PHENOTYPIC AND GENETIC DIVERSITY, AND HSP70 GENE POLYMORPHISM OF THE HELMETED GUINEA FOWL IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in this or any other University.

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DEDICATION

This work is dedicated to my loving wife Joan Kahombi Majanga and my sons Elvis, Ryan and Leon whose unconditional love, support and devotion has seen me through this journey of realizing my best self.

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LIST OF ABBREVIATIONS AND ACRONYMS

ASALs	Arid and Semi-arid Lands
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
bp	Base pairs
cDNA	Complimentary Deoxyribonucleic acid
D-Loop	Displacement Loop
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
ddNTP	Dideoxynucleotide Triphosphate
EDTA	Ethylene Diamine Tetra-acetic Acid
GPS	Global Positioning System
FAO	Food and Agricultural Organization
GRP78	Glucose-regulated Protein 78
Hap	Haplotype
Hg	Haplogroup

HSC70	Heat Shock Cognate Protein 70
HSF	Heat Shock Factor
HSP	Heat Shock Proteins
HSP7	Heat Shock Protein 70
HVS	Hyper Variable Segment
IBR	Institute for Biotechnology Research
kDa	Kilodalton
MEGA	Molecular Evolutionary Genetic Analysis
MJ	Median Joining
ML	Maximum Likelihood
µl	Microlitre
mtDNA	Mitochondrial DNA
MUPID	Mini Electrophoresis Unit
MUSCLE	Multiple Sequence Comparison by Log Expression
NAFIS	National Farmers' Information Service
ng	Nanogram
NJ	Neighbour Joining

nm	Nanometre
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TBE	Tris Boric Ethylene diamine tetraacetic acid

ABSTRACT

Little is known about the origin and genetic background of helmeted Guinea fowls despite their importance as a source of food, income, gifts, sacrifices, payment of dowries as well of being a source of manure. Heat stress is also one of the main problems affecting poultry production, especially affecting birds in the final phase of rearing causing mortality and economic losses. Understanding of genetic diversity in poultry provides information that would be used to conserve beneficial genotypes in the face of uncertainties brought about by global challenges such as climate change responsible for drought and heat stress in poultry. This study aimed to characterize local domesticated and wild helmeted Guinea fowls in selected regions in Kenya based on primary phenotypic traits, mitochondrial DNA (mtDNA) D-loop variations and polymorphisms in the heat shock protein 70 (HSP70) gene associated. Ninety (n=90) Guinea fowls selected from four domestic populations (n=70) in Western Kenya and a wild population (n=20) were scored for primary phenotypic characteristics. DNA was also extracted from blood collected from five populations of Guinea fowls comprising 13-21 individuals. Other than the wattle colour and head size, there is no marked difference between domestic and wild helmeted Guinea fowls of Kenya for the primary phenotypic traits considered. The 90 sequences were assigned to 25 distinct mtDNA and 4 HSP70 haplotypes. Most mtDNA haplotypes of the domesticated helmeted Guinea fowls were grouped into two main haplogroups; HgA and HgB. The wild population grouped into distinct haplogroups. Two haplotypes dominated across all populations of domesticated helmeted Guinea fowls; Hap2 and Hap4. The lack of population structure could suggest intensive genetic intermixing between the domestic populations. The differentiation of the wild population may be due to a clearly distinct demographic history that shaped its genetic profile. Overall, there was no significant ($P<0.05$) correlation between genetic variations and the geographic location in helmeted Guinea fowl populations in Kenya, indicating lack of a population structure within Kenya's domesticated helmeted Guinea fowls. Analysis of the Kenyan Guinea fowl population structure and history based on mtDNA D-loop variations complimented by archaeological and linguistic evidence supports the hypothesis that most domesticated helmeted Guinea fowls in Kenya are related to the West African domesticated helmeted Guinea fowls. More molecular work is recommended on a larger sample size to validate this work and identify more haplogroups not identified in this study.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The term "Guinea fowl" is a common name of the six species of gallinaceous birds of the family *Numididae*, which is indigenous to Africa. They are classified in the phylum *Chordata*, subphylum *Vertebra*, class *Aves* and order *Galliformes*. Hastings Belshaw (1985) classified Guinea fowls under the order *Galliformes* and family *Numididae*, but Howard and Moore (1984) placed them in the family *Phasianidae* and subfamily *Numidinae*.

