DIVERSITY, GENETIC BACKGROUND AND HSP70 GENE FUNCTIONAL POLYMORPHISMS FOR HEAT TOLERANCE IN INDIGENOUS CHICKENS IN KENYA

GRACE MORAA KENNEDY

MASTER OF SCIENCE

(BIOTECHNOLOGY)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2016

Diversity, Genetic Background and Hsp70 Gene Functional Polymorphisms for Heat Tolerance in Indigenous Chickens in Kenya

Grace Moraa Kennedy

A thesis submitted in partial fulfillment for the Degree of Master of Science in Biotechnology in the Jomo Kenyatta University of Agriculture and Technology

2016

DECLARATION

This research thesis is my original work and has not been presented for a degree or any other award in any institution.

Signature: …… Date: ...……

Kennedy Grace Moraa

This research thesis has been submitted for examination with our approval as supervisors.

Signature: …… Date: ...……

Dr. Sheila Ommeh

JKUAT, Kenya

Signature: …… Date: ...……

Dr. Emmanuel Ndiema

National Museums, Kenya

DEDICATION

This work is dedicated to my beloved parents, Mr. Rogers M Kennedy, Mrs. Jane N Kennedy, my brother James N Kennedy and my sister Deborah M Kennedy who have been there for me throughout the period of this study.

ACKNOWLEDGEMENT

I give glory and praise to God for His grace, peace, patience, strength and wisdom to undertake this project.

I am greatly indebted for the research grant JKU/2/4/RP/181 awarded to Dr. Sheila Ommeh by the Jomo Kenyatta University of Agriculture and Technology (JKUAT), Research Production and Extension Division (RPE) and a research grant iCMoB/01/2014 awarded to Ms. Grace Moraa by the African Union-african innovation-Jomo Kenyatta University of agriculture and technology and innovation center for molecular biology and Biochemistry without which it may not have been possible to do this work.

I am exceedingly grateful to my supervisors Dr. Sheila Ommeh and Dr. Emmanuel Ndiema for their guidance, encouragement and stimulating discussions constant encouragement throughout the planning and execution of this study.

Special appreciation also goes to the members of the indigenous poultry consortium for their invaluable assistance in the entire course of my study.

I sincerely thank the Institute for Biotechnology Research (IBR) for hosting me during the duration of the project and for their advice, helpful discussion and enlightening comments in my presentations. Lastly, I thank my family who were a source of inspiration to me during the entire period.

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LIST OF ACRONYMS AND ABBREVIATIONS

AEZs	Agro Ecological Zones
ASALs	Arid and Semi-Arid Lands
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
bp	Base Pair
D-loop	Displacement Loop
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
GPS	Global Positioning System
FAO	Food and Agricultural Organization
HSF	Heat Shock Factor
HSFs	Heat Shock Transcription Factors
HSPs	Heat Shock Proteins
HSP70	Heat Shock Protein 70
HVS	Hyper Variable Segment
IBR	Institute for Biotechnology Research
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MEGA	Molecular Evolutionary Genetics Analysis

MJ	Median Joining
mtDNA	Mitochondrial DNA
MUPID	Mini Electrophoresis Unit
MUSCLE	Multiple Sequence Comparison by Log- Expectation
mv	Median vectors
NJ	Neighbor Joining
PCR	Polymerase Chain Reaction
RAPD	Random amplified polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TBE	Tris Boric Ethylenediaminetetraacetic acid
µl	Microliter

ABSTRACT

The arid and semi-arid regions occupy the highest percentage of land mass in Kenya and are worst hit by the ongoing climate change across the horn of Africa. Indigenous chickens in the arid and semi-arid lands (ASALs) have a challenge of having to deal with heat stress. The crossbreeding programs in the 1970's to date have also led to the dilution of indigenous chickens which are known to be hardy and adapt well to the local climate as compared to commercial chickens. This study aimed to 1) characterize the phenotypes for heat tolerance in indigenous chickens of Kenya, 2) investigate the genetic background of indigenous chickens of Kenya 3) characterize the functional polymorphisms on the HSP70 gene in indigenous chickens of Kenya from Lake Turkana basin, Lake Victoria basin, Lamu archipelago and Mt. Elgon catchment. Data was collected from 296 indigenous chickens by the use of questionnaires. Blood samples were stored on FTA® cards. Analysis of variance (ANOVA) for the phenotypic traits was calculated in response to ambient temperature. There was significant relationship between the comb types and the ambient temperature. Similarly, the plumage density and the body temperature had significant relationship. All the other traits (skin color, shank color, comb color, earlobe color and the crested phenotype and feathered shanks phenotype) had no significant relationship to the ambient temperature. Indigenous chickens in Lake Turkana basin had the highest temperatures while those from Mt. Elgon catchment had the least temperatures. Lake Turkana basin had the highest outside temperature while Mt. Elgon catchment had the least temperature. For molecular work, heat shock protein 70 (HSP70) gene and the D-loop region of the Mitochondrial DNA (mtDNA) were amplified using PCR, purified and sequenced. Editing of the sequences was done and the sequences aligned. A total of 28 mtDNA haplotypes were mapped. Most of the samples were Haplotype 1 and most of them were from Lake Turkana basin. The other major haplotypes were 4, 7 and 26. Phylogenetic analysis for mtDNA clustered the 28 haplotypes in 4 out of the 9 reference haplogroups from GenBank. Most of the samples were haplogroup E with a high percentage comprising samples from Lake Turkana basin. None of the samples clustered in haplogroups C, G, F, H, and I. This information was clearly displayed in

the splits decomposition and the Network analysis. Analysis of molecular variance (AMOVA) clearly indicated greater diversity within populations at 78.06%. Three HSP70 haplotypes; AG, AC and GC were mapped. Phylogenetic analysis displayed haplotype GC as the ancestral haplotype since it was in the same clade with the ancestral red jungle fowl and it dominated in Lake Turkana basin. Haplogroup AG was dominant in Lamu archipelago while AC haplogroup had least frequencies in all the agro-climatic zones. The AMOVA values were highest within individuals at 89.62%. No recombination events were observed.

In conclusion this study successfully characterized the phenotypes implicated for heat tolerance. The origin of indigenous chickens in Kenya was also established and polymorphisms in the heat HSP70 implicated in heat tolerance was also established. These initial results should pave way towards first step to genetic breeding for heat tolerant indigenous chickens and conservation efforts of indigenous chickens.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Chickens are from Kingdom *Animalia,* Phylum *Chordate,* Class *Aves,* Order *Galliformes,* Family *Phasianidae,* Subfamily *Phasianinae,* and Genus *Gallus*. There are four species of chickens namely: *Gallus gallus* (red jungle fowl), *Gallus lafayettei* (Ceylon jungle fowl), *Gallus sonnerati* (Grey jungle fowl), and *Gallus varius* (green jungle fowl). Domesticated chickens belong to Subspecies *Gallus gallus domesticus* (Eriksson et al., 2008). Chickens were first domesticated in Asia as early as 8000 years ago and since then they have dispersed to the rest of the world (Mwacharo et al., 2011).

Africa and Asia are connected by a terrestrial route and ever since historical times, they have experienced important seafaring and maritime exchange of people, crops and livestock like indigenous chickens by the maritime traders (Boivin & Fuller, 2009). It's postulated with archaeological evidence that the process of livestock domestication spread southwards during human migration from Egypt to the Nile river basin then to East Africa (Macdonald et al., 1992).

In Kenya the earliest evidence of livestock domestication is Lake Turkana basin and through this lake basin, indigenous chickens found their way to Kenya at around 800 AD (Horton & Mudida, 1993). Since their introduction in Kenya, indigenous chickens have spread throughout the country and have adapted into various agro-climatic zones. There are twelve phenotypes of indigenous chickens in Kenya. Six of these phenotypes: frizzled, feathered shanks, naked neck, barred feathers, bearded and dwarf phenotypes were described by (Kingori, Wachira, & Tuitoek, 2010) while the rumpless, kuchi, mixed, plain feathered, crested and mottled were described by a study by (Moraa, et al., 2015). These ecotypes are spread throughout the seven agro-climatic zones in Kenya (Nyaga, 2007).

However, the local genetic pool was affected in the 1970's by the cockerel and pullet exchange program spearheaded by the national poultry breeding program in several Counties. This program led to genetic dilution of pure indigenous breeds in Kenya in the Counties affected (Nyaga, 2007). Most of the areas that were not affected by this program are in the Arid and semi-arid regions. These regions are considered very dry and are characterized by high ambient temperatures. However, there is a possibility that indigenous chickens in these areas still have traits for heat tolerance. Indigenous chickens in these regions, just like other organisms, have a group of highly conserved proteins that are produced when exposed to high temperatures. These proteins are known as the Heat Shock Protein 70 (HSP70s) and they protect the cells of the body against the toxic effects of heating thereby enabling their survival in heat stressed environments (Mazzi et al., 2003).

1.2 Statement of the problem

The cross breeding programs with exotic breeds that were not locally adapted to the environment, led to the genetic erosion of indigenous chickens. Consequently, there is a possibility that traits such as heat tolerance may have been historically lost in the indigenous chickens. If this *'ad hoc*' crossbreeding continues with the exotic breeds, then there is danger of a loss of genetic diversity of indigenous chickens unless efforts are not put in place to conserve them.

1.3 Justification of the study

The uncoordinated crossbreeding by the national poultry breeding programs with exotic breeds that continues to date could lead to genetic dilution of the indigenous chickens that have locally adapted to the environment since domestication. Therefore, there was need to carry out this study to identify the genetic diversity present in indigenous chickens in the arid and semi-arid regions of Kenya and design approaches to conserve them.

1.4 Objectives

1.4.1 General objective

To study the phenotypic diversity, genetic diversity and HSP70 gene functional polymorphisms for heat tolerance in indigenous chickens of Kenya.

1.4.2 Specific objectives

1. To characterize the phenotypes for heat tolerance in indigenous chickens in Kenya.
2. To characterize the genetic background of indigenous chickens in Kenya using mtDNA D-loop.
3. To characterize the functional polymorphisms on the HSP70 gene of indigenous chickens in Kenya.

1.5 Alternative Hypotheses

1. Indigenous chickens in Kenya have phenotypic traits implicated in heat tolerance.
2. Indigenous chickens in Kenya have a distinct genetic background.
3. There are functional polymorphisms in the HSP70 gene of indigenous chickens in Kenya.

1.6 Research questions

1. Are there phenotypes in indigenous chickens that are associated with heat tolerance?
2. Do indigenous chickens have distinct genetic background?
3. Are there polymorphisms in the HSP70 gene of indigenous chickens in Kenya?

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and domestication of chickens

Domestication of indigenous chickens is believed to have occurred in the Indus Valley 8000 years ago (Zeuner et al., 1963), but more recent archaeological evidences showed that a much earlier domestication occurred in China (West & Zhou, 1989). There are four *Gallus* species that are considered as progenitors of indigenous chickens; *Gallus gallus* (Red jungle fowl), *Gallus lafayettei* (Ceylon jungle fowl), *Gallus sonnerrati* (Grey jungle fowl) and *Gallus varius* (Green jungle fowl) (Singh et al., 2000). The red jungle fowl is one of the oldest domesticated birds and its popularity quickly spread to Europe. It was kept for cock fighting and in religious rituals (Singh et al., 2000). Indigenous chickens have since spread to the rest of the world.

The point of entry and dispersal of Indigenous chickens in Africa remains unsolved. It has been suggested that they arrived in Africa first through the Nilotes migration patterns in Egypt and dispersed along the Nile valley from lower Egypt in the 10^{th} and 14^{th} Century where the most ancient undisputed presence of domestic fowls have been found (Coltherd, 1966; Houlihan & Goodman, 1986). Indigenous chickens later dispersed via the Indian Ocean coastline and others southwards to Sudan, Ethiopia and Lake Turkana basin where the earliest livestock bones have been discovered in the Koobi Fora area on the eastern side of Lake Turkana (Macdonald et al., 1992; MacDonald & Edwards, 1993). Indigenous chickens could also have found their way to Kenya via the Bantu migration between 3000 and 2500 years ago. In this era, the Bantus expanded southwards from the Cameroon highlands and later from the equatorial forests of Central Africa between 2500-1500 years ago and settled in Uganda and the west side of Lake Victoria shores. They then dispersed to Kenya via the western regions (Marshall, 2000). Figure 2.1 shows the migratory patterns of indigenous chickens from Egypt and Cameroon to Kenya.

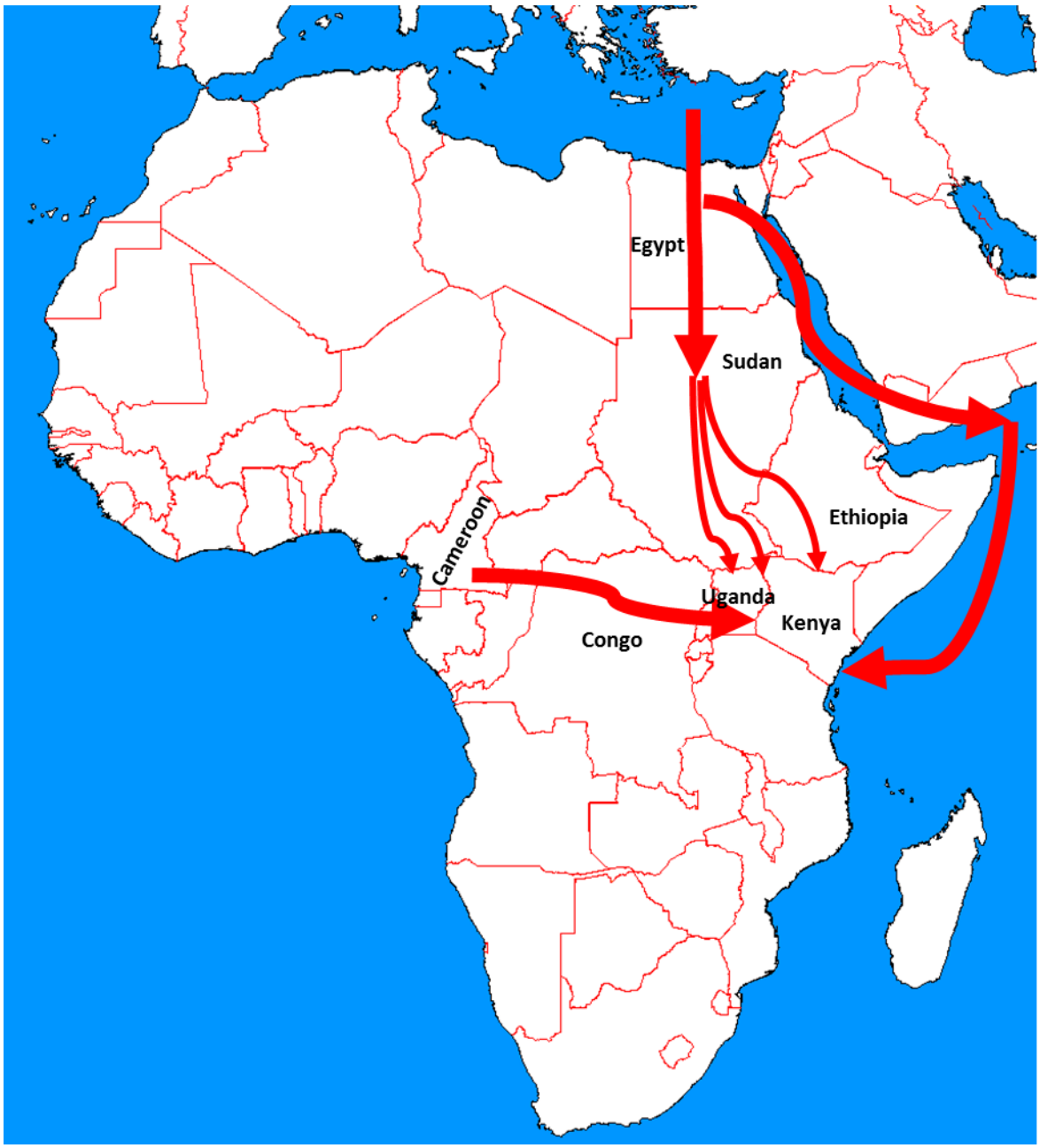


Figure 2.1: Migratory routes of indigenous chickens from Egypt and Cameroon to Kenya Source; (www.worldatlas.com)

2.2 Agro-climatic zones in Kenya

Climate change has become the most serious global challenge of our time, and the impacts are increasingly evident on the societies around the world ("National Climate Change Action Plan - Kenya | Environmental Migration Portal,"). Low

annual rainfall has led to severe drought across large parts of the horn of Africa. Kenya is one of the most vulnerable countries to climate change since the economic sectors and the livelihoods are already experiencing the manifestations of this problem. This has affected many sectors especially the agricultural sector. Kenya has seven agro-climatic zones and 70% of the land is ASALs ("The State of Food and Agriculture, 1996,"). Kenya is divided into the following agro climatic zones:

2.2.1 Zone I

This zone has no direct importance in agricultural production, however, it's a source of rain and some river streams and it's confined to mountains and immediate surroundings such as Mt. Kenya and Mt.Elgon.

2.2.2 Zone II

This zone is restricted to the highlands of Kenya at elevations between 1980 and 2700m and occurs as forests or open grasslands. It's found in the surroundings of Mt. Kenya, isolated parts of Rift Valley around Mau and Aberdare mountains and the surrounding of Mt.Elgon. The minimal rainfall is 1000mm.

2.2.3 Zone III

This zone occurs at elevations between 900-1800m as the previous zone but at times may be lower. However, it has lower rainfall of about 500-1000mm per annum. It occurs in the surroundings of Naivasha, vast parts of Laikipia and Machakos counties, and vast parts of counties in Central and Coast regions.

2.2.4 Zone V

This zone is much drier than the previous zone and occurs at lower elevations. Annual rainfall is 300-600mm. This zone is prevalent in northern Baringo, Turkana, lower Makueni and vast part of North Eastern counties.

2.2.5 Zone VI

This zone is considered as a semi-desert and it's the driest part of Kenya. Its annual rainfall is 200-400mm and is quite unreliable. This zone is found in Marsabit, Turkana, Mandera and Wajir counties.

2.2.6 Zone VII

This zone is represented by Chalbi desert in Marsabit County. The Chalbi desert is a salt desert with very sparse salty bushes as the only vegetation found. Pastoralists use it as a source of mineral lick for livestock during the rainy season.

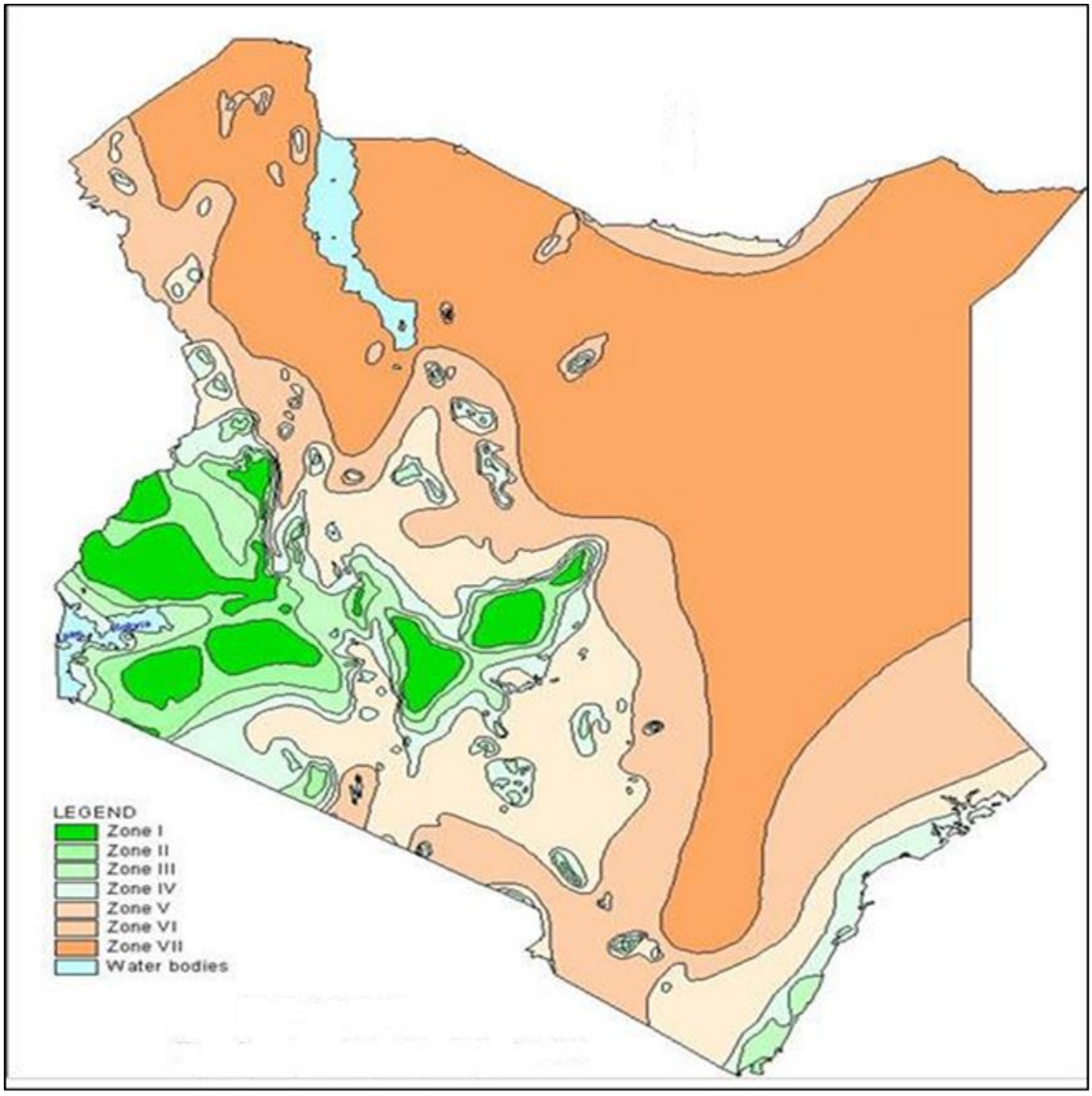


Figure 2.2: The agro-climatic zones of Kenya source; (“infonet - AEZs.)

2.3 Livestock adaptation to climate change

Adaptation is the ability of breeds to produce and reproduce in a given set of environments. It also forms the basis for the choice of particular breeds for specific environments (Barker, 2009). Adaptation traits have low heritability and are exposed to selection if by any chance the environment shifts (Hill & Zhang, 2009).

