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Genetic diversity and selection signatures within the major histocompatibility complex of local chicken ecotypes in Kenya

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Abstract

Diseases remain a major challenge in chicken population leading to reduced performance and economic losses. Whenever possible, chicken population should be reared under production systems with minimum predisposing factors to diseases and other shortcomings during their productive lifetime. Selection for resistance is an attractive option when responding to the diseases challenge. The chicken major histocompatibility complex (MHC) is a highly polymorphic region associated with immune response and disease resistance. The aim of this study was to investigate genetic diversity and as well as identify signatures of selection within the MHC, which help local chicken ecotypes to withstand disease and environmental challenges. A total of eight local chicken ecotype populations from different ecological zones in Kenya and two commercial layers (CL) chicken populations were assessed using single nucleotide polymorphisms (SNPs). Principal component analysis (PCA) showed that the two populations, local chicken ecotypes and commercial chickens were distinct. Expected heterozygosity ranged from 0.21 to 0.28 in local chicken ecotypes and was 0.26 in commercial layers. As expected, inbreeding was much higher in commercial chicken layers at 0.51 - 0.64 than indigenous chickens at 0.04 - 0.27. Selection signature analysis using fixation index (FST) detected two major regions of divergence in commercial and indigenous chickens, within the MHC region mapping to seven genes: KIFC1, ZNF 692, TRIM 7, TRIM 7.2, TRIM 39.2, BLEC 3 and YLEC 1, in local chicken ecotypes which are associated with immune response and disease resistance. This result shows that local chicken ecotypes possess significant genetic diversity within the MHC region, which can be exploited to improve the chicken breeds for disease resistance. However, a comprehensive study using a larger sample size is needed to provide more insight on the viability of the MHC region.

Keywords: *disease resistance; immune response; local chicken ecotypes; major histocompatibility complex; selection signatures*

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Introduction

Local chicken ecotypes play a significant role to smallholder farmers' livelihood, as they provide nutrients to families. They provide a cheap and reliable source of protein in the human diet as over 75% of the rural households keep them (Moges, 2009). The local chicken ecotypes are usually spread under varied agro-ecological and physical environments in Africa (Hogerwerf *et al.,* 2010). They are mainly kept as a source of revenue, food (meat and eggs), social, and cultural roles within the various Kenyan communities. Despite the low output obtained from local chicken ecotypes, characteristics such as disease tolerance/resistance make them more attractive to farmers in rural areas (Besbes, 2009). The foundation of each ecotype is the product of mutation, genetic drift, adaptation, and evolution with differing selection pressures imposed by environment, endemic parasites, and diseases, available diets and selection standards imposed by human. The ecotypes have developed adaptive capacities and can thus utilize the local available feedstuff and kitchen wastes within the reach of the local poultry farmers to yield affordable quality animal protein (Magothe *et al.,* 2012). The chicken ecotypes are reared in a variety of production systems characterized by differences in technical knowhow of the farmer. The key aspect that determines the system is whether the chicken is reared for subsistence or commercial purposes. Intensive production systems confine the chicken entirely where feed and water are offered, semi-intensive system partially confine the birds while the free-range system have their chicken scavenging with provision of night shelters only. Where night shelters are not provided, the chicken would perch on high areas and structures.

The local chicken ecotypes are mainly kept in extensive production system implying that they are not confined in pens besides being provided with inadequate feeds. They are exposed to infections with partial disease control measures being applied in the system (Mpenda *et al.,* 2019). The extensive production system in which the ecotypes are kept are disadvantaged by increased mortality rates because of infectious diseases that ultimately would cause production (eggs and meat) to be constrained (Moges *et al.,* 2010). High fatalities have been observed in chickens kept under unrestricted conditions due to bacterial and virus-related infections such as fowl typhoid, Mareks disease virus (MDV), Newcastle disease and infectious bursal disease (IBD) that they pick/get exposed to when they roam up and about the environment they are raised. Most of the farmers are incapable of controlling infections as they do not have the capability to implement biosecurity due to free ranging nature of production system and the constraining resources to buy vaccines (Alders *et al.,* 2018).