There are four genera of Guinea fowls; *Agelastes, Guttera, Acryllium* and *Numida* comprising six species (Crawford, 1990); (Ayorinde, 2004). The genus *Agelastes* comprises of two species, namely *Agelastes meleagrides* (white breasted Guinea fowl) and *Agelastes niger* (black Guinea fowl) (Ayorinde, 2004). The genus *Guttera* also comprises of two species; *Guttera plumifera* (plumed Guinea fowl) and *Guttera pucherani* (crested Guinea fowl) (Ayorinde, 2004). *Acrylium* (vulturine Guinea fowl), consists of one species, *Acryllium vulturinum* while *Numida* (helmeted Guinea fowl) comprises a single polytypic species; *Numida meleagris* and 9 subspecies (Crowe *et al*, 1986). Guinea fowl phenotypic characteristics are described and shown below (Table 1.1 and Figure 1.1)

Table 1.1: Description of Guinea fowl phenotypic characteristics

Guinea fowl species	Main phenotypic characteristics	Reference
Agelates meleagris - (Figure 1.1a) white breasted	Black plumage, small featherless white breasted red head, , long black tail, greenish brown beak and grayish feet.	-BirdLife International., 2008; Botchway, 2013
	-Body length is 45cm.	-BirdLife International., 2008;
	-Found in West African forests of Cote d'Ivoire, Ghana, Guinea, Liberia and Sierra Leone	-Botchway, 2013
Agelastes niger (Figure 1.1b) black	-Featherless head, short crests of black down feathers and plumage.	-BirdLife International, 2008
	-Found in humid forests of Central Africa.	Botchway, 2013
	-Possess large toes for grasping and tiny feet that aid in flight.	BirdLife International, 2008
Acryllium vulturinum (Figure 1.1c) Vulturine	-Largest, measuring 61-71cm	(Jacob & Pescatore, 2011; Botchway, 2013)
	-Long, glossy-blue cape, white extending from the neck and cobalt blue breast, looks like the vulture	
	Black plumage with finely spangled white spots, short rounded beaks and a tail longer	BirdLife International, 2008
	Can stay longer without water	Martinez, 1994
	Found in East Africa	
Guttera pucherani (Figure 1.1d) Crested	-*F*ound in open forest, woodland and forest-savanna medley	Clements, 2010
	-Body length of about 50cm and A black plumage with dense white spots.	
	-Black crest on top of its head which varies from small curly feathers to down feathers.	BirdLife International, 2008
Guttera plumifera Figure 1.1e Plumed	-Naked head and neck with a small fold of skin at the back of the head, wattles, long straight black crest and black plumage with white spots	BirdLife International., 2008
	-Body length is 45 to 51cm, found in the humid forest of Central Africa	BirdLife International., 2008
Numida meleagris Figure 1.1f Helmeted	Found in a range of sub-Saharan, open country vegetation types	Crowe et al., 1986
	-Reared commercially in Europe, America and Asia	Dei & Karbo, 2004; Botchway, 2013
	-Body length is 53 to 63cm in length, has a bony helmet, naked gray neck and wattles on either side of the beak.	Crawford, 1990

Figure 1.1: Species of Guinea fowls within the four genera; (a) *Agelastes meleagrides* (white-breasted Guinea fowl), (b) *Agelastes niger* (black Guinea fowl), (c) *Acryllium vulturinum* (vulturine Guinea fowl), (d) *Guttera pucherani* (crested Guinea fowl), (e) *Guttera plumifera* (plumed Guinea fowl), and (f) *Numida meleagris* (helmeted Guinea fowl). Source: (BirdLife International., 2008; Moreki, 2009)

Crowe et al. (1986) classified helmeted Guinea fowls into nine well marked subspecies falling into three groupings;

West African- *N.m. galeata* and *N.m. sabyi,*

East African- *N.m. meleagris* and *N.m. somaliensis*

Central-South African- *N.m. reichenowi, N.m. mitrata, N.m. marungensis, N.m. papillosa* and *N.m coronata.*

N.m. meleagris (bristle nosed Guinea fowl) are found around Lake Chad and Sudan. *N.m. sabyi* (filoplume-necked Guinea fowl) were originally found in Morocco while *N.m. galeata* (grey breasted Guinea fowl) are found in Cameroon, Senegal and Nigeria. *N.m. marungensis* (Marunga helmeted Guinea fowl) are found in the Democratic Republic of Congo and Zambia. *N.m. damarensis* (Namibian Guinea fowl) are found in Namibia and Western Botswana. *N.m. coronata* (helmeted Guinea fowl) are found in South Africa and Southern Botswana. *N.m mitrata* (mitred Guinea fowl) are found in Mozambique, Zimbabwe and Zambia and finally *N.m. reichenowi* (Reichenowi's Guinea fowl) are found in Uganda, Kenya and Tanzania (van Niekerk, 1993).