2.3.1 Physiological stress and thermoregulatory control in chicken

Heat stress interferes with the physiology of chicken leading to increased mortality rates, suppressed appetite and feed intake, as well as reduced reproduction and production capacity. Heat stress is an important factor in determining specific environments for various livestock breeds (Coble et al., 2013; Etches et al., 2008 (Zwald, Weigel, Fikse, & Rekaya, 2003). The ability to thermo regulate is highly dependent on the anatomical and physiological factors. Such factors include: body weight, skin color, plumage density and metabolic heat production among other factors.

Decreased feather coverage provides relative heat tolerance. Chicken suffer high temperature because the feather coverage hinders internal heat dissipation (Yalcin et al.,1997). This is a challenge especially to chickens found in hot environments especially in the arid and semi-arid lands (ASALs). Several studies have been conducted on the various chicken phenotypes to assess their performance in hot environments in the world and other regions in Africa. A study conducted by (Cahaner et al., 2008) indicated that the naked neck gene can be used to alleviate heat stress. The naked neck genotype has been shown to affect heat dissipation traits like the wattle length, shank length, comb type, rectal and surface temperature measurements (C. F. Chen et al., 2008).

The frizzled genotype has also been studied in chickens exposed to high ambient temperatures. This gene has been proven to have beneficial effects in decreasing heat stress in poultry production (Zerjal, Gourichon, Rivet, & Bordas, 2013). Apart from the naked neck and the frizzled gene, the dwarf gene has been shown to have no value with regard to chicken tolerance to chronic heat stress (Deeb & Cahaner,

2001). In addition to genotypic studies, phenotypic studies on differences in adaptation among various chicken ecotypes have been widely conducted in other regions. Studies by Duguma, (2006) and Reta, (2009) characterized some indigenous chicken ecotypes of Ethiopia. These two studies looked at some morphological traits such as plumage colour, earlobe colour, comb colour, skin colour among other traits in various indigenous chicken ecotypes in Ethiopia. These studies indicated that chickens found in the highland areas of Ethiopia differed from those found in the lowlands. Guni and Katule, (2013) characterised some indigenous chickens in selected southern highlands of Tanzania. This study looked at some morphological traits and it revealed that frequencies of various plumage forms and colours vary from one location to another. All these studies did not characterise the morphologies of indigenous chickens based on adaptation to hot environments and no such study has ever been conducted in Kenya. This study therefore aimed to describe the phenotypic traits in indigenous chickens found in hot environments in Kenya.

2.4 Molecular markers

A molecular marker/genetic marker is a particular segment of DNA that is a representative of the differences at the genome level. In the recent past, several DNA markers have been developed and utilized in the genetic diversity analysis (Dodgson, Cheng, & Okimoto, 1997). Molecular markers may or may not correlate with the phenotypic expression of a trait. They are advantageous over other conventional phenotype based alternatives since they are stable and easily detectable in all the tissues regardless of the growth, differentiation or defense status of a cell. Molecular markers are also not confounded by the environmental effects and provide useful information about genetic diversity (Karp, 1997).

Genetic diversity evaluation within and between the different chicken populations, both native and commercial has been carried out by the use of several DNA marker systems. The fundamental DNA technology developments over the recent past such as restriction enzymes coupled with Southern-blot hybridization, sequencing and PCR have contributed to the burst of applications in multiple research areas such as diversity and genetic variation in chickens (Hillel et al., 2007).

2.4.1 Restriction fragment length polymorphism (RFLP)

This is a modification of the Southern blot technique whereby the whole genomic DNA or its fraction is cut with restriction enzymes, transferred into a membrane and hybridized with radio-labeled or fluorescent probes. This method has low polymorphism and can be used in linkage and physical mapping (Botstein, White, Skolnick, & Davis, 1980; Y. Chen & Lamont, 1992; Dunnington et al., 1990) characterized the genetic variation among 13 of the highly inbred chicken lines by RFLP.

2.4.2 DNA fingerprinting

This is a widespread derivative of the restriction enzyme-based method for detecting multiple anonymous loci across the whole genome that is either multilocus minisatellite or multilocus microsatellite. This method was used in chicken diversity studies in the last decade because no preliminary sequence information about these anonymous multilocus markers is necessary. This method is time consuming but it's still useful in species for which no or little sequence information is available. Many studies have been conducted to determine the intraline and interline characteristics of chickens and comprehensive surveys of relatedness in the redjungle fowl, layers and broiler strains and the white leghorn based on DNA fingerprints, have been described (Dunnington et al., 1990; Kuhnlein, Zadworny, Dawe, Fairfull, & Gavora, 1990; Kuhnlein et al., 1990; Ponsuksili, Wimmers, & Horst, 1998; Siegel et al., 1992)

2.4.3 PCR-based techniques

2.4.3.1 Random amplified polymorphic DNA markers (RAPD)

This method employs single short primers of random sequence, which produce multiband patterns similar to DNA fingerprints. This method was used to study chicken genetic diversity in the 1990′s (Dunnington et al., 1990). However, because of poor PCR reproducibility and dominance mode of inheritance, they are no longer used as the markers of choice. (Smiths, Jones, Bartlett, & Nestor, 1996) used RAPD markers to analyze relatedness in chickens and turkeys. Also other studies were

conducted by (Y. Chen & Lamont, 1992; Dunnington et al., 1990); (Lamont et al., 1992) to characterize the genetic variation in highly inbred chicken lines using RAPD.

2.4.3.2 Amplified fragment length polymorphism (AFLP)

This method is ideal for population genetics. It involves the restriction of genomic DNA, followed by the ligation of adopters complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. In the past years this method was frequently used to identify markers associated with traits under selection in vertebrate species (Herbergs, Siwek, Crooijmans, Van der Poel, & Groenen, 1999) This method has been used to enhance marker density in the East Lansing reference chicken genome map, using a backcross family derived from a red jungle fowl by white leghorn mating with white leghorn as the recurrent parent (Knorr, Cheng, & Dodgson, 1999).

2.4.3.3 Microsatellites

These are single-locus markers sometimes known as simple sequence repeats (SSRs). Prior sequence information of the flanking regions is necessary to develop these markers. It's useful in genetic diversity studies since it's highly polymorphic. This has been used in the analysis of the genetic relationships between various populations of domestic chickens and jungle fowls whereby microsatellite allele variations in chickens was observed (Romanov & Weigend, 2001; Takahashi, Nirasawa, Nagamine, Tsudzuki, & Yamamoto, 1998; Vanhala, Tuiskula-Haavisto, Elo, Vilkki, & Maki-Tanila, 1998; Zhang, Leung, Chan, Yang, & Wu, 2002; Zhou & Lamont, 1999)

2.4.3.4 Single nucleotide polymorphism (SNP)

This is a minimal DNA variation that occurs as a replacement of a single nucleotide with one of the three other possible nucleotides. This is the most common class of genetic polymorphism often outside of the coding region. It has recently become the method of choice for biodiversity studies due to its abundance and applicability for

high throughput analysis (Schmid et al., 2006). Muir et al, (2008) did a genome wide assessment of the world genetic diversity among chickens in his study, he was able to characterize successfully in terms of allele diversity the rate of allele loss. The genetic diversity of 4 Indonesian native chicken populations by the use of autosomal SNP marker was done (Nishida et al., 2012)

2.4.3.5 Assessment of biodiversity using mtDNA as a marker for genetic background

The mtDNA is a circular molecule that is on average 16,775 base pairs in size in several animal species (Desjardins & Morais, 1990) and has a maternal mode of inheritance. The displacement loop region (D-loop) of the mtDNA is highly polymorphic and contains the elements that control the replication of the molecule. These features make the displacement-loop region attractive for phylogenetic studies.

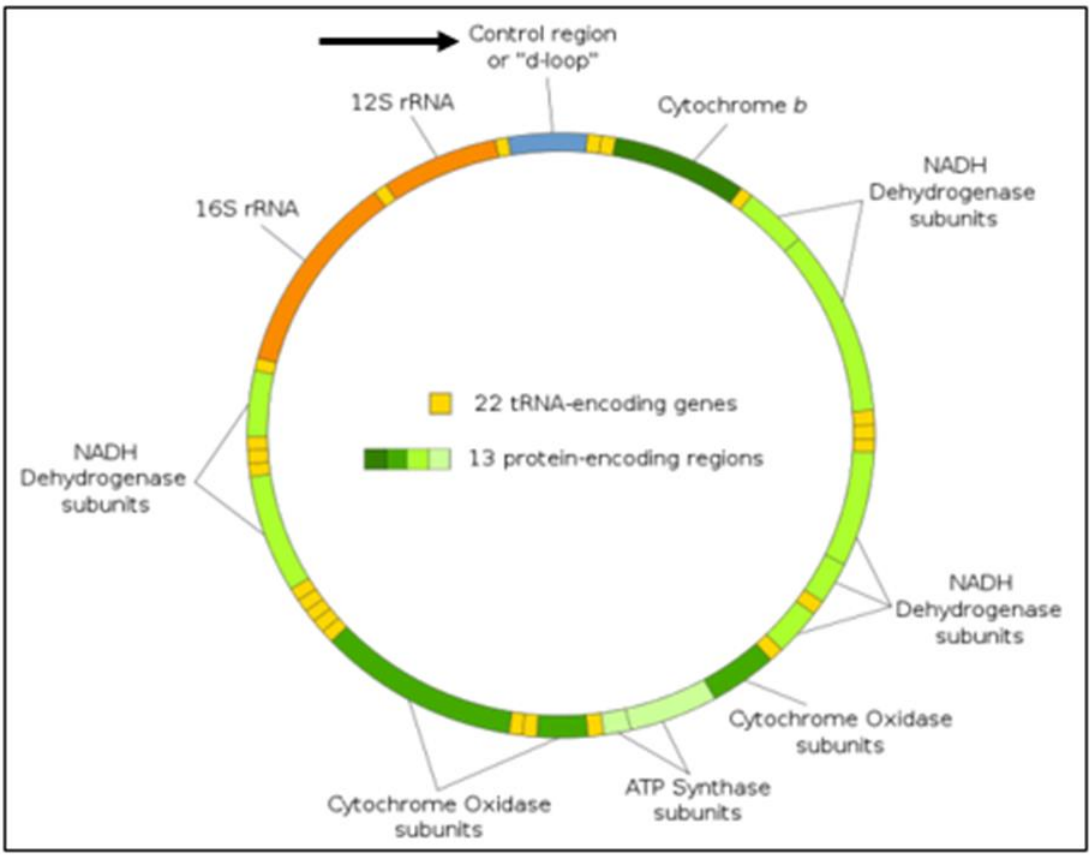


Figure 2.4: mtDNA molecule showing mtDNA D-loop region. Source;
(wikipedia.org)

One of the first attempts to look into the problem of the genealogical origin of the present domestic chickens at the molecular level was undertaken by (Fumihito et al., 1996) who studied the D-loop region of various *Gallus* species, including red jungle fowl (*Gallus gallus*) and diverse domestic breeds. Based on sequence differences, (Fumihito et al., 1996)) suggested that the origin of domestic chickens is monophyletic, a conclusion that supported Darwin's hypothesis (Litchfield, 1915). A study by (Cramer et al., 1896) was the first to hypothesize that the red jungle fowl was the direct ancestor of the chicken.

In the study by (Fumihito et al., 1996)), a rather small number of wild chicken samples were studied, and they concluded that continental subspecies of red jungle

fowl from Thailand is the maternal origin of all chickens. This study lacked samples from both domestic and wild subspecies of red jungle fowls from China and India. A study by (Liu et al., 2006), analyzed the mtDNA hyper-variable segment for a larger number of domestic chickens across Eurasia and the red jungle fowl from Southeast Asia and china. They were able to identify nine divergent clades in which seven clades contained both the red jungle fowl and the domestic chickens. These results indicated that there are several ancestral chicken populations that contributed to the maternal genetic makeup of the *gallus* species. This supported the theory of multiple origins of domestic chickens in South and South East Asia.

A study carried on by (Eriksson et al., 2008) using mtDNA and SNP indicated a hybrid origin of domestic chickens. From their results, it was evident enough that the yellow skin gene in domestic chickens originated from the grey jungle fowl and not from the red jungle fowl, which is presumed to be the sole wild ancestor of domestic chickens. (Shen et al., 2010) analyzed complete mitochondrial genomes from 34 *Galliforme* species including 14 new mitochondrial genomes and 20 published mitochondrial genomes. This analysis recovered robust phylogenetic relationships among the *Galliformes.*

In Africa, studies on mtDNA have been done in various countries. In Malagasy, a study to characterize indigenous chickens using mtDNA was carried out by (Razafindraibe et al., 2008). Two haplogroups were identified and this indicated a dual geographic and genetic origin for the indigenous Malagasy chickens. In South Africa, (Mtileni et al., 2011) carried out a study on mtDNA of conserved and field chicken populations and 18 haplotypes were identified. These chickens were found to be polymorphic from China, Yunnan, Southeast Asia and the Indian subcontinent. In Nigeria, 35 haplotypes were identified in a study carried out by (Adebambo et al., 2010) to determine the origins and diversity of local Nigerian chickens by use of mtDNA. From this study, it emerged that the Indian sub-continent was likely to be the origin of Nigerian chickens. Muchadeyi et al. (2008) carried out a study on Zimbabwean chickens in 5 agro-ecological zones. In this study, 34 haplogroups were identified and the results indicated the existence of two maternal lineages

evenly distributed among the 5 ecological zones. In Tanzania, a study was carried out on mtDNA to determine the genetic diversity of chicken population by (Lyimo et al., 2014). Two haplogroups of different genealogical origin were identified. This study proved that they originated from the Indian subcontinent and in Southeast Asia. Mwacharo et al. (2011) reported multiple introductions of chickens into East Africa resulting in five distinct haplogroups of different maternal origins.

Samples selected by Mwacharo et al. (2011) were from the areas that were previously affected by the cockerel and pullet exchange program in 1970′s. These areas included: Kilifi, Taita, Muranga, Meru, Marsabit, Kitui, Nairobi, Kisii, Homabay, Kakamega, Nandi and Naivasha (Mwacharo et al., 2011).

The current study is unique since the samples selected were from areas that were not affected by the cockerel and pullet exchange program. The samples locations were from the following four agro-climatic zones of Kenya; Lamu archipelago, Mt. Elgon catchment region, Lake Victoria basin and Lake Turkana basin.

2.5 Heat shock proteins role in thermo tolerance

When living organisms are exposed to a variety of physiological stresses such as non-lethal temperatures between 40°C - 42°C, the synthesis of most proteins is suppressed. However, a small number of highly conserved proteins are rapidly synthesized. This response is known as heat shock response and the induced proteins are called heat shock proteins (HSPs), (Hightower & Nover, 1991). These proteins play a major role in the protection of organisms from the toxic effects of heating. In eukaryotes, the heat shock response is regulated mainly at the transcription level by heat shock transcription factors (HSFs) (Wu, 1995).

Studies have shown that heat shock factor (HSF) is crucial to the acquisition of induced thermal tolerance in fruit fly (Jedlicka, Mortin, & Wu, 1997), mouse embryo fibroblast cells (McMillan, Xiao, Shao, Graves, & Benjamin, 1998), and in chicken DT40 cells (Tanabe et al., 1998). HSFs activate heat shock gene in response to elevated temperatures. A study carried out by (Nakai, 1999) indicated that in chickens, HSF3 as well as HSF1 bind to DNA when the cells are exposed to heat

shock . In another study carried out by (Tanabe et al., 1998), it emerged that HSF3 is necessary for burst activation of heat shock genes in chicken B-lymphocyte DT40 cells. Since HSF3 is ubiquitously expressed in most developing tissues at high levels, HSF3 may be a dominant factor for heat shock response in chickens (Kawazoe, Nakai, Tanabe, & Nagata, 1998).

A study was recently carried out by Akaboot et al. (2012) to genetically characterize the fowl population using HSP70 and microsatellite markers. In this study, the genetic structure of the red jungle fowl, local domestic chickens and commercial chickens that were undergoing different selection and breeding strategies were identified. In another study carried out by (Xia, Gan, Luo, Zhang, & Yang, 2013), acute heat stress on the ducks was implemented and the expression levels of HSP70 messenger RNA (mRNA) assayed. It emerged that HSP70 was present in all the tissues that were tested. This study revealed that ducks HSP70 was heat inducible. This was in line with other reports (Maak, Melesse, Schmidt, Schneider, & Von Lengerken, 2003). (Zhen, Du, Xu, Luo, & Zhang, 2006) conducted a study in chicken HSP70 and the results indicated that the levels of HSP70 in the chickens liver was significantly higher than in the muscles under normal growth conditions. However, during acute heat stress, the levels were higher in the brain.

This will be the first study of its kind to be conducted in Africa since no molecular studies have been carried out on chickens HSP70 as impacted by heat tolerance in Africa.

CHAPTER THREE

MATERIALS AND METHODS

3.2 Study areas

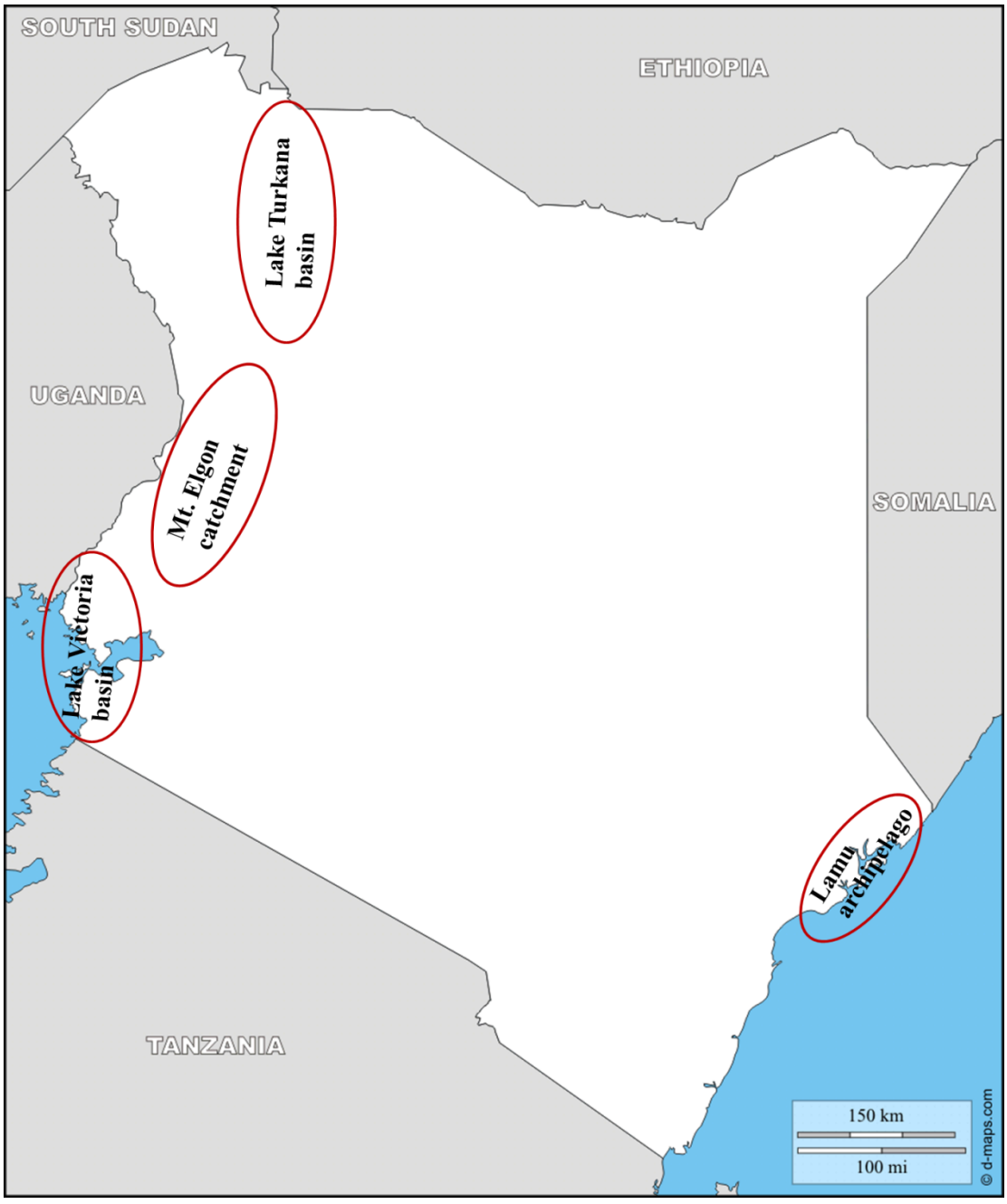


Figure 3.1: Map of Kenya showing the study areas, source; (www.worldatlas.com)

This study was conducted in Lake Turkana basin, Lamu archipelago, Lake Victoria basin and Mt. Elgon catchment. These were the preferred areas since Mt. Elgon catchment and Lake Victoria basin are highlands that experience low temperatures as compared to Lake Turkana basin and Lamu archipelago which are arid and semi-arid lands that experience high temperatures. Further, these areas were not affected by the cockerel and pullet exchange program.

3.2.1 Mt. Elgon catchment

Mt. Elgon is located between 0°27'38.76" South latitude and 34°6'41.26" East longitude. It comprises zone I and II of the agro-climatic zones of Kenya. These two zones are considered wet and they are characterized by an annual rainfall of 1000 mm per annum and an annual temperature of 27.0°C and a minimum of 15°C (Jaetzold & Schmidt, 1983).

3.2.2 Lake Victoria basin

Lake Victoria basin lies in zone III of the agro-climatic zones of Kenya and receives an annual rainfall of 500-1000 mm with an annual temperature of 28°C and a minimum temperature of 17°C. It is situated between 0°24'41.74" South latitude and 34°9'51.55" East longitude.

3.2.3 Lamu archipelago

Lamu is located between 2°16'10.41" South latitude and 40°54'2.31" East longitude. It lies within zones IV of the agro-climatic zones of Kenya, this zone is considered dry and humid and receives an annual rainfall of 800 mm and an annual temperature of 30°C and a minimum of 26°C.

3.2.4 Lake Turkana basin

Turkana basin is positioned between 3°37'36.55" South latitude and 36°0'8.35" East longitude. It lies within zone VI and zone VII of the agro-climatic zones of Kenya. These zones receive annual rainfall of 200-600 mm. Lake Turkana is also characterized by an annual temperature of 34°C and a minimum of 30°C (Paron,

Olago, & Omuto, 2013). These zones are considered as semi desert and they are the driest part of Kenya.