The major histocompatibility complex (MHC) is a gene region possessed by all higher vertebrate species. The MHC, B complex consists of several clusters of highly polymorphic genes associated with disease resistance (Lamont, 1998) and immune response (Juul-Madsen *et al.,* 2002; Nikbakht & Esmailnejad, 2015). Genetic diversity within the MHC of local chicken ecotypes is extensive and may provide a basis for breeding chickens that are adapted to various disease challenges (Mpenda *et al.,* 2019). The chicken immune system is divided into two: non-specific and specific immune mechanisms. Non-specific immune mechanisms include innate or inherent ways chicken resist disease (Butcher & Miles, 2015), while specific mechanisms are composed of specialized cells and processes that eliminate pathogens or prevent their growth. Specific immunity has two aspects of immunity that is, humoral carried out by antibodies and cell mediated immunity (Erf, 1995). Non-specific immunity comprises a broad spectrum of defense mechanisms, which include physical, and biochemical barriers that prevent invasion of both infectious and non-infectious agents (Erf, 1995). These include features such as body temperature, anatomic features, normal microflora, respiratory tract cilia and genetic factors (Butcher & Miles, 2015). Other factors may include age, nutrition, compliment, interferon and the environment as described by Butcher & Miles (2015). The specific immunity is mainly acquired and characterized by specificity, heterogeneity, and memory (Butcher & Miles, 2015).

Populations with genetic diversity can be safeguarded against infectous diseases and those with a higher variation are likely to be more torelant to diseases than similar populations. Unlike commercial chickens, local chicken ecotypes have not been subjected to rigorous artificial selection, but are exposed to harsh environmental conditions with predisposure to diseases. This implies that they may habour genes responsible for disease resistance within the MHC. Its well known that local chicken ecotypes are genetically diverse (Mpenda *et al.,* 2019). However, little information is available about genetic diversity within the MHC which may confer disease resistance to these local ecotypes. Most studies on genetic diversity within the MHC of local chicken ecotypes in Africa uses microsatellite markers and mitochondrial DNA loop region which has resulted in limited information in disease resistance (Mpenda *et al.,* 2019). Single nucleotide polymorphisms (SNPs) have been used in this study to decipher how genomic variations are associated with resistance within the MHC. SNP genotyping has made it possible to evaluate/examine genomic regions exhibiting deviations from neutrality (Onzima *et al.,* 2018). Admixture events, population bottlenecks and migration forces may have a profound effect on genetic variability which may reduce or increase variation.

The method used to detect selection signatures in this study was the fixation index (F_{ST}) which has been useful to differentiate between populations. F_{ST} is popular in detecting selection signatures of multiple populations. Barreiro *et al.,* (2008) illustrated that local positive selection tends to increase F_{ST} while negative selection tends to decrease the parameter. The main advantage of F_{ST} is that it's able to reveal actual variants under selection (Gholami, 2014). Therefore, this study was aimed at assessing diversity within the MHC of local chicken ecotypes and selection signatures. The results would be the first step in developing a criterion for resistance to diseases in local chicken ecotypes breeding plan.

Ethics statement

The DNA from chicken used in this study was part of a previous study that adhered to the ethics guidelines (Ngeno *et al.,* 2014). Therefore, no ethical statement was required.

Materials and methods

Sampling procedure

A total of eight (8) chicken ecotypes representing 8 different populations of locally reared chicken ecotypes were obtained from different geographical regions in Kenya: Kakamega (KK) and Bondo-Siaya (BN) in the Western region, West Pokot (WP) and Turkana (TK) in the North Rift region, Bomet (BM) and Narok (NR) in the South Rift region, Lamu (LM) and Taita Taveta (TT) in the Coastal region. Commercial exotic layers (CL) sequences which were unrelated were included in the study (source of sequences was INCIP).

DNA extraction and PCR amplification of LEI0258 and MCW0371

Genomic DNA was extracted using the standard phenol-chloroform method (Ngeno *et al.,* 2014). LEI0258 and MCW0371 were determined using PCR as described by Fulton *et al.,* (2006). These markers are located within the MHC region on chromosome 16 between the BG and BF region making them good candidates for MHC investigation. The markers LEI0258 and MCW0371 were examined together. SNP alignment and discovery pipeline was performed according to Altmann *et al.,* (2012). A total of 11,788 SNPs were obtained which upon further filtering, using PLINK software (Purcell *et al.,* 2018), 11,223 SNPs remained.

