GENETIC DIVERSITY OF INDIGENOUS CHICKEN (*Gallus domesticus*) POPULATION IN KENYA

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Student declaration

This thesis is my original work and has not been presented for an award of a degree in any other university.

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Dedication

I dedicate this work to my beloved parents, siblings and researchers who are committed to see an improvement in the livestock production sector in our country and the rest of the world.

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I would like to express my heartfelt gratitude to my supervisors for their commitment in guiding me throughout the whole period of research. I would also like to appreciate the Department of Animal Production staff members, my friends and family for their continuous encouragement to see me through with the program.

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Abbreviations

AMOVA – Analysis of Molecular Variance

AnGR – Animal Genetic Resources

APA – American Poultry Association

CARI- Central Avian Research Institute

cM – centi Morgan

CRD – Chronic Respiratory Disease

DNA – Deoxyribonucleic Acid

EDTA – Ethylene Di-amine Tetra acetic Acid

FAO - Food and Agricultural Organization

GenAlEx – Genetic Analysis in Excel

IFPRI – International Food Policy Research Institute

ISAG – International Society for Animal Genetics

InCIP – Indigenous Chicken Improvement Program

ICGSC – International Chicken Genome Sequencing Consortium

MLFD – Ministry of Livestock and Fisheries Development

MoLD – Ministry of Livestock Development

NCD – Newcastle Disease

NPDP - National Poultry Development Programme

PCoA – Principle Coordinate Analysis

PCR – Polymerase Chain Reaction

PIC – Polymorphic Information Content

RFLP – Restriction Fragment Length Polymorphism

SNP – Single Nucleotide Polymorphism

Abstract

This study was conducted with the aim of analyzing the diversity, relationship and population structure of the local indigenous chicken ecotypes in Kenya at the genetic level. Ecotypes from different regions were compared to identify regions which have been subjected to selection (selection signatures). A total of 384 free-ranging chicken sampled from eight counties in four geograpical regions (Western, North Rift, South Rift and Coast) in Kenya were genotyped using 12 microsatellite markers. Identification of signatures of selection was done using whole genome-resequencing data. The total number of alleles for all codominant data was 140, while the mean number of different alleles (N_A) was 8.094 ± 0.516 and the effective number of alleles (N_E) 4.452 ± 0.297. Observed (H_O) and expected (H_E) heterozygosities were 0.714 ± 0.011 and 0.726 ± 0.009 respectively for the whole population. The fixation indices – effects of sub-populations to the total population (F_{ST}), variance among subpopulations within groups (F_{IS}), and variance among groups relative to total variance (F_{IT}) for all populations were 0.029, 0.066 and 0.093 respectively. A total of 21 private alleles were observed in all populations. Bomet and West-Pokot ecotypes were closely related (0.997) than the rest of the population while the most distantly related were Lamu and Narok (0.779). The studied chicken population showed two clusters at K=8 when analyzed using STRUCTURE software. On identifying selection signatures, a total of 36,026 SNP variants were identified in these chicken populations with 30 Z-transformed outlier values ranging between 5 and 8 all in the 8th chromosome. Ninety nine percent (99%) of the variants were modifiers. Of the total biotype, 50.96 were in the non-coding region, 26.22 in the protein coding region. The consequences were expressed in upstream and downstream regions of the DNA, intron, intron non-coding transcript region and intergenic regions. The study concluded that with this rich genetic diversity, management and conservation measures should be undertaken for sustainable utilization of the indigenous chicken and reduce genetic dilution.

Key words; indigenous chicken, genetic diversity, microsatellite markers, selection signatures

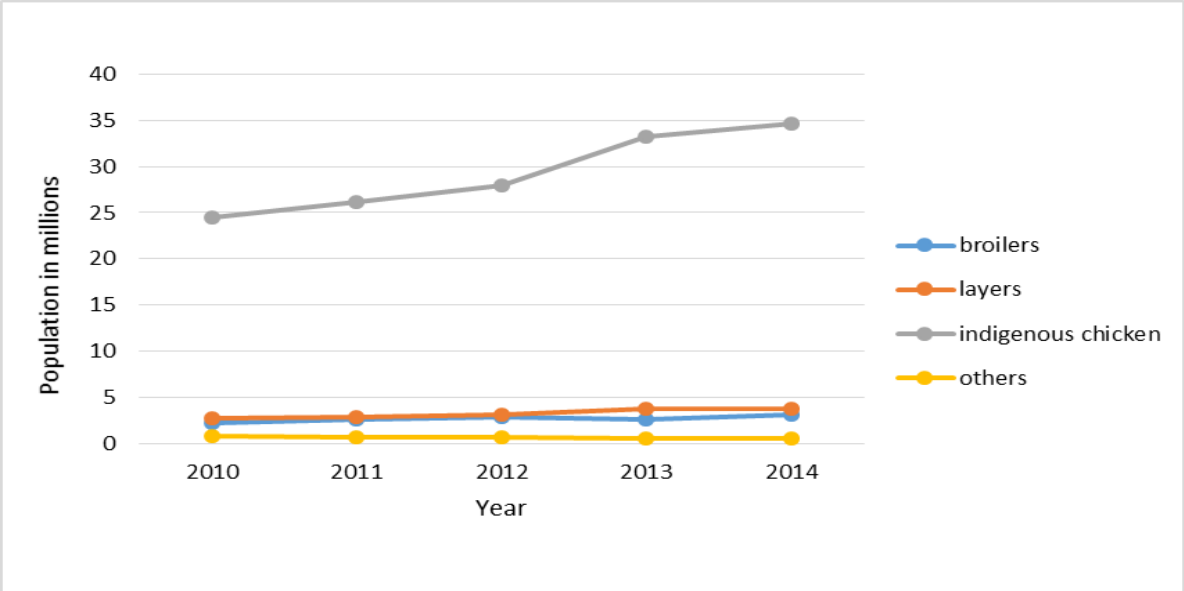
CHAPTER ONE

1.0. INTRODUCTION

1.1. Background information

In the developing countries, indigenous chicken have been used as a source of income, cash reserves, nutrition to the family, for religious ceremonies and celebrations (King'ori *et al.*,2010). Indigenous chicken are hardy, disease resistant, survive with less nutrition levels, require minimum management practices, and can endure severe conditions (Mwacharo *et al*., 2013a). These chickens when free ranging, can feed on materials ranging from waste feeds, cereal residues in farms, insects, weed seeds, and green leaves from crops like beans, kales and from grass among others (Apuno *et al.,* 2011). With these characteristics, they make the best domestic animal species for the low resource households.

In Africa, these indigenous chicken populations exceed 1.6 billion and make up to over 80% of the total chicken population (Hassaballah *et al.*, 2015). Chicken in Kenya totaled up to around 32.5 million in 2014 with the indigenous chickens accounting for the highest number at 25 million (MOLD, 2015). This is 77% of the total chicken population in the country.



Source; MoLD, 2015

Figure 1; Graph showing the trends in chicken populations in Kenya from 2010 to 2014

The number of chicken in the country has been increasing yearly with a notable increase in 2013 and 2014. Indigenous chickens are mainly kept under free ranging systems and others under backyard production system (Omiti *et al.,* 2009; King'ori *et al.,* 2010). The households obtain the breeding stock from friends and neighbors as gifts or from the local market (FAO, 2007). The ratio of cocks to the hens in these households can be roughly 1:7. Approximately, 50% of the total indigenous chicken population is immature (Nyaga, 2007). The indigenous hens can lay about 60 eggs per year and about 40 % of the birds reared are slaughtered annually with an average of 1.5 kilograms per bird (King'ori *et al.,* 2010). Today, households keep indigenous chicken from 5 to about 20 birds for eggs and meat production (Omiti and Okuthe, 2009; Gueye, 1998).

Africa as a continent is believed to have less standardized poultry breeds compared to other regions in the world (FAO, 2007). In Kenya, indigenous chicken have been mainly kept in the rural villages and are adapted to the local conditions and environment (King'ori *et al*., 2010; Khobondo *et al*., 2014). They are fairly distributed in the whole country except in the very arid areas (FAO, 2007). These geographically and agro-ecologically isolated chicken have been subjected to variable local climatic conditions and chicken from different region are believed to be unique (Ng'eno *et al.,* 2014; Zanetti *et al*., 2011; Mogesseh, 2007).

The indigenous chickens exhibit high genetic diversity because they are raised in diversified ecological habitats. The diversity can also be due to subjection to different diets, parasites and diseases (Ng'eno *et al.,* 2014).

1.1.1. Selection signatures

The spread of indigenous chicken in Africa is as a result of trade and exchange of cultural values between communities over the years (Storey *et al.,* 2012). The different population diversity was developed over time as the chickens were selected for the many morphological traits, appearance, as well as production characteristics. Adaptation to the different environmental conditions, breeding practices and geographic isolation resulted in wide variation and differentiation of the chicken breeds (Ng'eno *et al*., 2014).

Artificial selection causes variations within the chicken genome. This results to loss in nucleotide diversity at and near the selected loci. (Muir *et al.*, 2008). Since selection affects specific genomic regions in an organism, identifying these regions in chicken will help in understanding their selection, domestication history and the molecular pathways underlying the phenotypic traits and breeding goals (Elferink *et al.,* 2012). It will also aid in understanding the evolutionary genetics since selection tends to cause specific change in patterns of variation in selected and neutral loci linked to them. Genomic footprints caused by selection can be used as signatures to identify loci under selection (Kreitman, 2000).

Identification of selection signatures provides interests in evolutionary genetics because it gives guidance about how evolution has been shaping genomes to function at different genomic regions (Nielsen, 2005; Schlötterer, 2003). Previously, identification of genomic regions affecting traits of economic and biological importance has been done using Quantitative Trait Loci mapping (Stainton *et al.,* 2014). This approach however has a limitation as it requires extensive phenotypic information (Elferink *et al.,* 2012).

An alternative approach is to use the genomic information to identify regions showing signatures of selection associated with the trait of importance or interest (Rubin *et al.,* 2010, Elferink *et al.,* 2012). This allows pinpointing new selection signatures and distinguishes those related to modern breeding objectives and to earlier post domestication constraints. Response to selection on traits like color, morphology, adaptation to new environments among others can be revealed with identifying the signatures of selection.

1.2. Statement of the problem

Indigenous chicken are an important source of livelihood in the rural set up (King'ori *et al.,*2010). They require low capital, less space to be reared, are adapted to and can survive well under harsh conditions, and can be kept by all social groups in the society (Apuno et al., 2011). The ability to survive in almost all regions in Kenya provide an opportunity to help in improving the welfare of people in Kenya by keeping them.

Over time, the Kenyan human population has been increasing steadily. The increase in number of people has in turn resulted to an increase in demand for protein products which the easy and relatively cheap products are from poultry. Chickens are the main species in the Kenyan poultry industry. With the high demand for chicken products, the government and farmers have been coming up with ways to improve on the productivity of the indigenous chicken. Since the 1960s to date,latest being the introduction of Kuroiler breeds from India, attempts are being made to substitute breeds or to crossbreed to improve on indigenous chicken productivity (Ng'eno *et al*., 2014).

Indiscriminate crossbreeding within and between ecotypes and with exotic breeds and artificial selection widely practiced by farmers (Mwacharo *et al*., 2007). This leads to dilution of the

indigenous chicken genes. Their high intake and offtake rates promoted by their prolific nature increases the risk of loss of important genes (Khobondo *et al.*, 2015). This goes against the global move to conserve indigenous genetic resources.

Artificial selection of the indigenous chicken for imperative economic traits results in unique signatures of selection on the chicken genome. Accessing the high density genotypes of theses chicken will enable precise identification of the regions that have undergone positive assortment. These findings will aid in the elucidation of selection mechanisms and also identify the candidate genes of significance to breeding programs, which is yet to be done in the country.

Many studies have been done on the morphological attributes of the indigenous chicken in Kenya (Khobondo *et al.,* 2015; Nwachukwu et al., 2006; Ndirangu et al., 1991). The studies revealed a huge variability of the phenotypic traits of these indigenous chicken (Ng'eno et al., 2014). However, there is insufficient information that gives insight into the underlying genetic diversity and population structure of the chickens. There is need to genetically characterize these chickens so as to associate the phenotypic traits to the genetic make-up.

1.3. Justification

Africa is among the least in poultry keeping globally at 8.08% of the total world poultry population. Kenya is not left as it one of the African countries (Ng'eno *et al*., 2014). The Kenyan human population is increasing at a high rate leading to demand for more proteins. With the rise in health concerns, a lot of people in the country are opting for white quality meat which indigenous chicken happens to be the favourite/ most preferred. With the high demand, many people are keeping their own indigenous chicken without considering any breeding strategies posing a threat to loss of some important traits of these chickens (Mwacharo *et al.*, 2007). In addition, studies on genetic diversity

on indigenous chicken in Kenya have not been widely done. Opportunities exist for sustainable improvement of indigenous chicken in Kenya through proper selection and use of well-established breeding programs (Khobondo *et al*., 2015).

Assessing the genetic difference will help in defining the different strains of indigenous chickens and the extent to which they are differentiated. This study will help improve on the assessment of genetic diversity, genetic purity and also trace the evolutionary history of these birds. The information on indigenous chicken diversity will also be useful in genetic improvement for enhanced production, maintenance and management plans, sustainable utilization and conservation of the breeds. It will also provide an avenue to further the study of genetics of chicken and add to the library of knowledge on indigenous chicken in the country.

1.4. Research Questions

i. Is there genetic distinctiveness among the different Kenyan indigenous chicken populations?
ii. Do signatures of selection exist at specific loci among the Kenyan indigenous chicken populations?

1.5. Research Objectives

1.5.1. Overall Objective

The goal of this study is to contribute to improved indigenous chicken production and conservation in Kenya through the assessment of genetic diversity and identification of signatures of selection

1.5.2. Specific Objectives

i. To genetically characterize indigenous chicken populations in Kenya

ii. To identify the selection signatures within local Kenyan chicken populations.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Background Information

The domestic chickens (*Gallus domesticus*) are known to be widely spread in the sub-Saharan Africa (Mwacharo *et al.,* 2013a; Khobondo *et al.,* 2014*;* Menge, 2008). They are believed to have descended from the wild jungle fowl which was first domesticated in South and South East Asia (Hassaballah *et al.,* 2015; Gifford – Gonzalez and Hanotte, 2011). This dates back to 2000 B.C. The domestic fowl belongs to the genus *Gallus,* family *Phasianidae* and order *Galliformes.* There are four species close to the domestic chicken. They are the Red jungle fowl (*Gallus gallus*), the Ceylon (*Gallus lafayettei*), the Grey (*Gallus sonneretii*) and the Green jungle fowl (*Gallus varius*) (Abebe *et al.,* 2013, Menge, 2008). The Ceylon is also known as the Sri Lankan jungle fowl. Of the four species, based on molecular and archaeological evidence, the red jungle fowl is believed to be the closest feral kin to the domestic chicken and the principal progenitor (Mwacharo et al., 2013b). However, the extent to which these species contribute to the domestic chicken is not well known (Dessie *et al.,* 2012, Abebe *et al.,* 2013). The evidence of multiple ancestral origins of the of domestic chicken is also reported by a study done revealing the yellow skin colour in domestic chicken is controlled by a gene present in the grey jungle fowl. The Red jungle fowl has a white skin colour (Eriksson *et al.,* 2008).

