-	-	-	1	1	

*NB: In bold are the alleles with the highest frequencies

Other information from the Sanger sequences of this genomic DNA using the LEI0258 markers are a variable number of tandem repeats (VNTRs), single nucleotide polymorphisms (SNP), and insertion-deletion (indel) as shown in Table 3 and Figure 2. In the region, the polymorphisms found are as follows:

- Upstream ("on the left") of the tandem repeat, there is a
  - ✓ TT/– indel at positions -32 and -31,
  - ✓ a G/A SNP at position -30 and
  - ✓ a G/A SNP at position -13.
- Within the tandem repeat;
  - ✓ R13 (ATGTCTTCTTTCT) is the count of R13 units,
  - ✓ R12 (TTCCTTCTTTCT) is the count of R12 units and
  - ✓ a C/T SNP at position 3 in the last R12, transforming TTCCTTCTTTCT into TTTCTTCTTTCT as seen in YEC002C
- In the region downstream ("on the right") of the tandem repeat,
  - ✓ there is a T/C SNP at position +1,
  - ✓ an insertion of ATTTTGAG between positions +9 to +16 (none of the FEC had this insertion),
  - ✓ a small indel -/A at position +19, an A/T SNP at position +25, and a T/A SNP at position +32

All the sequences listed in Table 3 were submitted to the International Nucleotide Sequence Database Collaboration (INSDC) with Bioproject accession numbers PRJEB58853 (ID: 923574). These 55 sequences listed are with accession numbers ranging from OX406934 to OX406988 (Table 3).

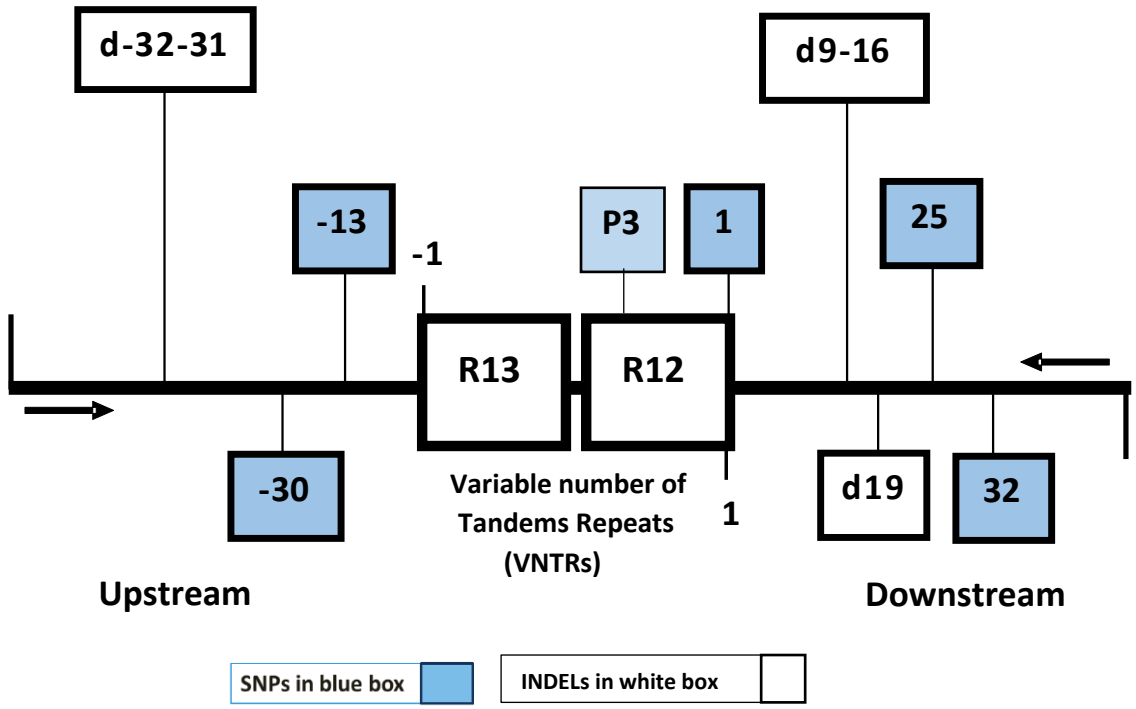


Figure 2. The positions of a variable number of tandem repeats (VNTRs) – R13 & R12 in between the upstream and downstream flanking regions, 5 SNPs (in blue boxes), and 3 indels (denoted with d in white boxes) within the LEI0258 locus (P3 is the SNP in position 3 of the last R12 VNTRs)

The R13 and R12 are independent repetitions of two repeat motifs of 13 and 12 base pairs R13: ATGTCTTCTTTCT and R12: TTCCTTCTTTCT. The R13 with a 13 bp repeat unit was found with a frequency of one in more than 90% of the studied alleles and a frequency of 15-25 in the remaining 10% of the alleles. The number of R12 motifs in the individual sequences ranged from 1 to 19. The upstream region is just before the R13 VNTRs, which numbered from -86 to -1 and 1 to 76 for the downstream region immediately after the R12 VNTRs. In all, there was a total of five SNPs and three indels in the upstream and downstream regions (Figure 2).

