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Original Research Article

Genetic variation of the major histocompatibilty complex-B haplotypes in Nigerian local chicken populations

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Abstract

To understand the genetic basis and mechanism underlying the differences in the level of immunity among and within chicken populations in Nigeria, it is important to start from the Major Histocompability Complex (MHC) region particularly as it serves as a reservoir for genes of the immune system. The B-complex of chicken major histocompatibility complex, located on microchromosome 16, consists of gene classes responsible for immunity through antigen presentation to T cells. A highly polymorphic tandem repeat marker (LEI0258) located within the B-complex has been a marker of choice for genotyping to identify major histocompatibility complex-B haplotypes and to study the genetic diversity of chicken populations. This study was carried out to determine the genetic variations, at the LEI0258 locus, in three Nigerian local chicken populations; Normal feather, Frizzle feather and Naked neck. The allelic and genotypic profiles of each representative from each population were determined through polymerase chain reaction amplification of the repeat region. The genetic diversity parameters, analysis of molecular variance and evolutionary relationship were determined using GenAlex, FSTAT, Arlequin and POPTREEW, respectively. 76% of the entire population was heterozygous at the LEI0258 locus. Analysis of molecular variance revealed that large proportion of the total variations across populations was due to variation between individuals (79%), whereas variations among the populations and among individuals within populations only accounted for less than 1% and 21%, respectively. Using Anak Titan as an exotic outgroup, the evolutionary relationship among the Nigerian local chicken populations was studied and a Nei-based dendrogram showed two major clades separating the exotic population from the Nigerian local chicken populations. The identified diversity at the locus could be exploited for usage in further breeding programmes especially for disease resistance and fitness in locally adapted chicken populations in Nigerian.

Keywords: Chickens; immunity; diversity; microsatellite; allele size.

INTRODUCTION

Individuals in a population differ in the way they respond to stimuli in their environment. One of such responses that is of importance to the poultry industry is the capacity for a normal immune response to diseases in the environment. The B-complex of chicken Major Histocompatibility Complex (MHC) is a "minimal essential" region (Kaufman et al., 1995; Miller and Taylor Jr, 2016) located on micro-chromosome 16 and consist a linkage group of three classical gene loci; BF, BL and BG which encode the MHC classes I, II and IV molecules (Miller et al., 2004).

Before the advent of molecular biological tools, chicken MHC-B haplotypes were identified by serological methods that use series of chicken allo-antisera (Briles and Briles, 1982; Fulton, 2020). Then, a standardised MHC-B haplotype nomenclature was created in 1982 (Briles et al., 1982) which was later updated by Miller et al. (2004) with sequences of genes within the B-region. Approximately 20 serologically-derived B-haplotypes have been well-defined using closely related populations, majorly White Leghorn chickens (Rogers and Kaufman, 2008). This fact makes this method abound with cross-reactivity (Fulton et al., 1995; Fulton et al., 2016), which tends to provide a biased result when applied to outbred populations (Kroemer et al., 1990).

A tandem repeat nucleotide sequence (LEI0258) close to the BF/BL region was identified by McConnell et al. (1999) and its differences in allele size detectable by rapid, inexpensive and more

accurate method was used to study its association with serologically defined MHC haplotypes in birds (Fulton et al., 2006). Interestingly, LEI0258 alone distinguished most of the MHC-B haplotypes and further discrimination between haplotypes with similar LEI0258 allele was feasible using an additional marker, MCW0371 located 10,560 bp downstream of LEI0258, as well as indels (i.e. insertions and deletions) and Single Nucleotide Polymorphisms (SNPs) within these markers (Fulton et al., 2006).

In addition, owing to the hypervariability of the LEI0258 marker, several studies have been carried out to study the genetic diversity and the polymorphic nature of the MHC region in different chicken populations, such as local chicken population of South Africa (Ncube et al., 2014), Kenya (Ngeno et al., 2014), Cameroon (Hako Touku et al., 2015) and China (Han et al., 2013).