Domestication of chicken first started purposely for the sport of cock fighting which spread throughout the world over time (Khobondo *et al.,* 2014). Many factors have led to the dispersion and variation of the chicken species/breeds. The chicken later adapted to the different conditions they are exposed to (FAO, 2007). The highly adaptive characteristics of the chicken to their environment with minimal input requirements for their production made them adopt the term

native or indigenous or local chicken (Kaya and Yildiz, 2008, Abebe *et al.*, 2013). Currently, there are more than 350 chicken breed combinations known and for the purpose of adopting standards of excellence and classifying chicken breeds, the American Poultry Association (APA) was established in 1873.

2.2. Origin of indigenous chicken in Kenya

Domestic chicken are believed to have been introduced in Africa through Egypt from the Middle East between 1425 B.C and 1123 B.C (Mwacharo *et al.*, 2011; Magothe and Kahi, 2010; Maina, 2000). They reached the western part of Kenya with human migration from Egypt towards the south at around 100 B.C and later to the eastern part at 50 A.D. In 100 A.D, the early Greco-Roman East Coast trade led to introduction of more chicken to the country's coastal region (Mwacharo *et al*., 2013a; Gifford – Gonzalez and Hanotte, 2011). With this, all chicken types found in Kenya were introduced. Since the introduction, the chickens have been left to mate randomly and scavenge for their feed. They receive minimal healthcare and have adapted to the local climatic conditions. No significant breed has been developed but the chickens are currently being crossbred with exotic chicken to improve on their meat and egg production (Nyaga, 2007, Magothe and Kahi, 2010).

2.3. Importance and use of indigenous chicken

Poultry is an important source of meat, eggs and income in the rural areas. In the developing countries, they have been branded as a tool for poverty alleviation and food security (Magothe and Kahi, 2010). They are valued for their capability to scavenge, ability to tolerate diseases, provide good quality meat and eggs among others (Mwacharo *et al*., 2013). They are easy to market and are a reservoir for genes which influence productive adaptability. In Kenya, indigenous chicken account for more than 71% of total poultry meat and eggs produced (Bett *et al.*, 2012). They also

play an important role in social cultural obligations, entertainment, funeral rights and spiritual cleansing among others (Kingori *et al.,* 2010). Their manure is used for agricultural purposes for the small-scale farmers in the rural areas.

2.4. Indigenous chicken production systems

2.4.1. Free range production system

In the free range production system, indigenous chickens are the most commonly reared (Omiti and Okuthe, 2009). The chicken kept in this system have a variety of uses for both urban and rural areas. In the rural areas, the chickens are raised under the free scavenging system where the chicken look for their own feed (Von Braun *et al.,* 2008). An approximate of 90% of the rural households keep their chickens in this system (King'ori, 2010). Since they are an element in a mixed farming system, they are kept alongside other livestock species, whose mix hinge on the agro climatic zones.

The chickens in this system are a mix of species with varying flock ages. The numbers of these birds are affected by a variety of factors which include feed availability, diseases, environmental conditions and the economic status of the market. Their distribution and flock sizes are influenced by the social, cultural, biophysical and economic environment. Chicken kept under free range production system are mainly found in the rural areas where there is enough space to roam (Omiti and Okuthe, 2009).

2.4.1.1. Housing

For free range poultry production, the kind of housing mostly depends on the economic class of the households. Most of the rural households sleep with the chicken in their houses at night and release them in the morning (King'ori *et al.,* 2010). Some communities leave their birds to sleep

on trees at night while others have a separate house next to the main house to ensure the safety of their chicken. There is no comprehensive housing system described for the free range poultry production system (Nyaga, 2007; Blum, 2008). For places where chicken houses are constructed, the structure is raised about a metre above the ground to protect the birds from predators, harsh weather conditions - rain, high temperatures, cold and faecal matter (Khobondo *et al*., 2014; King'ori *et al*., 2010). Some make simple houses under their grass thatched granaries by surrounding the place with mud or material that cannot be removed by wild animals leaving an entrance/door for the chickens only.

2.4.1.2. Biosecurity, health practices and service providers

Biosecurity measures in this production system are very minimal (Omiti and Okuthe, 2009). With the birds free ranging, they are allowed to interact with other flocks, wild birds and the domestic animals. The birds can come into contact with the neighbours' flocks because there are no fences for every household. Vaccination is only done by a very small percentage of farmers while others do it in case of a disease outbreak in the locality. Health service providers are rare and there is no regular disease control program. New Castle Disease (NCD) is the common disease in the backyard system alongside helminthosis and salmonellosis (Njue, 2002). Neem leaves and *Aloe spp*. are the commonly used herbs to cure diseases (Njue, 2002). There exist a good number of service providers in the free range production system but their interaction with the farmers is not optimal. They include Agro vets, community animal health workers, government extension staffs, veterinarians and animal health assistants.

2.4.1.3. Marketing and other uses of indigenous chicken products

In the free range production system, there is no structured way of marketing the poultry products, which are essentially meat and eggs. The chicken are sold mainly at the local markets when there

is need for cash or when there is a disease outbreak (King'ori *et al.,* 2010). Ng'eno *et al.* (2014) in his studies reported that indigenous chicken contribute 46.7% and 58.3% annually for total eggs and poultry meat production in the country. Of the total eggs produced in this system, 50% of the eggs are eaten or sold. The remaining percentage is for natural hatching.

2.4.2. Semi intensive system

This is an amalgamation of both the extensive and intensive systems. The chickens are enclosed in a specific unsheltered area where they are provided with shelter, feed and water being in the house. The chickens are free to move within the confined area during the day but roost in the house at night for security and welfare purposes. Water and feed are housed to avoid spillages and wastage from rain, wind and wild birds. The system can be in the rural or urban areas (Sonaiya and Swan, 2004).

2.4.3. Intensive production system

This sector is also known as the industrial and integrated production system, classified as sector one in the country (Magothe *et al*., 2012; Nyaga, 2007). In intensive production system, exotic chicken are reared indoors in large numbers and have strict biosecurity measures at farm level. The farms are located near capital and major cities and depend on good roads and the market for their products (King'ori *et al.,* 2010). Most of the inputs for these farms are from the market where firms are given contracts to supply different items including feeds, equipment, medication, vaccines and technical information to the farms. The parent stocks are mainly imported from the developed countries having better performing breeds and their product is mainly for the urban centres and for export (Dessie *et al*., 2012). Human and animal traffic is minimal and there is no contact with other birds. Kenchic and Sigma are the largest producers of poultry products in Kenya (Nyaga, 2007).

2.5. Genetic improvement

2.5.1. Production performance

Several studies have been done on the productive and reproductive performance of indigenous chicken in the different production systems (Olwande *et al.*, 2010; King'ori *et al.*, 2010; Ogali, 2011). At the rural farms, egg production ranges from 40 – 100 eggs per year and are laid in clutches of 12 – 20 eggs. The chicken lay 3-4 times a year with the average weight of an egg ranging between 25-49 grams (Olwande *et al.,* 2010). Naked neck chickens are believed to lay the heaviest eggs, about 52g (Olwande *et al.,* 2010). The low number of eggs laid per year is due to the chickens brooding behaviour. The chickens spend time sitting on the eggs and later after hatching, they brood the chicks.

Indigenous chicken in most regions in Kenya are reared for meat production. Approximately 50% of the farmers leave the eggs to hatch naturally (Upton, 2000). The indigenous chickens are sold 5-6 months of age weighing between 1.3 – 1.8 kilograms. When dressed, they weigh 72% of their live weights compared to 75% for commercial culled layers and 80% for broilers (King'ori *et al.,* 2010). However, indigenous chicken meat dominates the poultry meat produced in Kenya. Processing and packaging is being embraced with time to add value to the chicken.

As per the report by Aviana Africa (2015), the annual poultry meat production in Kenya is 20 tonnes worth 40 million US dollars while the eggs produced annually are 1.3 billion worth 115 million US dollars. Indigenous chicken contribute the highest percentage of this produce.

2.5.2. Constraints to Village Chicken Rearing

Disease and Health Constraints

The most common poultry diseases are New Castle Disease (NCD), coccidiosis, Chronic Respiratory Disease (CRD), fowl pox, salmonellosis, and fowl typhoid (Omiti and Okuthe, 2009; Ssewanyana *et al.,* 2006). NCD is the most devastating. It is reported as the most important disease in developing countries (Ssewanyana *et al.,* 2006). Diseases like NCD, CRD and infectious coryza spread mainly during the dry seasons and seasons of festivities while salmonellosis, fowl typhoid, fowl pox and coccidiosis are prevalent during the rainy seasons (Magothe *et al.,* 2012). Marek's and Infectious Bursal Disease (IBD) commonly known as Gumboro, which affect most commercial breeds are rare in indigenous Kenyan chicken. Parasites are common in the poultry population. Lice, fleas, ticks and mites are the common external parasites while the helminths and coccidia are the common internal parasites (Magothe *et al.,* 2012). In his study on helminths infestation in the semi-arid areas of Kenya, Mungube *et al*. (2008) reported a 93% helminth infection rate on adult indigenous chicken. Biosafety measures are rarely observed by rural farmers. Treatment of infectious diseases is mainly herbal which include *Aloe vera*, croton, tea leaves and milk weed (Magothe *et al.,* 2012). Parasites are controlled by use of paraffin, oil, pesticides and ash, which are not very effective and may sometimes affect the welfare of the chickens (Magothe *et al.,* 2012).

Limited access to veterinary services

Farmers in the rural set up find it challenging to access a veterinary doctor as their production is less intensive, the services are costly and also a perception that vets do not have the expertise in chicken health and production (Sambo *et al.,* 2014).

Nutritional Constraints

Most farmers rarely supplement their chickens. The chickens are always released to scavenge for their food including chicks (King'ori *et al.,* 2010). Kitchen wastes and food remains are the

common additions when the chickens need supplementation. Feed concentrates from mills are expensive hence not affordable to the farmers. Some who supplement their birds formulate their own rations which do not provide balanced nutrients due to lack of expertise (Ogali, *2011)*

Technical Constraints

In disease control techniques, housing structures and operations, equipment handling and management and collective marketing initiatives are among the key factors considered. Purchase of inputs should be done jointly as opposed to each farmer purchasing their inputs reducing the economies of scale (Justus *et al.,* 2013)

Breeding Constraints

Inbreeding is common in indigenous chicken farms since most farmers do not control breeding. A breeding protocol should be adopted which should entail the breeding goal, selection criteria, a breeding scheme, records, genetic evaluation and monitoring for progress, genetic response and propagation.

Marketing Constraints

Marketing of indigenous chicken is done as a private sector activity which involves a marketing chain. The chain has the producers, traders, processors and the consumers (Nyaga, 2007). Purchase is done at farm level by a primary collector or at the weekly held markets in the region where the consumer will end up purchasing the raw form of the product or processed, which is mainly through cooking. Sale of the products is based on profitability, availability of stocks. The market prices only fluctuate depending on seasons but the system is well developed and stable (Upton, 2000). Prices of birds depend on their weight, size and health. Indigenous chicken eggs are smaller and lighter than those of commercial birds but fetch higher prices.

Marketing challenges included lack of means of transportation, harsh climatic conditions, theft of chicken at the market and unavailable market. The indigenous chickens are cheaper to purchase than the exotic ones which lead to a loss for the farmers.

2.5.3. Past improvement efforts

2.5.3.1. Genetic Upgrading in Kenya

In the year 1976, the cockerel exchange program was started in 12 districts in Kenya and later on in 1980, nine other districts embraced the program. The program was a strategy by the National Poultry Development Programme (NPDP) which aimed at increasing egg and meat production, farmers' income and protein intake by commercializing indigenous chicken (Magothe and Kahi, 2010). More districts got involved in the program and by 1994, the total number of districts added up to 75. The local cocks were killed and in exchange, a hybrid cock was placed. The hybrids were Rhodes Island Red and the Light Sussex. Farmers recruited were to keep 10 -15 pullets in their farms. This program was meant to improve the production of indigenous chicken in the provinces of Kenya through the slightly improved cross bred chicken but the project was later terminated after 18 years of implementation. This was as a result of insufficient supply of pure bred cockerels and pullets which led to the use of terminal breeds meant for the layer and broiler industries. FAO (2007) reports that the extent to which these genetic types of the crossbred used is not known among the free ranging chicken but there is some element of Rhode Island Red and the Light Sussex blood in a number of the indigenous chicken in the different regions of the country.

Such a case was also experienced in Malawi where due to the inability to survive in the prevailing backyard conditions, the cockerels and their progenies could not survive leading to low production in the industry (Safalaoh, 2001). All attempts to replace the indigenous flock with the exotic breeds and transform the systems of production to intensive ones failed because factors such as social and

cultural effects were not considered. The program was not widely adopted as it posed challenges such as high production costs, biodiversity erosion and biosafety concerns (Nyaga, 2007).

2.5.4. Future prospects

Most households in the rural are characterized by high poverty level. Their production enterprises require low starting capital and input costs. A system of production that increases productivity with low capital and input requirements should be figured out and development projects be strategized for these areas. Sustainable utilization of indigenous chicken genotypes should also be embraced to avoid loss of special alleles in pursuit of increased productivity (Mwacharo *et al.,* 2013).

2.5.4.1. Utilizing locally adapted chicken genotypes

Domestic chicken have diverse traits that can be utilized in specific environments or conditions (Magothe and Kahi, 2010, Ngeno *et al., 2014*). Scientists having conducted several experiments have identified specific genes which promotes the utilization of indigenous chicken. The genes are categorized as feather reducing, genes for small body size and genes controlling plumage colour (FAO, 2010). The naked and frizzled feather ecotypes can thrive well in hot environments. They have a better feed conversion, grow faster and have a superior mature body weight, higher egg production and disease tolerance in the tropics compared to others. With these traits, they can be utilized for meat production (Nwachukwu *et al.,* 2006). The dwarfed genotypes have efficient feed utilization ability and lay bigger eggs than birds of their size (Rashid *et al.,* 2005). Kuchi does well in hot humid lowlands while the bearded and shanky feathers perform in cold environments (Magothe and Kahi, 2010). Chicken with large single comb sizes are favoured by hot climatic conditions as the combs allow for efficient heat regulation.

Crosses between the naked neck and frizzling genes have been done by the Central Avian Research Institute (CARI), Izatnagar in India to improve on the tropical adaptability for broilers and layers (Tixier-Boichar *et al.,* 2009). Egypt has selected its Fayoumi breed for their resistance to coccidiosis and Marek's disease (Tixier-Boichar *et al.,* 2009).

For proper and efficient utilization of these genotypes, a holistic approach should be embraced to incorporate productivity, socio cultural and conservation issues among others to ensure improved performance of indigenous chicken in the country.