Table 3. Polymorphisms identified within the LEI0258 alleles of the MHC haplotypes in Nigerian locally-adapted chickens

Chicken ID	Consensus size (bp)	Upstream Position			Number of repeats		In R12	Down-stream Position					Genbank Accession Number
		-32-31	-30	-13	R13	R12	3 in the last R12	+1	+9+16	+19	+25	+32	
		TT/Δ	G/A	G/A			C/T	C	ATTTTGAG	-/A	A/T	T/A	
YEC014B	189	-	-	-	1	3	Δ	-	Δ	-	-	-	OX406934
YEC053B	188	-	-	-	1	3	Δ	-	Δ	-	-	-	OX406935
FA20B	209	-	-	A	1	3	-	-	Δ	-	-	-	OX406936
NC182B	191	-	-	-	1	3	Δ	-	Δ	-	-	-	OX406937
NC181B	195	-	-	-	1	1	Δ	-	Δ	-	-	-	OX406938
YEC002C	193	-	-	-	1	3	Δ	-	Δ	-	-	-	OX406939
FA01A	236	-	-	-	1	5	-	-	Δ	-	-	-	OX406940
NC51B	204	-	-	-	1	5	-	-	Δ	A	-	-	OX406941
NC162B	213	-	-	-	1	5	-	-	Δ	-	-	-	OX406942
YEC008B	240	-	-	-	1	5	-	-	Δ	-	-	-	OX406943
NC81B	280	-	-	-	1	5	-	-	Δ	-	-	-	OX406944
FA130B	240	-	-	-	1	9	-	-	-	-	-	A	OX406945
FA47B	230	-	-	-	1	7	-	-	-	-	-	-	OX406946
NOC203-F	249	-	-	-	1	7	-	-	-	-	-	-	OX406947
FA05A-1	255	-	-	-	1	8	-	-	Δ	-	-	A	OX406948
FEC004B	229	Δ	-	-	1	8	-	Δ	-	-	-	-	OX406949
FEC40A	248	Δ	-	-	1	8	-	Δ	-	-	-	A	OX406950
FEC40B	248	-	-	-	1	8	-	Δ	-	-	-	A	OX406951
FA18A	280	-	-	-	1	12	-	-	-	-	T	-	OX406952
NC32B	291	Δ	A	-	1	13	-	-	-	-	-	-	OX406953
NC51A	297	-	-	G	1	11	-	-	-	-	-	-	OX406954
FA44A	308	Δ	A	-	1	12	-	-	-	-	-	-	OX406955
NC161A	306	Δ	A	-	1	12	-	-	-	-	-	-	OX406956
NC271B	307	Δ	A	-	1	12	-	-	-	-	-	-	OX406957
NOC102-F	309	-	-	-	1	12	-	-	-	-	-	-	OX406958
FEC048A	372	-	-	-	1	12	-	-	-	-	T	-	OX406959
NC151A	430	-	-	-	1	12	-	-	-	-	-	-	OX406960
NC132A-1	296	-	-	-	1	12	-	-	-	-	-	-	OX406961
NC62A	477	-	-	-	1	12	-	-	-	-	T	-	OX406962
FEC042	297	Δ	A	-	1	13	-	-	-	-	-	-	OX406963
NC151B	324	Δ	A	-	1	12	-	-	-	-	-	-	OX406964

Table 3. Continued

FEC004A	326	-	-	-	1	15	-	-	-	-	T	-	OX406965
FEC1403-F	330	Δ	-	-	1	12	-	-	-	-	-	-	OX406966
YEC1401	330	-	-	-	1	15	-	-	-	-	-	-	OX406967
FEC1601	331	Δ	A	-	1	12	-	-	-	-	-	-	OX406968
NOC502	331	-	-	-	1	12	-	-	-	-	-	-	OX406969
NC32A	313	-	-	-	1	19	-	-	-	-	-	-	OX406970
YEC003	314	-	-	-	1	15	-	-	-	-	-	-	OX406971
YEC053A	358	-	-	-	1	15	-	-	-	-	-	-	OX406972
NC232B	368	-	-	G	1	15	-	-	-	-	-	-	OX406973
FAC701-F	346	-	-	-	1	15	-	-	-	-	T	-	OX406974
FEC044B	390	-	-	-	1	16	-	-	-	-	T	-	OX406975
NC73A	348	-	-	-	1	16	-	-	-	-	T	-	OX406976
YEC025B	373	-	A	-	1	15	-	-	-	-	-	-	OX406977
FA130A	387	-	-	-	1	18	-	-	-	-	T	-	OX406978
FA35B	378	-	-	-	1	18	-	-	-	-	T	-	OX406979
NC172A	387	Δ	A	-	1	12	-	-	-	-	-	-	OX406980
YEC025A	416	-	-	-	16	5	-	-	-	-	-	-	OX406981
YEC026A	415	-	-	-	16	5	-	-	-	-	-	-	OX406982
NC133B	423	-	-	-	1	7	-	-	Δ	-	-	-	OX406983
NC302A	427	-	-	-	15	8	-	-	-	-	-	-	OX406984
NC212A	418	-	-	-	15	8	-	-	-	-	-	-	OX406985
YEC022A	482	-	-	-	22	3	-	-	-	-	-	-	OX406986
YEC138A	488	-	-	-	25	3	-	-	-	-	-	-	OX406987
YEC030A	515	-	-	-	25	3	-	-	-	-	-	-	OX406988