Numerous studies have been carried out on the variations that exist within the MHC-B complex of different chicken populations. Adequate adaptive genetic variations are necessary for species to respond and thrive well under dynamic environment changes (Manjula et al., 2020). However, in this context, the Nigerian local chicken populations have not been studied. Chicken serves as one of the most important animal genetic resources in the country, and the local types play significant socio-cultural and economic roles, most especially among the rural farmers. Nigeria local chicken is one of the basic sources of dietary protein and good source of income especially among the rural, semi-urban and some urban dwellers as it is preferred for its portability, short generation interval and organoleptic properties. Although having low performance compared with their exotic counterparts, their products (egg and meat) are readily available. These chickens are hardy, immune and are able to withstand adverse weather conditions that may include temperature extremes of the arid regions and high humidity of the tropics, which affect their production and predispose them to disease vectors and parasites (Ilori et al., 2016). They possess untapped and great genetic resources with high genetic variance in their performance that are necessary potentials for their improvement through breeding programmes (Ilori et al., 2016, 2017). There is a paucity of information on the genetic diversity of the major histocompatibilty complex-B haplotypes in the locally adapted chicken populations of Nigeria. We hypothesised that genotype would influence the diversity of this haplotype in our local chicken. Therefore, this study was carried out with the aim to identify the genetic variation of the MHC-B haplotypes present in Nigerian local chicken populations. This is crucial as it serves as a preliminary step in understanding the genetic basis and mechanism underlying the differences in the level of immunity among and within these chicken populations, since the MHC region particularly serves as a reservoir for genes of the immune system.

MATERIALS AND METHODS

Blood sampling and DNA extraction

Sampling was done in the three Nigerian local chicken populations; the Naked neck (n = 42; 19 males and 23 females), the Frizzled feather (n = 44; 20 males and 24 females) and the Normal feather (n = 44; 22 males and 22 females) that were 20 to 25 weeks old and came from three geographical regions of Nigeria in South-West (Ogun and Oyo States), North-West (Kaduna and Sokoto States) and North-East (Bauchi and Yobe States). The birds are being maintained on free range or semi-intensively reared and fed with household waste and cereals while also allowed to scavenge on farm remains and insects. Blood sample was also taken from one Anak Titan exotic broiler chicken being maintained intensively at the University Research Farm and used as an outgroup for the genetic analysis. Venous blood was collected from the brachial veins of a total of 130 healthy chickens and then stored on Whatman FTA filter cards (Whatman International Limited) while methylated spirit was used to disinfect the area around the bleeding site in the chicken used.

Whatman FTA filter cards containing blood samples were stored at room temperature before DNA was extracted from them by punching ten 1-mm discs from each card into 1.5mL tube using Harris Uni-Core 1.25 MM Punch (Novolab-labware.com) and the DNA extracted using the boiling method. Extracted DNA was quantified for concentration and purity using Nanodrop spectrophotometry and gel electrophoresis in congruence with protocol reported by Desjardins and Conklin (2010). The manuscript does not contain clinical studies or patient data and the study was conducted following the code of ethics for animal experimentation with prior approval by the Federal University of Agriculture, Abeokuta, Nigeria Animal Ethics Committee.

Polymerase chain reaction and microsatellite genotyping

The MHC-B region of each extracted DNA was studied using the highly polymorphic microsatellite marker, LEI0258, where CACGCAGCAGAACTTGGTAAGG and AGCTGTGCTCAGTCCTCAGTGC are the forward and reverse primers respectively (McConnell et al., 1999). Amplification of LEI0258 marker was carried out using 0.5 µL each of forward and reverse PCR primers, 20 ng of genomic DNA and 6.3 µL of One Taq Hot Start 2X Master Mix (New England BioLabs) in a final volume of 12.5 µL. Initial denaturation was done at 94 °C for 15 minutes, followed by 35 cycles of 94 °C for

45 seconds, 60 °C for 1 min 30 seconds, and 72 °C for 1 minute with a final extension of 72 °C for 15 minutes (Chazara et al., 2013).

Amplicons were separated on a 4% agarose gel containing ethidium bromide. Ten microlitres of each PCR product was mixed with 2 µL of purple gel loading dye and then loaded in each well. Electrophoresis was carried out at 60 V for 4 hours. The gels were then viewed in a gel documentation system to reveal the amplified fragments and their sizes by comparing them with a 100 bp DNA ladder.

Data Analysis

Alleles were identified by their sizes (bp) and genotyped by scoring against the 100 bp size DNA ladder (i.e. as a standard) using GelAnalyzer, version 2010a (Lazar, 2010). Data generated were subjected to initial analysis in Microsoft Excel.

Other softwares including microsatellite toolkit (Park, 2001) and GenAlex (Peakall and Smouse, 2012) were used to determine total number of alleles (*Na*), allele frequencies, effective number of allele (*Ne*), observed heterozygosity (*Ho*), expected heterozygosity (*He*) and the polymorphic information content (*PIC*) for each population at the LEI0258 locus. B-haplotype(s) of each bird was determined by comparing its allele sizes with the allele sizes of well-defined haplotypes (Fulton et al., 2006). The input files for FSTAT and Arlequin were generated from these softwares for further analysis.