2.6. Chicken diversity

2.6.1. The chicken genome

The first genome sequence draft with its analysis was published in 2004 for a single female Red jungle fowl (Eriksson *et al.*, 2012). The female bird was chosen for sequencing because of its heterogametic sex chromosomes Z and W as opposed to the male chromosome which is homogametic (ZZ). The chicken genome/karyotype has a diploid chromosome number with 10 pairs of macro chromosomes, one pair of the sex chromosomes and 28 micro chromosomes, totalling to 39 pairs. The size of the genome is estimated to be 1.2×10^9 base pairs and is approximately a third of most mammalian genomes (Groenen *et al.,* 2000, Singh, 2000). The micro chromosomes have a higher purine content, gene density and recombination rate compared to the micro chromosomes (ICGSC, 2004).

2.6.2. Techniques for assessing chicken diversity

2.6.2.1. Morphological characterization of indigenous chicken in Kenya

There are distinct indigenous chicken ecotypes in Kenya. They vary in body sizes, plumage colour, skin colour, comb type, wattles, earlobes, beaks among others. In their study, Ng'eno *et al.* (2014)

noted the large head appendages of cockerels compared to the hens. The average length and height of combs is 6.36 and 4.88 cm for cocks and 3.64 and 1.63 cm for hens (Khobondo *et al.,* 2015; Ndirangu *et al.,* 1991). Almost all wattles and combs for the chicken population are red with a number having spotted wattles. A large number have red earlobes, whereas some have white or mottled red. Beak colour varies with black and yellow being common and their skin being cream, yellow, off white or red. At maturity, the cocks weigh heavier than the hens, 2.6 and 1.8 Kilograms respectively but the naked neck are heavier than the feathered chickens (Nwachukwu *et al.,* 2006; Ndirangu *et al.,* 1991). The feet and toes are black or cream in colour.

2.6.2.2. Molecular techniques for the assessment of poultry genetic diversity

Rationale for molecular genetic approach: Over the years, characterization has been done on many animal species basing on their morphological and production characteristics (Khobondo *et al.,* 2014; Apuno *et al.,* 2011; Alemu, 2004). Breeds have been characterized by their external features- physical body measurements, reproductive traits, production traits, survival traits, among others because it is easy and less costly compared to genetic characterization (Magothe *et al.,* 2012; Maina, 2000; Alemu, 2004). The limitation with characterization based on the phenotype alone is that phenotypic variation may not directly correspond with the genetic variation of the organism. The phenotype of an organism is as a result of its genetic component, the environment, the interaction and correlation between the environment and genes (Mogesse, 2007). Different traits can be polygenetically inherited and can also be influenced by varying environmental factors. More so, natural selection is based on the individual's phenotype thus makes it hard to make meaningful conclusions by comparing breeds phenotypically.

Initially, to characterize an organism genetically, protein markers were used. This method was used to identify the approximate sizes of molecules run in gel electrophoresis. The markers provide

a scale which is used to estimate the length and size of the fragments. The fragments are then identified by their sizes. Gel electrophoresis was used for visualizing the genes that had been characterized (Alemu, 2004). The problem with protein markers is the inability to differentiate between closely related breeds.

2.6.3. Molecular techniques in the study of genetic diversity

Molecular markers or DNA markers are the common tool used to characterize and assess the closeness between populations and within populations (Tixier-Boichard *et al.,* 2009; Ya-Bo *et al.,* 2006). Many organisms, animals and plants, both of the wild and those kept by man are being characterized genetically (FAO, 2007). Restriction fragment length polymorphism, amplified fragment length polymorphism , single nucleotide polymorphism, Randomly amplified polymorphic DNA, mitochondrial DNA, are among the many different techniques applied to generate data for genetic analysis. Microsatellite markers help in studying populations, estimating genetic distances and constructing phylogenetic trees by analysing their evolutionary relationships (Abebe *et al.,* 2013). The data can also be analysed by statistical tools to determine whether the population is in Hardy Weinberg equilibrium and linkage disequilibrium, allele frequencies between and within populations, the population structure among others.

2.6.3.1. DNA markers (mtDNA)

Mitochondrial DNA is found in the mitochondria of eukaryotes. It is made up of a haploid globular molecule and is maternally inherited in all mammals with 37 genes in total, most being transfer and ribosomal ribonucleic acids (tRNA and rRNA) (Hoque *et al*., 2011). Thirteen of the 37 genes are involved in oxidative phosphoration. Part of the mtDNA is noncoding and is the most varying in mammals since rapid changes in sequence and length occur in this region (Thuo, 2015). MtDNA shows high polymorphism levels and mutation rates. It is effective when assessing genetic

differentiation patterns in populations and defining evolutionary significant units (ESU) (Storey *et al*., 2012).

2.6.3.2. Restriction Fragment Length Polymorphism (RFLPs)

A restriction fragment is a fragment of a DNA obtained from cutting the DNA strand by a restriction endonuclease. This action can result to removal or creation of a new restriction site depending on the exposed end of the DNA (Al-Samarai, 2015). RFLPs can be used to identify specific region where a gene for a specific disease lies on a chromosome in a family and determine those at risk of being affected by the disease (Lateef, 2015).

2.6.3.3. Randomly Amplified Polymorphic DNA (RAPD)

This technique works with the principle that single short oligonucleotide primers are used to amplify random sequences from a DNA template (Lateef, 2015). The primers bind on the different loci exhibiting polymorphism. They are simple to use and do not depend on the prior DNA sequence information (Al-Samarai, 2015). The disadvantage with this technique is that it does not give information on heterozygosity and has problems with reproducibility.

2.6.3.4. Microsatellites

Microsatellite markers also known as Simple Sequence Repeats (SSRs), Variable Number of Tandem Repeats (VNTRs) or Simple Sequence Length Polymorphisms (SSLPs) are found in the nuclear genome ranging from one to six nucleotides in length (Gholizadeh and Mianji, 2007). They are tandem repeats of 5 to 20 times, the number varying in different populations for the whole DNA of within individuals alleles (Al-Samarai, 2015). Microsatellites can be used in genetic studies to identify linkage in families and populations. The advantage with microsatellite markers is that they are more variable and informative than RFLPs, RAPDs and AFLPs (Lateef, 2015). They are multi allelic and can also be used in analysing parentage and relatedness of groups or

individuals. The limitations of these markers are among others cost, time consumption and laborious (Han *et al.,* 2013).

2.6.3.5. Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms are sequence polymorphisms caused by single nucleotide mutation at specific locations in the DNA chain (Hoque *et al.*, 2011). The mutation can be a transition, insertion, inversion or a deletion. These markers are becoming common because they are abundant, genetically stable and responsive to high throughput programmed analysis (Stainton *et al*., 2015). They analyse diversity or variations among different species and breeds. They however are not as informative as the microsatellites. This limitation can however be curbed by use of higher number of markers, also referred to as SNP chips (Werner et al., 2002).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Sampling

Blood samples were collected from eight counties in four geographical regions in Kenya; Kakamega, and Siaya from the western region, West Pokot and Turkana from the North Rift, Bomet and Narok from the south Rift, Lamu and Taita Taveta from the coastal region (Figure 2). Ninety six (96) birds were sampled from each county giving a total of 768 birds from the eight counties.

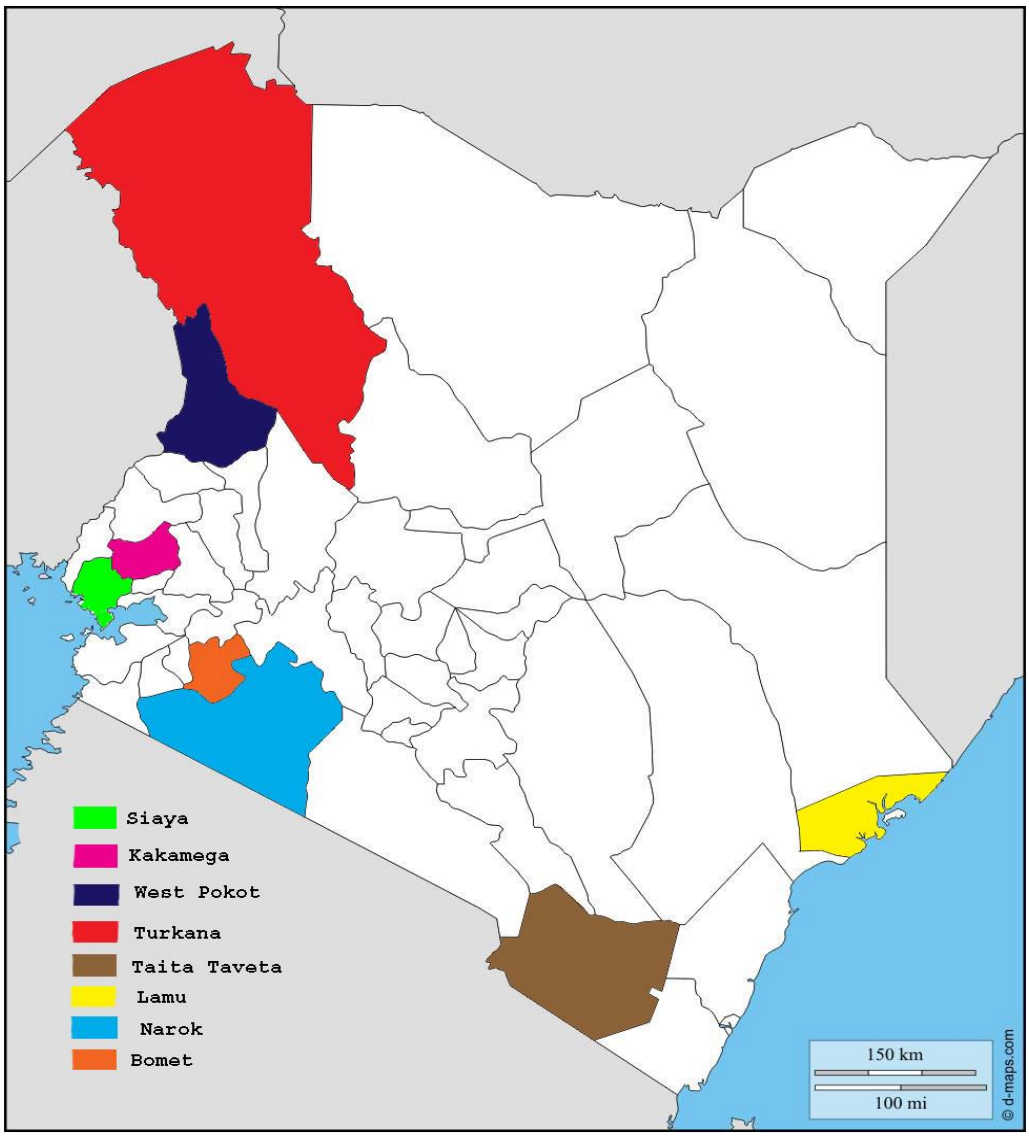


Figure 2; Map of Kenya showing the counties from which chicken samples were obtained

Two mature chickens were randomly sampled from each household, each household distanced by a minimum of 5 Km from the next. All the birds that we sampled were free ranging indigenous chicken. The chicken were physically restrained to avoid injury. Two millilitres (2ml) of blood was extracted from their wing veins for every bird sampled and stored in EDTA tube. The blood samples were stored in Nucleosave blood storage cards and dried overnight in a laminar flow hood

over the night. Laboratory analysis for the extraction of DNA, its purification and amplification was done at the International Livestock Research Institute (ILRI), Kenya.

3.2. DNA extraction and Polymerase Chain Reaction

The genomic DNA was extracted from the blood by the standard phenol-chloroform extraction method (Ng'eno *et al.,* 2014). Twelve autosomal microsatellite loci which are among the 30 suggested markers by the International Society for Animal Genetics (ISAG) and Food and Agricultural Organisation (FAO) project for surveying chicken biodiversity (FAO, 2012) were used for genotyping. Genotyping was done for a single chicken per household to reduce chances of sampling closely related birds i.e. out of 96 samples collected in each county, 48 samples per county were genotyped. For all the markers, the PCR protocol described by Spinella *et al.,* (1999) and Fulton *et al.,* (2006) was used for amplification. Table 1 shows the primer sequences, annealing temperatures and the amplification protocol used. Genotyping for SNP identification was done using the PCR – RFLP protocol as described by Ngeno et al., 2014.

Table 1; Primer sequences, chromosome, fluorescent label annealing temperatures (°C) and observed number of alleles for the microsatellite markers used in this study

Marker	chromosome	Fluorescent dye	Annealing temp (°C)	Observed no. of alleles	Primer sequence
ADL0268	1	Vic	55	6	Forward: CTCCACCCCTCTCAGAACTA Reverse: CAACTTCCCATCTACCTACT
MCW0111	1	Ned	55	10	Forward: GCTCCATGTGAAGTGGTTTA Reverse: ATGTCCACTTGTCAATGAT
MCW0183	7	Vic	55	10	Forward: ATCCCAGTGTCGAGTATCCGA Reverse: TGAGATTTACTGGAGCCTGCC
MCW0206	2	Ned	55	7	Forward: CTTGACAGTGATGCATTAAATG Reverse: ACATCTAGAATTGACTGTTCA
MCW0222	3	Fam	55	7	Forward: GCAGTTACATTGAAATGATTCC Reverse: TTCTCAAAACACCTAGAAGA
MCW0034	2	Pet	55	12	Forward: TGCACGCACTTACATACTTAGAGA Reverse: TGTCCTTCCAATTACATTCATGG
MCW0037	3	Ned	55	8	Forward: ACCGGTGCCATCAATTACCTATTA Reverse: AAAGCTCACATGACACTGCGAAA
MCW0067	10	Vic	55	7	Forward: GCACTACTGTGTGCTGCAGTTT Reverse: GAGATGTAGTTGCCACATTCCGAC
MCW0069	26	Pet	55	10	Forward: GCACTCGAGAAAACTTCCTGCG Reverse: ATTGCTTCAGCAAGCATGGGAGG
MCW0081	5	Pet	55	7	Forward: GTTGCTGAGAGCCTGGTGCAG Reverse: CCTGTATGTGGAATTACTTCTC
LEI0258	16	Fam	55	46	Forward: CACGCAGCAGAACTTGGTAAGG Reverse: AGCTGTGCTCAGTCCTCAGTGC
MCW0371	16	Ned	55	10	Forward: TTTCATGGCATCCTAAGATGG Reverse: CTGCTCCGAGCTGTAATCCTG

Source: Ng'eno *et al.,* 2014

3.3. Microsatellite DNA amplification and genotyping

An approximate of 50 nanograms of the extracted DNA was used in the amplification procedure. For amplification of the DNA, the twelve microsatellite primers were used. A multiplex PCR amplification was conducted in a 20 µl solution containing 6 µl of eluted DNA, 10x PCR buffer (5mM Magnesium Chloride), 2.5mM dNTPs, 3mM of each microsatellite marker primers and 2,5µl Taq polymerase. The amplification process was conducted using the Applied Biosystems

9700 thermal Cycler Gene Amp. The initial temperature for denaturation was set at 94^0 C for 5 minutes followed by 30 cycles for denaturation at 94^0 C for 30 seconds, primer annealing was done for 1 minute at 55^0C and extension for 30 seconds at 72^0 C. The final extension was done for 7 minutes at 72^0C to complete the protocol.