Δ - indel (insertion-deletion)
R12 and R13 – Variable number of tandem repeats

Population structure of the NLAC population

The haplotype network among the NLAC (Figure 3) illustrated the possible structure of the chicken populations. There were three major clusters (1-3) as well as three minor clusters (4-6). Cluster 1 represents the four populations and is, therefore, the main cluster with NC representing 50% of the cluster while FEC and YEC were about 20% each of the clusters and FA is about 10% of the major cluster 1. FA represents about 70% of cluster 2 while NC and YEC were about 10% and 20% respectively. FEC was not found in cluster 2. Cluster 3 grouping NC (60%) and FA (40%) represents only the improved breeds which were carriers of both local and exotic genes; this explains the reasons why this cluster is far away from other clusters because of the exotic genes which are more commonly found in the improved breed population studied. Minor clusters 4 and 6 were quite similar as they only contain the NC, FA, and YEC in 60, 20, and 20%, respectively. The number of bars between two consecutive nodes/clusters represents mutations. Cluster 5 is a unique cluster with only the *Fulani* haplotypes observed, the reason for this could be attributed to the fact that Fulani chickens sampled in this study were reared in isolated *Kraal* settlements of the Fulani tribes, and these chickens hardly crossbreed with other chicken populations.

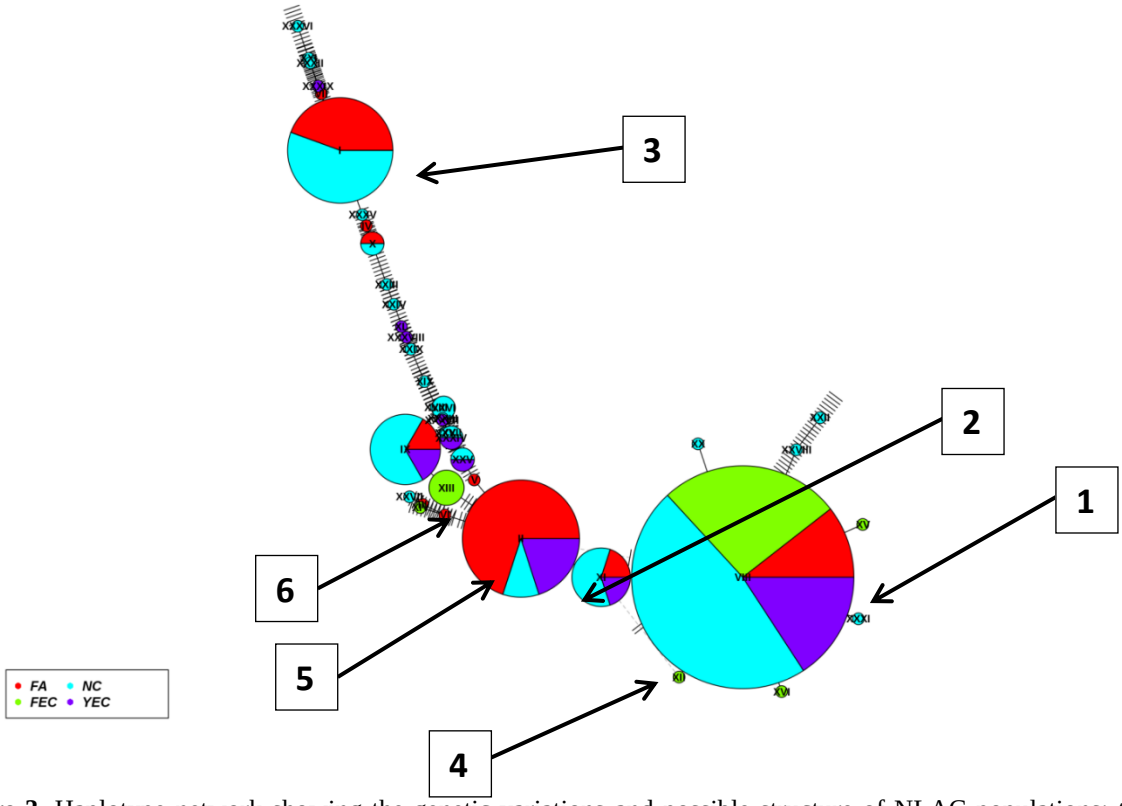


Figure 3. Haplotype network showing the genetic variations and possible structure of NLAC populations; the number of bars between two consecutive nodes/clusters represents mutations (the main nodes/clusters are numbered 1-6)