FSTAT 2.9.3.2 (Goudet, 2002) was used to determine allelic richness (i.e. the corrected mean allele number in the standardized sample size, *AR*) and Wright's *F*-statistics [i.e. inbreeding coefficient for the total population (F_{IT}), inter-population genetic differentiation (F_{ST}) and within-population inbreeding coefficient (F_{IS})]. Analysis of molecular variance was determined using Arlequin 3.5.1.3 according to the procedure of Weir and Cockerham (1984). Gene flow (N_m) was also calculated using the equation below:

$$Nm = \frac{0.25(1 - FST)}{FST}$$

The genetic relationship among populations was estimated by constructing a dendrogram based Nei's D_A genetic distances (Nei, 1972) using UPGMA method implemented in POPTREEW (web version) with 1000 bootstraps (Takezaki et al., 2014). The dendrogram was rooted using an exotic population (Anak Titan) as an out-group population.

RESULTS

A total number of 46 distinct alleles were discovered, with allele sizes ranging from 124 to 482 base pairs (Table 1). From the 46 alleles present in the three populations, a total of 55 genotypes were generated, and approximately 76% of the entire population was heterozygous at the LEI0258 locus. Allele sizes and their corresponding frequencies across and within the three chicken populations are shown in Table 2. The most occurring allele was 206, occurring 13 times across populations and representing 10% of the entire number of alleles.

A summary of the diversity parameters is shown in Table 3. The effective numbers of alleles (Ne) for each population were 12.308 for Normal feather, 21.511 for Frizzle feather, 15.474 for Naked neck, and 24.744 for the overall population. For allelic richness (AR), the Frizzle feather population with an AR value of 27.149, possessed the greatest number of alleles compared to the Normal feather and Naked neck populations which had AR values of 20.000 and 21.423, respectively.

Within the populations, expected heterozygosity (He) ranged from 0.919 to 0.954 with the highest observed in the Frizzle feather population. All He values were lower than the observed heterozygosity (Ho) within each population while values of 0.966 and 0.762 for the He and Ho, respectively, were observed across the populations. Marker LEI0258, used in this study was discovered to be highly informative, having a PIC value above 0.900 within each population and overall population.

As shown in Table 1, the genetic differentiation of the three populations taken together (F_{IT}), among populations genetic differentiation (F_{ST}) and the within-population inbreeding coefficient (F_{IS}) values were 0.207, 0.023 and 0.189, respectively. The gene flow (N_m) value of 10.620 clearly revealed that there has been transfer of genetic materials at the MHC region among the three subpopulations.

Table 4 shows the Analysis of Molecular Variance (AMOVA) among and within the Nigerian local chicken populations. This revealed that 0.5% of the total variation can be explained by the variation among the populations while the variations among individuals within populations and those between individuals explained 21% and 79% of the total variation, respectively.

Figure 1 shows a dendrogram revealing the relationship among the three local chicken populations, using an exotic population (Anak Titan) as an out group. The dendrogram is based on Nei's D_A genetic distances, in Table 5, using UPGMA method with 1 000 bootstraps. As expected, it was revealed that the local chickens are mutually closely related but distant to the exotic birds (Anak Titan). However, among the local chicken populations, the dendrogram showed that the Normal and Frizzle feather populations formed a cluster revealing some level of closer genetic relatedness between these two populations than with the Naked neck.

Table 1. Allelic pattern, gene flow and *F*-statistics across populations for LEI0258 locus

Locus	Chromosome	N_A	ASR	N_m	F_{IS}	F_{IT}	F_{ST}
LEI0258	16	46	124 – 482	10.620	0.189	0.207	0.023

N_A, total number of allele; ASR, allele size range; N_m,gene flow; F_{IS},within population inbreeding; F_{IT}, total inbreeding; F_{ST}, among population genetic differentiation.