Table 3.1: **Summary of sampled locations**

Agro climatic zone	Population	Number of samples
Lake Turkana basin	Elmolo, Ilolo, chireirich	37
	Ileret, baulo, telesgaye	24
	Kapua, kalokol	10
	Lorengerlup	13
	Kainuk, westpokot	28
	Total	112
Lamu Archipelago	Ndau, Kiwayu, Vumbe, kizingitini	30
	Faza, tchundwa town, tchundwa village	46
	Patte, Siu	27
	Total	103
Lake Victoria Basin	Rusinga	12
	Mfangano	12
	Total	24
Mt. Elgon catchment	Busia	19
	Bungoma	25
	Transnzoia	13
	Total	57
	Total samples	296

3.3 Study design

This was a stratified random cross sectional study. Field surveys were conducted in remote villages that were not part of the cockerel and pullet exchange program in the four agro-climatic zones. A rural participatory approach was used where interviews

were conducted at the farmers' houses with the assistance of local agricultural extension officers. A pre-tested questionnaire (appendix 6) was used to obtain the farmers information as well as to record the various morphological forms and body temperature of the indigenous chickens. A total of 296 adult indigenous chickens were characterized from 13 populations in the four agro climatic zones of Kenya.

3.4 Data collection

Phenotypic attributes studied included; skin color, shank color, comb type, comb color, earlobe color, wattle color, plumage density, presence of a crest and feathers in the shank. Body temperature was obtained by placing a thermometer under the wing of the bird. Ambient temperature and global positioning system (GPS) were also recorded. For the molecular work, blood was drawn from the wing vein of the 296 indigenous chickens and stored on FTA® cards. Two mature birds (a cock and a hen) were sampled per flock. The sampling characteristic and strategy followed were described previously (Mwacharo et al., 2007)

3.5 Molecular analysis

3.5.1 DNA extraction for mtDNA and HSP70

Genomic DNA was extracted from air dried blood preserved on FTA® classic cards (Whatman biosciences) (Gutiérrez-Corchero et al., 2002). Five 1.2 mm discs were punched from each FTA® sample and placed in eppendorf tubes. 1ml of 100mM (Tris with 0.1% SDS at pH 8) was added and gently agitated after every five minutes on a vortex for 30 minutes at room temperature. This was spun briefly to settle the discs and supernatant discarded. 500 µl of 1.5M Guanidine Thiocyanate was added and gently vortexed frequently for a total of 10 minutes then discarded. 500 µl of triple distilled water was added and this was gently vortexed frequently for 10 minutes. This step was repeated three times and the water discarded. 50 µl of triple distilled water was added and placed in a water bath at 90 ^{0}C and heated for 20 minutes. This was left to cool at room temperature for 30 minutes. The supernatant containing DNA was spun and transferred into clean eppendorf tubes. The correct concentration was determined by nanodrop 1000 spectrophotometer and the DNA

integrity checked using 260/280 ratio. This generated 50-70 µl of DNA, 1µl was used for PCR reaction and the rest was stored at -20 ^{0}C. The samples were then diluted to a working stock of 50ng/µl for PCR.

3.5.2 PCR amplification for mtDNA

PCR reactions were performed in a final volume of 10µl containing 3.8µl of double distilled water; 1µl of template genomic DNA, 5µl of thermoscientific^{TM} DreamTaq^{TM} Green Master Mix (2X), 0.2µl Primer (forward + reverse, 20pmol/µl). mtDNA D-loop region was amplified via primers AV1F2 (5'-AGGACTACGGCTTGAAAAGC-3') and CR1b (5'-CCATACACGCAAACCGTCTC-3') (Mwacharo et al., 2010). Amplification was carried out in a Veriti 9901 96 Well Fast gradient Systems thermo cycler. The amplification program started with one cycle of initial denaturation at 94^{0}C for 3min followed by 5 cycles of 15 seconds at 94^{0}C, 30 seconds at 60^{0}C for primer annealing and 30 seconds at 72^{0}C for elongation. The final extension was done at 72^{0}C for 10 minutes.

For electrophoretic analysis, two percent (2%) agarose gel in 1X Tris Boric Ethylenediaminetetraacetic acid (TBE) buffer was prepared by adding 2g of agarose to 100 ml 1X TBE and weighed. The solution was then heated in a microwave at short intervals of 15-30 sec with occasional shaking until it boiled and became clear. This was left to cool to 55^{0}C. The gel was then poured on the tray of the mini electrophoresis unit (MUPID) to solidify, bubbles were removed, after which the combs were fixed and the gel allowed to set. After solidifying, the combs were removed and 1X TBE buffer added on the mini electrophoresis unit to cover the gel. DNA preparations were loaded onto the 2% agarose gel using 1X TBE buffer (89Mm Tris, 89mM boric acid, 2Mm Na_2 EDTA) in a voltage of 80V for 25 minutes. The gels were stained with gel red and visualized under UV light (BTS-20 model, UVLtec Ltd., UK). Sequencing was done in 20µl volumes at Macrogen using Sanger ABI 3730 method (Sanger and Coulson, 1975) (Appendix 3).

3.5.3 PCR amplification for HSP70 gene

PCR reactions were performed in a final volume of 10µl containing 3.8µl of double distilled water; 1µl of template genomic DNA, 5µl of thermoscientific^{TM} DreamTaq^{TM} Green Master Mix (2X), 0.2µl Primer (forward + reverse, 20pmol/µl). The first 360bp region of HSP70 gene was amplified via primers HSPF(5'AACCGCACCACACCCAGCTATG3') and HSPR (5'CTGGGAGTCGTTGAAGTAAGCG-3') (Akaboot et al., 2012). Amplification was carried out in a Veriti 9901 96 Well Fast gradient Systems thermo cycler. The amplification program started with one cycle of initial denaturation at 94^0C for 3min followed by 5 cycles of 15 seconds at 94^0C, 30 seconds at 60^0C for primer annealing and 30 seconds at 72^0C for elongation. The final extension was done at 72^0C for 10 minutes.

For electrophoretic analysis, two percent (2%) agarose gel in 1X Tris Boric Ethylenediaminetetraacetic acid (TBE) buffer was prepared by adding 2g of Agarose to 100 ml 1X TBE and weighed. The solution was then heated in a microwave at short intervals of 15-30 sec with occasional shaking until it boiled and became clear. This was left to cool to 55^0C. The gel was then poured on the tray of the mini electrophoresis unit (MUPID) to solidify and bubbles were removed after which the combs were fixed and the gel allowed to set. After solidifying, the combs were removed and 1X TBE Buffer added on the mini electrophoresis unit to cover the gel. DNA preparations were loaded onto the 2% agarose gel using 1X TBE buffer (89Mm Tris, 89mM boric acid, 2Mm Na_2 EDTA) in a voltage of 80V for 25 minutes. The gels were stained with gel red and visualized under UV light (BTS-20 model, UVLtec Ltd., UK). Sequencing was done in 20µl volumes at macrogen using Sanger ABI 3730 method (Sanger and Coulson, 1975) (Appendix 3).

3.6 Data analysis

3.6.1 Phenotypic data analysis

Phenotypic data were analyzed using Excel spread sheet software package 2013 to compute frequencies of occurrence of each trait. ANOVA tests in R core statistics

software version 3.1.2 (Ihaka & Gentleman, 1996) was used to determine significant relationships between various traits and the ecosystems in response to outside temperature. A box plot in R was used to show if any relationship existed between the ambient temperature and the various agro climatic zones. A conditioning plot was used to determine if there was any relationship between outside temperatures in the four agro climatic zones to the body temperatures of indigenous chickens.

3.6.2 mtDNA data analysis

3.6.2.1 Editing mtDNA trace files

The raw sequences for mtDNA were manually edited using Chromas Lite 2.1 (Avin, 2012). The forward and reverse sequences were aligned to determine the accurate sequence. The accurate sequence was then aligned against reference sequence (accession number X52392), (Desjardins & Morais, 1990) from GenBank using Clustal X 2.1.1 (Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997). Subsequent analyses were restricted to the first 374 bp incorporating the first hyper variable segment (HVS1). The primer sequences were trimmed out and consensus sequences generated. These consensus sequences were used in subsequent analysis.

3.6.2.2 mtDNA haplotype analysis

Mitochondrial DNA haplotypes were manually constructed and confirmed with DnaSP 5.10 (Librado & Rozas, 2009). Distribution frequencies of the various haplotypes in various populations were displayed by the help of pie charts constructed using Excel spreadsheet 2013 (Liengme, 2015). This was important as it showed how various haplotypes were distributed in the various agro climatic zones of Kenya.

3.6.2.3 Phylogenetic analysis of the mtDNA haplotypes and network profiles of the major clades

A phylogenetic tree involving the mtDNA haplotypes that were discovered in the four agro climatic zones was constructed using the maximum likelihood tree algorithm as implemented in MEGA6 (Tamura, Stecher, Peterson, Filipski, &

Kumar, 2013) following 1000 bootstrap replications. The model used for mtDNA was T92+G with a gamma shape parameter of 0.1. The affinity of indigenous chickens to the other avian species was revealed by the maximum likelihood tree incorporating nine avian species downloaded from GenBank. The resultant tree was viewed and edited by FigTree version 1.4.2. To test the robustness of the phylogenetic analyses sequence clusters were detected by the analysis of phylogenetic networks from uncorrected p-distances with the phylogenetic splits decomposition network implemented with SplitsTree version 4.13.1 (Huson & Bryant, 2006). The phylogenetic network diagrams produced from these analyses were used to validate the haplotypes.

To investigate the possible relationships among the haplotypes and link the populations under study to the presumed centers of domestication, median joining networks were constructed following the algorithms of (Bandelt, Forster, & Röhl, 1999) using the Network version. 4.6.1.3 software package. In addition, network analysis included nine haplotypes from GenBank representing the nine clades (Clades A-I) in the Chinese and Eurasian region (Liu et al., 2006).

3.6.2.4 mtDNA Population genetic variability and structure

Genetic variation (nucleotide diversity, haplotype diversity and nucleotide differences) for each population were calculated using Arlequin 3.5.2.2 software (Excoffier et al., 2005). The population genetic structure was assessed by the nested analysis of molecular variance (AMOVA). The groupings used for AMOVA were as follows: Among groups, among populations, within groups and within populations. Significance testing was performed using 10000 coalescent simulations in Arlequin 3.5.2.2 software (Excoffier et al., 2005).

3.6.3 HSP70 data analysis

3.6.3.1 Editing HSP70 trace files

The raw HSP70 sequences were edited manually using Chromas Lite 2.1 (Avin, 2012) and aligned using Clustal X 2.1.1 (Thompson et al., 1997) and MUSCLE version 3.8.31 (Edgar, 2004) against reference sequence obtained from GenBank (accession number: AY143693), (Xia et al., 2013). These were visualized using seaview version 4 (Gouy, Guindon, & Gascuel, 2010) and Clustal X version 2.1 (Thompson et al., 1997). Subsequent analyses for HSP70 were restricted to 360pb region which corresponds to position 52,784,305-52,784,620 of the Galgal4 Chromosome 5 (NP_001006686). The primer sequences were trimmed out and consensii sequences generated. These consensii sequences were used for subsequent analysis.

3.6.3.2 Haplotype analysis for HSP70

Heat shock protein 70 haplotypes were constructed manually and confirmed with DnaSP 5.10 software (Librado & Rozas, 2009). The distribution frequencies of the various haplotypes in various populations were displayed on pie chats constructed using Excel spreadsheet 2013 (Liengme, 2015).

3.6.3.3 Phylogenetic analysis for HSP70

A multiple sequence alignment was first done on the haplotypes using MUSCLE version 3.8.31. Thereafter, a phylogenetic tree involving the HSP70 haplotypes observed in the four agro climatic zones and the ancestral red jungle fowl and other avian species was inferred using the maximum likelihood tree algorithm as implemented in MEGA6 (Tamura et al., 2013). The model used was K2+G with a gamma shape parameter of 0.1 to model the nucleotide substitution pattern and rate of evolution following 1000 bootstrap replications.

The affinity of indigenous chickens to the other avian species was revealed by the maximum likelihood tree incorporating nine avian species downloaded from GenBank. The resultant tree was viewed and edited by Dendroscope version 3.3 (Huson & Scornavacca, 2012). To test the robustness of the phylogenetic analysis sequence clusters were detected by the analysis of phylogenetic networks from uncorrected p-distances with the phylogenetic splits decomposition network implemented with SplitsTree version 4.13.1 (Huson & Bryant, 2006). The phylogenetic network diagrams produced from these analyses were used to validate the haplotypes.

3.6.3.4 Population genetic diversity for HSP70

Genetic variation (nucleotide diversity, haplotype diversity, expected heterozygosity and observed heterozygosity) for each population were calculated using Arlequin v 3.5.2.2 software (Excoffier et al., 2005). The population genetic structure was assessed by the nested analysis of molecular variance (AMOVA). The groupings used for AMOVA were as follows: Among populations, among individuals within populations and within individuals. Significance testing was performed using 10000 coalescent simulations in Arlequin 3.5.2.2 software (Excoffier et al., 2005).

CHAPTER FOUR

RESULTS

4.1 Phenotypic characterization of indigenous chickens of Kenya

4.1.1 Phenotypes observed

A total of 11 phenotypes in the four agro-climatic zones were observed. These phenotypes varied across the various agro-climatic zones. These phenotypes included the naked neck, kuchi, plain feathered, mottled, crested, barred feathered, frizzled, feathered shanks, mixed, rumpless and bantam phenotypes. Some phenotypes were common to some agro-climatic zones while others were shared between agro-climatic zones. The pictures of the phenotypes are shown in figure 4.1.

Figure 4.1: Sampled indigenous chickens phenotypes

Key: A: Naked neck, B: Kuchi, C: Plain feathered, D: Mottled, E: Crested: F: Barred feathers G: Frizzled H: Feathered shanks I: Mixed, J: Rumpless, K: Bantam

4.1.2: Distribution of indigenous chicken phenotypes in the four agro climatic zones of Kenya

A total of 11 phenotypes that varied in frequency in the four agro climatic zones were sampled. Most chickens sampled were of the mixed phenotype which is characterized by the presence of more than one "phenotype" in one chicken. Bantam phenotype which is characterized by a small body was only observed in Lake Turkana basin. All chickens sampled from Lamu archipelago were of the kuchi phenotype. Crested and feathered shank phenotypes were frequent in Mt. Elgon catchment though in low frequencies. Other phenotypes like the rumpless, mottled, naked neck and frizzled were observed in very low frequencies across all the agro climatic zones (Table 4.1).

Table 4.1: Proportionate (%) occurrence of phenotypes of indigenous chicken in various agro-climatic zones

	Agro climatic zones									
Phenotypes	Lamu archipelago (n=103)	%	Lake Turkana basin (n=94)	%	Lake Victoria basin (n=24)	%	Mt. Elgon catchment (n=75)	%	Total	%
Frizzled	2	50	0	0	0	0	2	50	4	0.84
Bantam	0	0	39	100	0	0	0	0	39	8.21
Rumpless	0	0	0	0	0	0	1	100	1	0.21
Kuchi	103	100	0	0	0	0	0	0	103	21.7
Mixed	59	26.3	83	37.1	18	0.08	64	0.28	224	47.2
Naked neck	2	0.13	5	0.33	3	0.20	5	0.33	15	3.16
Plain feathered	8	0.40	9	0.45	0	0	3	0.15	20	4.21
Crested	0	0	6	0.27	1	0.05	15	0.68	22	4.63
Mottled	0	0	7	0.58	3	0.25	2	0.17	12	2.53
Barred Feathers	1	0.05	3	0.14	6	0.29	11	0.52	21	4.42
Feathered shanks	0	0	1	0.07	0	0	13	0.93	14	2.95

4.1.3 Morphological traits important for heat stress in indigenous chickens of Kenya

Indigenous chickens of Kenya varied in morphological traits such as comb type, comb colour, earlobe colour, wattle colour, skin colour, shank colour and plumage densities in the various agro climatic zones in Kenya. This information is summarized in table 4.2.

4.1.3.1 Comb types

Six comb types were observed in indigenous chickens in Kenya: single, strawberry, rose, walnut, cushion and pea were observed in the four agro-climatic zones. Out of the total proportion studied, single comb was predominant in Mt. Elgon catchment, Lake Victoria basin and Lake Turkana basin. Strawberry comb was predominant in Lamu archipelago at 68.9%. Additionally, all comb types were observed in this region. Mt Elgon catchment and Lake Victoria basin had only single and strawberry comb types. Lake Turkana basin had single, cushion, rose and strawberry combs. This information is summarized in table 4.2

4.1.3.2 Comb colors

Three comb colors were observed: red, pale and black. Red combs were predominant in Mt. Elgon catchment, Lake Turkana and Lake Victoria basin at 90.6%, 85.1% and 70.8% respectively. However, pale color was common in Lamu archipelago at 51.5% (Table 4.2). The black comb was only observed in indigenous chickens from Lake Turkana basin though in very low frequency.

4.1.3.3 Ear lobe colors

Four earlobe colors were observed; red, white, yellow and black. White was the predominant earlobe color in indigenous chickens from all the agro climatic zones. This was followed by yellow and red earlobes. Low frequencies of black earlobes were observed in Lake Turkana basin and Mt. Elgon catchment at 2.13% and 1.33% respectively (Table 4.2).

4.1.3.4 Wattle colors

Three wattle colors were observed; red, pale and black. Red wattles were common in Mt. Elgon catchment, Lake Victoria and Lake Turkana basins. Most chickens in Lamu archipelago had pale wattles at 51.4% (Table 4.2). Pale wattles were also observed in the other agro climatic zones. Black wattles were only observed in Mt Elgon catchment but in very low frequencies.

4.1.3.5 Skin colors

Differences in skin color were observed across the four agro climatic zones. White, yellow, cream and grey skin colors were observed. White was the predominant skin color in Lamu archipelago, Lake Turkana basin and Mt. Elgon catchment at 96.1%,90.4%,61.3% respectively (Table 4.2). Yellow was the predominant skin color in Lake Victoria basin. All the skin colors were observed in indigenous chickens from Mt. Elgon catchment.

4.1.3.6 Shank colors

Six shank colors (white, yellow, cream, black, grey and green) were observed across the four agro climatic zones (Table 4.2). Overall, yellow shanks were most common across all the agro climatic zones. White shanks were also frequent in all the agro climatic zones. Green shanks were only observed in Mt. Elgon catchment though in low frequencies. Black shanks were observed in all the zones except Lake Turkana basin. Lamu archipelago was the only region without grey shanks. Cream shanks were common in Lake Turkana basin and Lamu archipelago though in small frequencies.

4.1.3.7 Plumage density

Normal plumage densities were observed across four agro climatic zones except Mt. Elgon catchment. All chickens from Mt. Elgon catchment had dense plumage. Scarce plumage was only observed in Lamu archipelago at a frequency of 1.94% (Table 4.2)

Table 4.2: Proportionate (%) occurrence of morphological traits of indigenous chickens in various agro-climatic zones.

		Agro-climatic zones			
Character	Expression	Mt.Elgon catchment (n=75)	Lake Victoria basin (n=24)	Lake Turkana basin (n=94)	Lamu Archipelago (n=103)
Comb type	Single	96%	95.8%	84%	3.88%
	Walnut	0%	0%	0%	7.77%
	Cushion	0%	0%	1.06%	0.97%
	Pea	0%	0%	0%	12.6%
	Rose	0%	0%	11.7%	5.83%
	Strawberry	1.33%	4.17%	3.19%	68.9%
Comb colour	Red	90.6%	70.8%	85.1%	48.5%
	Pale	9.33%	29.1%	13.8%	51.5%
	Black	0%	0%	1.06%	0%
Earlobe colour	Red	21.3%	45.8%	11.7%	22.3%
	Yellow	32%	45.8%	5.32%	5.83%
	Black	1.33%	0%	2.13%	0%
	White	45.3%	8.33%	80.8%	71.8%
Wattle colour	Red	89.3%	70.8%	85.1%	48.5%
	Pale	9.33%	29.2%	14.8%	51.4%

	Black	1.33%	0%	0%	0%
Skin colour	White	61.3%	29.2%	90.4%	96.1%
	Yellow	36%	70.8%	8.5%	3.9%
	Cream	1.33%	0%	1.1%	0%
	Grey	1.33%	0%	0%	0%
Shank colour	White	33.3%	25%	68.1%	43.7%
	Yellow	52%	70.8%	20.2%	54.4%
	Grey	8%	4.16%	6.38%	0%
	Black	4%	0%	4.26%	1.94%
	Green	2.67%	0%	0%	0%
	Cream	0%	0%	1.06%	0.97%
Plumage density	Dense	82.7%	0%	0%	0%
	Normal	17.3%	100%	100%	98.1%
	Scarce	0%	0%	0%	1.94%

4.1.4 Relationships of various traits in response to outside temperature in the various agro-climatic zones of Kenya

Various traits were analyzed using R core statistical package in order to show any significant relationship between the various traits in response to the outside temperature. Statistical analysis revealed that there was significant interaction between comb types, and plumage density to the outside temperature in the various agro-climatic zones at $P \leq 0.05$.

This study did not show significant interaction between the comb, earlobe, wattle, skin and shank colors to the outside temperature. There was no significant relationship between the crested and the feathered shank phenotype to the outside temperature following the statistical analysis. The ANOVA p-values are indicated in table 4.3.

Table 4.3: ANOVA summary results of various traits in response to outside temperature

Phenotypic trait response to outside temperature	p-value
Comb type	0.05*
Comb colour	1.00
Earlobe colour	1.00
Wattle colour	1.00
Skin colour	0.10
Shank colour	1.00
Plumage density	0.001***
Phenotype crested	0.10
phenotype feathered shank	0.66

4.1.5 Relationship between outside temperature and the various agro-climatic zones of Kenya

The relationship between the outside temperature and the various agro climatic zones was shown by the use of a box plot. Using R core statistics package, a box plot was used to determine whether outside temperature across the four agro-climatic zones was uniform. Lake Turkana basin had temperature that ranged between 32^0C -42^0C. Lamu archipelago had temperature ranges of between 29^0C - 40^0C. Lake Victoria basin had temperature ranges of between 27^0C - 30^0C while Mt. Elgon Catchment recorded temperature ranges of 17^0C - 25^0C. This information is indicated in figure 4.2.