Genetic diversity

Genetic diversity was determined by computing observed heterozygosity and inbreeding coefficients using PLINK (Purcell, 2018). The observed heterozygosity estimates per population was computed from observed genotype frequencies obtained from PLINK (Purcell, 2018) as follows: (N-O)/N (where N is number of non-missing genotypes and O is number of observed homozygous genotypes for each given individual).the inbreeding coefficient (F) was computed by utilizing PLINK based on observed

$F= f_i+(1- f_i)(p2-q2)$

Where f_i is the probability of individual I being homozygous by descent, 1- fi is homozygous by

chance for a specific SNP with known allele frequencies p and q (Purcell, 2018)

PCA structure was used to describe the genetic structure of local chicken ecotypes. Filtered SNPs were converted to VCF formats using PLINK, whereby phylogenetic trees were constructed using MEGA software (Tamura *et al.,* 2018) with bootstraps of 1000 replicates.

Selection signatures

The local chicken ecotypes and the CL were grouped as two different populations in analyzing the selection signatures. The analysis was performed as described by Porto-neto *et al.,* (2013) whereby F_{ST} statistics were calculated between the local chicken and exotic chicken populations. Genomic regions with SNPs of a high proportion were identified and analysed further. Estimation of SNP F_{ST} was based on a pure drift method as described by Nicholson *et al.,* (2002). Individual SNPs were grouped within genomic windows of two, five and 10 SNPs. A window of five was used utilising the kernell regression method (Brito *et al.,* 2017). Analysis was performed using R software. The F_{ST} values were smoothed across a chicken reference genome assembly Galgal 4 using a local variable band width (R package lockern). Identification of the top and bottom 2.5% SNPs within smoothed fixation index (sF_{ST}) qualified as putative selection regions. The package ggplot2 in R was used to display the smooth F_{ST} graphs.

Functional annotation

Functional annotation of genomic variants detected in signatures of selection anaysis were determined using variant effect predictor (VEP 87) (McLaren *et al.,* 2016). SIFT prediction was used to predict substitutions of amino acids which had an effect on protein function using annotations found in Ensemble 75. BED Tools v 2.17.0 (Quinlan & Hall, 2010) was used to intersect regions high in smothed fixation index (sF_{ST}) within the VEP file. Misense variants were extracted and their associated genes analysed for gene ontology.

Results

Expected heterozygosity (He) ranged from 0.215-0.288 for local chicken ecotypes and exotic layers (Table 1). The highest heterozygosity was observed from the Taita Taveta (TT) ecotypes at 0.288, followed by Bondo (BN) and Turkana (TK) ecotypes where both were at 0.281. Some level of inbreeding was observed within the MHC. It was higher in exotic chicken and ranged from 0.513-0.638 than in the local chicken ecotypes which ranged from 0.017 for the TK ecotype to 0.269 for the Bomet (BM) ecotype (Table 1).

Table 1. Expected heterozygosity and inbreeding of local chicken ecotypes and commercial exotic chicken layers

Population	Inbreeding	Expected heterozygosity
CL1	0.638	0.255
CL2	0.513	0.263
BM	0.269	0.261
KK	0.042	0.215
LM	0.051	0.258
NR	0.043	0.273
BN	0.111	0.281
TK	0.071	0.281
TT	0.121	0.288
WP	0.017	0.278

Genetic *structure within the MHC ecotypes*

Genetic structure was inferred using PCA that accounted for 15.58% PCA 2 and 16.75% PCA 1

(Figure 1) and accounted for 25% the total variation. PCA clustered the eight ecotypes into four (4) groups namely LM and NR in the first group, exotic chicken (CL1 and CL2) and BM ecotype in the second group while KK, BN, TK, TT clustered in the third group and WP clustered into the fourth group.