Discussion

In this study, the MHC polymorphisms of the NLACs were obtained using the LEI0258 MHC-linked microsatellite marker. This microsatellite marker LEI0258 (accession number – Z83781) is mapped to chromosome 16 (McConnell *et al*., 1999). As expected for a normal microsatellite marker, the LEI0258 alleles display a significant range in sizes (Adebabay, 2018); this is evident in this study because out of 200 samples sequenced, 48 distinct alleles were found. The distribution of the LEI0258 microsatellite alleles among the populations was shown to be appropriate by the observed allele frequencies, which ranged from 1.9 to 29.2%. Of all these alleles, there were seven alleles with low frequencies below 2% namely; 196, 220, 241, 297, 320, 383, and 444 bp. In small populations, these rare alleles may be susceptible to loss due to selection pressure or genetic drift. The most prevalent allele, 309 bp, may be important for an individual's survival or fitness if they have it (Ncube *et al.*, 2014). The present study found allele sizes to vary between 188 bp and 530 bp as found in Table 3 and this corroborates the findings obtained by Mwambene *et al.* (2019); Chazara *et al.* (2013) and Fulton *et al*. (2006) in different chicken populations. Fulton *et al*. (2006) reported the allele size of North American and

European layer-type chickens ranged from 182 bp to 552 bp and a total of 26 alleles were identified for the LEI0258 marker. Chazara *et al.* (2013) stated that 79 different alleles were identified among the African, Asian, and European chickens ranging from 181 to 552 bp allele sizes. Moreover, among 10 Tanzanian chicken ecotypes, the size of the sequenced alleles ranged from 249 bp to 552 bp with a total of 30 alleles (Mwambene *et al.*, 2019).

Furthermore, the existence of numerous frequent and common alleles in all chicken groups under study revealed close links between NLAC populations. The high allelic polymorphism throughout the examined chicken populations is implied by the huge numbers and wide range of allele sizes at this marker. To illustrate the heterogeneity in chicken populations depending on their production settings, diseases resistance (tolerance) or susceptibility, origins, dispersion, and level of interactions within and among the NLAC population; there may be variances in allele counts between populations (Oladejo *et al*., 2021a).

Sequence information from the LEI0258 includes the varying numbers of tandem repetitions (VNTRs). R13 and R12 were the two primary VNTRs. A single frequency was detected for the R13 with the 13-bp repeat unit "ATGTCTTCTTTCT." in more than 90% of the samples similar to what was found in the wild Red jungle fowl, Leghorn and Chinese indigenous chickens (Wang *et al*., 2014) and 15-25 frequencies in the remaining 10% of the samples. This is consistent with other studies with more frequencies of the R13 motifs (Chazara *et al*., 2013; Nikbakht *et al*., 2013; Han *et al.,* 2013). The number of R12 motifs (TTCCTTCTTTCT) in the individual sequences ranged from 1 to 19 which is also comparable to the study by Mwambene *et al.* (2019). The pattern of the motif used in this present study corroborated with the pattern of arrangement of the nucleotide bases used by Chazara *et al*. (2013) with the two VNTRs starting with 'ATG' and 'TT' and both ending with 'CT' respectively. On the other hand, this is a bit different in the order of arrangement in Fulton *et al*. (2006) in which the R13: CTATGTCTTCTTT and R12: CTTTCCTTCTTT with the 'CT' starting these independent repeat motifs and ending with 'CTTT' (Oladejo *et al.*, 2021a). In all, there was a total of five SNPs and three indels in the upstream and downstream regions of the LEI0258 marker in NLAC which is a pointer to how polymorphic the LEI0258 sequences of the Nigerian locally adapted chickens are. Although a SNP was detected in position 3 of the last R12, from the literature, SNPs were neither reported nor commonly found inside the VNTRs. This is therefore a novel polymorphism situated in the VNTR.

Serologically, B-haplotypes from the LEI0258 alleles are useful to determine or study chicken populations that confer either susceptibility or resistance to certain common diseases in chickens. For the same MHC haplotypes from different chicken populations and sources, there was a strong correlation between LEI0258 alleles and the serologically characterized MHC haplotypes (Fulton *et al*., 2006). The most common allele size 309 found in 18 out of 48 alleles originating from three different populations in this study was associated with well-defined haplotypes: B10, B24, B26, and B76 (Fulton *et al*., 2006).

These 17 alleles correspond individually with one to four MHC B-haplotypes each (last column of Table 2). From the table, allele 193 corresponds with four haplotypes with the B15.1 haplotype being the major haplotype with a defined influence on either disease resistance or susceptibility as reported in previous studies (Fulton *et al.,* 2006).