Electrophoresis was then conducted for the PCR products and further genotyping was performed. The microsatellite genotyping was done by diluting the PCR products about twenty times. The genotyping reaction blend contained 1 µL of thinned PCR products, 10 µL of Hi-Di™ Formamide (Applied Biosystems, USA) and 0.1 µL of GeneScan™-500 LIZ™ size standard marker (Applied Biosystems, USA). The mixture was then denatured after dilution and fragment analysis performed using capillary assay in Genetic Analyzer 3130xl (Applied Biosystems, USA). Gene mapper version 3.7 (Applied Biosystems, USA) was used to identify the microsatellite genotypes.

3.4. Statistical Analysis

3.4.1. Genetic variability

3.4.1.1. Allelic diversity and heterozygosity

Total number of alleles for individual markers (N_A), mean number of alleles (MNA), number of private alleles by locus and by population, allelic patterns for codominant data across populations and genetic diversity, Allele frequencies, H_O and H_E were computed using GenAlEx software version 6.502 (Peakall and Smouse, 2012).

3.4.1.2. Fixation indices/ inbreeding coefficients

Differentiations in populations fixation indices inclusive of genetic variation within sub population (F_{IS}), which reflects/ indicates the level of inbreeding (F) within sub populations ($F_{ST)}$ and the total population (F_{IT}) were estimated using GenAlEx version 6.502 software (Peakall and Smouse, 2012).

3.4.1.3. Hardy-Weinberg Equilibrium (HWE)

The deviations from HWE were determined using GenAlEx software version 6.502 (Peakall and Smouse, 2012). Each locus for the population was tested for its degree of freedom, chi square test, probabilities and their significance level given at probabilities less than 0.05, 0.01 and 0.001. This was to determine the evolutionary force behind variation in the chickens.

3.4.1.4. Genetic distances

Nei's genetic distance and genetic identity per population were computed using GenAlEx software version 6.502 (Peakall and Smouse, 2012) where a population matrix was used to compare the distances between the different populations and indicate their relationships. The results were compared to Nei's unbiased genetic distances and identity calculated to assess their genetic distances.

3.4.1.5. Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance was used to test the within individuals variation, among population and among individuals variation values and their respective percentages using GenAlEx software version 6.502 (Peakall and Smouse, 2012).

3.4.1.6. Mantel test

The Mantel Test was used to test for the association or correlation between two distance matrices (both genetic and geographic distances). It uses the Pearson Product moment correlation coefficient *r* to compute the significance of the correlation in the matrices. This test was used to assess the association between the populations sampled at different regions and indicate their relationships. It was carried out using Isolation By Distance Web Service (IBDWS) software version 3.16 (Keambou *et al.,* 2014; Jensen *et al.,* 2005) at a significance level calculated from

10000 randomizations. The test examines whether geographically proximal populations were also more closely related genetically and whether geographic scale shapes genetic relatedness.

3.4.2. Population structure

The data was converted to structure format from GENALEX using CONVERT version 1.31 (Glaubitz, 2005) and structure analysis carried out using STRUCTURE software version 2.3.4 (Pritchard *et al.*, 2010). Missing data was represented as -9. Correlated allele frequencies and probability in estimation of genetic cluster (K) using the data were performed at 20 runs for each value of K. A random configuration was done before iterations on the program. Induced correlations between the state of the Monte Carlo Markov Chain (MCMC) during the runs were eliminated or reduced significantly by running several simulations. The burn in length was set at 50000 with 100000 Monte Carlo Markov Chain replications and 8 iterations. Bayesian clusters numbers for the populations were set at K=8 (Sethuraman, 2013). Admixture model within structure was used to generate results. STRUCTURE HARVESTER version 0.6.93 (Earl and vonHoldt, 2012) was used to analyse the output and plot the means and variance in likelihood per K. Visualization of the Q values (membership coefficient matrix, commonly termed as the individual Q-matrix values) extracted from STRUCTURE was done using Distruct software version 1.1 (Rosenberg, 2004).

3.4.2.1. Principal Coordinate Analysis (PCoA)

The Principal co-ordinate analysis (PCoA) was carried out using GenAlEx 6.5 based on Nei's genetic estimations. The graph was viewed using the same software to show the distribution of the eight populations sampled.

3.5. Selection signatures

3.5.1. Single Nucleotide Polymorphism (SNP) Calling

The sequences were first trimmed using Sickle Master to remove errors in the sequenced data. The chicken reference genome was then indexed in the server using bwa version 0.7.12 software. The trimmed data was then aligned to the reference genome and the Fastq data saved as .sam file. The fourth step was viewing the file which was converted to .bam file. The output was then sorted and duplicates removed using Samtools 1.3. This was then indexed and realignment done for insertions and deletions using Genomic Analysis Toolkit (GATK). The final step was calling SNPs to identify the variations/ selection signatures.

In analyzing for the selection signatures in the indigenous chicken in Kenya, the chickens in Rift Valley region were grouped into two, the Northern region and the southern region. The northern region was a combination of West Pokot and Turkana while the southern region had Bomet and Narok. These formed two populations needed for the analysis. To identify the regions transformed/modified by selection, a three step technique was developed; first the fixation indices values (F_{ST}) between the two populations was calculated using VCFtools (Marcketta and Auton, 2014). The windows size of 200Kb sliding 100Kb each time over the whole genome was used to generate the values. False positives were reduced by removing windows with less than 10 variants in every step. Z transformation was done to normalize the skewed distribution of the F_{ST} as described by Rubin *et al*. (2010). Regions affected by selection were identified in the third step. Windows with transformed F_{ST} values less than five were considered not qualified whereas Z transformed (ZF_{ST}) values above 5 standard deviations were affected by selection.

Variant Effect Predictor VEP75 (Mc Laren *et al.*, 2010) was used to check on the genes in the affected regions and to predict if the substitution of the amino acids had any effects on the protein function.

CHAPTER FOUR

4.0. RESULTS

4.1. Genetic diversity

4.1.1. Overall estimates of the various genetic diversity parameters of local Kenyan chicken

The mean number of samples per population was 46.79 ± 0.147. The number of different alleles (N_A) for the local chicken in Kenya was 8.094 ± 0.516. Effective number of alleles was 4.45 ± 0.297. The overall observed and expected heterozygosities were 0.714 and 0.726 respectively (Table 2).

Table 2; Overall means and standard errors of different genetic diversity parameter estimates for the Kenyan local chicken taken as a single population.

Genetic diversity parameters	Mean	Standard error
Sample number (N)	46.792	0.147
Number of different alleles (N_A)	8.094	0.516
Number of effective alleles (N_E)	4.452	0.297
Shannon's Information Index (I)	1.563	0.046
Observed heterozygosity (H_O)	0.714	0.011
Expected Heterozygosity (H_E)	0.726	0.009
Unbiased Expected Heterozygosity (uH_E)	0.734	0.009
Number of founders (F)	0.016	0.009

4.1.2. Genetic diversity parameters of the eight ecotypes

On average, the populations sampled from Bondo had the highest number of different alleles, effective alleles, observed and expected heterozygosities and Shannon's Information Index. Taita Taveta had the least values for all the alleles and heterozygosities except for the number of different alleles (Na).Lamu population had the least number of different alleles (Table 3).

Table 3; Mean and standard errors of the different genetic diversity parameters estimated for eight indigenous chicken populations in Kenya

Populations		**Na**	**Ne**	**I**	**Ho**	**He**	**uHe**	**F**
Bomet (bm)	Mean	7.750	4.379	1.563	0.716	0.730	0.738	0.020
	SE	1.349	0.710	0.131	0.031	0.026	0.026	0.025
Kakamega (kk)	Mean	8.583	4.867	1.627	0.731	0.743	0.752	0.013
	SE	1.760	0.994	0.147	0.026	0.026	0.027	0.026
Lamu (lm)	Mean	7.083	4.134	1.485	0.704	0.711	0.718	0.012
	SE	1.221	0.784	0.125	0.038	0.026	0.026	0.034
Narok (nr)	Mean	8.333	4.495	1.584	0.711	0.726	0.734	0.020
	SE	1.588	0.939	0.137	0.028	0.026	0.026	0.026
Bondo (bn)	Mean	8.833	4.971	1.654	0.734	0.754	0.762	0.027
	SE	1.632	1.026	0.135	0.030	0.023	0.023	0.021
Turkana (tk)	Mean	8.167	4.186	1.520	0.700	0.713	0.721	0.021
	SE	1.576	0.834	0.132	0.031	0.024	0.024	0.021
Taita Taveta (tt)	Mean	7.667	3.799	1.460	0.690	0.688	0.696	**-0.004**
	SE	1.345	0.657	0.120	0.029	0.030	0.030	0.018
West Pokot (wp)	Mean	8.333	4.785	1.611	0.728	0.741	0.749	0.023
	SE	1.509	0.904	0.139	0.045	0.028	0.028	0.037

See Table 2 for definition of parameters estimated

4.1.3. F Statistics results

The total F_{ST}, F_{IS} and F_{IT} for all populations was 0.029, 0.066 and 0.093 respectively. Inbreeding coefficient for each locus ranged between - 0.02 (for locus MCW0371) and 0.048 (for locus MCW69). The highest number of migrations Nm (19.355) was registered at locus MCW81 which resulted to least F_{ST} value of 0.013. Locus MCW111 had the least Nm (4.168) and highest F_{ST} value (Table 4). Of the 12 microsatellites loci, four had negative inbreeding coefficients over all

populations which indicate reasonably high heterozygosities for the four loci. This, however, is contrary to the expected result where inbreeding is expected to affect all loci. There is therefore a possibility of selection taking place at neutral loci thus resulting to this observation. The F_{IT} values ranged between 0.002 and 0.088 for all the loci. G statistics revealed almost similar values. Locus MCW111 had the highest genetic differentiation (G_{ST}) value over all loci while MCW81 had the least.

Table 4; F-Statistics (F_{IS}, F_{ST} and F_{IT}) and Estimates of Number of migrants for the 12 microsatellite markers used to assess genetic diversity of the local Kenyan chicken

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
ADL268	0.047	0.088	0.044	5.495
MCW111	0.032	0.086	0.057	4.168
MCW183	0.040	0.080	0.041	5.837
MCW206	0.033	0.060	0.028	8.611
MCW222	0.001	0.049	0.047	5.019
MCW34	-0.018	0.021	0.039	6.190
MCW37	0.036	0.077	0.042	5.697
MCW67	-0.016	0.039	0.054	4.399
MCW69	0.048	0.066	0.018	13.472
MCW81	-0.009	0.003	0.013	19.355
LEI0258	0.021	0.046	0.025	9.653
MCW0371	-0.020	0.002	0.021	11.518
Mean and SE	0.016±0.008	0.051±0.009	0.036±0.004	8.285±1.318

F_{IT} - the inbreeding coefficient of an individual (I) relative to the total (T) population.

*F_{IS} - the inbreeding coefficient of an individual (**I**) relative to the subpopulation (**S**).*

F_{ST} - the effect of subpopulations (S) compared to the total population (T).

The total number of alleles for all codominant data was 140, with locus LEI0258 registering the highest number of alleles (46). The number of loci with private alleles was found to be 7 The local chicken ecotypes which had the highest number of loci with private alleles were Turkana , Bondo, Kakamega, Narok and Taita-Taveta all having two loci with private alleles. In-terms of population, a total of 21 alleles were private. Allele 396 and 308 at locus LEI0258 had premier frequencies at

0.067 and 0.052 respectively, for Turkana and Lamu populations. Loci MCW34, MCW67, MCW81 and MCW0371 had higher heterozygosity values than the rest, registering a negative inbreeding coefficient (Table 5).

Table 5; Summary of numbers of private alleles at specific loci and their frequencies for the local Kenyan chickens

Population	Locus	Allele	Frequency
Bomet	MCW69	173	0.011
Kakamega	MCW67	187	0.011
Kakamega	LEI0258	432	0.010
Lamu	LEI0258	308	**0.052**
Lamu	LEI0258	406	0.021
Lamu	LEI0258	458	0.021
Lamu	LEI0258	471	0.010
Narok	MCW183	320	0.012
Narok	LEI0258	445	0.021
Narok	LEI0258	482	0.010
Bondo	MCW222	305	0.021
Bondo	LEI0258	453	0.010
Bondo	LEI0258	475	0.021
Bondo	LEI0258	479	0.010
Turkana	MCW111	91	0.010
Turkana	MCW111	118	0.010
Turkana	LEI0258	396	**0.067**
Turkana	LEI0258	550	0.011
Taita Taveta	MCW37	175	**0.032**
Taita Taveta	LEI0258	371	0.011
West Pokot	MCW222	300	0.022

4.1.3. Hardy Weinberg Equilibrium

All the 12 loci were tested for HW equilibrium. Of the 12 loci for all the 8 populations, 7 loci showed a deviation from the HWE with 8 at $p<0.05$ and 12 at $p<0.001$. This stands for 20.8% of the total population sampled. The rest (5) was not significant with the Chi Square tests hence obeyed the HWE theory (Table 6).

Table 6; Chi-Square values, probabilities and level of significance for Hardy-Weinberg Equilibrium test (all loci tested for every population)

Pop	Locus	Degree of freedom	Chi Square	Probability	Significance
Bomet	MCW206	15	44.070	0.000	***
Kakamega	MCW34	**55**	78.306	0.021	*
Kakamega	MCW37	15	29.095	0.016	*
Kakamega	MCW67	15	49.461	0.000	***
Narok	MCW111	15	53.116	0.000	***
Narok	MCW67	10	47.668	0.000	***
Bondo	MCW111	21	36.115	0.021	*
Bondo	MCW222	10	57.245	0.000	***
Bondo	MCW81	21	66.775	0.000	***
Bondo	LEI0258	**325**	386.887	0.010	*
Turkana	ADL268	15	29.326	0.015	*
Turkana	MCW111	21	100.637	0.000	***
Turkana	MCW67	10	48.129	0.000	***
Turkana	MCW0371	**36**	70.067	0.001	***
Taita Taveta	MCW67	15	50.040	0.000	***
Taita Taveta	MCW69	15	28.353	0.019	*
West Pokot	ADL268	15	30.546	0.010	*
West Pokot	MCW222	10	47.489	0.000	***
West Pokot	MCW37	21	59.976	0.000	***
West Pokot	MCW67	10	21.563	0.017	*
Key: * P<0.05, ** P<0.01, *** P<0.001,					

4.1.4. Mean allelic patterns across populations

Taita Taveta chicken population showed the least mean values for the number of different alleles, private alleles, common alleles and expected and unbiased expected heterozygosity. The chicken in Bondo had the highest values for means. Lamu had the least number of different alleles but had the highest number of private alleles together with Bondo population at 0.333. This was higher than the least number of alleles given by the populations sampled from Bomet and West Pokot. Unbiased expected heterozygosities for all populations fell between 0.696 (Taita Taveta) and 0.762 (Bondo).