Two types of helmeted Guinea fowls are found in Kenya based on their wattle colours. These include the red wattle and the blue wattle (National Farmers' Information Service, 2014). The red wattle helmeted Guinea fowl is the most commonly domesticated Guinea fowl in Kenya (National Farmers' Information Service, 2014). The blue wattle helmeted Guinea fowl is occasionally domesticated and found in fewer numbers among farmers though they are the most numerous in the wild and occupy almost every ecological zone, from the coast to the shores of Lake Victoria in Kenya (National Farmers' Information Service, 2014).

It is estimated that Kenya has 32 million poultry species out of which 76% consist of free-ranging indigenous chicken, while 22% are commercial layers and broilers

(Government of Kenya Agricultural Sector Development Strategy 2010–2020, 2010). Other poultry species like duck, turkey, pigeon, ostrich, Guinea fowl and quail make up only 2.2% of the total poultry production though it is encouraging to note that they are also becoming increasingly important as a food source (Government of Kenya Agricultural Sector Development Strategy 2010–2020, 2010).

Guinea fowls as an emerging livestock are a ready source of animal protein (meat and eggs), income, funerals, gifts, sacrifices, payments of dowries as well as being a source of manure for soil enrichment (Teye and Adam, 2000; Dei and Karbo, 2004; Agbolosu et al., 2015).Their lean meat with its characteristic fine flavor is relished by the local population (Kayang et a*l*., 2010). In most parts of Africa, Guinea fowl are mainly reared under extensive (free-range or traditional) systems at subsistence level with low levels of input resulting in low productivity(Weimann et al., 2016).

Climate change has also 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, 2012). Low annual rainfall has led to severe drought across large parts of the horn of Africa. This crisis peaked in the early 2011 with families losing their crops and livestock and even wildlife dying due to lack of pasture and failure by these animals to adapt to the harsh climatic conditions. Although 2012 experienced an increase in the annual rainfall, it was not sufficient to enable people and wildlife recover from the devastating impact of drought during the previous year. Kenya is one of the most vulnerable countries to climate change with the economic sectors and livelihoods already experiencing the manifestations of this problem. Climate change is expected to have a great impact on the Horn of Africa since the changing climate poses a major impact on the arid and semi-arid regions where most pastoralists reside (Said et al, 2013). In order to cope up with an unpredictable future, genetic resources that are capable of readily responding to directional forces imposed by a broad spectrum of environments must be maintained (Kayang et al., 2010).

The advent of molecular techniques has led to an increase in studies that focus on the genetic characterization of livestock using genetic markers (Giovambattista et al., 2001). As a tool used in evaluating genetic variation, molecular markers provide useful information for analyzing population genetic structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and parentage.

Genetic variation is the basis of plant and animal breeding and selection. Genetic characterization of domestic animals is therefore the first step in considering the sustainable management or conservation of a particular population. Since the 1990's, molecular markers have played a leading role in the characterization of diversity. The genetic characterization of breeds requires knowledge of genetic variation that can be effectively measured within and between populations.

The genetic characterization of domestic animals is part of the Food and Agriculture Organization (FAO) global strategy for the management of farm animal genetic resources (FAO, 2004). This strategy places a strong emphasis on the use of molecular methods to assist in the conservation of endangered breeds and also determine the genetic status of breeds. Mitochondrial DNA (mtDNA) D-loop is a widely used genetic marker for studying origin and diversity of species (Semik & Krawczyk, 2011), while functional polymorphisms in the heat shock protein 70 (HSP70) gene have been postulated to be associated with heat stress in birds (Iwamoto et al., 2005; Iwamoto et al., 2008; Gaviol et al., 2008). Analysis of mtDNA variations is therefore expected to help understand the origin and genetic background of the helmeted Guinea fowl. HSP70 marker was likewise chosen on the basis that climate change which leads to increase in global temperatures causes heat stress that can be measured by levels of heat stress proteins such as HSP70..

1.2 Statement of the problem

There is limited information on diversity and genetic background of helmeted Guinea fowls in Africa, with studies having been carried out only in a few countries, namely Ghana, Nigeria and Sudan. Lack of regulation in the utilization of Guinea fowls has also seen a lot of poaching of wild Guinea fowls from game parks and reserves which are then sold into the local markets. This poses a major threat to the conservation efforts for these important birds. Kenya is also one of the countries in the proximity of the Horn of Africa experiencing climate change leading to severe drought especially in the ASAL areas of the Northern Frontier Districts. This crisis had its worst effects in the year 2011 with farmers losing a lot of livestock due to the drought. Climate change has thus become the most serious global challenge of our time, and the impacts are increasingly evident on societies around the world. In this study, primary phenotypic characteristics that could be related to environmental adaptations were assessed. mtDNA D-loop region was also used as a DNA marker to study origin and diversity of species, while HSP70 gene polymorphism was employed to study heat tolerance in Guinea fowls.