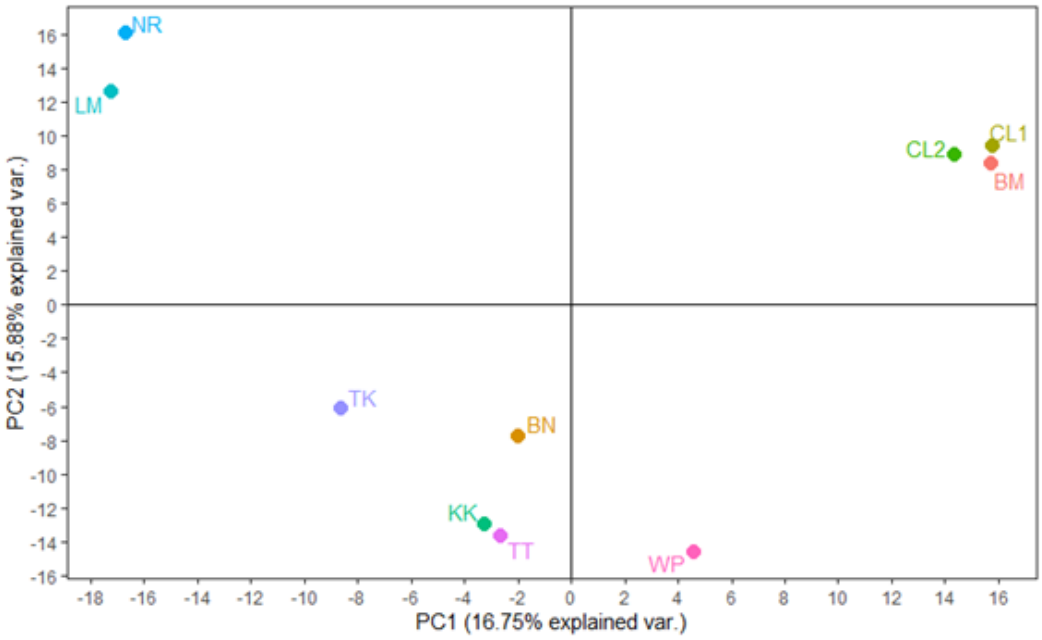

Figure 1. PCA showing clustering of the studied population (eight Kenya local chicken ecotypes and two exotic commercial layers). Each coloured point represents an ecotype

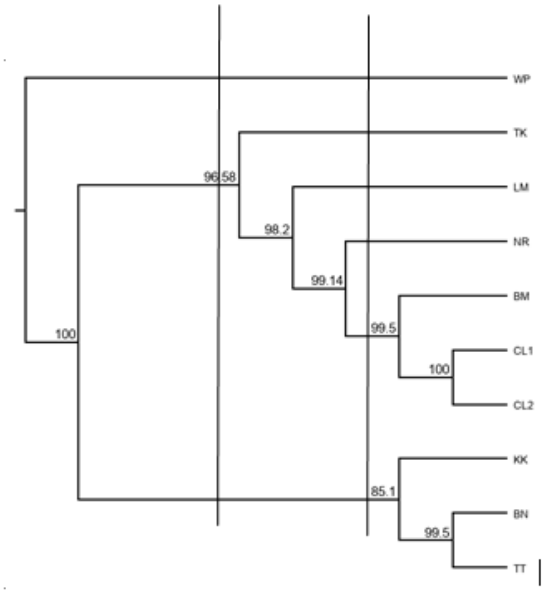

Figure 2. The neighbour joining tree of eight (8) local chicken ecotypes and two (2) commercial chicken layers. Numbers along the branches represent bootstrap values (1000) replicates

phylogenetic analysis

The phylogenetic tree of the studied populations formed three clusters. Bootstrap values ranged from 85-99.5. Cluster one was composed of WP. Cluster two had BN, KK, and TT. Cluster three (3) was composed of LM, NR, TK, BM, CL1 and CL2 while WP and TK were on their own clusters which were independent of each other.