4.1.5. Principal Coordinate Analysis

The chickens from Lamu and Taita Taveta were on the right side of the axis 1 mainly in the second quadrant. The ecotype from Narok concentrated on the left side of axis 1 on the first quadrant. The Turkana ecotype concentrated on the third quadrant while Taita-Taveta concentrated on the fourth quadrant. The remaining populations were distributed uniformly in the four quadrants. Plotting Principal Coordinate Analysis 2 versus 3 depicted a similar result to that of 1 versus 2.

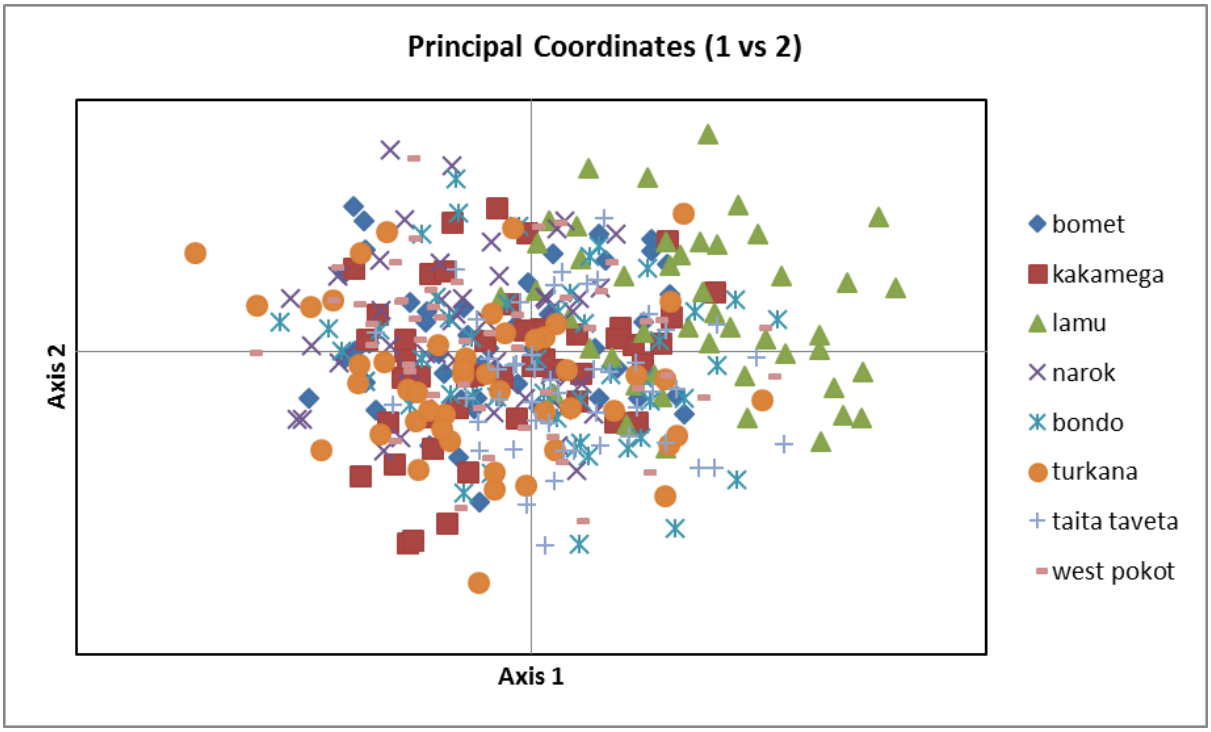


Figure 3; Scatter diagram showing principal coordinate analysis for 8 chicken ecotypes in Kenya

4.1.6. Genetic variance analysis

The mean pairwise F_{ST} estimates by AMOVA revealed the inbreeding coefficient among populations to be 3% (0.132), among individuals variation was 6% (0.297) and within individuals' variation at 91% all at 99% CI as calculated using GENALEX software. Wrights F statistics at all loci was found to be 0.029 for F_{ST}, 0.066 variation for F_{IS} and 0.093 for F_{IT}. The maximum F_{ST} value found was 0.254 with the number of migrations as 8.47.

Table 7; Analysis of Molecular Variance (AMOVA) table showing the percentage variations among populations, among individuals and within individuals

Source	Degrees of freedom	Sum of squares	Mean of Squares	Estimated Variance	%
Among Populations	7	122.216	17.459	0.132	3%
Among Individuals	376	1795.094	4.774	0.297	6%
Within Individuals	384	1605.000	4.180	4.180	91%
Total	767	3522.310		4.609	100%

4.1.7. Nei's Unbiased Genetic Distance- Genetic relationships

According to Nei's unbiased genetic distance using the pairwise population matrix, chicken from Bomet were closely related to the West Pokot population (0.003) than any other chicken populations (Table 9). Others closely related were chicken from Kakamega, Bondo, Turkana and Taita Taveta with West Pokot at 0.009, 0.012, 0.013 and 0.013 respectively (Table 9). The ecotype from Narok were also closely related to Bomet (0.008), this being the second closest ecotypes after Bomet West Pokot ecotypes. The most distant was Lamu from Narok at 0.249, Lamu from Turkana at 0.232 and Kakamega from Lamu at 0.231. This is confirmed with Nei's genetic Identity matrix where chickens sampled from Bomet were 99.97% identical to those in West Pokot. Lamu and Narok were 77.9% identical which is a high value though the least value in Nei's genetic identity matrix for this study. The same results are reflected in the pairwise population F_{ST} values matrix as given in table 10.

Table 8; Pairwise Population Matrix of Nei's Unbiased Genetic Identity (upper right diagonal) and genetic distance (lower left diagonal) of the local chicken ecotypes in Kenya

Population	Bomet	Kakamega	Lamu	Narok	Bondo	Turkana	Taita Taveta	West Pokot
Bomet	-	0.989	0.822	0.992	0.983	0.979	0.897	0.997
Kakamega	**0.011**	-	0.794	0.974	0.98	0.978	0.877	0.991
Lamu	0.196	0.231	-	0.779	0.834	0.793	0.818	0.794
Narok	**0.008**	0.027	0.249	-	0.972	0.963	0.886	0.983
Bondo	0.017	0.021	0.181	0.028	-	0.972	0.921	0.988
Turkana	0.022	0.022	0.232	0.037	0.028	-	0.895	0.987
Taita Taveta	0.108	0.131	0.201	0.121	0.082	0.111	-	0.878
West Pokot	**0.003**	**0.009**	0.231	0.017	**0.012**	0.0132	**0.13**	-

Table 9; Pairwise population F_{ST} values for the chicken ecotypes in Kenya

Population	Bomet	Kakamega	Lamu	Narok	Bondo	Turkana	Taita Taveta	West Pokot
Bomet	0.000							
Kakamega	0.007	0.000						
Lamu	0.037	0.042	0.000					
Narok	0.007	0.010	0.046	0.000				
Bondo	0.008	0.009	0.034	0.010	0.000			
Turkana	0.009	0.009	0.044	0.012	0.010	0.000		
Taita Taveta	0.027	0.029	0.042	0.029	0.022	0.027	0.000	
West Pokot	0.006	0.007	0.041	0.008	0.007	0.008	0.029	0.000

4.1.8. G Statistics for loci

With 999 permutations and 1000 bootstraps, loci MCW81 and MCW69 had the least inbreeding coefficient within subpopulations relative to the whole population at 0.3% and 0.8% respectively. The rest of the loci had among population differentiation (G_{ST}) values above 1%, the highest at 47% and 45% for MCW111 and MCW67 loci. This was also reflected in Nei's and Hedrick's

standardized fixation indices (G_{ST}) values. The Nei's fixation index ($G_{ST)}$ was 0.026. Hendrick's G_{ST} values further corrected for bias for small population was slightly high (0.112).

4.1.9. Mantel's Test

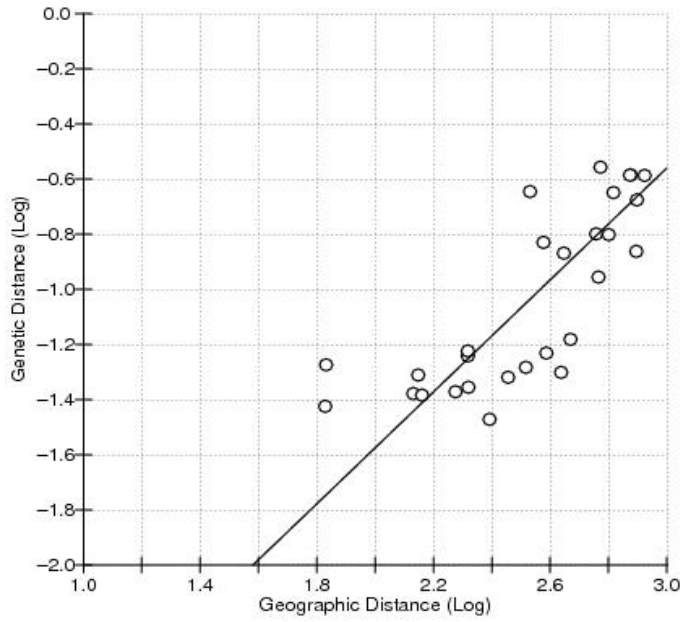


Figure 4; A regression analysis between genetic distance and geographic distance for eight indigenous chicken populations in Kenya (r=0.7406, R2 =0.5485)

The results from Mantel test depict a direct/linear correlation between genetic distance and geographical distance for the chicken ecotypes. This concludes that the ecotypes which are near each other tend to be genetically more similar than expected by chance, and this genetic difference increases as the geographic distance between the populations increases.

4.1.10. Population structure

The outcome for population clusters in structure are as shown in figure 6 below. At K=2, the individuals from Lamu formed a distinct cluster while the Taita-Taveta population was an admixture of the two clusters. This changed as it formed a distinct cluster at K=3 resulting to three population clusters. The clusters remain to be three up to K=8, where the individuals express a

mixture with Lamu and Taita-Taveta forming different clusters. The resultant cluster number for the chicken populations in the sampled areas is three, with the third population being a mixed cluster. Chicken which were geographically close depicted the same pattern and tended to form a single cluster. Kakamega and Bondo ecotypes portrayed a similar pattern from K=1 to k=8, same to Bomet with Narok ecotypes.

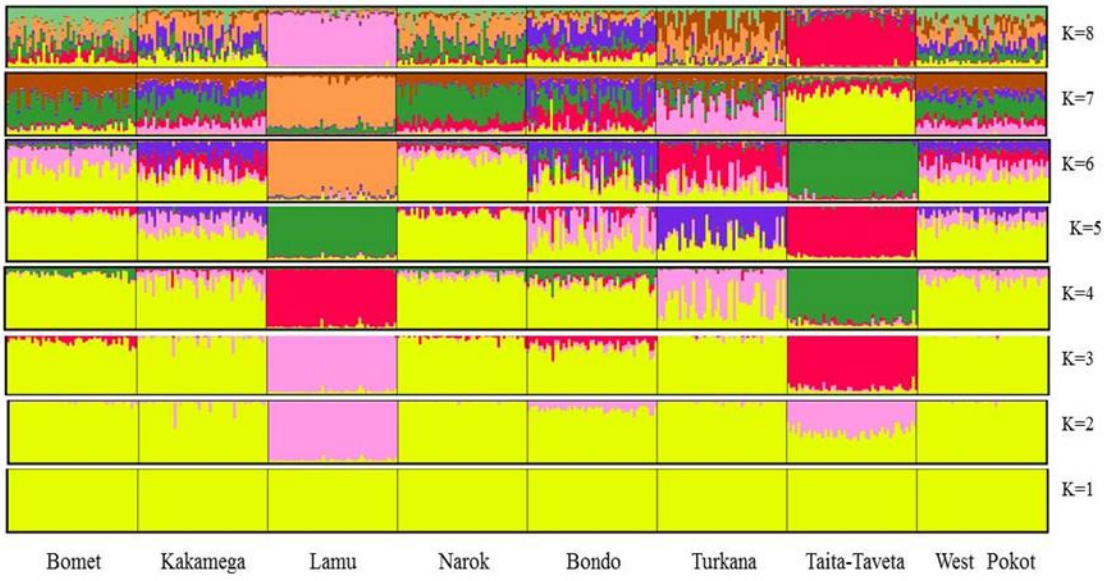


Figure 5; STRUCTURE clustering analysis of 8 indigenous chicken populations.

Table 10;Values of K, each number repeats, Mean log of likelihood with their standard deviations, difference in means of consecutive likelihood K values (Ln'(K)), absolute values of second order change rate |Ln"K| and most likely cluster number of eight indigenous chicken in Kenya

K	Mean LnP (K)	Stdev LnP (K)	Ln'(K)	|Ln"(K)|	Delta K
1	-15322.23	0.43	—	—	—
2	-14913.56	5.22	408.67	300.58	**57.55**
3	-14805.47	14.42	108.09	132.62	9.20
4	-14830.01	75.19	-24.54	14.10	0.19
5	-14868.65	87.79	-38.64	174.47	1.99
6	-15081.75	383.07	-213.10	84.99	0.22
7	-15209.87	385.77	-128.12	21.88	0.06
8	-15316.11	548.27	-106.24	—	—

The maximum likelihood number of clusters were calculated according to Evanno Method (2005). The Delta K value was determined by dividing the second order rate of change value by the standard deviation of the log likelihood. The highest delta K value (57.55) indicated the probable cluster number (K = 2) of the populations (Table 11, Figure 6).

4.2. Signatures of selection

A total of 36,026 markers were identified as variants in the indigenous chicken DNA. These were the markers identified after a SNP calling approach. To identify regions that had undergone recent selection, Z transformed F_{ST} outlier values were identified from the total 36,026 SNPs. Values that ranged between 5 and 5.99 were grouped as for loci with strong signatures of selection while those ranging between six and eight as loci with decisive selection signatures (Table 12). A total of 14 of the 30 identified outlier markers had values ranging between six and eight. The remaining 16 markers had Z transformed F_{ST} values falling between 5 and 5.99. These markers were found in chromosome 1, 4, 8 and 14. The eighth chromosome had all the outlier markers above 5 for the chicken population sampled.

Table 11; Z transformed F_{ST} values above six standard deviations depicting regions that have been affected by selection

chromosome	bin start	bin end	number of variants	weighted F_{ST}	mean F_{ST}	mean of weighted-F_{ST}	stdev of weighted F_{ST}	z trans-formed F_{ST}
8	8700001	8900000	714	0.77305	0.6205	0.0094	0.1197	6
8	8800001	9000000	787	0.75764	0.5958	0.0094	0.1197	6
8	8900001	9100000	377	0.80447	0.6566	0.0094	0.1197	7
8	9000001	9200000	377	0.94459	0.8696	0.0094	0.1197	8
8	9100001	9300000	508	0.95375	0.8950	0.0094	0.1197	8
8	9200001	9400000	506	0.92904	0.8505	0.0094	0.1197	8
8	9300001	9500000	457	0.91882	0.8279	0.0094	0.1197	8
8	9400001	9600000	374	0.83003	0.6693	0.0094	0.1197	7

8	9900001	10100000	382	0.66960	0.5119	0.0094	0.1197	6
8	10000001	10200000	342	0.67950	0.5280	0.0094	0.1197	6
8	10100001	10300000	288	0.69981	0.5597	0.0094	0.1197	6
8	10200001	10400000	333	0.71649	0.5807	0.0094	0.1197	6
8	10300001	10500000	346	0.69803	0.5651	0.0094	0.1197	6
8	10600001	10800000	617	0.69558	0.4706	0.0094	0.1197	6

The z transformed values went up to negatives and upon plotting the following (figure 7) was obtained.