1.3 Justification

Because of inadequate information on helmeted Guinea fowl diversity and genetic background in Kenya, and also to mitigate against global challenges such as climate change that could have an impact on their survival and distribution, there is need to understand environmental adaptations that have enabled them to survive under the harsh ASAL conditions. This could be achieved with adequate information on their phenotypic and genetic background and with the study of variations in genes associated with heat tolerance. Characterization of specific primary phenotypic traits and analysis of the mtDNA D-loop variations is expected to help in understanding the phenotypic and genetic diversity of this species. Identification of variations in the heat shock protein 70 gene related to heat stress is likewise expected to help in understanding how

the helmeted Guinea fowl has adapted to local environmental conditions such as heat stress arising from increasing environmental temperatures due to climate change. Information generated from this study is expected to support conservation efforts and also develop breeding programs aimed towards mitigating the effects of climate change. Characterization and conservation of these genetic resources is thus necessary to ensure future food security and wildlife conservation.

1.4 Null hypotheses

- There is no significant difference in phenotypic traits measured among Kenyan helmeted Guinea fowls across regions.
- Helmeted Guinea fowls of Kenya are not genetically diverse based on mtDNA D-loop variations.
- Polymorphisms do not exist in HSP70 gene in helmeted Guinea fowls of Kenya.

1.5 Research questions

- Are there major differences in phenotypic traits among helmeted Guinea fowls in Kenya?
- Based on mtDNA D-loop variations, are helmeted Guinea fowls of Kenya genetically diverse?
- Are there HSP70 gene polymorphisms in helmeted Guinea fowls of Kenya?

1.6 Objectives

1.6.1 General objective

To study phenotypic diversity, genetic diversity and HSP70 gene polymorphisms in helmeted Guinea fowls of Kenya.

1.6.2 Specific objectives

- To establish phenotypic variations among helmeted Guinea fowls of Kenya.
- To identify mtDNA D-loop variations in helmeted Guinea fowls of Kenya.
- To identify HSP70 single nucleotide polymorphisms in helmeted Guinea fowls of Kenya.

CHAPTER TWO

LITERATURE REVIEW

Research work on the genetic variation of gallinaceous birds is becoming important in the characterization of the genetic structure of local populations (Kayang et al., 2010). This serves as an important first step to reveal the uniqueness among populations and to identify valuable genetic resources for conservation through breeding programmes (Kayang et al., 2010). Such studies are facilitated by primary phenotypic characterization and use of molecular tools, particularly analysis of mitochondrial DNA (mtDNA) which is a widely used genetic marker to the study of origin and diversity of species. Additionally, heat shock protein 70 (HSP70) gene polymorphisms have been postulated to be associated with prevention of heat stress in many organisms, including birds (Morimoto et al., 1986; Maak et al., 2003; Iwamoto et al., 2008).

2.1 Overview of helmeted Guinea fowls

Helmeted Guinea fowls are opportunistic omnivores that inhabit open Savanna and mixed Savanna-bush Crowe & Crowe (1985). They are timid and usually gregarious in the non-breeding season and monogamous as breeders. Darkness and presence of perches reduce the bird's timidity since it likes to hide and remain motionless when it is frightened (Crawford, 1990). Females, especially during the breeding season, emit a characteristic two note "back wheat" "back wheat" call while males respond with a single note. Both sexes have a rattling alarm call. Males are slightly larger than females though they exhibit almost no sexual dimorphism. Adult body size ranges from 0.7-2.0kg (Long, 1981). The crown of the head carries a bony helmet with a horny sheath, and a pair of wattles hangs from the gape. The nares (nostrils) are exposed, but in subspecies inhabiting hot dry areas, the nares are surrounded with warts or cartilaginous bristles. Blood supply to the helmet, wattles and cere (fleshy covering at the base of the upper beak) may have importance in thermoregulation. The legs are long and powerful,

lacking a spur. Plumage is monotypic. The ground colour is black, with white spots intermeshed with white vermiculation; the spots on the outer margins of the secondaries are enlarged to form bars. Their incubation time is 27-28 days with clutch sizes varying between 6-10 eggs (Moreki, 2009).