Table 2. LEI0258 allele sizes and frequencies across and within the Nigerian local chicken populations

	Within population frequency (%)						Within population frequency (%)			
Allele size (bp)	Allele count	Frequency (%)	NF	FF	NN	Allele size (bp)	Allele count	Frequency (%)	NF	FF	NN
124	1	0.79	2.50	-	-	262	1	0.79	2.50	-	-
128	5	3.97	2.50		9.52	270	1	0.79	-	-	2.38
140	5	3.97	-	2.27	9.52	276	3	2.38	-	4.55	2.38
146	1	0.79	-	-	2.38	278	10	7.94	7.50	9.09	7.14
148	1	0.79	-	2.27	-	292	2	1.59	5.00	-	-
150	2	1.59	-	2.27	2.38	304	1	0.79	-	2.27	-
152	2	1.59	2.50	2.27	-	306	2	1.59	-	-	4.76
154	2	1.59	-	-	4.76	312	1	0.79	2.50	-	-
156	1	0.79	-	2.27	-	318	5	3.97	2.50	6.82	2.38
158	2	1.59	2.50	2.27	-	320	1	0.79	-	2.27	-
162	3	2.38	5.00	-	2.38	346	2	1.59	-	-	4.76
168	1	0.79	-	-	2.38	348	1	0.79	-	2.27	-
170	2	1.59	-	4.55	-	356	4	3.17	2.50	2.27	4.76
174	1	0.79	-	-	2.38	360	4	3.17	5.00	4.55	-
188	1	0.79	-	2.27	-	392	6	4.76	5.00	6.82	2.38
194	2	1.59	-	4.55	-	416	1	0.79	-	-	2.38
198	4	3.17	5.00	2.27	2.38	418	3	2.38	5.00	2.27	-
204	1	0.79	-	2.27	-	444	1	0.79	-	2.27	-
206	13	10.32	20.00	2.27	9.52	470	1	0.79	2.50	-	-
210	2	1.59	5.00	-	-	474	1	0.79	-	2.27	-
220	4	3.17	5.00	2.27	2.38	482	1	0.79	-	2.27	-
230	5	3.97	-	-	11.90	252	2	1.59	-	4.55	-
234	1	0.79	-	2.27	-	258	10	7.94	10.00	9.09	4.56

Frequency (%), percentage of each allele in the entire population; NF, percentage of corresponding allele size present in Normal Feather population only; FF, percentage of corresponding allele size present in Frizzle Feather population only; NN, percentage of corresponding allele size present in Naked Neck population only.

Table 3. Genetic diversity indices for the three Nigerian local chicken populations at the LEI0258 locus of the MHC-B complex

Population	N	N_A	Ne	AR	UA	He	Ho	G	PIC	F_{IS}
NF	22	20	12.308	20.000	6	0.919	0.700	0.949	0.914	0.262
FF	22	29	21.511	27.149	14	0.954	0.864	0.978	0.952	0.117
NN	21	22	15.474	21.423	9	0.935	0.714	0.964	0.932	0.259
Overall	65	46	24.055	24.744	-	0.966	0.762	0.968	0.957	0.210

NF, Normal feather; FF, Frizzle feather; NN, Naked neck; N, sample size; N_A, number of alleles; Ne, effective number of alleles; AR, allelic richness; UA, number of unique alleles; He, expected heterozygosity; Ho, observed heterozygosity; G, gene diversity; PIC, polymorphic information content; F_{IS}, inbreeding coefficient

Table 4. Analysis of molecular variance of LEI0258 marker across the Nigerian chicken populations

Sources of variation	d.f.	SS	MSS	VC	Percentage of variation (%)
Among populations	2	1.386	0.693	0.003	0.54
Among individuals within populations	60	34.995	0.583	0.101	20.87
Between individuals	63	24.000	0.381	0.381	78.60
Total	125	60.381		0.485	

d.f., degrees of freedom; SS, sum of square; MSS, mean sum of square; VC, variance component.

Table 5. Nei's D_A genetic identity (above diagonal) and genetic distance (below diagonal) among Nigerian local chicken populations

	NF	FF	NN
NF		0.536	0.526
FF	0.623		0.405
NN	0.643	0.904	

NF, Normal feather; FF, Frizzle feathes; NN, Naked neck.