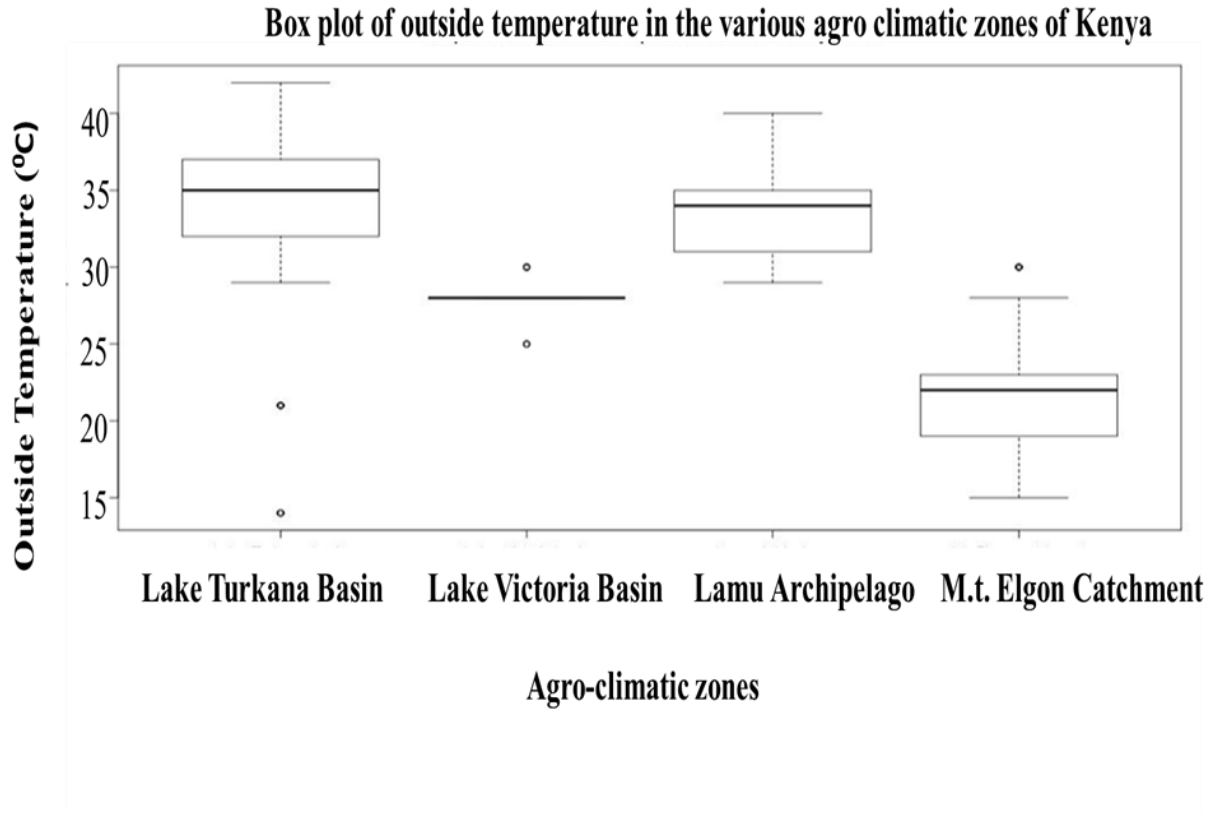


Figure 4.2: Box plot of outside temperature in the various agro-climatic zones

4.1.6 Relationship between body temperature of indigenous chickens' and outside temperature

A conditioning plot of the outside temperature in the various agro climatic zones and the body temperature of the indigenous chickens is shown in the figure 4.3. R core statistical package was used to obtain a conditioning plot of body temperature against outside temperature in the four agro-climatic zones. It was shown that chickens in Lake Turkana basin had very high temperature above 40 ^{0}C. This was closely followed by chickens from Lamu archipelago. Chickens from Mt. Elgon catchment had the least temperature.

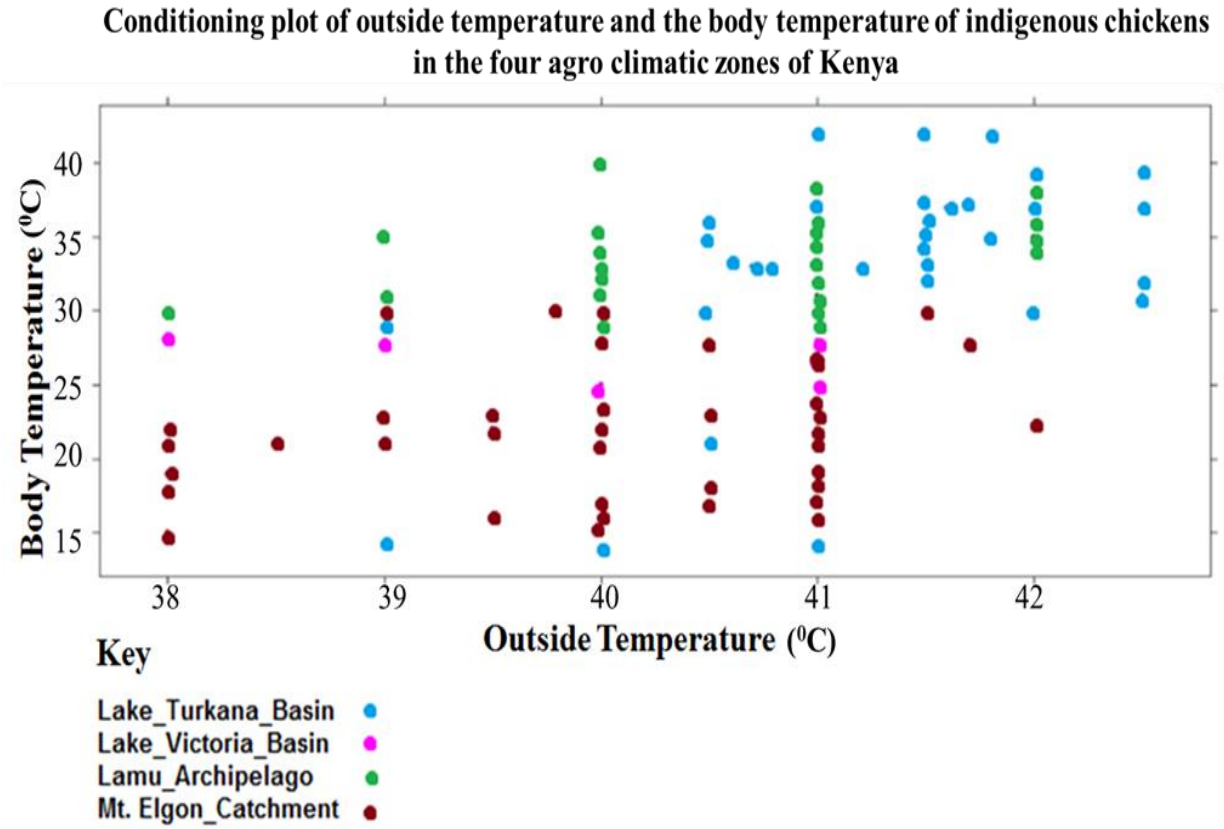


Figure 4.3: A conditioning plot for outside temperature and body temperature of indigenous chickens

4.2 Characterization of the genetic background of indigenous chickens of Kenya using mtDNA D-loop

After DNA extraction amplification was done on all the 296 samples. Figure 4.4-figure 4.7 displays a 2% agarose gel electrophoresis with representative results from the four agro-climatic zones that were obtained with primers targeting the D-loop region of mtDNA in indigenous chickens of Kenya.

4.2.1 Gel picture showing PCR Amplification of mtDNA samples

The amplicons size was 900 base pairs. This showed positive amplification. All the 296 indigenous chicken samples showed positive amplification. Samples from International Livestock Research Institute were used as positive control while water was used as a negative control. The gel images with representative samples from Mt. Elgon catchment, Lake Turkana basin, Lamu archipelago and Lake Victoria basin are shown in figures 4.4, 4.5, 4.6, and 4.7 respectively.

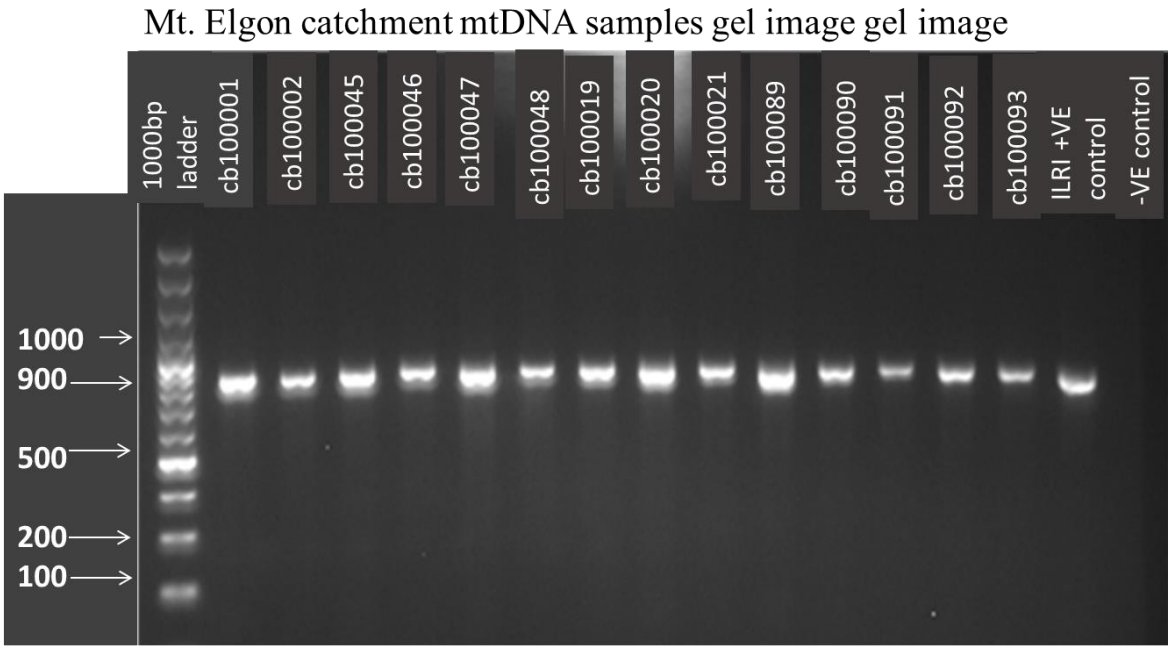


Figure 4.4: Gel picture showing mtDNA amplification in indigenous chickens in Kenya from Mt. Elgon catchment.

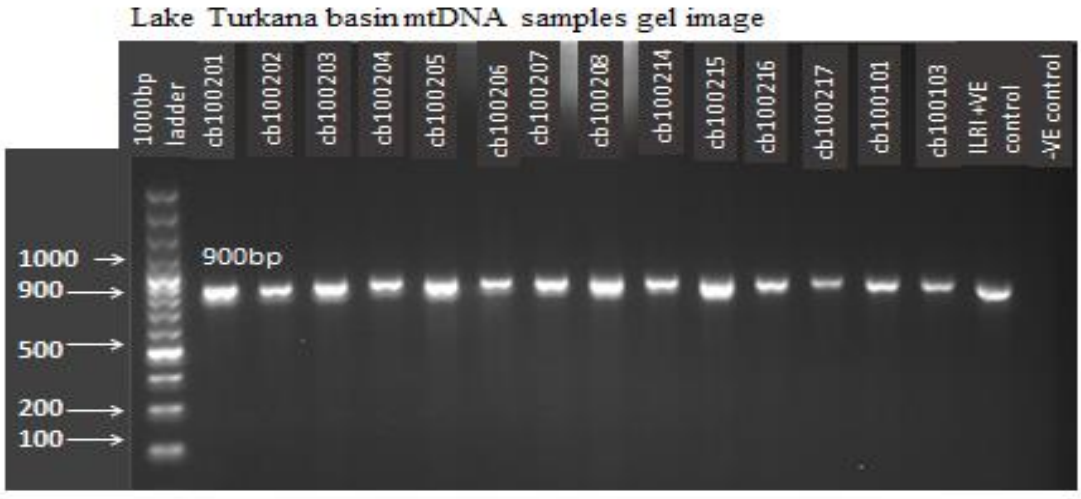


Figure 4.5: Gel picture showing mtDNA amplification in indigenous chickens in Kenya from Lake Turkana basin.

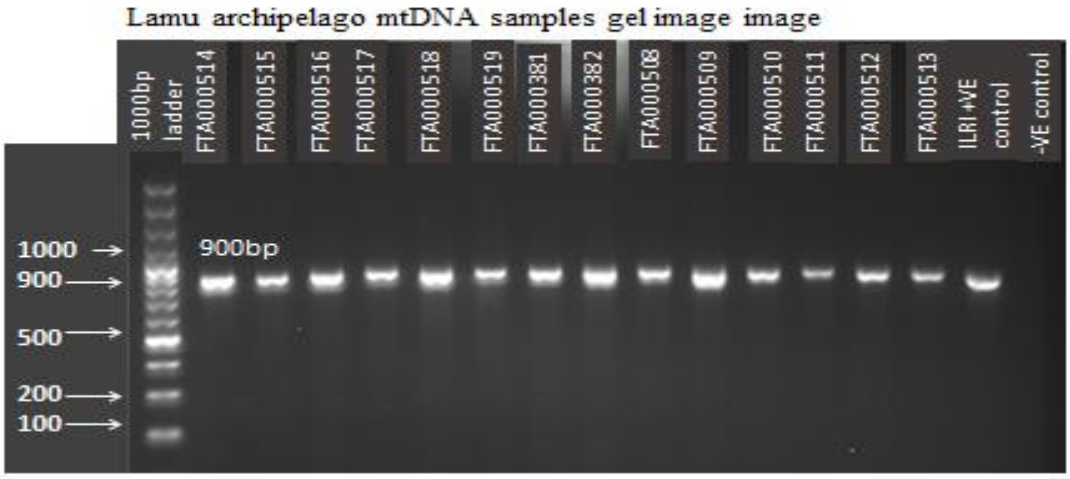


Figure 4.6: Gel picture showing mtDNA amplification in indigenous chickens in Kenya from Lamu archipelago.

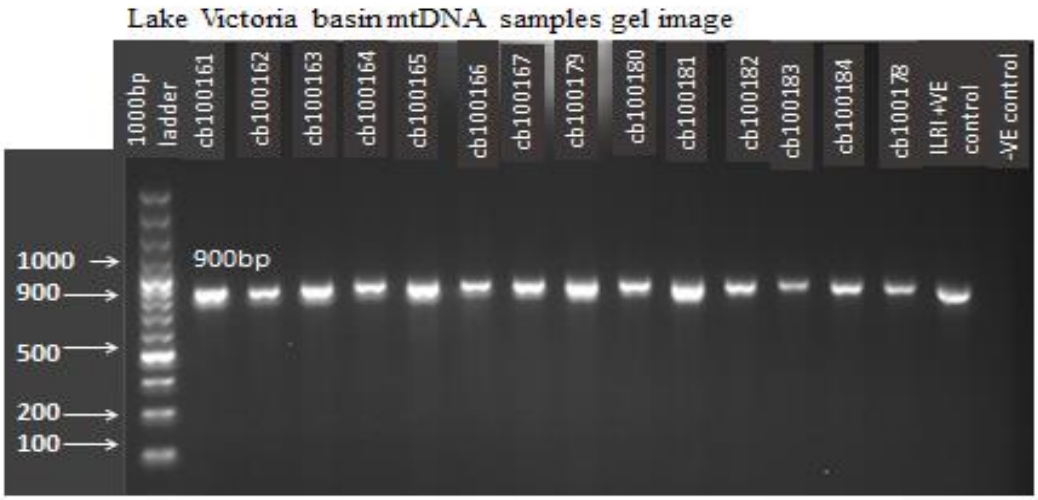


Figure 4.7: Gel picture showing mtDNA amplification in indigenous chickens in Kenya from Lake Victoria basin.

4.2.2 Editing of mtDNA chromatograms

After sequencing, the chromatograms were manually edited by the use of Chromas Lite 2.1. Figure 4.8 shows polymorphism result at various locations. This has been indicated by the arrows. The first location was position 227 where there is a transition where a purine base (adenine) is replaced by another purine (guanine). Position 256 and 257 shows a transition between pyrimidine bases (thymine) with another thymine base (cytosine). The arrows show positions of nucleotide differences. The various samples varied at various locations along the chromatogram. A total of 28 variable sites were observed.

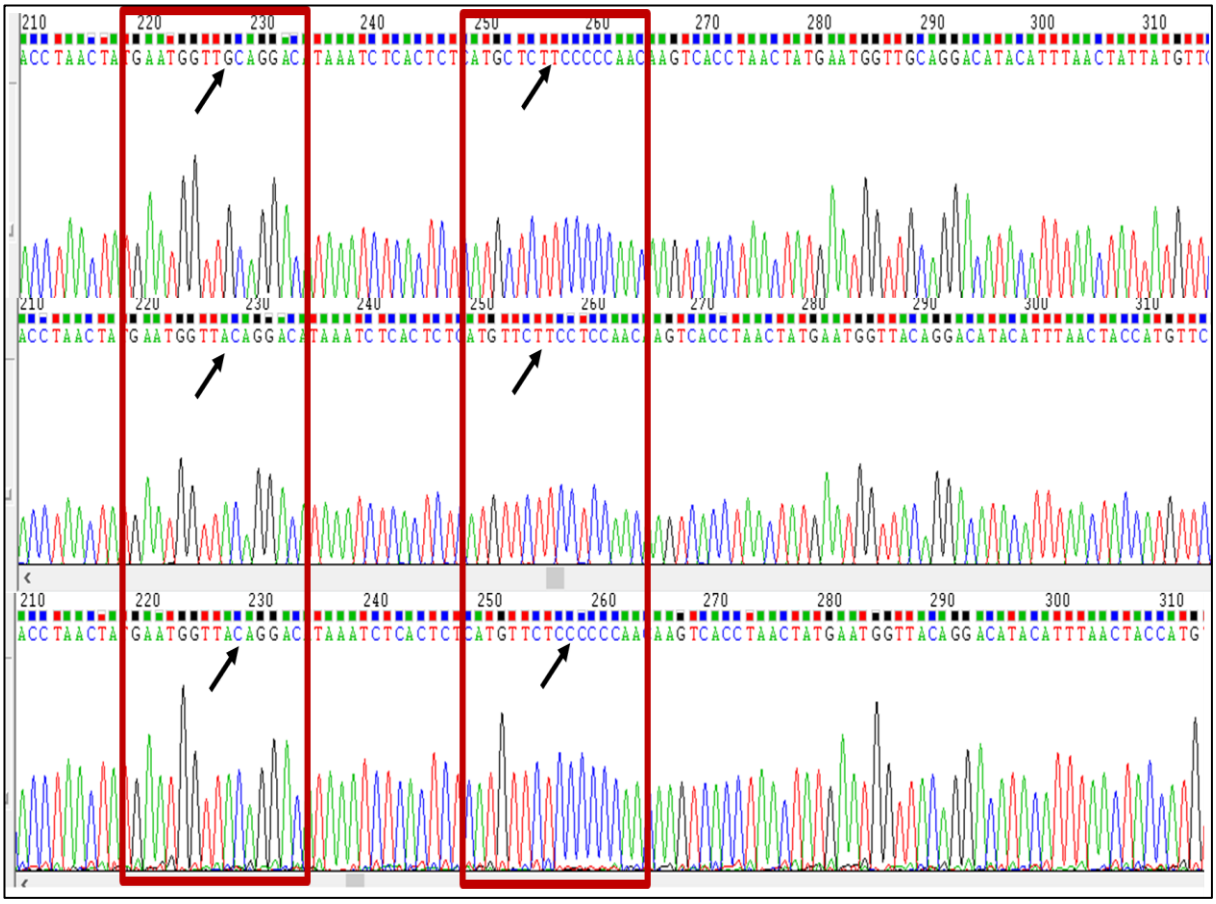


Figure 4.8: mtDNA chromatograms showing variable regions along the chromatogram

4.2.3 Multiple sequence alignment of mtDNA with reference sequences

A multiple sequence alignment of all the 296 samples plus reference sequences from GenBank was done with Clustal X2 software .This was done to confirm variable regions that were earlier on shown in the sequence chromatograms (figure 4.8). Variable sites are clearly indicated by the arrows and this is clearly indicated in figure 4.9. This confirms the transitions in the purine and pyrimidine bases at different positions as was previously shown (figure 4.8) by the chromatograms.

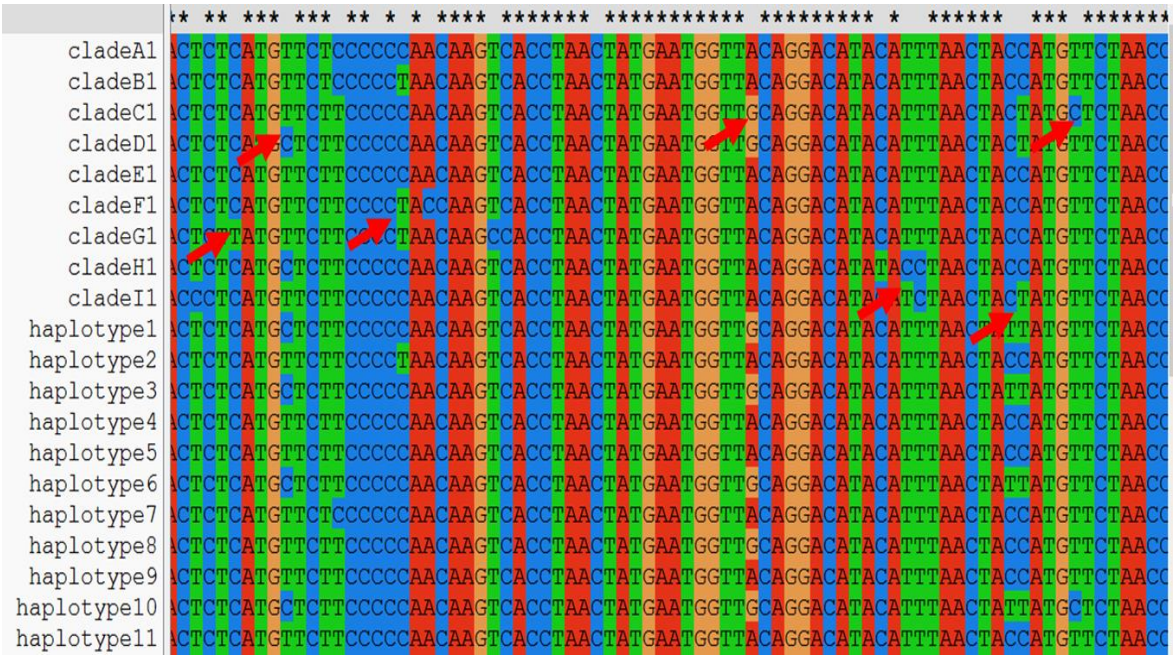


Figure 4.9: An alignment of some of the 28 haplotypes and 9 clades from GenBank.