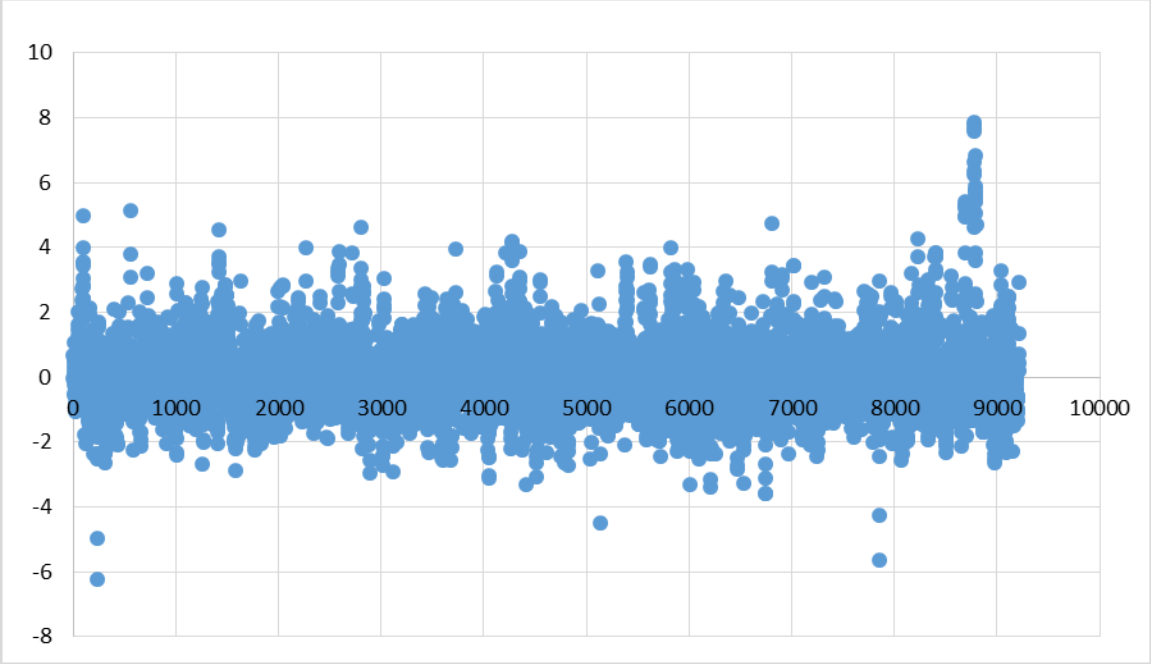


Figure 6; Scatter diagram of chromosome number (vertical axis) against Z transformed value (horizontal axis)

Figure 8 shows the impact of the variations (SNPs) in the genetic codes of the sampled chicken. Most of the transformations in the genetic makeup of the chicken had an influence on the traits as a larger percentage (99%) were modifiers of the genes (Figure 8). Variants that had the largest consequences were upstream variants, downstream variants, intergenic variants, intron variants and intron non coding transcript variants. The rest had an insignificant influence on the chicken chromosome.

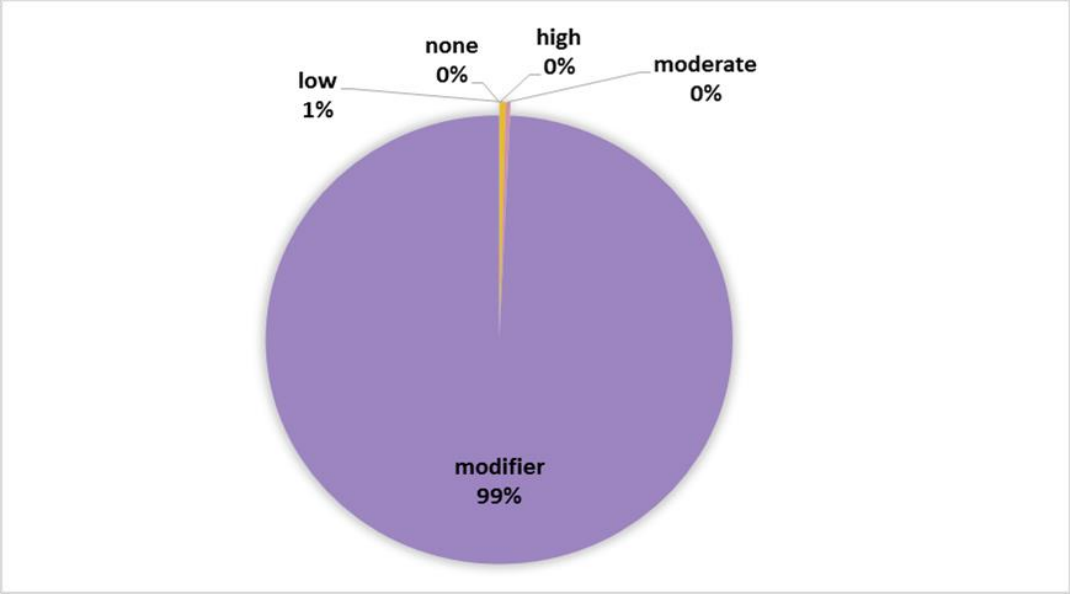


Figure 7; Pie chart showing the impact of the variations (SNPs) in the genetic codes of the sampled chicken

Of the total SNPs, the major consequent variants were five; the upstream gene variants (21.84%), downstream gene variants (20.99%), intergenic variants (18.10%), intron variant (17.44%) and the intron variants, non-coding transcript variants (17.99%). This totalled to 96.36 % of the total variants. The remaining 3.64% was for missense variants, splice acceptor variants for coding and non-coding transcripts, 3' and 5' UTR variants, synonymous variants and splice region variants.

Genes identified to be influenced by the variations included Olfactomedin 3 (*OLFM3*), Amylase alpha 1A (*AMY1A*), Collagen alpha 1 (XI) (*COL11A1*), *RNPC3*, Chromosome 1 Open Reading Frame 27 (*C1orf27*), Translocated Promoter Region (*TPR*), Proteoglycan 4 (*PRG4*) and Programmed Cell Death (*PDC*) genes.

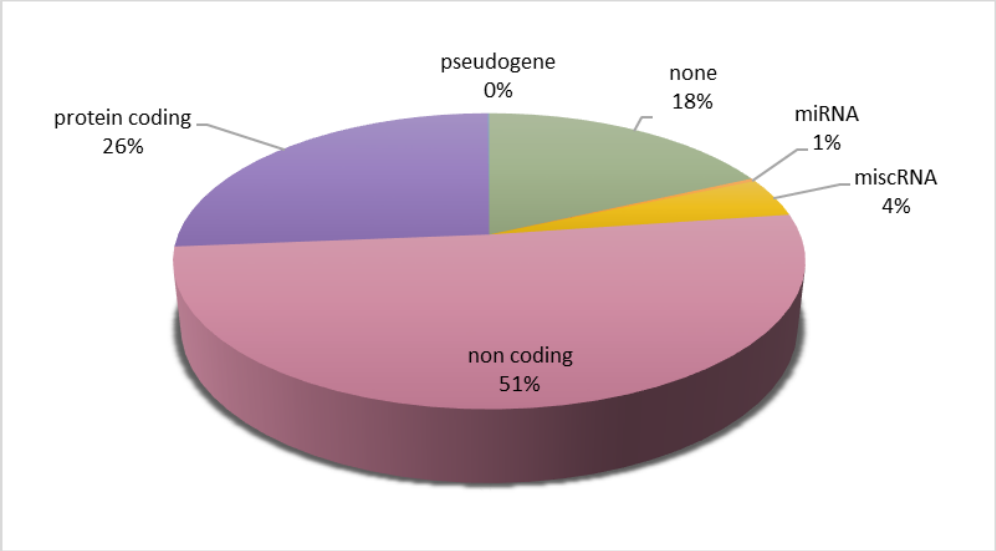


Figure 8; Pie chart showing the type of variant/SNPs with frequencies over all variants

CHAPTER FIVE

5.0. DISCUSSION

5.1. Genetic diversity

This study investigated the genetic diversity and relationships of eight indigenous chicken populations in Kenya. This was done using 12 microsatellite markers, all recommended by ISAG except LEI0258 and MCW0371. The overall expected (H_E) and observed (H_O) heterozygosities for all populations and loci were high, 0.726 ± 0.009 and 0.714 ± 0.011, respectively (Table 2). These values are a reflection of how diverse the indigenous chickens in Kenya are within their ecotypes and between populations. These estimates are higher than those reported by Mwacharo *et al*. (2007) for H_O (0.58) and H_E (0.66) estimated using autosomal markers. This difference can be due to factors such as cross breeding between populations, gene flow across boundaries, different sample numbers and types of markers used. In his study, Mwacharo *et al*. (2007) did not use LEI0258 and MCW0371 which are Major Histocompatibility Complex (MHC) related markers.

Cross breeding leads to increased heterozygosity among populations and sub-populations (Keambou *et al*., 2014). Gene flow across boundaries introduces new unique alleles in a population leading to an increase in heterozygosity for the whole population (Keambou *et al*., 2014). Gene flow may be considered an influential factor as there has been an increase in marketing of indigenous chicken in the country where different populations were transported to other areas in search of market. An increase in demand for indigenous chicken has also resulted to farmers outcrossing different ecotypes to get a better breed that is more productive than the existing chickens without considering breeding practices.

Sampling from a small population in very geographically isolated areas may also be a factor that resulted to having low heterozygosity values reported by Mwacharo *et al*. (2007). Isolated areas have reduced migration rates into the population thus have a more homozygous population. Sampling closely related individuals in an area and not taking distance into consideration may also lead to a low heterozygote count.

Genetic diversity studies in many African countries have reported lower H_O and H_E values than those reported in this study. The estimates for H_O and H_E were, respectively, were 0.62 and 0.62 in Tanzania (Lyimo *et al.*, 2013), 0.60 and 0.62 in Cameroon (Keambou *et al.,* 2014), and 0.55 and 0.57 in South Africa (Mtileni *et al.*, 2011). However, Ethiopia reported a higher H_E (0.82) than the Kenyan population but with a low H_O (0.76) (Mogesseh *et al.,* 2007). This reflects the potential for high genetic variation within their populations.

The observed number of alleles doubled the number of effective alleles 8.094 and 4.45, a similar case reported by Mwacharo (2007) with the values 8.83 and 3.43 respectively. Of all the chicken ecotypes, Bondo chicken population had the highest mean number of alleles (N_A), H_O and H_E and Lamu had the least (Table 3). Geographically close ecotypes had closely related values than those that are distant. This is attributed to the high likelihood of sharing of genetic material between the two populations. The small value for Lamu can be attributed to the geographic seclusion of this area from the rest which limits/hinders introduction of new genes into these populations. The allelic and heterozygosity values for Lamu, however, are high compared to Tanzania (H_E 0.58 for kuchi breed) and Sudan (H_E 0.552) (Lyimo *et al*., 2013; Berima *et al*., 2013).

Most developed countries have lower values because of active artificial selection done on breed lines of specific traits (Abebe *et a*l., 2013). Stabilizing and directional selection can lead to reduced heterozygosity, fixation of alleles and even worse, may result to extinction of specific alleles in

the population. Historic population bottlenecks and founder effects can also be the cause of low variations (Kreivi *et al.,* 2005). This is because the bottleneck reduces the population to a small number which rarely reflects the actual genetic make-up of the former population. Taita Taveta had the smallest number of founders (-0.004).

5.1.1. F statistics and AMOVA

Wrights F Statistics F_{IT}, F_{ST} and F_{IS}, 0.093, 0.029 and 0.066 respectively were almost similar to those estimated by Dana *et al*. (2011) on Ethiopian chicken (0.095, 0.033 and 0.064, respectively). This was however different from Chinese chicken, respectively, 0.18, 0.164 and 0.02 (Chen *et al.,* 2008) and Cameroonian chicken, respectively, 0.13, 0.08 and 0.03 (Keambou *et al.,* 2014). The coefficient of inbreeding, the probability that genes chosen at random at any locus in the DNA are identical by ancestry, was high within individuals (91%).

The inbreeding coefficient estimates were low in the sub population the individual belongs (6.6%) and to the entire population (9.3%). These values depend on the degree of relationships of the individuals within the population as well as the restricted population size. A small effective number of alleles for the sample leads to a larger genetic drift in frequencies from one generation to the next one (Kreivi *et al.*, 2005). In this study, this value was almost half the total number of alleles observed (Table 2).

Four of the 12 microsatellite markers exhibited excess heterozygosity (negative inbreeding coefficient) (MCW34 -0.018, MCW67 -0.016, MCW81 -0.009 and MCW0371 -0.020). Excess heterozygosity comes about after a population has recently experienced a genetic bottleneck or when heterozygotes have a selective advantage. At loci, it arises as a result of high mutations rates at the specific locations (Cornuet and Luikart, 2002). High inbreeding coefficients as reflected by locus MCW69 and ADL268 can be due to fixation of one type of allele in all breeds, presence of

different homozygotes alleles for different breeds at the same locus or effects of null alleles in the population (Zanetti *et al.,* 2011).

AMOVA results obtained showed an almost similar variation among populations, individuals and within individuals (Table 8) as the indigenous chicken population in Cameroon 4.27%, 10.36% and 85.36% (Keambou *et al.,* 2014). The figures were, however, smaller than those for Ethiopian chicken which had 96.6% within population variations (Dana *et al.,* 2011). The among ecotype variations for Ethiopian indigenous chicken was similar to those for Kenya (Dana *et al.,* 2011).

By population, Lamu and Turkana had the highest frequency for private alleles for locus LEI0258. This marker had 46 alleles out of the total 140, accounting for 32.86%. High polymorphism in this locus is also seen in Italian chicken with 16 alleles (Zanetti *et al.,* 2011), Ugandan and Egyptian chicken both having 21 alleles (Wei *et al.,* 2011).This marker is said to be located on micro-chromosome 16 in the B region of the Major Histocompatibility Complex in the chicken genome. The MHC region is believed to control disease resistance, immune responses and other productive and reproductive traits in chicken (Nikbakht and Esmailnejad, 2015). This results in high polymorphism of the genes in the locus (Ng'eno *et al*., 2015).

The percentage of loci with private alleles was 23.57% (33 of 140 alleles), a value similar to that reported in Ethiopian chicken (24%) (Dana *et al.,* 2011) and double the value reported in Cameroonian chicken (10.62%) (Keambou *et al*., 2014). The private alleles bring out the uniqueness of a population or ecotype. This helps in identifying whether the populations under study are genetically different or not.