Consistently, the allele 193 found in this study has been corresponding with the B15.1 haplotype (Fulton *et al.,* 2006) and it is found only in the YEC. This B15.1 haplotype was reported to be associated with resistance to avian infectious bronchitis disease (Bacon *et al.,* 2004), poor resistance to avian leucosis (Bacon *et al.,* 1981), poor immune response to infectious bursal disease (Bacon *et al.,* 1981) and susceptibility to Marek's disease (Briles *et al.,*1977), Rous sarcoma (Nordskog and Gebriel, 1983) and *Salmonella enteritidis* (Cotter *et al.,* 1998). The Allele 194 BW3 haplotype is found though in small percentages across the 3 out of four populations except in the FEC. Allele 194 bp which is BW3 and 261 bp allele (B15) conferred poor immune responses against infectious bursal disease (Esmailnejad *et al.,* 2017). Allele 206 found only in the YEC is the allele size for both B17 and B13 haplotypes, although these two haplotypes are serologically distinct (Fulton *et al.,* 2006). The B17 haplotype is associated with poor resistance to Marek's disease (Schat *et al.,* 1994) while the B13 (allele 205) haplotype is associated with resistance to *Escherichia coli* (Macklin *et al.,* 2002), poor resistance to avian infectious bronchitis disease (Bacon *et al.,* 1981), poor resistance to avian leucosis (Bacon *et al.,* 1981) and Rous sarcoma (Schierman and Collins, 1987), moderate resistance to infectious bursal disease (Bacon *et al.,* 1981) and susceptibility to Marek's disease (Briles *et al.*, 1977) and Avian Influenza (Boonyanuwat *et al.,* 2006).

Even though allele 249 corresponds with haplotypes B15.2, B22, and B73, none of these haplotypes had been categorically defined to confer important disease susceptibility/resistance in the literature. Although B15, B15.1, and B15.2 are haplotype variants; these haplotypes are serologically similar with unique allele sizes for the LEI0258 marker (Fulton *et al.,* 2006). The allele 261 which is found only in the improved chickens is corresponding with haplotypes B2, B15, and B29. Haplotype B2 is associated with resistance to Marek's disease (Briles *et al.,* 1977), good immune response to infectious bursal

disease (Bacon, 1987), susceptibility to avian leucosis (Bacon *et al.,* 1981) Rous sarcoma (Collins *et al.,* 1977). The B23 haplotype is found to be susceptible to Marek's disease (Schat *et al.,* 1994). Furthermore, the B21 (allele 357) haplotype found in the FEC and NC in this study are known to be associated with resistance to avian influenza (Boonyanuwat *et al.,* 2006), poor resistance to avian leucosis (Bacon *et al.,* 1981) strong resistance to Marek's disease (Blankert *et al.,* 1990), good immune response to infectious bursal disease (Bacon *et al.,* 1981) and susceptibility to Avian infectious bronchitis (Bacon *et al.,* 2004) and *E. coli* (Macklin *et al.,* 2002). The B1 (allele 393) haplotype is found only in the FEC and is associated with a high mortality rate to *Salmonella enteritidis* (Pevzner *et al*., 1975) and promotes tumor growth to Rous sarcoma (Nordskog and Gebriel, 1983). The B6 (allele 443) haplotype found only in the NC is associated with resistance to Marek's disease (Briles *et al.,*1977) as well as with tumor regression to Rous sarcoma disease (Schierman and Collins, 1987). The haplotypes B12 (allele 487), B12.2 (allele 474,) and B12.3 (allele 513) are all haplotype variants and found in the YEC. These B12 haplotypes were reported by Bacon (1987) to be linked with a poor immune response to infectious bursal disease but strong resistance to avian leucosis (Bacon *et al.,* 1981) and suppressing tumor growth to Rous sarcoma (Plachy, 1984).

Other important haplotypes reported by other scientists known to confer notable influence on diseases include: B19 (allele 539) haplotype is found in birds susceptible to avian infectious bronchitis (Juul-Madsen *et al.,* 2002) and Marek's disease (Briles *et al.,* 1977, Blankert *et al.,* 1990) as well as with those with strong resistance to avian leucosis (Bacon *et al.,* 1981); B18 (allele 247) haplotype was associated with susceptibility to *S. enteritidis (*Cotter *et al.,*1998); B5 (allele 295) haplotype is associated with poor resistance to avian leucosis (Plachy, 1984), poor immune response to infectious bursal disease (Bacon, 1987) and susceptibility to Marek's disease (Briles *et al.,* 1977); B4 (allele 182) haplotype is associated with strong resistance to infectious bursal disease (Juul-Madsen *et al.,* 2002) whereas B3 haplotype correlates with susceptible to Marek's disease (Briles *et al.,*1977). Summarily, haplotypes B2, B12, and B21 in this study are economically important haplotypes as they were reported to be associated with resistance to infectious poultry diseases especially avian influenza in locally adapted chickens as reported by Wang *et al.* (2014) and Esmailnejad *et al.* (2017).