4.2.4 Distribution of mtDNA haplotypes from the four agro-climatic zones of Kenya

The various mtDNA haplogroups discovered were distributed in the various agro-climatic zones. Some haplotypes were common in specific agro-climatic zones. This information is displayed in table 4.4.

Table 4.4: Distribution of mtDNA haplotypes in thirteen populations from four agro-climatic zones of Kenya.

	L. Turkana Basin						Mt. Elgon Catchment		L. Victoria basin		Lamu archipelago			
	Elmo, Ilolo, chir	Ileret, Baulo, telesgaye	Kapua, Kalokol,	Lore	Kainuk, westpokot	Trans nzoia	bungoma	busia	rusinga	mfangano	Ndau, Kiwayu, Vumbe, kizingitini	Faza, Tchundwa town, Tchundwa vill	Patte, siu	Total
HAP1	31	21	5	7	10	4	14	7	9	5	1	2	2	118
HAP2	5													5
HAP3	1								1		3	16	1	22
HAP4		1									4	5	1	11
HAP5		1					2	1						4
HAP6			1					2						3
HAP7			2		1	5		4	1	1				14
HAP8				3								1	5	9
HAP9				1		1	2		1	3				8
HAP10				2				2		1				5
HAP11					4									4
HAP12					3									3
HAP13					6									6
HAP14					4									4
HAP15						1		1						2
HAP16						1					2	3	1	7
HAP17							1							1
HAP18							1							1
HAP19							5							5
HAP20								1						1
HAP21								1						1
HAP22										1				1
HAP23										1				1
HAP24											2			2
HAP25											5	15		20
HAP26											1		15	16
HAP27											1	3		4
HAP28											1	1	1	3
Total	37	23	8	13	28	12	25	19	12	12	20	46	26	281

Hap-haplotype, chir-chireirich, Lore- lorengerlup, Tchundwa vill-Tchundwa village, Elmo-Elmolo

In total, 28 variable sites that defined the 28 haplotypes were observed in the indigenous chickens from the four agro-climatic zones of Kenya. A major haplotype (haplotype 1) was dominant in all populations. This haplotype was widely distributed in Lake Turkana basin indigenous chicken populations. Haplotypes 11, 12, 13 and 14 were found only in Kainuk and West pokot in Lake Turkana population

Haplotypes 17, 18, 19 and haplotypes 20, 21 were only observed in Bungoma and Busia populations respectively in Mt. Elgon cathment. Haplotypes 22 and 23 were only observed in Mfangano in Lake Victoria population as shown in table 4.4. The other haplotypes were observed in two or more agro-climatic zones.

4.2.5 Pie chart distribution of the various mtDNA haplotypes in the four agro-climatic zones

Figure 4.10 shows pie charts distribution of some of the mtDNA haplotypes. From the figure it is evident that haplotype 1 is frequently distributed in all the agro climatic zones. Haplotype 1 dominated in Lake Turkana basin indigenous chicken populations. Haplotype 3,4,7,25 and 26 were fairly distributed in all the agro-climatic zones of Kenya (figure 4.10). The remaining haplotypes were in low frequencis in all agro-climatic zones as shown in appendix 4

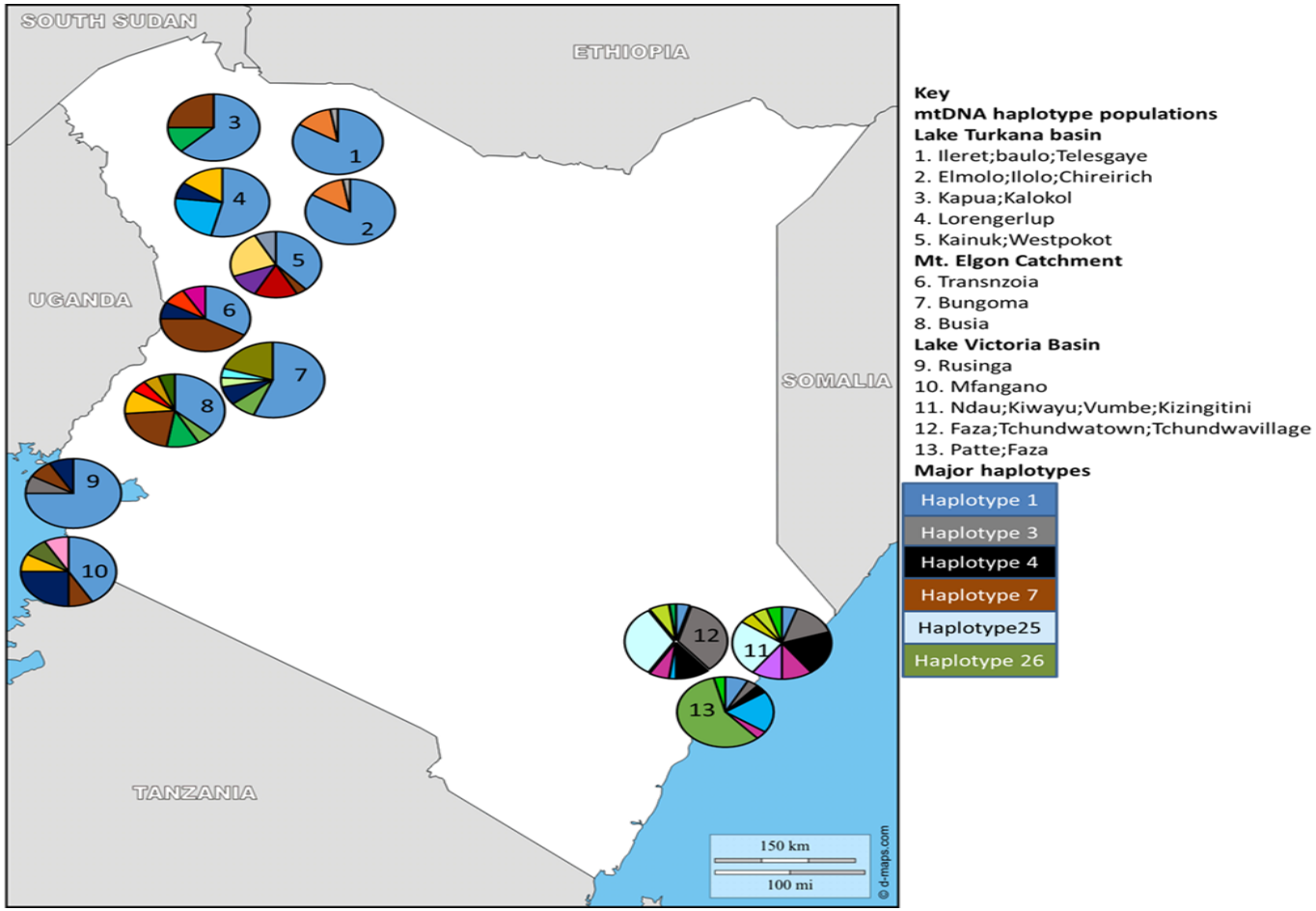
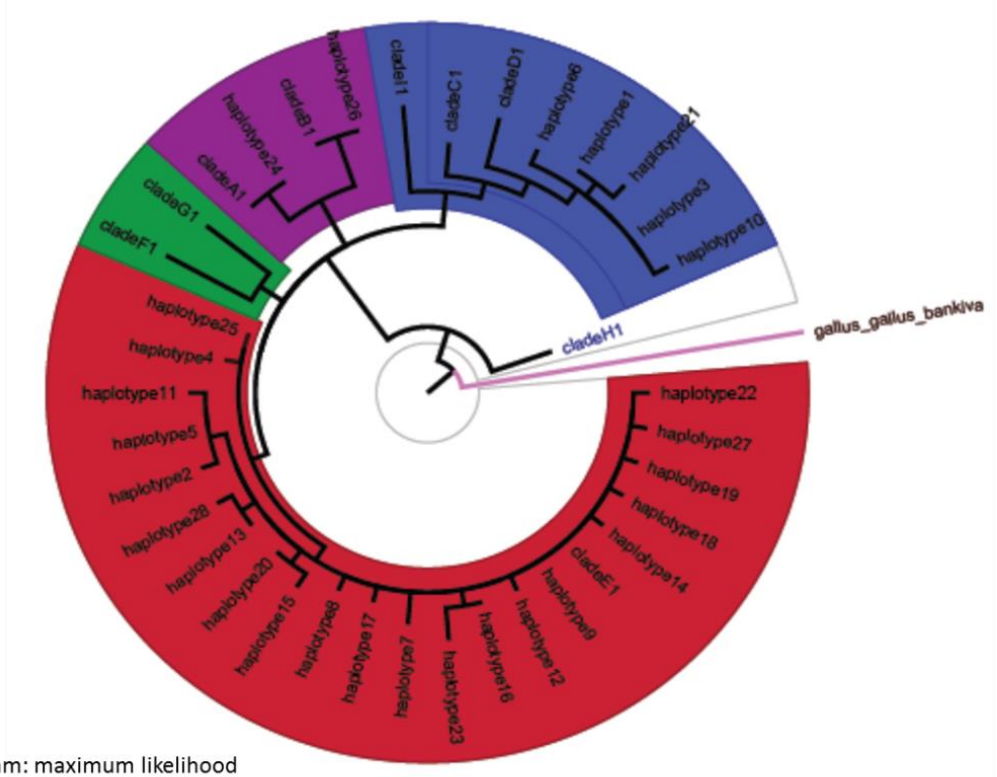


Figure 4.10: major mtDNA haplotype distribution in the various agro-climatic zones of Kenya

4.2.6 Phylogenetic analysis of mtDNA haplotypes

4.2.6.1 Maximum likelihood tree of mtDNA and reference populations

A rooted maximum likelihood tree that was edited in Fig tree shows clustering of the 28 haplotypes with reference haplogroups and an outgroup (*Gallus gallus bankiva*) from GenBank as shown in figure 4.11.



Algorithm: maximum likelihood

Model: Hasegawa-Kishino-Yano with

5 categories of Gamma distribution.

Gamma shape parameter : 0.14

Bootstrap: 1000 > 50

Figure 4.11: Rooted maximum likelihood tree of the 28 haplotypes of indigenous chickens with reference sequences.

These results reveal 4 divergent clades; clade A, B, D, and E into which the 28 haplotypes of the sampled indigenous chickens in Kenya samples clustered. None of the samples clustered into clades C, F, G, H and I. Most of the samples clustered into

clade E followed by haplogroup D which had five haplotypes. Haplogroup A and B had single haplotypes each as shown in figure 4.11.

4.2.6.2: A splits decomposition network of mtDNA with reference populations

Figure 4.12 shows a splits decomposition network of the 28 mtDNA haplotypes, reference sequences and *Gallus gallus bankiva* outgroup. The algorithim used was the Uncorrected_P NeighborNet with Equal Angle. Different colors show clustering of the 28 haplotypes of the indigenous chickens in Kenya into various clades. None of the 28 haplotypes clustered in clade C, G, F, H and I. This is also indicated in figure 4.11. Most of the haplotypes clustered into clade E followed by clade D whereas Clades A and B had single haplotypes

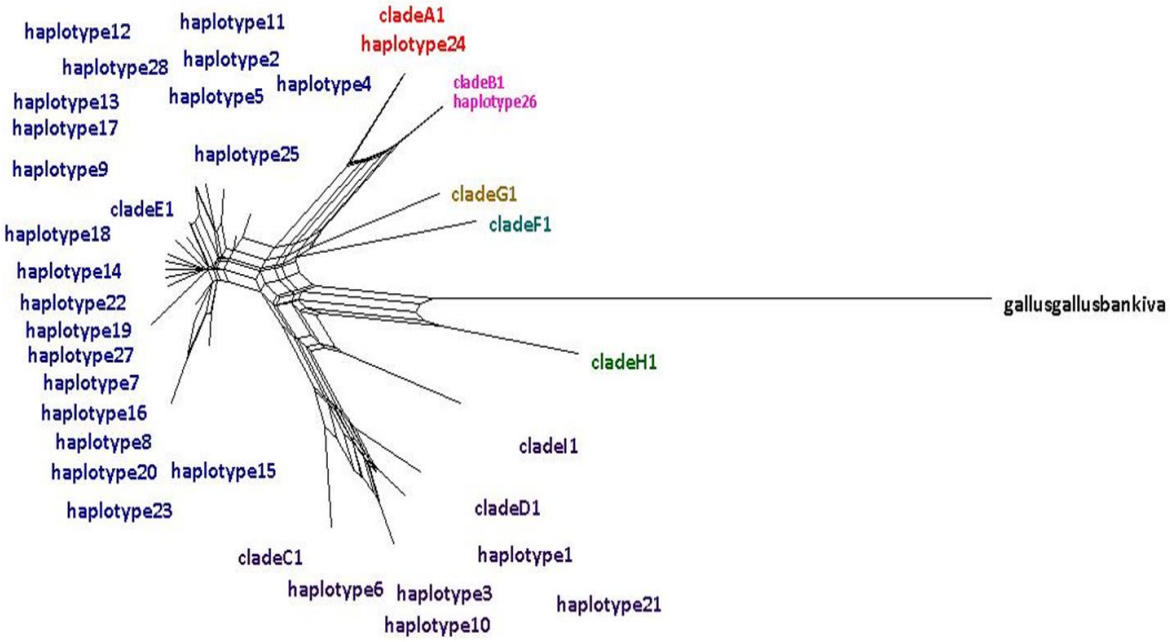


Figure 4.12: A splits decomposition network of the 28 mtDNA haplotypes with the 9 clades and *Gallus gallus bankiva* outgroup

4.2.6.3: A network of mtDNA haplotypes with reference populations

The 28 haplotypes observed in the 4 agro-climatic zones of Kenya clustered into 4 out of the 9 main clades of (Liu et al 2006). Clade E centered on haplotype 27 and was made up of haplotypes from all the four agro-climatic zones. Most of the

samples in this clade were from Turkana basin. Distances between haplotypes in this clade ranged from one to five mutations. Clade A and B consisted of one haplotype each. None of the 28 haplotypes made up clade C,G, F H and I as shown in figure 4.13.

4.2.5.3: A network of mtDNA haplotypes with reference populations

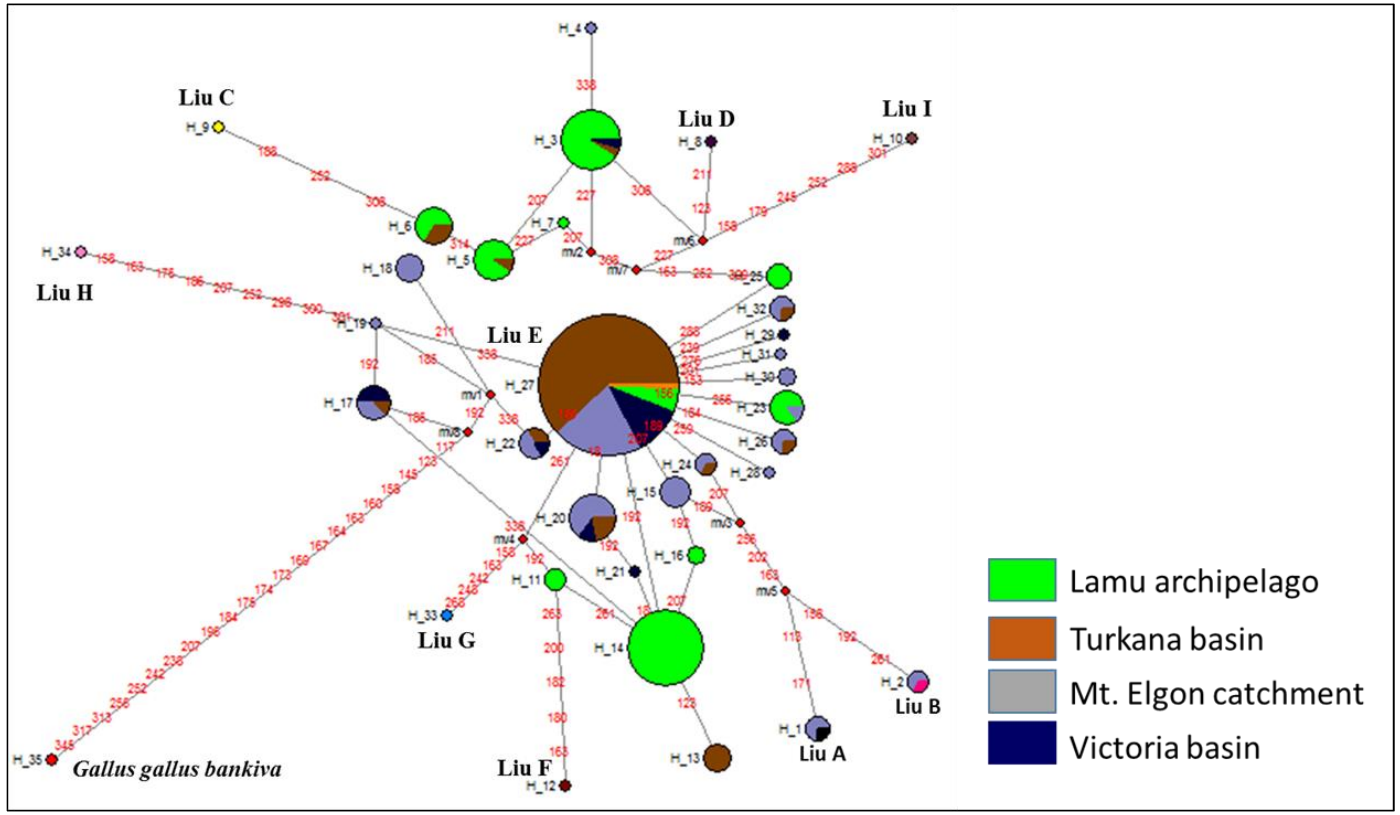


Figure 4.13: Network analysis of the mtDNA haplotypes

4.2.7: Diversity indices of mtDNA haplotypes

All the haplotypes from the 13 populations were polymorphic with the number of haplotypes ranging from 3 to 9 (Table 4.5). The study populations showed a wide range of nucleotide (0.024845+/-0.021751-0.160150+-0.091934) and haplotype (0.1700+/-0.1025-0.8895+/-0.0416) diversities (Table 4.5). The low values of nucleotide diversity were for Kapua Kalokol populations while the highest nucleotide diversity was seen in the Ndau, Kiwayu, Vumbe and Kizingitini populations. Haplotype diversity was low in Turkanas ileret, baulo, telesgaye population (0.17) and high in Lamu archipelagos ndau, kiwayu, vumbe, kizingitini (0.89). Ndau Kiwayu, Vumbe and Kizingitini had the highest number of haplotypes. Lake Turkana population had the least number of haplotypes. Tajimas D values ranged from - 0.27918 in mfangano to 1.58270 in Ndau, Kiwayu, Vumbe and Kizingitini population

Table 4.5: mtDNA haplotype diversity indices of indigenous chickens from four agro-climatic zones in Kenya

Population	Sample size	Number of polymorphic sites	Number of haplotypes	Tajimas D	haplotype diversity	Nucleotide diversity
Lake Turkana basin						
Elmolo, Ilolo, Chireirich	37	8	3	-1.68167	0.2868±0.0880	0.028743± 0.593763
Ileret, Baulo, Telesgaye	23	8	3	-2.2.299	0.1700± 0.1025	0.024845± 0.021751
Kapua, Kalokol	8	2	3	-0.44794	0.6071± 0.1640	0.024235± 0.023361
Lorengerlup	13	11	4	0.13907	0.6795± 0.1116	0.130952± 0.079611
Kainuk, Westpokot	27	13	7	-0.37047	0.8006±0.0506	0.107245± 0.064330
Mt. Elgon catchment						
Transnzoia	12	6	5	-1.19623	0.7576± 0.0927	0.048701± 0.036333
Bungoma	25	12	6	-1.24887	0.6567± 0.0903	0.071786± 0.046606
Busia	19	7	8	-1.38872	0.8304± 0.0657	0.041771± 0.031413
Lake Victoria basin						
Rusinga	12	9	4	-2.01608	0.4545± 0.1701	0.053571± 0.039012
Mfangano	12	5	6	-0.27918	0.8030± 0.0959	0.054654± 0.039604
Lamu archipelago						
Ndau, Kiwayu, Vumbe, kizingitini	20	11	9	1.58270	0.8895± 0.0416	0.160150± 0.091934
Faza, Tchundwatown, Tchundwavillage	46	12	8	1.49447	0.7662±0.0392	0.146066± 0.082310
Patte, Siu	26	12	7	0.77754	0.6431± 0.0934	0.138242± 0.079897

4.2.8: Analysis of molecular variance for the mtDNA haplotypes

Variations in mtDNA haplotypes from the four agro-climatic zones was assesed at three hierarchical levels: among groups, among populations, within groups and within populations using analysis of molecular variance (AMOVA) of pairwise differences as implemented in ARLEQUIN version 3.5.1.2. Based on pairwise differences in AMOVA across all the populations studied, variation within populations was 78.06% of the total variation while 18.8% was due to variation among groups and 3.14% variation among populations within groups (Table 4.6).

Table 4.6: Population genetic structure of mtDNA haplotypes from AMOVA

Source of variation	df	variation	percentage variation	p value
Among groups	4	0.29534	18.8	0.00079±0.00027
Among populations within groups	8	0.04936	3.14	0.02030±0.00147
Within populations	267	1.2261	78.06	0.00000±0.00000

4.3 Characterization of the functional polymorphisms on the HSP70 gene in indigenous chickens in Kenya

The 296 samples were amplified for HSP70 gene with the relevant primers and visualized under UV light. The gel image in figure 4.14 - figure 4.17 displays a 2% agarose gel electrophoresis showing a 360bp fragment of representative results.