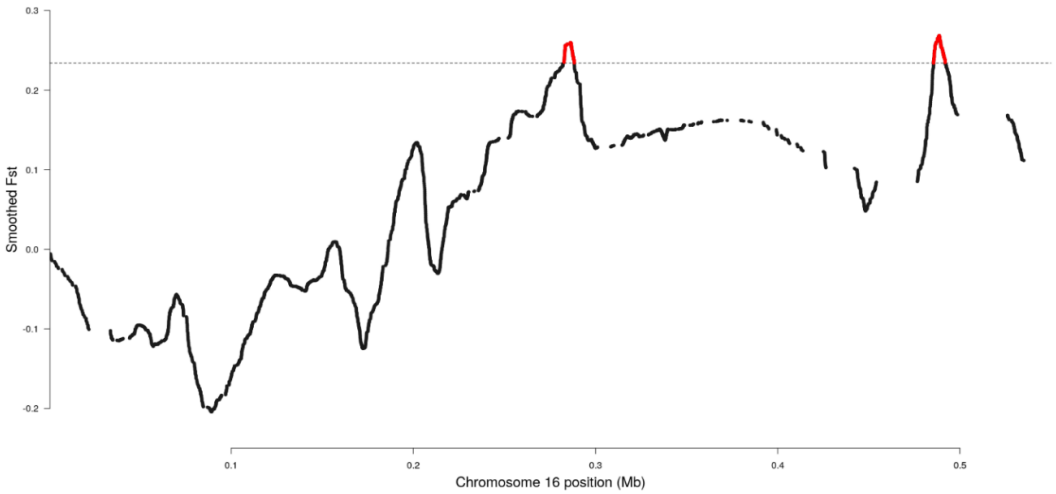

Figure 3. Smoothed F_{ST} comparing local chicken ecotypes and exotic commercial chicken layers. The top 2.5% are highlighted in red corresponding to the regions under selection while the lower 2.5% are not

Signatures of selection

The F_{ST} analysis of local chicken ecotypes and the CL exhibited peaks and troughs in chromosome 16 at position 0.3 and 0.5 respectively (Figure 3). These regions in local chicken ecotypes contained a number of genes namely *TRIM 7.2, TRIM 39.2, ZNF 692, TRIM 7, KIFC1, BLEC 3* and *YLEC 3*. The RNA *gga mir 6566* was also found in this region.

Discussion

Genetic diversity within the MHC region

To date there is little evidence that a concrete evaluation of diversity within the MHC in local chicken ecotypes has been carried out in Kenya (Khobondo, 2018). We hypothesized that local chicken ecotypes would exhibit genetic diversity within the MHC (Ngeno *et al.,* 2014). The birds used in this study were sampled from different geographical regions of Kenya and were exposed to diverse pathogenic environments. Genetic variation measured in-terms of expected heterozygosity was greater within the MHC of all local chicken ecotypes when compared to the commercial chicken layers. The high values of the expected heterozygosity for the local ecotypes can be attributed to long term natural selection and historic mix of strains as described in an earlier study by Okumu *et al.,* (2017) and possibly because of the difference in management systems with chicken from the free range management system exhibiting a greater genetic diversity when compared to the exotic commercial lines (Granevite *et al.,* 2007). The high levels of exhibited inbred lines among the commercial layers is mainly due to the intense inbreeding levels that are observed within the MHC attributable to the tight selection schedules that are undertaken by breeders in pursuit of high egg production from the crop of offspring raised (Burt, 2005).

Genetic clustering is important in identifying whether a whole population is treated as a single population or whether it should be subdivided. Using SNPs, local chicken ecotypes were clustered into three (3) groups. The BN and KK clustered together probably because of their geographic closeness, which has made it easy for interactions of chicken ecotypes from the two regions thus sharing common environmental and disease pressure (Westerdahl, 2007). The Taita Taveta population cluster could be related to BN