Conclusion

There were high allelic variability and genetic polymorphisms observed via the atypical LEI0258 microsatellite in describing the MHC-B region. These were detected in the four chicken populations of NLAC studied as 5 SNPs and 3 indels were found in the flanking regions. Also, when compared with standard, defined serological haplotypes; haplotype B2 (261 bp) is associated with resistance to Marek's, and B21 (357 bp) haplotype found in the FEC and NC is associated with resistance to avian influenza disease. The comprehensive reference set of alleles and haplotypes is now available to identify and classify MHC populations. The present study was able to identify novel alleles and haplotypes in which their importance has not been previously identified. Allele 249 corresponds with haplotypes B15.2, B22, and B73 of which none of these haplotypes had been previously categorically or defined to confer any important disease (avian influenza or Marek's disease) susceptibility/resistance.

Acknowledgment

"This work was supported by the BecA- ILRI Hub through the Africa Biosciences Challenge Fund (ABCF) program. The ABCF Program is funded by the Australian Department for Foreign Affairs and Trade (DFAT) through the BecA- CSIRO partnership; the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill & Melinda Gates Foundation (BMGF); the UK Department for International Development (DFID); and the Swedish International Development Cooperation Agency (Sida)."

References

Addisu H, Kyallo M, Yohannes T, Sendeku W, Getu A, Dagnachew S, Dejen M, Wolde Y, Engdaw F, Kidane A, Dessie T, Getinet MT, Githae D & Roger P. 2020. Genetic diversity and population structure of indigenous chicken ecotypes (*Gallus gallus domesticus*) in Ethiopia using LEI0258 microsatellite. International Journal of Poultry Science, 19: 102-110. DOI: 10.3923/ijps.2020.102.110

Adebabay KB. 2018. Whole genome-based characterization of indigenous chicken populations in Ethiopia. PhD thesis. Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia. 496 Pages.

Adebambo AO, Mobegi VA, Mwacharo JM, Oladejo BM, Adewale RA, Ilori LO, Makanjuola BO, Afolayan O, Bjørnstad G, Jianlin H & Hanotte O. 2010. Lack of phylogeographic structure in Nigerian village chickens revealed by mitochondrial DNA D-loop sequence analysis. International Journal of Poultry Science, *9*(5), 503-507. DOI: 10.3923/ijps.2010.503.507

Ajayi FO. 2010. Nigerian indigenous chicken: A valuable genetic resource for meat and egg production. Asian Journal of Poultry Science, 4:

164-172. DOI: 10.3923/ajpsaj.2010.164.172

Ajibike AB, Adeleye OO, Ilori BM, Osinbowale DA, Adeniyi OA, Durosaro SO, Sanda AJ, Adebambo OA & Adebambo AO. 2017. Genetic diversity, phylogeographic structure and effect of selection at the mitochondrial hypervariable region of Nigerian chicken populations. Journal of Genetics, 96(2): 959-968. DOI: 10.1007/s12041-017-0860-1

Apuno AA, Mbap ST & Ibrahim T. 2011. Characterization of local chickens (*Gallus gallus domesticus*) in Shelleng and Song Local Government Areas of Adamawa State, Nigeria. Agriculture and Biology Journal of North America, 2(1): 6-14. DOI: 10.5251/abjna.2011.2.1.6.14

Bacon LD, Hunter DB, Zhang HM, Brand K & Etches R. 2004. Retrospective evidence that the MHC (B haplotype) of chickens influences genetic resistance to attenuated infectious bronchitis vaccine strains in chickens. Avian Pathology, 33(6): 605–609. DOI: 10.1080/03079450400013147

Bacon LD. 1987. Influence of the major histocompatibility complex on disease resistance and productivity. Poultry Science, 66(5): 802–811. DOI: 10.3382/ps.0660802

Bacon LD, Witter RL, Crittenden LB, Fadly A & Motta J. 1981. B-haplotype influence on Marek's disease, Rous sarcoma, and lymphoid leukosis virus-induced tumors in chickens. Poultry Science, 60(6): 1132–1139. DOI: 10.3382/ps.0601132

Blankert JJ, Albers GA, Briles WE, Ginkel MV, Groot AJ, Te Winkel GP, Tilanus MG & Van der Zijpp AJ. 1990. The effect of serologically defined major histocompatibility complex haplotypes on Marek's disease resistance in commercially-bred White Leghorn chickens. Avian Diseases, 34(4): 818–823. DOI: 10.3389%2Ffimmu.2022.908305

Boonyanuwat K, Thummabutra S, Sookmanee N, Vatchavalkhu V & Siripholvat V. 2006. Influences of major histocompatibility complex class I haplotypes on avian influenza virus disease traits in Thai indigenous chickens. Animal Science Journal, 77(3): 285–289. DOI: 10.2307/1591368