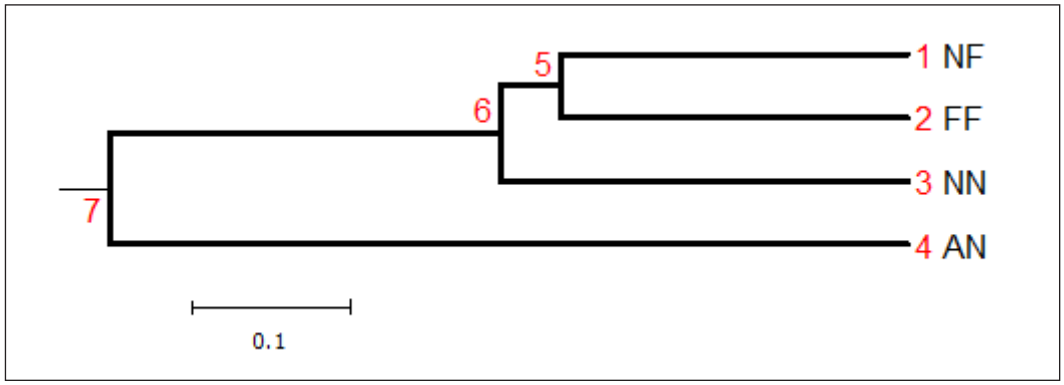

Figure 1. Dendrogram based on Nei's (D_A) genetic distance using UPGMA method
NF, Normal feather; FF, Frizzle feather; NN, naked neck; AN, Anak Titan.

DISCUSSION

The LEI0258 allele size range of 124 to 482 bp found across the Nigerian local chickens differs from those found in other African chicken populations such as 188 to 448 bp found by Ncube et al. (2014) in South African, Malawian and Zimbabwe village chicken populations, 194 to 550 bp reported by Ngeno et al. (2014) in Kenyan local chicken populations, and 166 to 494 bp in Cameroonian native chicken populations (Hako Touku et al., 2015). Allele 206 with frequency of 10.32% and alleles 258 and 278, having the same frequency of 7.94%, were the alleles frequently found across the populations, which probably means that individuals bearing these alleles possess high fitness or survival advantage as reported by Kuhnlein et al. (1997).

Within each population and across populations, high level of heterozygosity values was observed which were slightly lower than the expected heterozygosity values. This observation was the same with most African chicken populations except for some Kenyan local populations; Siaya and Lamu (Ngeno et al., 2014), those studied by Izadi et al. (2011) and some of the populations studied by Chazara et al. (2013).

Since microsatellite markers are regarded as informative when their PIC value is greater than or equal to 0.5 (Botstein et al., 1980; Chatterjee et al., 2008), the within and across populations PIC values which are all above 0.9 showed that the LEI0258 marker is highly informative for revealing genetic differentiation within and among the Nigerian local chicken populations. The N_m value of 10.620 derived in these populations revealed a significant level of gene flow among the Nigerian chicken populations; similar result was observed when other microsatellite markers from non-functional DNA regions were used to study gene flow among Nigerian chicken populations (Olowofeso et al. 2016).

The result on inbreeding coefficient (F_{IS}) across the Nigerian local chicken populations showed that some level of inbreeding has been ongoing among the populations, which confirms the same results observed by 8 out of 9 neutral markers used by Olowofeso et al. (2016). The inbreeding result observed among these populations was also justified by the gene flow value derived in this study; this might be as a result of genetic intermixing between populations

due to human migrations and exchange through trade as reported by Adebambo et al. (2010). In addition, the AMOVA results revealed that a large proportion of the total variation in these three populations was due to variation between individuals (78.593 %) whereas variation among the populations accounted for less than 1% of the variation in the entire population, thereby agreeing with the above observations that there was little genetic differentiation among the Nigerian local chicken populations. These low and high genetic variances observed among populations and between individuals, respectively, were also observed when other chicken populations were studied at the LEI0258 locus (Hako Touku et al., 2015).

As expected, the exotic population (used as an outgroup) stands out in a cluster as a distinct strain in the phylogenetic tree. Among the three Nigerian local chicken populations, Normal feather and Frizzle feather populations were found in the same sub-clades showing that they share common recent ancestor. In other words, the Normal feather and Frizzle feather populations were probably closely related to each other than the Naked Neck population which was found in a separate sub-clade. Interestingly, this result supported reports from previous studies that used different microsatellite markers (Ohwojakpor et al., 2012) and biochemical markers (Adeleke et al., 2011).

CONCLUSION

In conclusion, this study was able to confirm the genetic diversity previously observed in Nigerian local chicken population by neutral and biochemical markers using functional marker LEI0258. Out of all the alleles found in Nigerian chicken populations, it was only allele 474 that has been allocated to the well defined B-haplotypes such as B12.2 and B71 (Fulton et al., 2006). This allele, which was found only in the Frizzle feather population, and can further be discriminated to a specific B-haplotype using additional marker MCW0371 or sequence information. Alleles 206, 258 and 278, which were mostly found in the population, should be further studied for disease resistance in order to validate their fitness in the Nigerian local chicken populations.

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