and KK due to common breed source during the Cockerel Improvement Program introduced in Kenya in 1976 as indicated by Ngeno *et al.,* (2014). It can also be that these regions could at one point be exposed to specific diseases, which may have influenced their MHC diversity (Jeffery & Bangham, 2000; Mwacharo *et al.,* 2013; Lyimo *et al.,* 2013). Further, the prolonged exposure of the local ecotypes to the commercial hybrids may have led to genetic introgression within the MHC of the local ecotypes (Okumu *et al.,* 2017). Previous studies have indicated South East Asia and South Asia as the source of chicken in Kenya whose entry points were coastal regions (Mwacharo *et al.,* 2011). The WP ecotype cluster on its own on the PCA and phylogenetic tree since it is different within the ecological zones thus having divergent disease pressures than from neighboring counties. The people inhabiting this region have minimal interaction with neighboring (regions) communities due to cultural differences, harsh terrain and poor road network which can lead to reduced gene flow hence resulting to genetic uniqueness of their chicken (Lyimo *et al.,* 2014; Chen *et al.,* 2008). The rest of the ecotypes CL1, CL2, LM, NR, TK and BM clustered together. This may be due to improved pullet/cockerel exchange program in the 1950s (Ngeno *et al.,* 2014). These pullets and cockerels originated from Asia and Europe, thus both commercial layer and local chicken ecotypes obtained genetic material from them (Lymo *et al.,* 2014). Clustering and sub clustering of local chicken ecotypes can be attributed to inbreeding as described earlier by Okumu *et al.,* (2017), and exchange of MHC genes sharing ancestral origins. In addition to inbreeding, the overlap may result from unrestricted mating of chickens domiciled from diverse backgrounds, making them share a common ancestry (Walugembe *et al.,* 2019). Chickens were sampled from different geographical locations with some areas being far off. However, the phylogenetic tree does not show distinct grouping with respect to geographical proximity. Clustering and sub clustering can also be attributed to human migration and international trade especially in the East Africa coast (Fuller *et al.,* 2011). The Indian Ocean trading networks led to maritime and terrestrial transfers of domestic and non-domestic animal and plant material among them chicken (Fuller *et al.,* 2011, Mwacharo *et al.,* 2013).

Phylogenetic analysis

The phylogenetic tree derived during this study showed 8 chicken populations that clustered into 5 major clusters that would have individuals from mixed clusters of the ecotypes. This illustrated a shared common ancestry among the ecotypes that were considered in the study. The mixed clustering and relatedness would be attributable to the government-initiated exchange program in the year 1976. The program was aimed at improving the local chicken breeds by crossing them with exotic cocks (Okumu *et al.,* 2017). The mixed populations could also be due to the interaction of the poultry farmers from various regions of the country through trade, agricultural shows, use of chicken as gift or as dowry and displacements among other factors as this would facilitate genetic likeness (Mwacharo *et al.,* 2007). The NR's ecotypes were further from the ecotypes in the cluster two. This uniqueness in NR could be attributed to disease and worm resistance that has been built by the local ecotypes to the environment they are raised. Kaingu *et al.,* (2010), noted a habitual use of herbs while treating sick and worm-infested chicken. This use of herbs by the local inhabitants of Narok County of Kenya could facilitate their resistance and adaptation to diseases and parasites resistance and should be studied so as to provide information which would facilitate their conservation and utilization.

The LM's ecotypes notably clustered on their own which is possibly due to isolation of the Indian Ocean's population since the county forms an island. Such barrier would limit genetic interaction of such ecotype with the other chicken populations. This predisposes them to other disease pressure when compared to those in the mainland. This makes them genetically unique because of this geographical, physical, and economic isolation when compared to rest of the population in the country (Ngeno *et al.,* 2014).

The ecotypes from BM were noted to cluster closely to the CL1 and CL2 that were exotic. This is possibly because of the initial effects of the Kenyan government-initiated program that brought in genes that mixed with the local types causing genetic distancing from the rest of the chicken flock of the counties under study. Studies

to further outline the main cause of the clustering should be undertaken.

Selection signatures

Selection signatures of both local chicken ecotypes and exotic layers presented an opportunity to understand historical selection and genes of the two populations. The identification of selection signatures aided in identifying genomic regions within the MHC under artificial and natural selection. These regions would be important to enhance ecotype survival across regions despite exposure to prevailing disease and harsh environmental conditions (Walugembe *et al.,* 2019). The distributed F_{ST} values in chromosome 16 which showed two peaks where local chicken ecotypes diverged from exotic layer chicken. The local chicken ecotypes used in this study were from different geographical locations characterized by a high disease incidences (Ngeno, 2015) and endoparasites (Kaingu *et al.,* 2010) which may have played a role in determining selection signatures. Natural selection may have played a role on local chicken ecotypes to withstand diseases under harsh conditions. We identified a region under selection pressure within the local chicken ecotypes MHC region containing a number of genes that has been linked to immune response and tolerance/resistance to disease, namely *TRIM 7.2, TRIM 39.2, ZNF 692, TRIM 7, KIFC1, BLEC 3* and *YLEC 3*. These selection patterns are highly amplified due to selection of the desired traits by farmers from the study areas that focused on improved mothering ability, survival traits and dual purpose (meat and eggs) while for the international breeding companies, their concern is majorly on meat or milk (Ngeno *et al.,* 2014).