5.1.2. Genetic distances

The Nei's genetic distance between populations was shortest between the Bomet and West Pokot ecotypes (0.003) with a genetic identity of 99.7% similarity. Others among the least distanced were Kakamega, Bondo, Turkana, and Taita Taveta to West Pokot. This is associated to the short distance between these regions. As a result, exchange of genetic material and genetic drift can easily occur within these populations. Lack of geographic barriers provide a suitable avenue for gene flow leading to genetic likeness between the chickens.

Geographic distance is negatively associated with genetic identity as the ecotypes far from each other depicted the least closeness to each other (Kreivi *et al.,* 2005). The Lamu population was furthest from Narok, Turkana and Kakamega with genetic identity of 77.9%, 79.3% and 79.4% respectively. Since there is a low likelihood that a population can be non-identical to another (0%), these values are considered to be high. Lamu is considered the most geographically isolated from the others.

5.1.3. Population structure

The genetic structure for all the populations resulted to two main clusters as yielded by Evanno method. The highest log likelihood value (Delta K) was found to be 57.55 at K=2. This was followed by a lower Delta K value of 9.2 at K=3. The Lamu ecotype formed its own cluster while the other cluster was as a result of seven populations coming together. The Taita Taveta ecotype almost formed its own cluster but this distinction faded as the precision for K value increased to 8. This is because the area is geographically distanced from the other counties (Abebe *et al.,* 2013). Market interactions, genetic relatedness of founder members and crossing of genes between populations are the possible causes of the resultant cluster of the seven ecotypes (Kreivi *et al.,* 2005, Dana et al., 2011).

These results for population structure were confirmed by Mantel's test where the graph displayed two clusters for all the populations (Figure 5). The high number of loci with private/unique alleles and the high private allele frequency had a significant contribution to forming a distinct population (Table 4 and 5). Moreover, as other populations deviated from the Hardy Weinberg's equilibrium, the Lamu population lingered in equilibrium.

5.2. Selection signatures

Indigenous chicken have been continuously selected to improve on their performance and productivity even after the domestication period. Through selection, populations develop different adaptive traits from the environment they are in. Markers identified as outliers in this study suggest that these loci in the genome could be influenced by the process of selection. The genes near these loci end up encoding proteins which are associated with the selected traits. Since most traits are influenced by a number of genes i.e are polymorphic in nature, selection that acts on these polymorphic traits end up changing the allele frequencies at many loci in the genome. Frequent variations for the specific traits end up being fixed in the genetic make-up leading to a distinction from the founder population. These alleles lead to a change in the phenotypic attributes of the individuals (Gutierrez et al., 2016).

The northern part of rift valley (West Pokot and Turkana) is largely a semi arid area and the occupants are pastoralists who keep chicken secondary to cattle and goats. Crop failure, extreme temperatures and harsh environment characterize this area (Opiyo *et al.,* 2012). The southern region, on the contrary, is arable and sustains crop production. The difference in diversity between chicken in these two regions could be due to adaptation to the different environmental conditions for their survival. Chicken in a hot climatic conditions end up having reduced feather density for instance, the naked neck chicken or frizzled feathers, less fat deposits under their skins (Cahaner,

2012). Since feed is also not readily available, small body size and and slower growth rates is evident contrary to the chicken in the southern region. The breeding goals of the two areas may also bring a difference.

Breeding goals like maximizing on egg production, fast growth rates, better feed conversion rates and hardiness may lead to having diverse chicken in these two regions as certain genes are favoured. Farmers in the semi arid areas prefer naked neck chicken because they are not affected by the extreme temperatures and perform better than other chicken (Cahaner, 2012). Selection for such genes end up to a genetically distinct population from the others due to fixation of the frequent and dominant gene.

5.2.1. Functional annotation of genomic variants

Variants are classified based on their prospective effects on genes. SNPs are known to be the most common genetic variation forms which affects the sequences of proteins.The variations affect protein functions, stability, and how the proteins interact with other proteins which may result to disorders or diseases (Yates et al., 2014). This study identified genes affected by the different forms of variations. However, the specific effects of these variations/ SNPs on the genes were not determined. The genes identified with the associated SNPs includes *OLFM3* which codes for the protein Olfactomedin 3, responsible for the development of new blood vessels from the existing ones in tumors (Sakuragi et al., 2005). Amylase alpha 1 A (*AMY1A*) which codes for the salivary amylase enzyme is essential in the carbohydrate metabolic process (Barlow, 2000) while *RNPC3* gene whose protein functions include nucleotide binding and the development of subcellular structures, cells, tissues and organs. *COL11A1* gene codes for the Collagen alpha 1 (XI) protein which plays a role in controlling lateral growth of collagen 11 fibrils during fibrillogenesis (Mao

and Nah, 2004). This gene is expressed in cartilage and non-cartilaginous embryonic tissues e.g heart, brain, skeletal muscle and skin.

In a study on effects of intronic variants on the transcriptional regulation of human dopamine transporter (*DAT 1*) gene, Greenwood and Kelsoe, (2003) identified that introns variants 9,12 and 14 contained enhancer elements which increased the expression of the *DAT 1* gene. Missense variants can weaken or strengthen protein to protein interactions leading to among many other effects, loss of salt bridges, steric clashes or modifications of translated proteins (Yates et al., 2014). The modification of the protein can render it nonfunctional or may cause replacement of an amino acid with another at the same position (destruction of a salt bridge and structure destabilization) which in most cases is expressed as a syndrome or disorder (Al Haggar et al, 2012). These changes in the genetic make-up may also result to altered phenotypes as the different biochemical pathways and complexes are affected.

SNPs effects can also be silent if their impact is not expressed on the phenotype. On the coding region of the gene, most SNPs do not have severe consequences due to the degeneracy of the gene code where different codons can code for the same amino acid eg leucine codons are UUA, UUG, CUU, or CUG codons and glutamine is coded by CAG and CAA. A change in one of the nucleotides would still result to the same amino acid hence have no negative effect expressed.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The indigenous chicken population in Kenya have a rich genetic pool compared to other countries. However, with the increasing rates of flow of genes across boundaries, areas with unique breeds of indigenous chicken might be at risk of losing the native strain adopted to that specific area due to cross breeding. To save these breeds, a sustainable breeding strategy should be developed alongside policies to regulate/control the flow of genes across boundaries for breeding purposes. The important traits of economic significance can also be harnessed and the specific traits fixed to come up with a better performing indigenous breeds or lines so as to improve the productivity of the poultry sector in the country.

6.2. Recommendations

A proper and sustainable breeding program should be developed to help in conservation of the indigenous genetic resource in the indigenous chicken. The conservation program can utilize the ability of different genotypes to match the different environments. This will in turn result to sustainable utilization of the chicken products without the need to concentrate on guessing which breeds to cross to improve on production.

Further studies can also be done to understand the effects of the variations in the Kenyan chicken genome and determine how these alterations lead to change in phenotype, resistance to infections and adaptability to the environment. This will help in effective prediction of the changes and their effects in other populations and species.

REFERENCES

Abebe, A. S. (2013). Analysis of the genetic diversity of local Swedish chicken breeds using microsatellite markers.

Avigdor Cahaner (2012). Adaptation of broilers to hot climate. World poultry congress, Salvador, Bahia, Brazil.

Alemu, T. (2004). Genetic characterization of indigenous goat populations of Ethiopia using microsatellite DNA markers (Unpublished, National Dairy Research Institute, Deemed University).

Al-Haggar M, Madej-Pilarczyk A, Kozlowski L, Bujnicki JM, Yahia S, Abdel-Hadi D, Shams A, Ahmad N, Hamed S, Puzianowska-Kuznicka M (2012). "A novel homozygous p.Arg527Leu LMNA mutation in two unrelated Egyptian families causes overlapping mandibuloacral dysplasia and progeria syndrome.". Eur J Hum Genet. 20 (11): 1134–40.

Al-Samarai, F. R., & Al-Kazaz, A. A. (2015). Molecular Markers: an Introduction and Applications.

Apuno, A. A., Mbap, S. T., 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.

Aviana Africa, (2015) livestock and poultry expo

Barlow Maureen Pugh, ed. (2000). Stedman's Medical Dictionary (27th ed.). Baltimore, Maryland, USA: Lippincott Williams & Wilkins. p. 65. ISBN 978-0-683- 40007-6.

Bett, H. K, M. P. Musyoka, K. J. Peters, and W. Bokelmann, "Demand for Meat in the Rural and Urban Areas of Kenya: A Focus on the Indigenous Chicken," Economics Research International, vol. 2012, Article ID 401472, 10 pages, 2012. doi:10.1155/2012/401472

Blum, L.S. (2008). Formative Research on Avian Influenza, Kenya.

Cabrera, C. P., Navarro, P., Huffman, J. E., Wright, A. F., Hayward, C., Campbell, H. ...& Haley, C. S. (2012). Uncovering networks from genome-wide association studies via circular genomic permutation. *G3: Genes| Genomes| Genetics*, *2*(9), 1067-1075.

Chen, G., Bao, W., Shu, J., Ji, C., Wang, M., Eding, H. & Weigend, S. (2008). Assessment of population structure and genetic diversity of 15 Chinese indigenous chicken breeds using microsatellite markers. *Asian australasian journal of animal sciences*, *21*(3), 331.

Dana, N., Hanotte, O., Jianlin, H., Dessie, T., vander Waaij, E. H., & van Arendonk, J. A. (2011). Microsatellite DNA analysis of genetic diversity and population structuring in Ethiopian chickens. *Breeding programs for indigenous chicken in Ethiopia*, 61.

Dessie, T., Dana, N., Ayalew, W., & Hanotte, O. (2012). Current state of knowledge on indigenous chicken genetic resources of the tropics: domestication, distribution and documentation of information on the genetic resources. *World's Poultry Science Journal*, *68*(01), 11-20.

Ding, F. X., Zhang, G. X., Wang, J. Y., Li, Y., Zhang, L. J., Wei, Y., ... & Hou, Q. R. (2010). Genetic diversity of a Chinese native chicken breed, Bian chicken, based on twenty-nine microsatellite markers. *Asian-Aust. J. Anim. Sci*, *23*(2), 154-161.

Earl, D. A. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation genetics resources*, *4*(2), 359-361.

Elferink, M. G., Megens, H. J., Vereijken, A., Hu, X., Crooijmans, R. P., & Groenen, M. A. (2012). Signatures of selection in the genomes of commercial and non-commercial chicken breeds. *PLoS One*, *7*(2), e32720.

Eriksson, J., Larson, G., Gunnarsson, U., Bed'hom, B., Tixier-Boichard, M., Strömstedt, L. ...& Jensen, P. (2008). Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. *PLoS Genet*, *4*(2), e1000010.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular ecology, 14(8), 2611-2620.

Fan, G., & Li, J. (2011). Regions identity between the genome of vertebrates and non-retroviral families of insect viruses. Virology Journal, 8, 511. http://doi.org/10.1186/1743-422X-8-511

FAO (2007). The structure and importance of the commercial and village based poultry systems in Kenya: An analysis of the poultry sector in Kenya. Food and Agriculture Organization of the United Nations, Rome, 93pp.

FAO, (2007). The state of the world's animal genetic resources for food and agriculture. Commission on genetic resources for food and agriculture. FAO Rome a CD-ROM.

FAO, (2012) - Yearbook, F. P. (2012). FAO. *Rome, Italy ISBN*, 978-92.

FAO, LPP, L. (2010). Network, IUCN-WISP. 2010. Adding value to livestock diversity: Marketing to promote local breeds and improve livelihoods. *FAO Animal Production and Health Paper*, *168*.

Faostat, F. (2013). Agriculture organization of the United Nations.

Fonteque, G. V., Battilana, J., Paludo, E., & Lima-Rosa, C. A. D. V. (2014). Genetic polymorphism of fifteen microsatellite loci in Brazilian (blue-egg Caipira) chickens. *Pesquisa Veterinária Brasileira*, *34*(1), 98-102.

Fulton, J. E., Juul-Madsen, H. R., Ashwell, C. M., McCarron, A. M., Arthur, J. A., O'Sullivan, N. P., & Taylor Jr, R. L. (2006). Molecular genotype identification of the Gallus gallus major histocompatibility complex. *Immunogenetics*, *58*(5-6), 407-421.

Gholizadeh, M., & Mianji, G. R. (2007). Use of microsatellite markers in poultry research. *International Journal of Poultry Science*, *6*(2), 145-153.

Gifford – Gonzalez, D., & Hanotte, O. (2011). Domesticating animals in Africa: Implications of genetic and archaeological findings. Journal of World Prehistory.

Glaubitz, J. C. (2005). CONVERT, version 1.31.

Goudet, J. (2002). FSTAT version 2.9. 3.2, a program to estimate and test gene diversities and fixation indices. *Institute of Ecology, Lausanne, Switzerland.*

Greenwood, T. A., & Kelsoe, J. R. (2003). Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. Genomics, 82(5), 511-520.

Groenen, M.A.M., Cheng, H.H., Bumstead, N., Benkel, B.F., Briles, W.E., Burke, T., Burt, D.W., Crittenden, L.B., Dodgson, J., Hillel, J., Lamont, S., De leon, A.P., Soller, M., Takahashi, H. and Vignal, A., (2000). A consensus linkage map of the chicken genome. *Genome Res.*10: 137- 147.

Guèye, E.F., (2000). The role of family poultry in poverty alleviation food security and the promotion of gender equality in rural Africa. *Outlook on Agriculture* 29: 129-136.

Han, B., Lian, L., Qu, L., Zheng, J., & Yang, N. (2013). Abundant polymorphisms at the microsatellite locus LEI0258 in indigenous chickens. *Poultry science*, *92*(12), 3113-3119.

Hassaballah, K., Zeuh, V., Lawal, R. A., Hanotte, O., & Sembene, M. (2015). Diversity and Origin of Indigenous Village Chickens (Gallus gallus) from Chad, Central Africa. *Advances in Bioscience and Biotechnology*, *6*(9), 592.

Hoque, M. R., Lee, S. H., Jung, K. C., Kang, B. S., Park, M. N., Lim, H. K., ... & Lee, J. H. (2011). Discrimination of Korean native chicken populations using SNPs from mtDNA and MHC polymorphisms. *Asian-Australasian Journal of Animal Sciences*, *24*(12), 1637-1643.

Hoshino Andrea Akemi, Juliana Pereira Bravo, Paula Macedo Nobile and Karina Alessandra Morelli, (2010). Microsatellites as Tools for genetic diversity analysis

ICGSC (2004).Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature, 432, 695–716.*

Jensen, J. L., Bohonak, A. J., & Kelley, S. T. (2005). Isolation by distance, web service. BMC genetics, 6(1), 13.

Justus, O., Owuor, G., & Bebe, B. O. (2013). Management practices and challenges in smallholder indigenous chicken production in Western Kenya. *Journal of Agriculture and Rural Development in the Tropics and Subtropics (JARTS)*, *114*(1), 51-58.