4.3.1 Gel electrophoresis showing PCR amplification of HSP70 gene in indigenous chicken populations

The primers targeted a region of 360 base pairs which is clearly indicated by the 100 base pair molecular weight DNA marker. The amplified region corresponds to position 52,784,305-52,784,620 of the Galgal4 Chromosome 5 of chicken HSP70 (NP_001006686). All the 296 samples amplified. Water was used as a negative control. The positive control was from the International Livestock Research Institute samples. The gel images with representative samples from Mt. Elgon catchment, Lake Turkana basin, Lamu archipelago and Lake Victoria basin are shown in figures 4.14, 4.15, 4.16, and 4.17 respectively

Mt. Elgon catchment HSP70 samples gel image

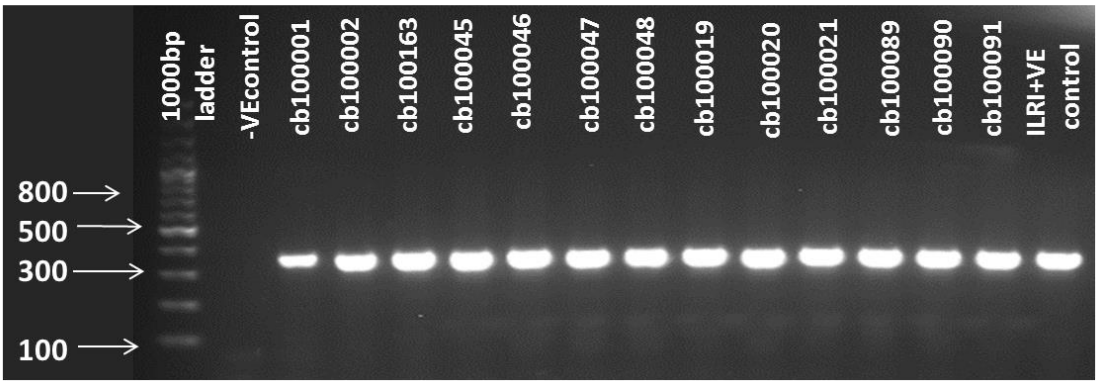


Figure 4.14: Gel picture showing HSP70 amplification in indigenous chickens in Kenya from Mt. Elgon catchment.

Lake Turkana basin HSP70 samples gel image

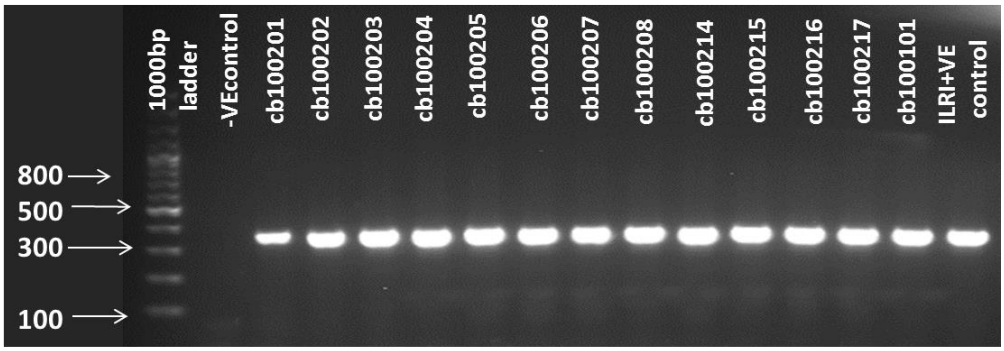


Figure 4.15: Gel picture showing mtDNA amplification in indigenous chickens in Kenya from Lake Turkana basin.

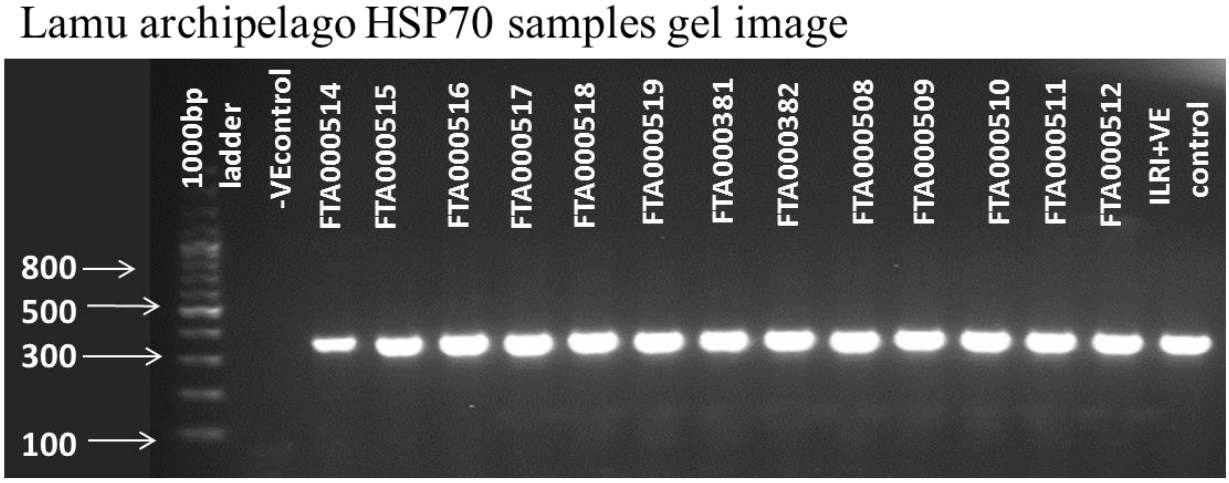


Figure 4.16: Gel picture showing mtDNA amplification in indigenous chickens in Kenya from Lamu archipelago.

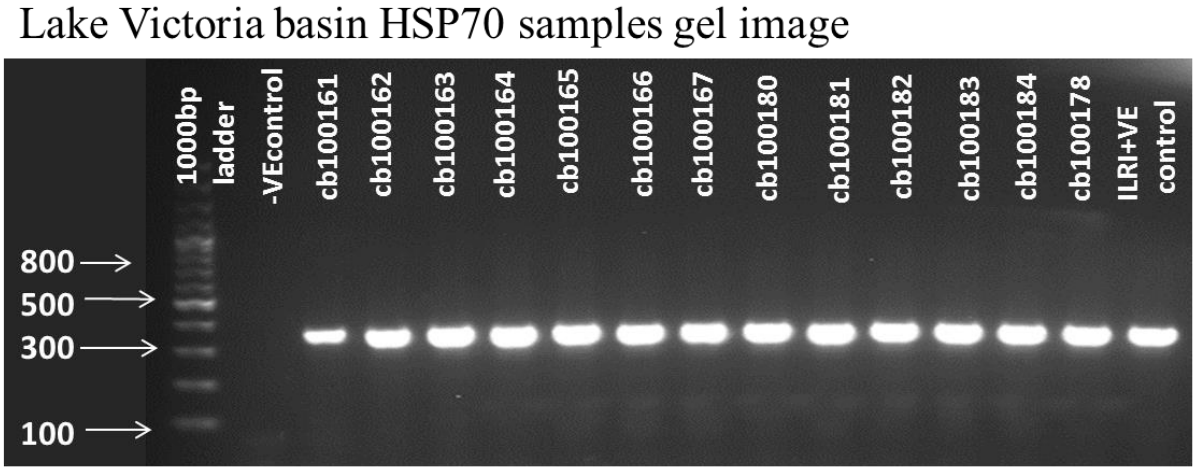


Figure 4.17: Gel picture showing mtDNA amplification in indigenous chickens in Kenya from Lake Victoria basin.

4.3.2 Editing of HSP70 chromatograms

After sequencing, the chromatograms were edited manually by the use of chromas lite 2.1 (Avin, 2012). Polymorphism within the chromatogram sequence was evident at position 153 and 171 as shown in figure 4.18. These two positions correspond to position 52784398 and 52784416 of the Galgal4 Chromosome 5 of chicken HSP70 (NP_001006686). Three homozygous sequences were observed at two locations in various samples. There were transitions and transversions at the two locations. The

cytosine base was replaced by an adenine or a guanine base in the transversion while in the transition adenine was replaced by a guanine.

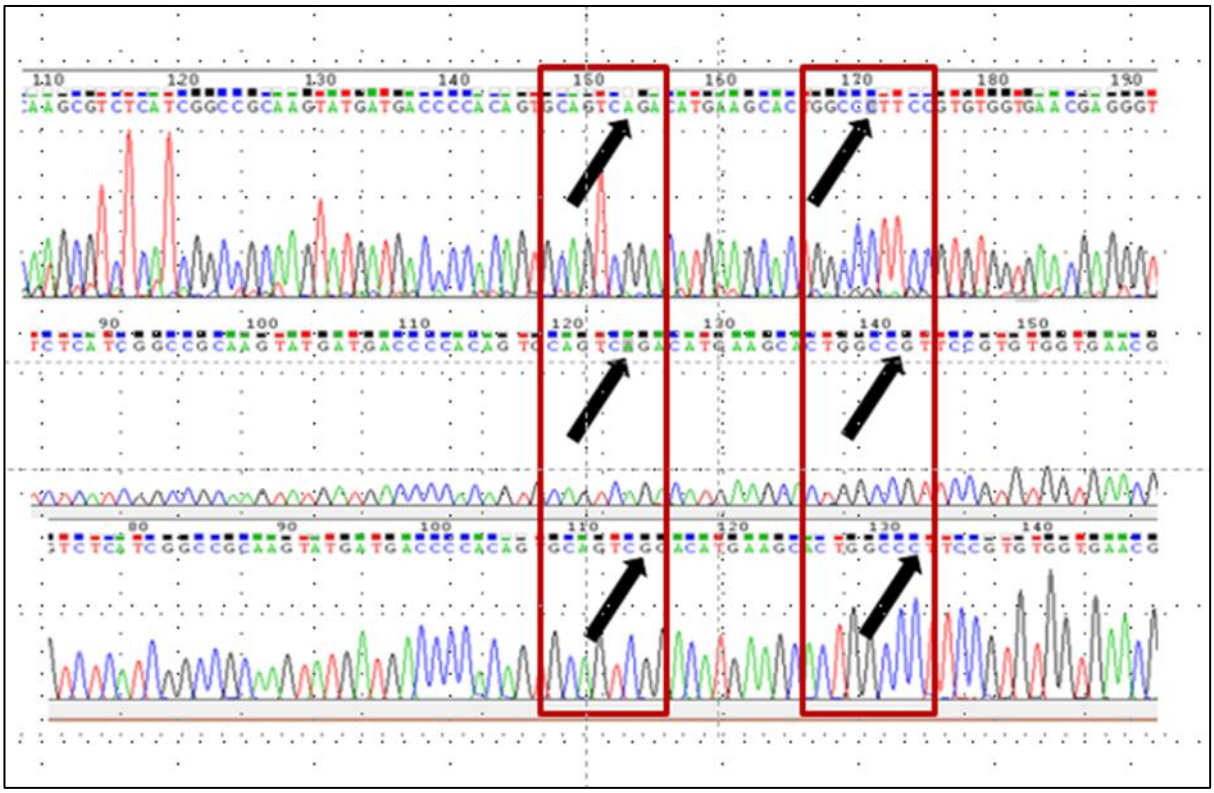


Figure 4.18: Chromatogram of HSP70 sequence showing variable regions.

Polymorphisms within the chromatogram sequence was evident as shown in figure 4.19. Three heterozygous sequences were observed in the various indigenous chicken population samples. The heterozygosity was observed at position 153 and 171 respectively that correspond to position 52784398 and 52784416 of the Galgal4 Chromosome 5 of chicken HSP70 gene (NP_001006686). The three heterozygous sequences were: RC, RS and AS. R and S are codes in chromas meant to replace adenine or guanine for R and cytosine or guanine for S.

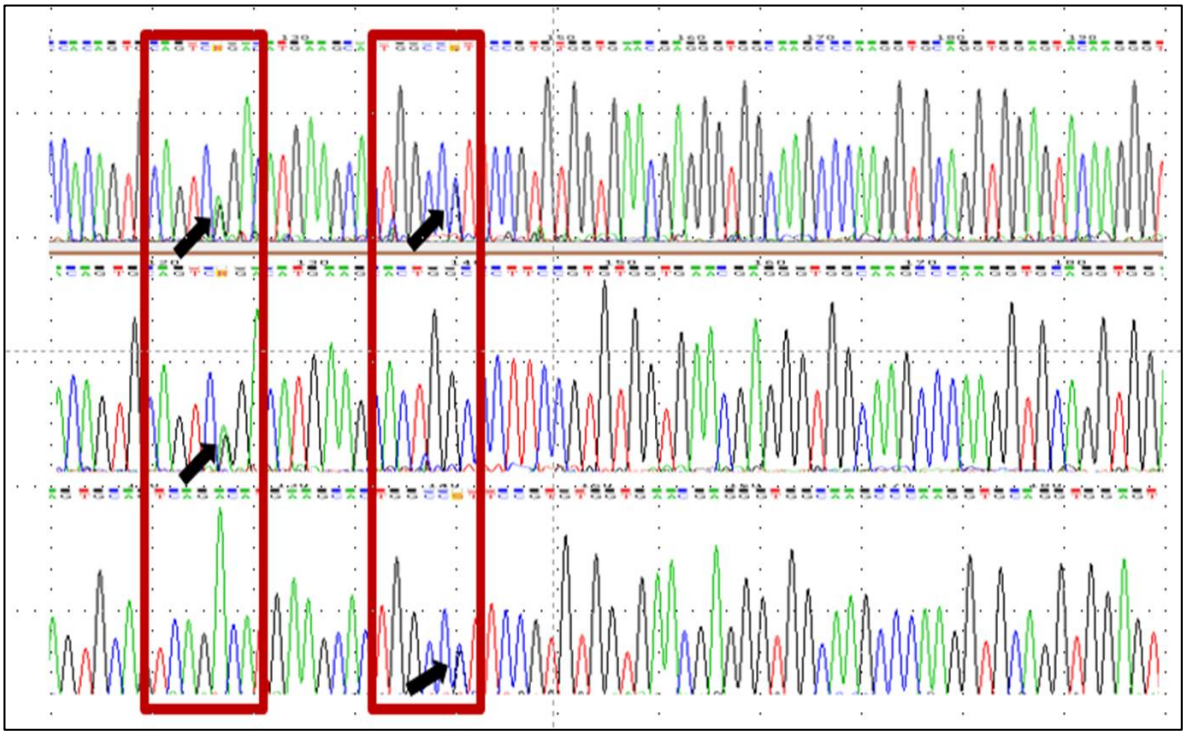


Figure 4.19: Chromatogram of HSP70 sequence showing locations of the two mutations.

4.3.3 Multiple sequence alignment of HSP70 with the red jungle fowl

4.3.3.1 Multiple sequence alignment of the homozygous, heterozygous and red jungle fowl sequences

An alignment of the edited sequences was done using Clustal X2 Version 2.1.

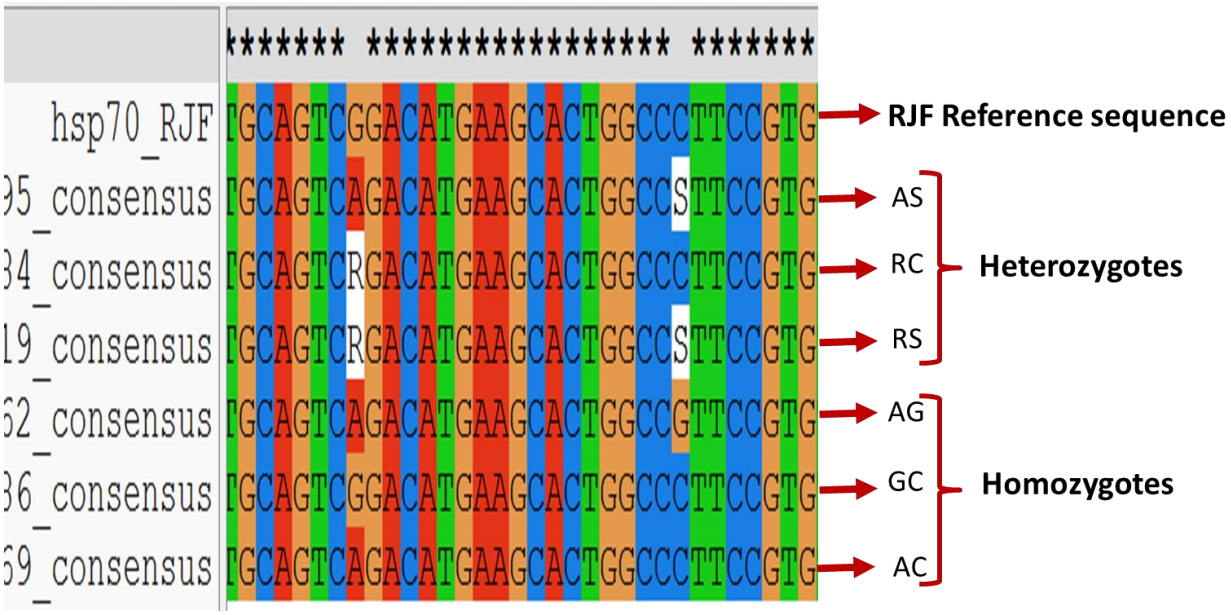


Figure 4.20: Multiple sequence alignment of the three HSP70 Heterozygotes and three HSP70 Homozygotes.

Figure 4.20 clearly shows the alignment of the three HSP70 homozygotes: AG, AC and GC and the three HSP70 heterozygotes: AS, RC and RS. The red jungle fowl was used as the reference HSP70 sequence. This shows areas of similarities and where the sequences vary.

4.3.3.2 An alignment of the red jungle fowl and the three indigenous chicken haplotypes

A multiple sequence alignment of the three HSP70 haplotypes and HSP70 sequence of the red jungle fowl was done by the use of Clustal X2 Version 2.1.

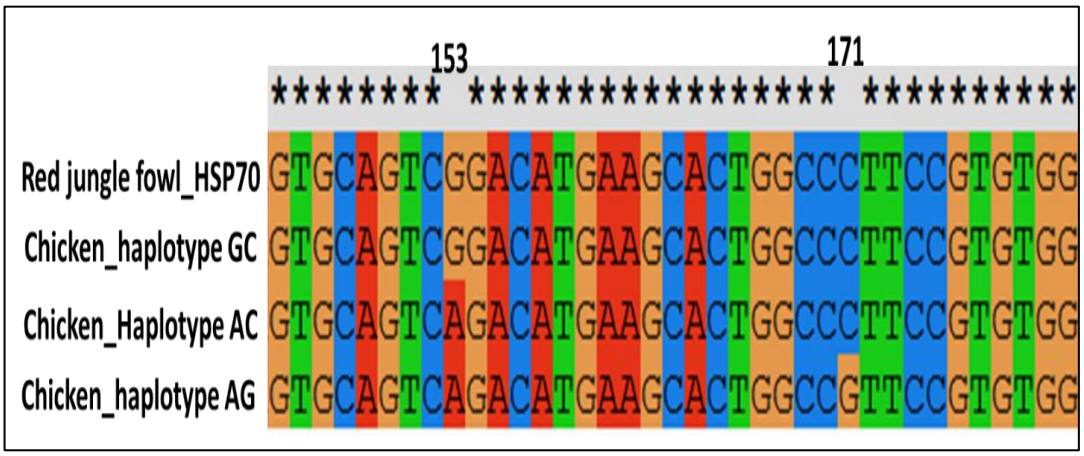


Figure 4.21: Multiple sequence alignment of the three HSP70 chicken haplotypes and the red jungle fowl HSP70 sequence.

Figure 4.21 shows the red jungle fowl and HSP70 haplotype GC have the same sequences that varied from haplotype AC and AG at position 153 and 171 respectively correspond to position 52784398 and 52784416 of the Galgal4 Chromosome 5 of chicken HSP70 (NP_001006686)

4.3.4 HSP70 haplotype distribution in the four agro-climatic zones in Kenya

Table 4.6 shows distribution of the 3 HSP70 haplotypes in the various agro-climatic zones in Kenya. Haplotype AC had the least frequencies in all the agro-climatic zones. Haplotype AG was dominant in all the agro-climatic zones except in Turkana basin. This haplotype was most frequent in Lamu archipelago (Table 4.6). Haplotype GC dominated in Lake Turkana basin and occurred in low frequencies in the other agro-climatic zones.

Table 4.7: HSP70 haplotype distribution in 13 populations from four agro-climatic zones of Kenya.

Population	Haplotype AC	Haplotype AG	Haplotype GC	Total
Lake Turkana basin				
Elmolo, Ilolo, chireirich	12	21	41	74
Ileret, baulo, telesgaye	5	22	13	40
Kapua, kalokol	1	8	9	18
lorengelup	0	7	19	26
Kainuk, westpokot	10	20	26	56
Mt. Elgon catchment				
transnzoia	3	13	10	26
bungoma	14	28	6	48
busia	6	13	17	36
Lake Victoria basin				
rusinga	5	12	7	24
mfangano	7	12	3	22
Lamu archipelago				
Ndau, Kiwayu, Vumbe, kizingitini	11	30	7	48
Faza, tchundwatown, tchundwavillage	18	60	6	84
patte_siu	16	31	5	52
Total	108	277	169	554

4.3.5 HSP70 haplotype distribution in four agro-climatic zones of Kenya

Figure 4.22 shows the distribution of the three HSP70 haplotypes in the four agro-climatic zones of Kenya. Haplotype GC dominated in Lake Turkana basin while haplotype AG dominated in Lamu archipelago (figure 4.22). Haplotype AC had the least frequencies across all the agro-climatic zones. Busia population in Mt. Elgon catchment also had haplotype GC as the dominant haplotype.