Functional annotation of genomic variants detected in signatures of selection anaysis

Under normal conditions, local chicken ecotypes are kept in low management conditions and highly predisposed to harsh environment of low feed, diseases, and parasites. In order for them to survive, selection candidate genes with potential for survival are exhibited to have been harboured within the MHC. The *TRIM 7.2* genes are associated with a molecular function of zinc iron binding (Chaves *et al.,* 2009). This gene binds to specific ligands within the MHC genome to perform catalytic, regulatory, and structural functions. Zinc is an important nutrient in poultry nutrition that plays a major role in antioxidation, growth and development, production, immunity and stress related issues (Naz *et al.,* 2016). The *TRIM 7* genes within the MHC are involved in initiation of glycogen synthesis as described by Skurat *et al.,* (2002) especially in day old chicks thus providing them with energy to withstand opportunistic diseases. The molecular function of *TRIM 39.2* gene is reported as a metal binding ligand (Mcconn *et al.,* 2014). *ZNF 692* was found to have a gene ontology (GO) function as a DNA binding transcription repressor activity and specific to RNA polymerase II (Jorge *et al.,* 2010). The *ZNF 692* is able to depress or decrease transcription. It is also involved in RNA polymerase II proximal promoter sequence-specific DNA binding and metal iron binding. Metal iron binding proteins are deemed to be important as they function by protecting enteric parasites from obtaining iron via oxidative stress and regulates iron absorption by pathogens (Drahansky *et al.,* 2016).

The roles of *KIFC1* gene were found to be microtubule binding, and microtubule motor activity whereby the gene is involved in moving proteins along microtubules (Hermo *et al.,* 2010). The gene is also involved in binding of ATP thus transports a diverse set of substrates across membranes hence playing an important role in protecting chicken from diverse environments (Liu *et al.,* 2013). The *BLEC 3* genes are an integral component of the membrane whose GO function is acting as a natural killer cell that is calcium dependent. The gene interacts with MHC class I molecules to either inhibit or activate cytotoxicity and cytokine reaction (Holmes *et al.,* 2014). The *YLEC 3* has not been characterized yet but an important component of the membrane. More work should be done to establish in details the function of the *YLEC 3* gene.

The micro RNA *gga-mir-6656* was found to be a protein inhibitor as it prevents expression of genes via translation repressions (Gu & Kay, 2010). While the roles of micro RNA (miRNA) within the MHC of local chicken ecotypes are not well defined they may function as pathways to host diseases such as Mareks disease virus (MDV), infectious larygotracheitis virus (ILA),

and avian leucosis virus (ALV) as described by Yao and Nair, (2014). This would explain the reason why chicken flocks die in huge numbers when predisposed to such infections. The miRNAs have also been found to play an important role in oncogenesis (Zhang *et al.,* 2007). More research targeting miRNAs within the MHC to reveal their roles in immune response or as a pathway to infectious diseases is recommended.

Conclusion

This study findings show that local chicken ecotypes possess a greater genetic diversity within the MHC compared to exotic chicken. Evidently, they have a moderate heterozygosity and a low inbreeding when compared to the exotic chicken. Results also revealed evidence of selection signatures within the MHC region of local chickens which may be important for survival during disease outbreaks. Genes of importance within the MHC were found to be directly or indirectly involved in disease resistance and immune response. The notable relative molecular co-ancestry could as well lead to an increased level of inbreeding especially in the subsequent generations. While improving on local chicken ecotypes, care should be taken to prevent loss of genetic diversity within the MHC. There is a need to implement an effective breeding strategy to prevent loss of genetic diversity within the MHC especially with the help of proper artificial selection even to the local flocks of chicken without compromising traits needed for immune response and disease resistance.

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