Jones, D. O., Cowell, I. G., & Singh, P. B. (2000). Mammalian chromo domain proteins: their role in genome organization and expression. *Bioessays*, *22*(2), 124-137.

Kaya, M., & Yıldız, M. A. (2008). Genetic diversity among Turkish native chickens, Denizli and Gerze, estimated by microsatellite markers. *Biochemical genetics*, *46*(7-8), 480-491.

Keambou, T. C., Hako, B. A., Ommeh, S., Bembide, C., Ngono, E. P., Manjeli, Y., ... & Skilton, R. A. (2014). Genetic diversity of the Cameroon indigenous chicken ecotypes. *International Journal of Poultry Science*, *13*(5), 279.

Khobondo J O, Okeno T O, Lihare G O, Wasike C B, and Kahi A K (2014). The past, present and future genetic improvement of indigenous chicken of Kenya. Animal Genetic Resources, 125.

Khobondo, J. O., Muasya, T. K., Miyumo, S., Okeno, T. O., Wasike, C. B., Mwakubambanya, R.& Kahi, A. K. (2015). Genetic and nutrition development of indigenous chicken in Africa. *Livestock Research for Rural Development*, *27*, 7.

Kingori, A. M., Wachira, A. M., & Tuitoek, J. K. (2010). Indigenous chicken production in Kenya: a review. *International Journal of Poultry Science*, *9*(4), 309-316.

Kreitman, M. (2000). Methods to detect selection in populations with applications to the human. *Annual review of genomics and human genetics*, *1*(1), 539-559.

Kreivi, M., Rautiainen, P., Aspi, J., & Hyvärinen, M. (2005). Genetic structure and gene flow in an endangered perennial grass, Arctophila fulva var. pendulina. Conservation Genetics, 6(5), 683-696.

Langella, O. (1999). POPULATIONS 1.2. 30 Population genetic software. *CNRS UPR9034. [Online.] Available at bioinformatics.org/~ tryphon/populations*.

Lateef, D. D. (2015). DNA marker technologies in plants and applications for crop improvements. Journal of Biosciences and Medicines, 3(05), 7.

Leroy, G., Kayang, B. B., Youssao, I. A., Yapi-Gnaoré, C. V., Osei-Amponsah, R., Loukou, N. E., … Rognon, X. (2012). Gene diversity, agroecological structure and introgression patterns among village chicken populations across North, West and Central Africa. *BMC Genetics*, *13*, 34.

Luikart, G., & Cornuet, J. M. (1998). Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data.*Conservation biology*, *12*(1), 228-237.

Lyimo, C. M., Weigend, A., Janßien-Tapken, U., Msoffe, P. L., Simianer, H., & Weigend, S. (2013). Assessing the genetic diversity of five Tanzanian chicken ecotypes using molecular tools. *South African Journal of Animal Science*, *43*(4), 499-510.

Magothe and Kahi, 2010. Indigenous chicken improvement in Kenya; past efforts and future prospects. Ministry of Livestock and Fisheries Development (MOLFD) (1996-2007). Various Animal Production Annual Reports.

Magothe, T. M., Okeno, T. O., Muhuyi, W. B., & Kahi, A. K. (2012). Indigenous chicken production in Kenya: I. Current status. *World's Poultry Science Journal*, *68*(01), 119-132.

Maina, J.O., (2000). Morphological and molecular characterization of Kenyan indigenous chickens. Unpublished Ph.D. thesis, Egerton University, Njoro, Kenya

Mao, J. J., & Nah, H. D. (2004). Growth and development: hereditary and mechanical modulations. American Journal of Orthodontics and Dentofacial Orthopedics, 125(6), 676-689.

Menge, E. O. (2008). Breeding goals for production systems utilizing indigenous chicken in Kenya (Doctoral dissertation, Egerton University).

Mogesse, H. H. (2007). Phenotypic and genetic characterization of indigenous chicken populations in Northwest Ethiopia. *Unpublished PhD Thesis, University of the Free State, Bloemfontein, South Africa*.

Moiseyeva, I.G., Romanov, M.N., Nikiforov, A.A., Sevastyanova, A.A. and Semyenova, S.K., (2003). Evolutionary relationships of Red Jungle Fowl and chicken breeds. Genetics Selection Evolution, 35: 403 – 423.

MOLD (2006). Animal Production Division Annual Report. Ministry of Livestock Development, Nairobi, Kenya.

MOLD (2015). Animal Production Division Annual Report. Ministry of Livestock Development, Nairobi, Kenya.

Mtileni, B. J., Muchadeyi, F. C., Maiwashe, A., Groeneveld, E., Groeneveld, L. F., Dzama, K., & Weigend, S. (2011). Genetic diversity and conservation of South African indigenous chicken populations. *Journal of Animal Breeding and Genetics*, *128*(3), 209-218.

Mungube, E. O., Bauni, S. M., Tenhagen, B. A., Wamae, L. W., Nzioka, S. M., Muhammed, L., & Nginyi, J. M. (2008). Prevalence of parasites of the local scavenging chickens in a selected semi-arid zone of Eastern Kenya. *Tropical Animal Health and Production*, *40*(2), 101-109.

Mwacharo, J. M., Bjørnstad, G., Han, J. L., & Hanotte, O. (2013a). The History of African Village Chickens: an Archaeological and Molecular Perspective. *The African Archaeological Review*, *30*, 97–114.

Mwacharo, J. M., Bjørnstad, G., Mobegi, V., Nomura, K., Hanada, H., Amano, T., ... & Hanotte, O. (2011). Mitochondrial DNA reveals multiple introductions of domestic chicken in East Africa. *Molecular phylogenetics and evolution*, *58*(2), 374-382.

Mwacharo, J. M., Nomura, K., Hanada, H., Han, J. L., Amano, T., & Hanotte, O. (2013b). Reconstructing the origin and dispersal patterns of village chickens across East Africa: insights from autosomal markers. *Molecular Ecology*, *22*(10), 2683–2697.

Mwacharo, J. M., Nomura, K., Hanada, H., Jianlin, H., Hanotte, O., & Amano, T. (2007). Genetic relationships among Kenyan and other East African indigenous chickens. *Animal Genetics*, *38*(5), 485-490.

Nei, M. (1977). F- statistics and analysis of gene diversity in subdivided populations. *Annals of human genetics*, *41*(2), 225-233.

Ngeno, K., van der Waaij, E. H., Megens, H. J., Kahi, A. K., van Arendonk, J. A. M., & Crooijmans, R. P. M. A. (2015). Genetic diversity of different indigenous chicken ecotypes using highly polymorphic MHC-linked and non-MHC microsatellite markers. *Animal Genetic Resources/Ressources génétiques animales/Recursos genéticos animales*, *56*, 1-7

Nielsen, R., Bustamante, C., Clark, A. G., Glanowski, S., Sackton, T. B., Hubisz, M. J., & Sninsky, J. J. (2005). A scan for positively selected genes in the genomes of humans and chimpanzees. *PLoS Biol*, *3*(6), e170.

Nikbakht, G. & Esmailnejad, A. Immunogenetics (2015) 67: 247. doi:10.1007/s00251-015-0832-7

Njue, S. W. (2002). Family poultry production in Kenya. In: *Proceedings, Kenya poultry workshop 18th-21st November 2002, Mombasa, Kenya*. Network for smallholder poultry development, Jan. 2003 (unpublished).

Nwachukwu, E. N., Ibe, S. N., Eiekwu, K. and Oke, U. K. (2006). Evaluation of growth parameters of main and reciprocal crossbred normal, naked neck and frizzle chickens in a humid tropical environment. Journal of Animal and Veterinary Advances, 5: 542 – 546.

Nwachukwu, E.N., Ibe, S.N. and Ejekwu, K., (2006). Short term egg production and egg quality characteristics of main and reciprocal crossbred normal local, naked-neck and frizzle chicken X exotic broiler breeder stock in a humid tropical environment. *Journal of Animal and Veterinary Advances*. 5 (7): 547-551.

Nyaga P., (2007a). The structure and importance of the commercial and village based poultry systems in Kenya. Food and Agricultural Organization of the United Nations.

Nyaga, P.N., (2007). Kenya poultry sector review. Food and Agriculture Organization, Rome, Italy.

Ogali, I. (2011). Performance and constraints of the poultry production system among FFS farmers in Pemba island, Tanzania.

Olowofeso, O., Wang, J. Y., Dai, G. J., Yang, Y., Mekki, D. M., & Musa, H. H. (2005). Measurement of genetic parameters within and between Haimen chicken populations using microsatellite markers. *International Journal of Poultry Science*, *4*(3), 143-148.

Olwande, P. O., Ogara, W. O., Okuthe, S. O., Muchemi, G., Okoth, E., Odindo, M. O., & Adhiambo, R. F. (2010). Assessing the productivity of indigenous chickens in an extensive management system in southern Nyanza, Kenya. *Tropical animal health and production*, *42*(2), 283-288.

Omiti, J. M., & Okuthe, S. O. (2009). An Overview of the Poultry Sector and Status of Highly Pathogenic Avian Influenza (HPAI) in Kenya*: Background Paper*. International Food Policy Research Institute (IFPRI).

Omiti, J., Otieno, D., Nyanamba, T., & McCullough, E. (2009). Factors influencing the intensity of market participation by smallholder farmers: A case study of rural and peri-urban areas of Kenya. *African Journal of Agricultural and Resource Economics*, *3*(1), 57-82.

Opiyo, F. E., Wasonga, O. V., Schilling, J., & Mureithi, S. M. (2012). Resource-based conflicts in drought-prone Northwestern Kenya: the drivers and mitigation mechanisms. Wudpecker Journal of Agricultural Research, 1(11), 442-453.

Osman, S. A. M., Sekino, M., Nishibori, M., Yamamoto, Y., & Tsudzuki, M. (2005). Genetic variability and relationships of native Japanese chickens assessed by microsatellite DNA profiling - Focusing on the breeds established in Kochi Prefecture, Japan. *Asian-Aust. J. Anim. Sci*, *18*, 755-761.

Peakall E and Smouse P R. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*, *28*, 2537-2539.

Pritchard, J. K., Wen, X., & Falush, D. (2010). Documentation for STRUCTURE software, version 2.3. University of Chicago, Chicago, IL.

Qing, W. M., Han Jian Lin, Wei Yan Ming, (2011). The Genetic Diversity of Egyptian and Ugandan Indigenous Chicken Populations. Web; http://www.dissertationtopic.net/doc/148866

Rashid, M. A., Howlider, M. A. R., Alam, J., Rashid, A., Kawsar, M. H., & Azmal, S. A. (2005). Effect of dwarfism on reproductive and meat yield parameters of crossbred chicken. *International Journal of Poultry Science*, *4*(6), 372-377.

Rosenberg, N. A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, *4*(1), 137-138.

Rubin, C. J., Zody, M. C., Eriksson, J., Meadows, J. R., Sherwood, E., Webster, M. T. & Hallböök, F. (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*, *464*(7288), 587-591.

Safalaoh, A.C.L., (2001). Village chicken upgrading programme in Malawi. World's Poultry Science. Journal, 57: 179 – 188.

Sakuragi M., Noriaki S., Makoto I., Masako K., Takayuki T.K. Harukazu N, and Yoshiki S. (2005).Functional analysis of chick ONT1 reveals distinguishable activities among olfactomedin-related signaling factors. *Mechanisms of Development 123 (2006) 114–123.* www.elsevier.com/locate/modo

Sambo, E., Bettridge, J., Dessie, T., Amare, A., Habte, T., Wigley, P., & Christley, R. M. (2015). Participatory evaluation of chicken health and production constraints in Ethiopia. *Preventive veterinary medicine*, *118*(1), 117-127.

Schlötterer, C. (2003). Hitchhiking mapping–functional genomics from the population genetics perspective. *Trends in Genetics*, *19*(1), 32-38.

Sethuraman, A. (2013). On inferring and interpreting genetic population structure-applications to conservation, and the estimation of pairwise genetic relatedness.

Sonaiya, E. B., & Swan, S. E. J. (2007). Small scale poultry production: technical guide (Vol. 1). Daya Books.

Spinella, D. G., Bernardino, A. K., Redding, A. C., Koutz, P., Wei, Y., Pratt, E. K., ... & McConnell, S. J. (1999). Tandem arrayed ligation of expressed sequence tags (TALEST): a new method for generating global gene expression profiles. *Nucleic acids research*, *27*(18), e22-i.

Ssewanyana E, Onyait AO, Ogwal Okot, Masaba J., (2006). Strategies for improving meat and egg productivity of indigenous chickens in Kumi and Apac districts, Uganda. Journal of agricultural sciences2 (12; 31-35)

Stainton, J. J., Haley, C. S., Charlesworth, B., Kranis, A., Watson, K., & Wiener, P. (2015). Detecting signatures of selection in nine distinct lines of broiler chickens. *Animal genetics*, *46*(1), 37-49.

Storey, A. A., Athens, J. S., Bryant, D., Carson, M., Emery, K., Higham, C. ...& Ladefoged, T. (2012). Investigating the global dispersal of chickens in prehistory using ancient mitochondrial DNA signatures. *PloS one*, *7*(7), e39171.

Tixier-Boichard, M., Bordas, A., & Rognon, X. (2009). Characterization and monitoring of poultry genetic resources. *World's Poultry Science Journal*, *65*(02), 272-285.

Upton, M., (2000). The livestock revolution-Implications for smallholder agriculture: A case study of milk and poultry production in Kenya. Livestock Policy Discussion Paper No. 1, Food and Agriculture Organization, Rome, Italy.

Von Braun, J., Ahmed, A., Asenso-Okyere, K., Fan, S., Gulati, A., Hoddinott, J., ... & van Rheenen, T. (2008). High food prices: the what, who, and how of proposed policy actions. Washington, DC: International Food Policy Research Institute.

Weigend S. and Romanov M. N., (2001). Current strategies for the assessment and evaluation of genetic diversity in chicken resources. World's Poultry Science Journal. 57 275-288.

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, 1358-1370.

Werner M, Sych M, Herbon N, Illig T, Konig IR, Wjst M. (2002). Large-scale determination of SNP allele frequencies in DNA pools using MALDI-TOF mass spectrometry. Hum Mutat, 20:57–64.

Ya-Bo, Y., Jin-Yu, W., Mekki, D. M., Qing-Ping, T., Hui-Fang, L., Rong, G., ... & Kuan-Wei, C. (2006). Evaluation of genetic diversity and genetic distance between twelve Chinese indigenous chicken breeds based on microsatellite markers. *Int. J. Poult. Sci*, *5*, 550-556.

Yates, Christopher M, and Sternberg, Michael JE (Aug 2014) Impact of Missense Variants on Protein–Protein Interactions. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net [doi: 10.1002/9780470015902.a0025699]

Zanetti, E., De Marchi, M., Abbadi, M., & Cassandro, M. (2011). Variation of genetic diversity over time in local Italian chicken breeds undergoing in situ conservation. *Poultry science*, *90*(10), 2195-2201.