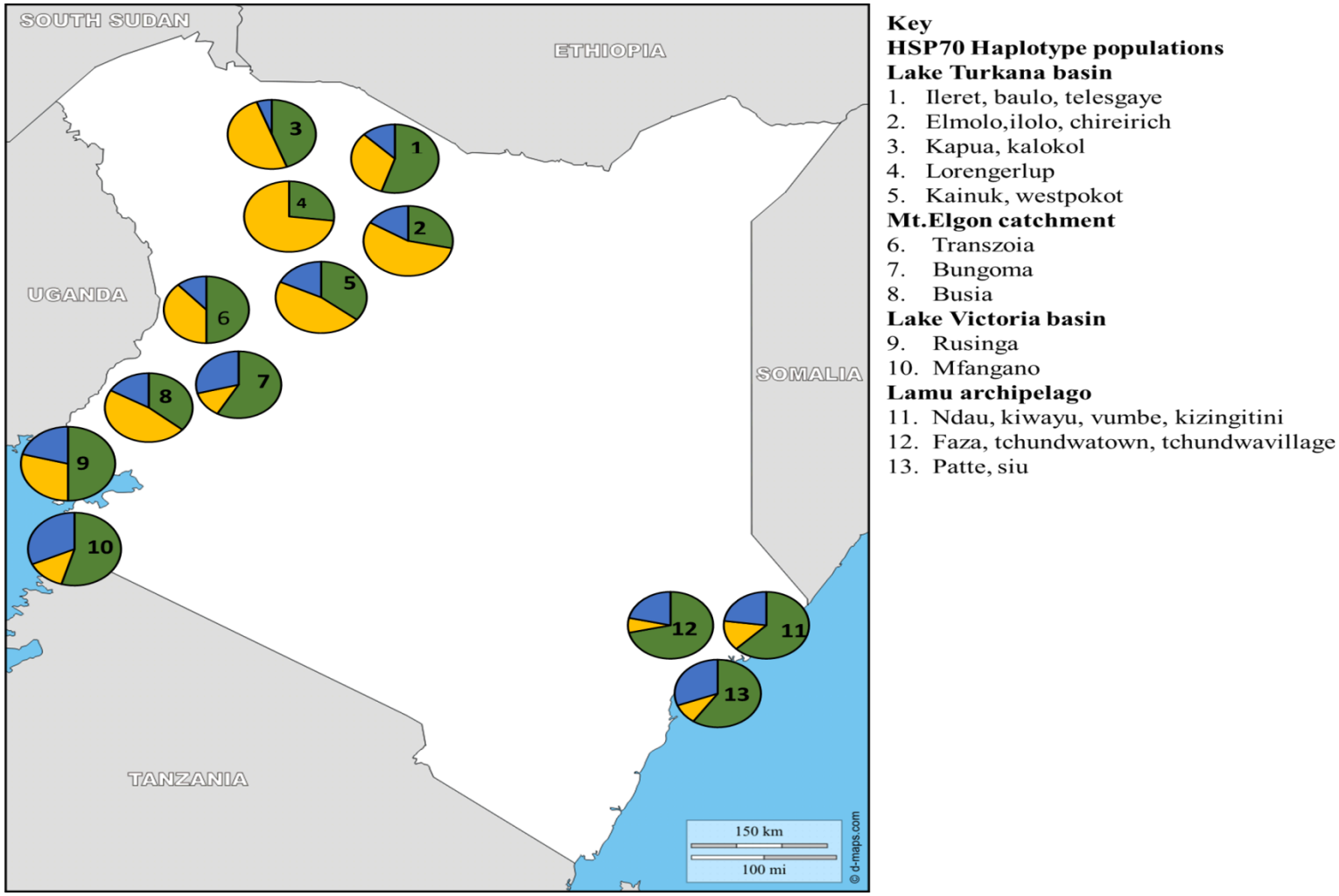
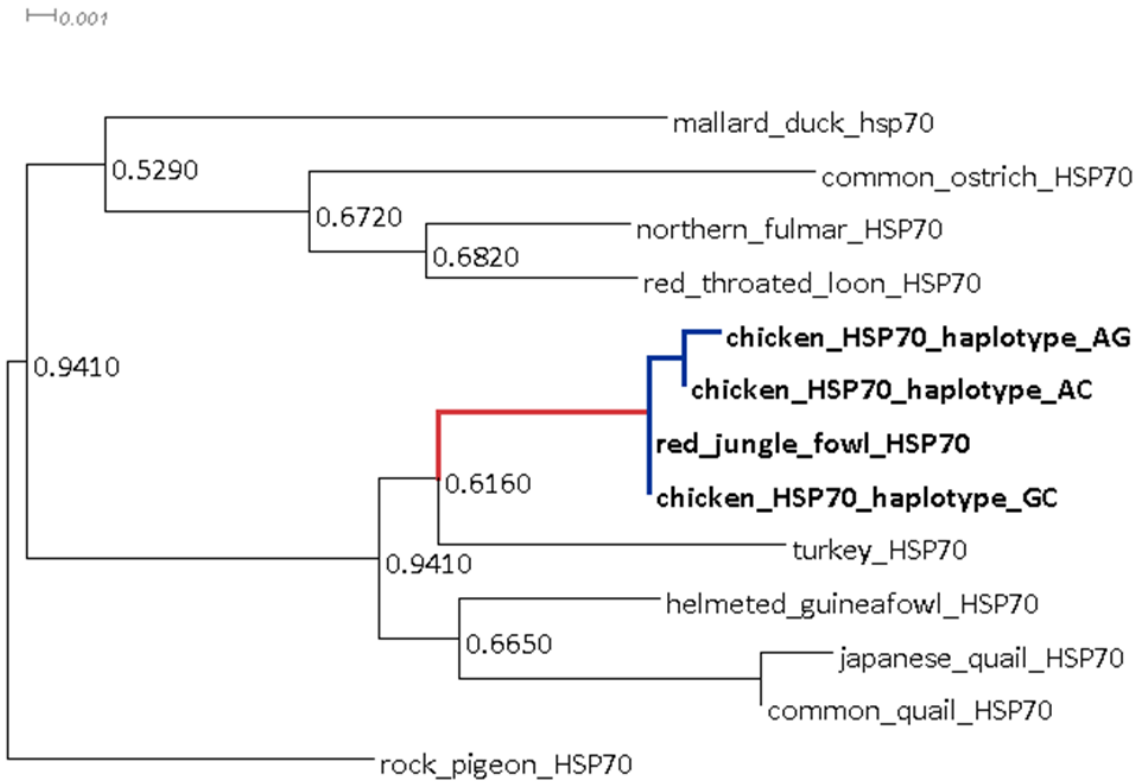


Figure 4.22: Pie chart distribution of the three HSP70 haplotypes in the four agro-climatic zones of Kenya

4.3.6 Phylogenetic analysis of HSP70 haplotypes with other avian species

4.3.6.1 Maximum likelihood tree of HSP70 and other avian species

To determine the model of sequence evolution and the rate of heterogeneity of the sequences MEGA Version 6 software was used.



Algorithm: Maximum likelihood
Model: K2+G
Gamma shape parameter: 0.1
Bootstrap: 1000

Figure 4.23: A rooted maximum likelihood tree of the three HSP70 haplotypes and other avian HSP70

Phylogenetic analysis of the three HSP70 haplotypes with other avian HSP70 sequences downloaded from GenBank showed clustering of the GC haplotype to the

ancestral red jungle fowl (Figure 4.23), while haplotype AG and AC clustered together. The tree was rooted with rock pigeon HSP70 sequence.

4.3.6.2 Splits decomposition of HSP70 and other avian species

A splits decomposition network of the three HSP70 chicken haplotypes with other HSP70 avian sequences generated in splitstree.

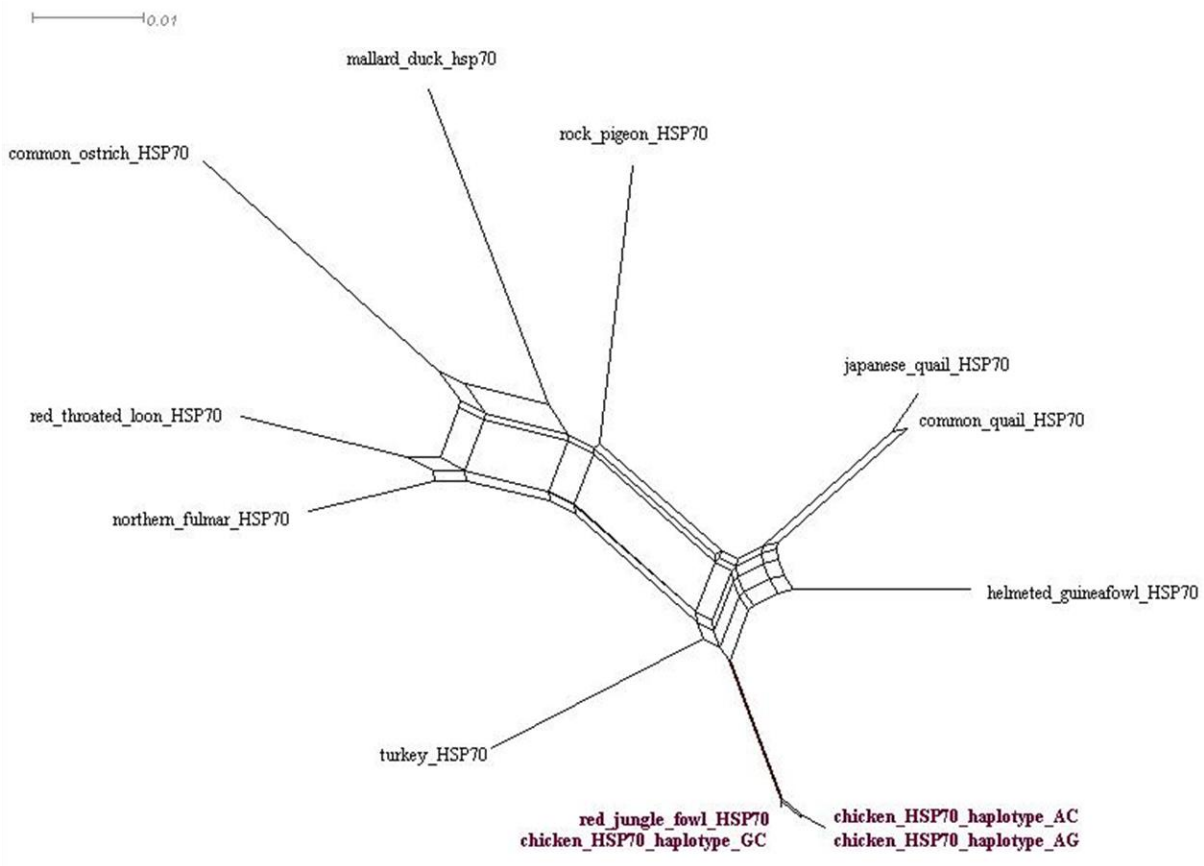


Figure 4.24: Phylogenetic network tree for the three HSP70 haplotypes with other avian HSP70 sequences.

The red jungle fowl clustered with the GC haplotype and were distant from haplotypes AG and AC which clustered together. The other avian species were distant from the three chicken HSP70 haplotypes (figure 4.24).

4.3.7 HSP70 diversity indices in the five populations from four agro-climatic zones of Kenya

Summary of the HSP70 diversity indices was calculated using Arlequin version.3.5.1.2. Lower estimates of expected heterozygosity were calculated for Lamu archipelago (0.4995) compared to the other populations (Table 4.7). Turkana basin population had the highest estimates of expected heterozygosity at (0.6305). Lake Victoria basin populations displayed the highest values for observed heterozygosity (0.7391). Lake Victoria basin populations had the least nucleotide diversity (0.00272) (Table 4.7).

Table 4.8: Diversity indices of HSP70 haplotypes in the sampled populations

Populations	Number of homozygotes	Number of heterozygotes	H_O	H_E	Pi
Turkana basin	64	94	0.5949	0.6305	0.00305
Mt.Elgon catchment	50	60	0.5456	0.6281	0.00294
Victoria basin	12	34	0.7391	0.6192	0.00272
Lamu archipelago	80	104	0.5652	0.4995	0.00283

H_E = Expected heterozygosity; H_O = observed heterozygosity; Pi=Nucleotide diversity

4.3.8 Analysis of Molecular Variance of HSP70 indigenous chicken populations

Variation in HSP70 among the 13 populations was assessed at three hierarchical levels: among groups, among individuals within populations and within individuals was measured with AMOVA of pairwise differences as implemented in ARLEQUIN v.3.5.1.2. Based on pairwise differences in AMOVA, variations within individuals was highest (89.62%) as opposed to variations among the populations (7.99%) and variations among individuals within the thirteen populations (2.39%) (Table 4.8). Variation among populations was very significant followed by variation within individuals and finally variation among individuals within the populations.

Table 4.9: HSP70 population genetic structure from AMOVA

Variance components	**Hierarchy**	**d.f.**	**variation**	**Percentage of variation**	***p*-value**
Among the populations	1	4	22.381	7.99	0.00000± 0.00000
Among individuals within the populations	2	549	164.923	2.39	0.21970± 0.00436
Within individuals	3	554	158	89.62	0.00337± 0.00057

CHAPTER FIVE

DISCUSSION

5.1 Characterization of phenotypes for heat tolerance in indigenous chickens in Kenya

5.1.1 Phenotypic characterization of indigenous chickens from the four agro-climatic zones of Kenya

Indigenous chickens in the study area varied phenotypically in plumage color, feather morphology and pattern, skin color, comb type. This is similar to what has been reported in various studies (Msoffe et al., 2001;Dana et al., 2010). Phenotypic characterization is the first step in considering sustainable management or conservation of a particular indigenous chicken population. It is therefore important to know how unique or different it is from other population phenotypes.

There is a substantial amount of phenotypic diversity for various traits in indigenous chickens in Kenya since some traits were dominant in hotter areas while others were prevalent in the cold regions. In addition the country has served as one of the gateways for domestic animal migration from Asia to Africa through the Indian Ocean and this has led to further impact on the diversity of Kenyan chickens. Their widespread distribution into all the agro-climatic zones indicates their adaptive potential to the local environmental conditions and other stresses such as heat stress.

5.1.1.1 Indigenous chicken phenotypes

A total of 11 phenotypes were recorded. Some phenotypes were only found in specific agro climatic zones while others were found in various agro climatic zones. Kingori et al. (2010) identified the frizzled, naked neck, barred feathers, feathered shanks, bearded and the dwarf phenotypes while Moraa et al. (2015), identified an additional 6 phenotypes: the rumpless, kuchi, mixed, plain feathered, crested and mottled phenotypes. The mixed phenotype which is characterized by the presence of more than one phenotype in a chicken had high frequencies in all the agro climatic zones sampled. Bantam phenotype was only observed in Lake Turkana basin.

Bantam phenotype is characterized by a small body size. The small body has been associated with better heat tolerance and heat evacuation (Chang et al., 2012), this may explain the reason why this phenotype was common in Lake Turkana basin which is characterized by high ambient temperature. Mt. Elgon catchment had a lot of the crested and feathered shank phenotypes. The crest and presence of feathers in the shank could be useful in the conservation of heat in chickens from Mt. Elgon catchment which experiences low temperatures. This could be true since studies have shown that feather coverage prevents heat loss in chickens (Yalcin et al., 1997). The naked neck phenotype was observed in low frequencies in some of the agro-climatic zones. Low frequencies of naked neck phenotypes have also been reported in previous studies in Tanzania (Guni & Katule, 2013), Ethiopia (Nigussie Dana et al., 2010) and in Botswana (Badubi, Rakereng, & Marumo, 2006). The frizzled and rumpless phenotypes were also observed in low frequencies in some agro-climatic zones. This reflects what was reported in Tanzania on the low frequencies of the frizzled and rumples phenotypes (Guni & Katule, 2013). It has also been reported in Botswana that the naked neck, dwarf and frizzled phenotypes occur at low frequencies and are at a high risk of extinction before they have even been characterized (Kgwatalala, Bolowe, Thutwa, & Nsoso, 2013).

5.1.1.2 Comb type and comb colors

This study has indicated that various comb types exist in various agro-climatic regions and in various chicken phenotypes. This is in agreement with what was reported by (Msoffe et al., 2001) who indicated that certain comb types occur more frequently in some chicken ecotypes than in others. Single comb type was the most frequent comb type in all the agro-climatic zones. Other research findings (Apuno, Mbap, & Ibrahim, 2011; El-Safty, 2012; Guni & Katule, 2013) reported the predominant occurrence of single comb type in indigenous chicken populations. Indigenous chickens in Lamu archipelago had all comb types. Lake Turkana basin also had most of the chickens exhibiting various comb types. The appearance of various comb types support that indigenous chickens are a valuable genetic resource that should be conserved.

This study shows that red and pale comb colors were predominant across all the agro climatic zones. This report reflects what was reported by (Guni & Katule, 2013) that Red and pale colors were common in Tanzanian chicken ecotypes, and (Faruque, Siddiquee, Afroz, & Islam, 2010) whose study showed the occurrence of red combs in Bangladesh chickens. However, there was no relationship in this trait to heat stress.

5.1.1.3 Earlobe and wattle colors

A total of four earlobe colors; red, white, yellow and black occurred across indigenous chickens in the four agro- climatic zones. Most of the chickens had white as the predominant earlobe color. The results of this study with regard to different earlobe colors in indigenous chickens are similar to those reported by (Egahi, Dim, Momoh, & Gwaza, 2010; Guni & Katule, 2013; Iqbal & Pampori, 2008; Msoffe et al., 2001). These studies however did not characterize earlobe colors in indigenous chickens in relation to heat stress. Variations in earlobe colors have been thought to be of genetic origin since earlobe color is dependent upon several genetic factors (Guni & Katule, 2013). This study has shown that there is no relationship between earlobe colors in indigenous chickens to heat stress.

Red, pale and black wattles were observed in indigenous chickens across the four agro-climatic zones. These results are similar to reports by (Kingori et al., 2010) on wattle colors in indigenous chickens. However Kingori et al. (2010) did not characterize wattle colors in relation to heat stress. Statistical analysis from this study have indicated that there is no relationship between wattle colors to heat stress in the various agro climatic zones of Kenya.

5.1.1.4 Skin color and shank color

White was the predominant skin color in all the agro-climatic zones except in Lake Victoria basin. These results are similar to those reported by (Guni & Katule, 2013; Kingori et al., 2010; Msoffe et al., 2001). However these results contrast with those from Dana et al. (2010) who reported yellow skin to be the most predominant skin color in Ethiopian chickens. All these studies done in the other parts did not

characterize skin color in relation to heat stress. This study characterized skin colors in relation to heat stress and there was no any relationship between the skin colors and heat stress in the various agro climatic zones of Kenya.

Yellow was the predominant shank color in indigenous chickens across the four agro-climatic zones. These results are similar to Reports by (Cabarles, Lambio, Vega, Capitan, & Mendioro, 2012; Guni & Katule, 2013; Msoffe et al., 2001). Conversely, these results differ from those observed by (Egahi et al., 2010) and (El-Safty, 2012) who reported black as the predominant shank color. In addition to yellow and black shanks, there were other shank colors observed; white, cream, grey and green. These results have shown variation in shank colors across the various agro climatic zones and this is in agreement with a research conducted by (Msoffe et al., 2001). However, these studies did not characterize shank colors in relation to heat stress. This study did not find any relationship between the various shank colors to heat stress in the various agro climatic zones of Kenya.

5.1.1.5 Plumage density

Normal, dense and scarce plumage densities were observed in indigenous chickens from all the four agro-climatic zones. Indigenous chickens in all agro-climatic zones with the exception of Mt. Elgon catchment had normal plumages. These areas with the exception of Mt. Elgon catchment are areas in the ASALs and thus do not need feather coverage to conserve heat since the areas experience high temperatures. Chickens from Mt. Elgon catchment which experiences low temperatures had dense plumage. This could be an advantageous trait that enables them conserve heat since feather coverage hinders heat loss (Yalcin et al., 1997).

5.2 Characterization of the genetic background in indigenous chickens in Kenya

5.2.1 mtDNA D-loop sequence variability and haplotype distribution patterns

Various studies have revealed extensive genetic diversity in indigenous chicken populations (Muchadeyi et al 2008; Mwacharo et al., 2010). This study analyzed the partial mtDNA D-loop sequences from 296 indigenous chickens sampled from 13

populations in four agro-climatic zones of Kenya to determine their genetic background. A total of 28 haplotypes were defined by 28 polymorphic sites. These were found distributed across the various populations.

Haplotype 1 was the most frequently observed. This haplotype was common in all the agro climatic zones. It is worth noting that most samples from Lake Turkana basin were of this haplotype. The rest of the haplotypes appeared in low frequencies and some of them were specific to particular agro-climatic zones while others were shared between the various agro-climatic zones. The nucleotide and haplotype diversity observed in this study were within the range of those observed in other chicken populations from Asia (Liu et al., 2006; Muchadeyi et al., 2008; Mwacharo et al., 2011; Oka et al., 2007; Silva et al., 2009).

5.2.2 Relationship of maternal haplotypes in a median-joining network

Haplogroup E was within 25 step mutations with the out group. Haplogroup E was connected to the other haplogroups by 1-2 median vectors (mv). The median vectors may represent either un –sampled haplotypes or haplotypes that were never introduced in these agro climatic zones or introduced but became extinct shortly upon arrival or later (Mwacharo et al., 2011). The star like pattern radiating from haplogroup E could represent ancestral haplotypes and this signals a rapid population expansion. The extent of haplotype sharing in the network indicated the absence of population structure in Kenya.

5.2.3 Maternal genetic structure

To reveal the maternal genetic structure across indigenous chicken populations from the four agro climatic zones, an analysis of molecular variance (AMOVA) was performed at three hierarchical levels. Genetic variation was highest within populations (78.06%) analyses indicated that the diversity of the haplogroups is found mainly within populations. This shows an independent arrival of the various haplotypes in the four agro climatic zones of Kenya. Mwacharo et al. (2010) also found variations within populations to be highest within populations for haplogroup A.

5.2.4 Phylogeographic relationships of mtDNA haplotypes with Asian haplotypes

Haplogroups A, B and E have been shown to be widely distributed (Liu et al., 2006). None of the 28 haplotypes clustered in haplogroup C, F, G, H and I. Clades A and D have been observed previously in village chickens from Southern Africa (Muchadeyi et al., 2008; Razafindraibe et al., 2008; Mwacharo et al., 2011), haplogroup D in West Africa (Adebambo et al., 2010), while haplogroups B, C and E were reported for the first time in the African continent (Mwacharo et al., 2011). Haplogroup A and D have been found to be related to geographically distinct Asian mitochondrial DNA haplogroups, primarily South Asia for haplogroup D and East Asia for haplogroup A (Liu et al., 2006; Mwacharo et al., 2011).

Haplogroup A was distributed only in Lamu chicken populations. This haplogroup has also been reported in Southern Africa (Muchadeyi et al., 2008) and Madagascar (Razafindraibe et al., 2008) but its absent in West Africa (Adebambo et al., 2010). It is speculated that this haplotype could have been derived from South and Southwest China and/or surrounding regions. Muchadeyi et al. (2008) speculates that it originates from Southeast Asia while Razafindraibe et al. (2008) speculated without corroborating evidence that the haplogroup may have originated from Indonesia following the Austronesian arrivals on the island. It is therefore unclear as to whether haplogroup A originated from South/Southwest China and/or surrounding areas or Southeast Asia. This haplotype could have been introduced via the trading contacts across the coast of East Africa with East Asia or an arrival in a single African coastal region and subsequent dispersal along the coastal area of the continent.