Briles WE, Stone HA & Cole RK. 1977. Marek's disease: Effects of B histocompatibility allo-alleles in resistant and susceptible chicken lines. Science, 195(4274): 193–195 DOI: 10.1126/science.831269

Chazara O, Chang CS, Bruneau N, Benabdeljelil K, Fotsa JC, Kayang BB, Loukou NG, Osei-Amponsah R, Yapi-Gnaore V, Youssao IA, Chen CF, Laan MP, Tixier-Boichard M & Bed'Hom B. 2013. Diversity and evolution of the highly polymorphic tandem repeat LEI0258 in the chicken MHC-B region. Immunogenetics, 65: 447–459. DOI: 10.1007/s00251-013-0697-6

Collins WM, Briles WE, Zsigray RM, Dunlop WR, Corbett AC, Clark KK, Marks JL & McGrail TP. 1977. The B locus (MHC) in the chicken: Association with the fate of RSV induced tumors. Immunogenetics, 5(1): 333–343. DOI: 10.1007/BF01570490

Cotter PF, Taylor Jr. RL, & Abplanalp H. 1998. B-Complex Associated Immunity to Salmonella enteritidis Challenge in Congenic Chickens. Poultry Science, 77(12): 1846 –1851. DOI: 10.1093/ps/77.12.1846

Davison TF. 2003. The immunologists' debt to the chicken. British Poultry Science, 44(1): 6-21 DOI: 10.1080/0007166031000085364

Esmailnejad A, Nikbakht G & Badavam M. 2017. LEI0258 microsatellite variability and its association with humoral and cell mediated immune responses in broiler chickens. Molecular Immunology, 90: 22–26. DOI: 10.1016/j.molimm.2017.06.027

Excoffier L & Lischer HEL 2011. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10(3): 564-567. DOI:10.1111/j.1755-0998.2010.02847.x

Fulton JE. 2020. Advances in methodologies for detecting MHC-B variability in chickens. Poultry Science 99: 1267–1274. DOI: 10.1016/j.psj.2019.11.029

Fulton JE, Juul-Madsen HR, Ashwell CM, McCarron AM, Arthur JA, O'Sullivan NP & Taylor R Jr. 2006. Molecular genotype identification of the *Gallus gallus* major histocompatibility complex. Immunogenetics, 58: 407–421. DOI: 10.1007/s00251-006-0119-0

Han B, Lian L, Qu L, Zheng J & Yang N. 2013. Abundant polymorphisms at the microsatellite locus LEI0258 in indigenous chickens. Poultry Science, 92: 3113–3119. DOI: 10.3382/ps.2013-03416

Ige AO, Salako AE, Ojedapo LO & Adedeji TA. 2013. Biochemical characterization of indigenous Fulani and Yoruba ecotypes chicken of Nigeria. African Journal of Biotechnology, 12(50): 7002-7008. DOI: 10.5897/AJB2013.12816

Izadi F, Ritland C & Cheng KM. 2011. Genetic diversity of the Major Histocompatibility Complex region in Commercial and non-commercial chicken flocks using the LEI0258 microsatellite markers. Poultry Science, 90: 2711–2717. DOI: 10.3382/ps.2011-01721

Juul-Madsen HR, Nielsen OL, Krogh-Maibom T, Rontved CM, Dalgaard TS, Bumstead N & Jorgensen PH. 2002. Major histocompatibility complex-linked immune response of young chickens vaccinated with an attenuated live infectious bursal disease virus vaccine followed by an infection, Poultry Science, 81(5): 649–656. DOI: 10.1093/ps/81.5.649

Kaufman J, Milne S, Göbel TW, Walker BA, Jacob JP, Auffray C, Zoorob R & Beck S. 1999. The chicken B locus is a minimal essential major histocompatibility complex. Nature, 401: 923-925. DOI: 10.1038/44856

Librado P & Rozas J. 2009. DnaSP v6: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451-1452. DOI: 10.1093/bioinformatics/btp187

Macklin KS, Ewald SJ & Norton RA. 2002. Major histocompatibility complex effect on cellulitis among different chicken lines. Avian Pathology, 31(4): 371–376. DOI: 10.1080/03079450220141642

McConnell SK, Dawson DA, Wardle A & Burke T. 1999. The isolation and mapping of 19 tetranucleotide microsatellite markers in the chicken. Animal Genetics, 30: 183–189. DOI: 10.1046/j.1365-2052.1999.00454.x

Miller MM & Taylor RL. 2016 Brief review of the chicken Major Histocompatibility Complex: the genes, their distribution on chromosome 16, and their contributions to disease resistance. Poultry Science, 95: 375–392 DOI: 10.3382/ps/pev379. DOI: 10.3382/ps/pev379

Mwambene PL, Kyallo M, Machuka E, Githae D & Pelle R. 2019. Genetic diversity of 10 indigenous chicken ecotypes from Southern Highlands of Tanzania based on Major Histocompatibility Complex-linked microsatellite LEI0258 marker typing. Poultry Science, 98(7):2734-46. DOI: 10.3382/ps/pez076

Ncube KT, Jooste PJ, Soma P, Dzomba EF & Muchadeyi FC. 2014. Polymorphism of the Major Histocompatibility Complex and Genetic Structure of Southern African Village Chicken Populations. International Journal of Poultry Science, 13(6): 357-363. DOI: 10.3923/ijps.2014.357.363

Ngeno K, Van der Waaij EH, Megens HJ, Kahi AK, Van Arendonk JAM & Crooijmans RPMA. 2015. Genetic diversity of different indigenous chicken ecotype using highly polymorphic MHC-linked and non- MHC microsatellite markers. Animal Genetic Resources, 56: 1–7. DOI: 10.1017/S2078633614000484

Nikbakht G & Esmailnejad A. 2015. Chicken major histocompatibility complex polymorphism and its association with production traits. Immunogenetics, 67:247–252. DOI:10.1007/s00251-015-0832-7

Nikbakht G, Atefeh E & Neda B. 2013. LEI0258 microsatellite variability in Khorasan, Marandi, and Arian Chickens. Biochemical Genetics, 51: 341–349. DOI: 10.1007/s10528-013-9567-z

Nordskog AW & Gebriel GM. 1983. Genetic aspects of Rous sarcoma-induced tumor expression in chickens. Poultry Science, 62(5): 725–732. DOI: 10.3382/ps.0620725

Oladejo OA, Oseni SO, Entfellner JBD, Kyallo M, Keambou CT & Pelle R. 2021a. Variability in the Alleles and B-haplotypes of the Major Histocompatibility Complex-linked LEI0258 microsatellite marker in four Nigerian locally adapted chicken populations. Proceedings of the 2nd Annual Conference of the Research and Development Committee of the Federal College of Animal Health and Production Technology, Ibadan, Nigeria. Pages, 107-112.

Oladejo OA, Oseni SO, Oguntunji AO, Ayoola MO, Alabi OM, Aderemi FA & Lawal TE. 2021b. Quantitative traits characterization of four Nigerian locally-adapted chickens. Proceedings of the 26th Annual Conference of Animal Science Association of Nigeria (ASAN) & 10th ASAN-NIAS Joint Annual Meeting, Uyo, Nigeria. Pages, 529–532.

Olufowobi OT, Ilori BM, Olowofeso O, Sogunle OM & Omotoso AO. 2020. Genetic variation of the major histocompatibilty complex-B haplotypes in Nigerian local chicken populations Agricultura Tropica et Subtropica, 53(4): 175–181. DOI: 10.2478/ats-2020-0017

Pevzner I, Nordskog AW & Kaeberle ML. 1975. Immune response and the B blood group locus in chickens, Genetics, 80(4): 753–759. DOI: 10.1093/genetics/80.4.753

Piertney SB & Oliver MK. 2006. The evolutionary ecology of the major histocompatibility complex. Heredity, 96: 7-21.

Plachy ÃJ. 1984. Hierarchy of the B (MHC) haplotypes controlling resistance to rous sarcomas in a model of inbred lines of chickens. Folia Biologica, 30 (6): 412–425.

Schat KA, Taylor Jr. RL & Briles WE. 1994. Resistance to Marek's disease in chickens with recombinant haplotypes to the major histocompatibility (B) complex. Poultry Science, 73(4): 502–508. DOI: 10.3382/ps.0730502

Schierman LW and Collins WM. 1987. Influence of the major histocompatibility complex on tumor regression and immunity in chickens. Poultry Science, 66(5): 812–818. DOI: 10.3382/ps.0660812

Tamura K, Stecher G, Peterson D, Filipski A & Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution, 30(12): 2725–2729. DOI: 10.1093/molbev/mst197

Tor NE, Adebambo AO, Wheto M, Sogunle OM, Ikeobi CO, Ajibike AB, Omotoso AO, Shems K, Durosaro S, Mendy EW, Oladejo O & Ifeanyi U. 2021. Genetic diversity and phylogeographic structure of West African village chickens based on a 920bp segment of the mtDNA D-loop. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS). 14(4): 26-36. DOI: 10.9790/2380-1404012636

Touko BH, Keambou CT, Han JM, Bembidé C, Skilton RA, Ogugo M, Manjeli Y, Osama S, Cho CY & Djikeng A. 2015. Molecular typing of the major histocompatibility complex B microsatellite haplotypes in Cameroon chicken. Food and Agriculture Organization of the United Nations Animal Genetic Resources, 56: 47–54. DOI: 10.1017/S2078633614000538

Wang H, Ma T, Chang G, Wan F, Liu X, Liu L, Xu L, Chen J & Chen G. 2014. Molecular genotype identification of different chickens: major histocompatibility complex. The Open Access Journal of Science and Technology, 2(8): 1-7. DOI: 10.11131/2014/101111