Haplogroup D was distributed in Mt. Elgon catchment, Lake Turkana and Lake Victoria basin chicken populations. This haplogroup could have been introduced at an earlier stage as compared to haplogroup A. This is because haplogroup A was geographically restricted to Lamu archipelago as compared to haplogroup D which was distributed across the three agro-climatic zones of Kenya. Similar results were also obtained by Mwacharo et al. (2010) whose results indicated a restricted geographical distribution of haplogroup A as compared to haplogroup D. This

haplogroup could have been introduced in Africa following terrestrial routes through the Middle East into Egypt from where it diffused southwards into Sudan and thereafter to Kenya, Ethiopia and Uganda. The second route is through the Arabian Peninsula/Gulf of Aden with subsequent dispersion westwards to Sudan and Southwards to Kenya and Uganda. The third route is directly to Coastal East Africa via the Indian Ocean trading route and subsequent movement inland towards Uganda, Sudan and Ethiopia.

Haplogroups B and C have been shown to have a similar geographical history (Mwacharo et al., 2011). Their origin remains uncertain but Liu et al. (2006) proposed that they originate from Yunnan province, and/or surrounding areas (Myanmar, Thailand). None of the 28 indigenous chicken haplotypes from the four agro-climatic zones of Kenya clustered in haplogroup C. Haplogroup B was only observed in indigenous chicken populations from Lamu archipelago and it was absent in indigenous chickens from the other agro-climatic zones.

It has been shown through studies that haplogroup B is identical to commercial brown and white egg layers (Muchadeyi et al., 2008; Mwacharo et al., 2011) . This could be as a result of the crossbreeding programs involving village chickens and commercial breeds to improve productivity. It is possible that this haplogroup may have been introduced via the commercial flocks through the crossbreeding programs. Similar introgression has also been shown to have occurred in Dutch fancy breeds on chicken based on mtDNA analysis (N. Dana et al., 2011), this haplotype could therefore represent signatures of recent introgression of commercial broiler and layer mtDNA haplotypes in indigenous chickens. A recent introgression theory is supported by lack of diversity of this haplogroup in East Africa and their presence in a single population.

Haplogroup E was common in the indigenous chickens from all the agro-climatic zones of Kenya. Most of the haplotypes in this haplogroup were from indigenous chickens from Lake Turkana basin. This haplogroup has also been reported in Ethiopia and Sudan (Mwacharo et al., 2011). This haplotype has never been reported in commercial chickens (Muchadeyi et al., 2008) therefore, a presence through cross

breeding with commercial flocks is not supported. This haplogroup has been shown to have a close relationship with chickens mitochondrial DNA sequences from Yunnan province in China (Liu et al., 2006) and this therefore supports an origin of the haplogroup in Yunnan province and/ or adjacent regions. There are two possible routes through which this haplogroup may have been introduced in the African continent. The first route is through the Horn of Africa via Mogadishu in Somalia where Chinese emissaries are reported to have received a Giraffe and Rhino as presents for their rulers (Duyvendak, 1939). The other route is via the Indian Ocean (Mwacharo et al., 2011)

5.3 Characterization of the functional polymorphisms on the HSP70 gene in indigenous chickens in Kenya

5.3.1 HSP70 haplotype distribution in the four agro-climatic zones in Kenya

There were three HSP70 haplotypes that were discovered in this study. Haplotype GC, AC and AG. All the haplotypes were present in all the agro-climatic zones but varied in their frequencies. Haplotype GC dominated in Lake Turkana basin. Lamu archipelago had haplotype AG in high frequencies. Mt. Elgon catchment and Lake Victoria basin had a fair distribution of the AG and AC haplotypes.

The haplotype variations observed in the various agro-climatic zones of Kenya could be attributed to the varying climatic conditions in these areas. Various studies have shown that polymorphisms in chicken HSP70 gene in different breeds has been found to be related to different resistance to heat stress (Zhang, Du, & Li, 2002). A study by Zhang, (2002) indicated that the AG and GC chicken HSP70 haplotypes were found to be advantageous to heat resistance of chickens. This is in agreement with results from this study since the GC haplotype was common in chickens from Lake Turkana basin which is an arid land and the AG haplotype was common in Lamu archipelago which is a semi-arid land. These two regions are characterized by very high temperatures therefore these two haplotypes could have been brought about by selection enabling indigenous chickens to survive the heat stress in these areas. Another study by (Hashmi, Hashmi, Glazer, & Gaugler, 1998) established a

relationship between polymorphisms of HSP70 gene of entomopathogenic nematodes and their geographic distribution and evolution.

5.3.2 HSP70 haplotype phylogeny

A maximum likelihood tree with the three HSP70 haplotypes and other avian HSP70 was constructed. The phylogeny revealed that the chicken HSP70 haplotype AG and AC fall in the same clade while haplotype GC and the red jungle fowl HSP70 fall in the same clade. Similar results were also evident in the splits decomposition network of the HSP70 haplotypes and the other avian species HSP70. Haplotype AG could be a recent introduction derived from the haplotype AC and GC. All the other avian species HSP70 were distant from the three HSP70 haplotypes and the red jungle fowl. These results clearly indicate that there still are some ancestral alleles present in indigenous chickens in Kenya.

5.3.3 HSP70 haplotype diversity

A study by Akaboot et al. (2012) reported low observed heterozygosity in various chicken breeds. Decreased homozygosity has been shown to occur from inbreeding which leads to loss of alleles since some alleles have been found to lack in commercial chickens' but are present in the red jungle fowl (Muir et al., 2008). High observed heterozygosity has been shown to be related to heat tolerance and heat susceptibility (Akaboot et al., 2012; Duangduen et al., 2008). This is in agreement with this study since all the other agro-climatic zones recorded quite high values for observed heterozygosity as compared to Mt. Elgon catchment which had the least values for observed heterozygosity.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study shows great diversity in indigenous chickens across the four agro-climatic zones of Kenya. The phenotypic diversity in indigenous chickens was high since they varied in various morphological attributes such as the skin color, shank colors, comb types and comb colors. Some of these traits could be attributed to heat tolerance like the various comb types that were dominant in the arid and semi-arid lands. Plumage density and the various comb types were shown to be significant to the outside and body temperature respectively. Lamu archipelago and Turkana basin had chickens with various comb types as compared to the comb types in the other agro climatic zones. This could be a survival mechanism to enable them dissipate excess heat through the varied combs.

Four D-loop mtDNA haplogroups in the indigenous chicken populations were discovered. Haplogroup E had most samples from Lake Turkana basin. It has been established that this haplogroup has never been reported in commercial chickens. This means that this haplogroup is purely indigenous and has not been seen in commercial flocks. The other haplogroups in this study are also shared with those of chickens from some other African countries and they could have found their way to Africa through the coastal line or through North Africa. It is also worth mentioning that these haplogroups may have originated from different regions of the world such as Yunnan, South and Southwest China and/or surrounding areas and the Indian subcontinent. This supports the theory of multiple origins of indigenous chickens in South and Southeast Asia.

Results from this study indicated that three chicken HSP70 haplotypes exist in the indigenous chicken populations sampled. Two of these haplotypes; The GC and AG haplotypes dominated in Lake Turkana basin and Lamu archipelago respectively. Lamu archipelago and Lake Turkana basin are characterized by high ambient temperatures, these two haplotypes could be advantageous to the indigenous

chickens enabling their survive in heat stress environments. Haplotype GC was also found to be the ancestral haplotype since it fell in the same clade with the ancestral red jungle fowl.

From these results it is clear that indigenous chickens in Kenya are diverse both in the genetic and phenotypic attributes. They were also shown to have originated from various parts of the Asian subcontinent and haplogroup E in mtDNA which was dominant in Lake Turkana basin has also been shown to be purely indigenous. It is also worth noting that HSP70 haplotype GC which is the ancestral haplotype dominated in indigenous chickens from Lake Turkana basin which is a heat stressed environment. This shows that despite the ongoing cross breeding programs, there are still some indigenous chickens' alleles present in Kenya especially in Lake Turkana basin which had unique mtDNA and HSP70 haplotypes that need to be conserved for the future.

6.2 Recommendations

1. These preliminary results should pave way for more studies on phenotypic characterization of indigenous chickens in all the agro-climatic zones in Kenya especially in the other arid and semi-arid lands to identify if there are more phenotypic traits that may be advantageous to heat stress in these environments.
2. The mtDNA results have indicated that there are unique haplotypes that exist and have a distinct genetic background. All indigenous chickens from the other agro-climatic zones of Kenya should also be characterized to know if other unique haplotypes exist and to determine their genetic background and come up with approaches to conserve them or utilize them in the breeding programs.
3. The HSP70 results indicated that the HSP70 GC and AG haplotypes dominated in heat stress environments of Lake Turkana basin and Lamu archipelago. These and could be used for breeding programs for heat

tolerance. This should lead to further studies focusing on desirable traits which can in turn be used for breeding programs for heat tolerance.

4. The crossbreeding programs should be discouraged since they lead to dilution of indigenous chicken populations which are unique and have good traits like heat tolerance as compared to commercial flocks. Efforts should also be put in place for the conservation of indigenous chickens since this study has clearly shown genetic diversity of indigenous chickens available in the country and hence could be utilized in indigenous chicken rearing e.g KARI kienyeji.

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APPENDICES

Appendix 1: GenBank accession numbers for mtDNA reference populations.

Organism	GenBank accession number
Liu A	AB114069
Liu B	AB007744
Liu C	AB114070
Liu D	AY588636
Liu E	AB114076
Liu F	AF512285
Liu G	AF515588
Liu H	D82904
Liu I	AB009434

Appendix 2: GenBank accession numbers for HSP70 reference populations.

Organism	GenBank accession number
Red jungle fowl	NC_006092.3
Common ostrich	XM_009675580.1
Mallard duck	XM_005022658.1
Japanese quail	AB259847.1
Common quail	EU622852.1
Northern fulmar	XM_009576438.1
Rock pigeon	XM_005506375.1
Helmeted guinea fowl	AB096696.1
Red throated loon	JJRM01051595

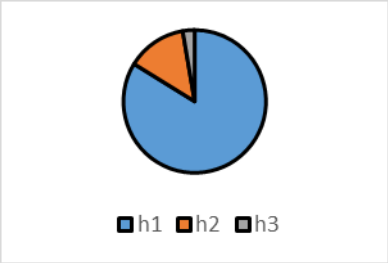
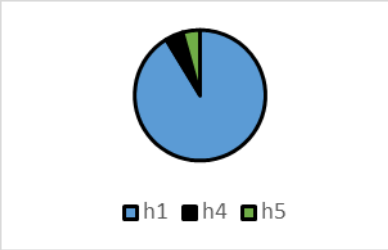
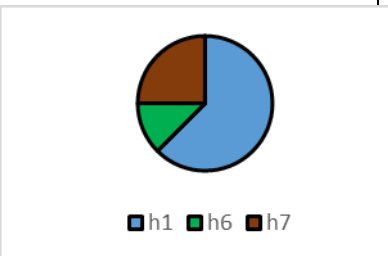
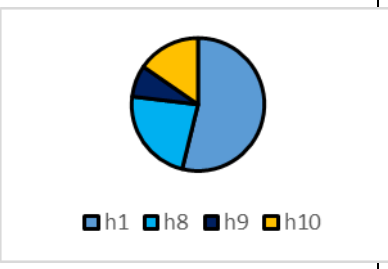
Appendix 3: Sanger sequencing method

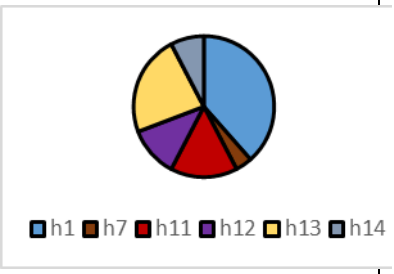
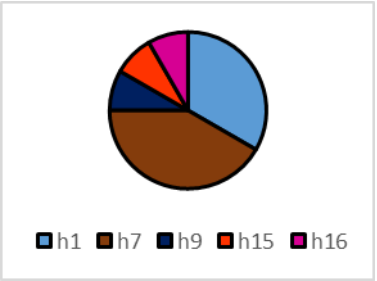
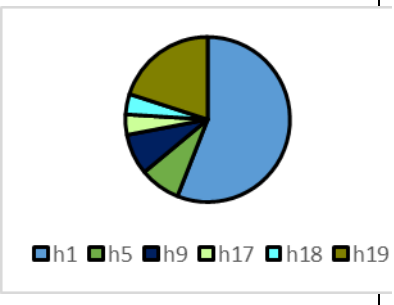
The sanger sequencing method is undertaken by following various steps. The first step is the preparation of the template DNA. This is done by purifying the PCR products to remove excess primers and the 4 dNTPs. Purification was done enzymatically. The next step is the cycle sequencing reaction by the sanger's dideoxy terminator method whereby the DNA, DNA polymerase, 4 dNTPs, 4 dideoxy terminator nucleotides fluorescently labeled with 4 different dyes and enzyme buffering containing Mg++, K+. Since only 1 primer is used for sequencing, this single primer binds to the complementary DNA strand and extends itself in a linear fashion. This extension goes on until by chance a particular ddNTP is incorporated depending on the complementary base. Because of the latter's dideoxy-configuration, the polymerase cannot add any other base to this fragment thus the extension is terminated. At the end of the 25 to 40 cycles depending on the size of the template, numerous fragments are generated having different lengths and a tagged nucleotide at the end. Stoichiometric manipulations of the reaction components ensures that the fragments of every possible length starting from n+1 to may be 1000 bases are generated, n is the number of bases in the primer. Since only one primer is used, this means that only one strand can be sequenced in one reaction and a primer cannot read itself. The next step that follows Is the post sequencing reaction cleanup which is necessary to remove excess dNTPs tagged ddNTP'S and salts from the reaction products. The purification is done using ABI's BigDye Xterminator kit. After this the samples are to go on the sequencer. All of the above steps were done in the 96- well plates.

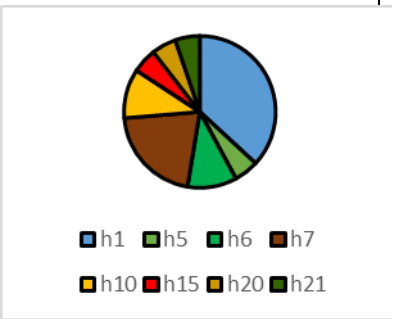
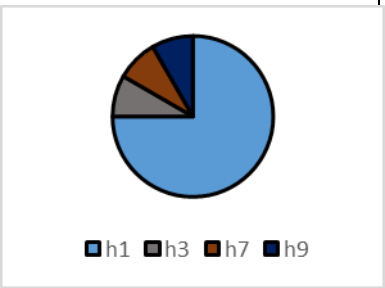
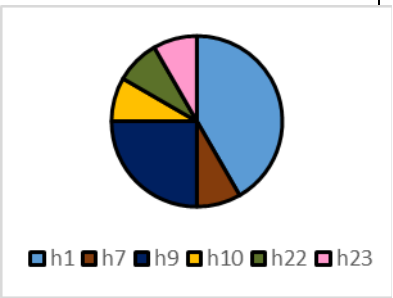
The next step that follows is the fragment separation by capillary electrophoresis on the ABI-capillary 3730 XL sequencer. The samples are electokinetically injected into the array of capillaries; the negatively charged fragments migrate towards the anode by size, the smallest ones move fastest. Their tagged ddNTP terminators can be reached as the fragment's base sequence. A laser beam excites the dye molecules as the fragments reach a detection window producing fluorescent signals that are collected from all 96 capillaries at once, spectrally separated and focused onto a

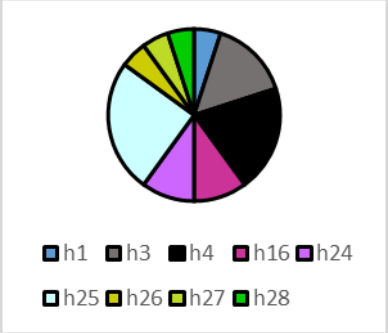
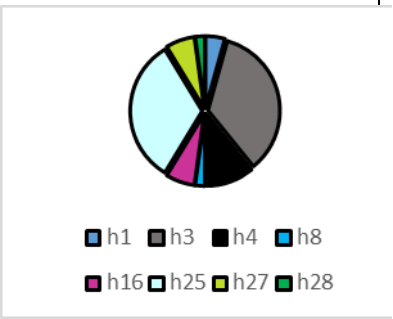
CCD camera. Very sophisticated optical and electronic devices produce a color read out that is translated with the help of sequence analysis software into a sequence. The data obtained is edited and blasted in NCBI GenBank for identification, and aligned against a reference sequence.

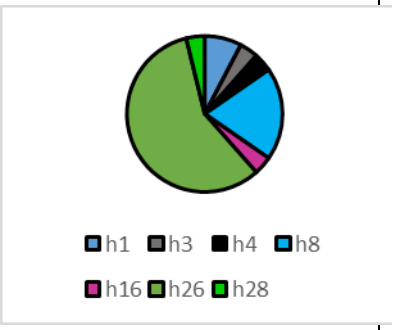
Appendix 4: Summary of mtDNA haplotype distribution in the four agro-climatic zones of Kenya

Population	Sample size	Haplotype frequency	Pie chart
Lake Turkana basin			
Elmolo;ilolo;chireirich	37	Hap1 - 31 Hap2 - 5 Hap 3 - 1	
Ileret;baulo;telesgaye	23	Hap 1 - 21 Hap 4 - 1 Hap 5 - 1	
Kapua;kalokol	8	Hap 1 - 5 Hap 6 - 1 Hap 7 - 2	
lorengerlup	13	Hap 1 - 7 Hap 8 - 3 Hap 9 - 1 Hap 10 - 2	

Kainuk;westpokot	27	Hap 1 - 10 Hap 7 - 1 Hap 11 - 4 Hap 12 - 3 Hap 13 - 6 Hap 14 - 2	
Mt. Elgon catchment			
transnzoia	12	Hap 1 - 4 Hap 7 - 5 Hap 9 - 1 Hap 15 - 1 Hap 16 - 1	
bungoma	25	Hap 1 14 Hap 5 - 2 Hap 9 - 2 Hap 17 - 1 Hap 18 - 1 Hap 19 - 5	

busia	19	Hap 1 - 7 Hap 5 - 1 Hap 6 - 2 Hap 7 - 4 Hap 10 - 2 Hap 15 - 1 Hap 20 - 1 Hap 21 - 1	
Lake Victoria basin			
rusinga	12	Hap 1 - 9 Hap 3 - 1 Hap 7 - 1 Hap 9 - 1	
mfangano	12	Hap 1 - 5 Hap 7 - 1 Hap 9 - 3 Hap 10 - 1 Hap 22 - 1 Hap 23 - 1	

Lamu archipelago			
Ndau;kiwayu;vumbe;kizingitini	20	Hap 1 - 1 Hap 3 - 3 Hap 4 4 Hap 16 - 2 Hap 24 - 2 Hap 25 - 5 Hap 26 - 1 Hap 27 - 1 Hap 28 - 1	
Faza;tchundwatown;tchundwavillage	46	Hap 1 - 2 Hap 3 - 16 Hap 4 - 5 Hap 8 - 1 Hap 16 - 3 Hap 25 - 15 Hap 27 - 3 Hap 28 - 1	

Patte;siu	26	Hap 1 - 2 Hap 3 - 1 Hap 4 - 1 Hap 8 - 5 Hap 16 - 1 Hap 26 - 15 Hap 28 - 1	

Appendix 5: Publication from this research work

Publication from this research work

2015: G K Moraa, P A Oyier, S G Maina, M Makanda, E K Ndiema, A E Alakonya, K J Ngeiywa, J Lichoti and S C Ommeh Assessment of phenotypic traits relevant for adaptation to hot environments in indigenous chickens from four agro-climatic zones of Kenya Livestock Research for Rural Development 27(10).

Appendix 6: Questionnaire for the phenotypic characterization of indigenous chickens in the four agro-climatic zones of Kenya

Farmers name…… Region …….

Location…… GPS……..

Enumerators name……. Date of interview……

Number of chickens…… Outside Temperature……..…….

How long has indigenous chickens been kept in the household? .....

Source of foundation stock…….

Age of indigenous chicken……

Do you feel the need to improve indigenous chicken production? Yes ( ) No ( )

Traits to improve in indigenous chickens……

What type of management system do you practice? Extensive ( ) semi-intensive ( ) intensive ( ) others, specify…….

Do you give supplementary food to your indigenous chickens? Yes ( ) No ( )

If you give feeds how frequently do you feed your birds daily? .....

How often do you cull your birds?.....

For what purpose do you cull your poultry? ( ) for consumption, ( ) sale, ( )sacrifice, ( ) others, specify……

Which factors determine which bird you will cull? ( ) poor productivity ( ) old age ( ) sickness ( ) others, specify……

Have you heard about the improved poultry production practices yes ( ) no ( )

If yes what is your major source of information on the improved poultry production practices…….

Morphometry

Age in months……

Sex; male ( ) female ( )

Shank color; white ( ), yellow ( ), green ( ), grey ( ), others……

Skin color; white ( ), yellow ( ), cream ( ), grey ( ), others……

Wattle color; Red ( ), pale ( ), others ……

Ear lobe color; red ( ), white ( ), yellow ( ), others……

Indigenous chicken phenotype; mixed ( ), kuchi ( ), feathered shank ( ), crested ( ),

mottled ( ), naked neck ( ), bantum ( ), rumples ( ), frizzled ( ), barred feathers ( ),

bearded ( ), others……

Plumage density; dense ( ) normal ( ) scares ( )

Comb type; single ( ), strawberry ( ), pea ( ), walnut ( ), cushion ( ), buttercup ( ), rose ( ) others …….

Comb color; red ( ), pale ( ), others ……

Body Temperature ……..

Other general issues

Do you intend to extend poultry production? Yes ( ) No ( )

If yes to what size……

What are your barriers to future expansion of poultry production? .....

What traits do you wish to see improved in indigenous chickens? .....

What do you think the government should do to improve poultry keeping particularly in the rural areas? .....