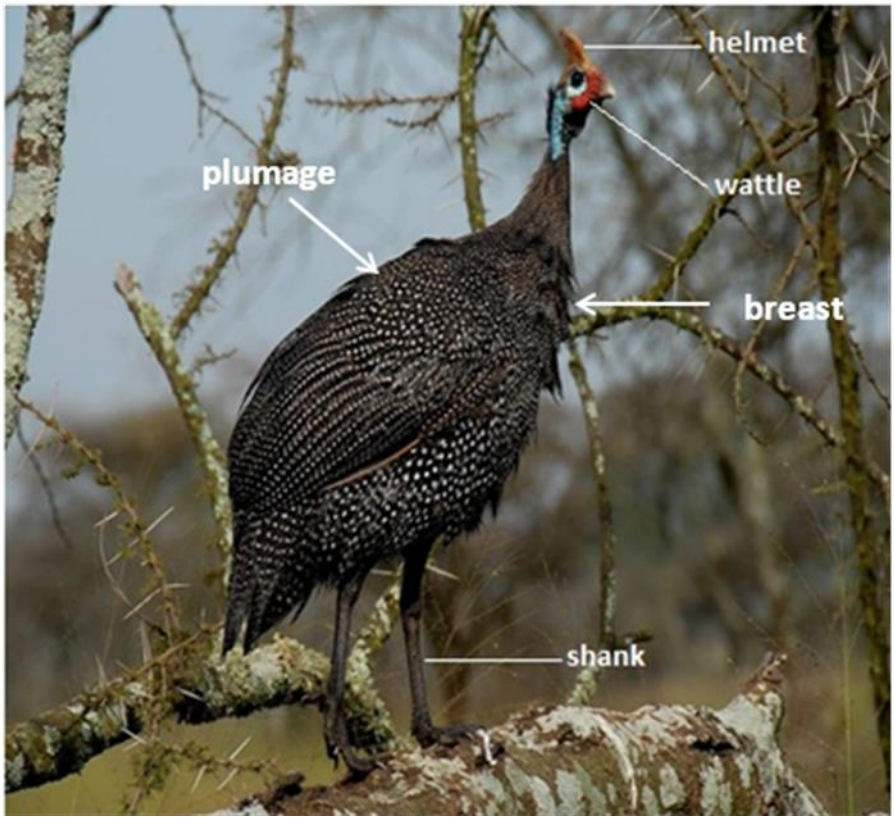


Figure 2.1: Labeled diagram of helmeted Guinea fowl (Source: en.wikipedia.org)

According to Crowe & Crowe (1985), the West African *N.m. galeata* subspecies is small to medium sized, and has a naked cere and rounded red wattles. *N.m. sabyi* is isolated in Morocco and differs very little from *N.m. galeata*. The East African *N.m. meleagris* and *N.m. somaliensis* subspecies are medium sized, have long bristles on the cere and rounded red wattles. The Central-South African groups are relatively large birds. They have a naked cere (except for *N.m. papillosa* which has warts around the nim) and triangular shaped blue wattles with red tips.

2.2 Geographical distribution of Helmeted Guinea fowl

Helmeted Guinea fowls occur naturally throughout most of sub-Saharan Africa with an isolated northern population of *N.m. sabyi* in Morocco (Crowe & Crowe, 1985). Many introductions have been made, some involving wild birds and others domestic stocks, and reintroductions have also been made to areas of Africa where they had been exterminated (Long, 1981). The population in Yemen was probably introduced long ago; it is similar to the East African subspecies, and for that reason, some researchers use the designation *ptilorhyncha* (Crawford, 1990). The population in Malagasy was probably also introduced; it is classified as *N. m. mitrata.* Many oceanic islands have also been stocked, although not all of them have been successful. Attempted repeated introductions in New Zealand, Australia, and the United States have been unsuccessful. There were Guinea fowl introductions in most islands of the Caribbean, sometimes with wild birds and at other with domestic stocks which became feral. Some of these introductions were attempted in the 16th century and others arrived as live provisions on African slave ships (Crawford, 1990). Populations flourished but many later became extinct because of hunting pressure and predation by the introduced mongoose. Viable wild or feral populations persist in Haiti, Dominican Republic, and Cuba (Crawford, 1990).

2.3 Domestication and early history of helmeted Guinea fowls

It is widely agreed that the domesticated Guinea fowl was derived from the helmeted Guinea fowl, *Numida meleagris*, of Africa (Crawford, 1990) with at least several independent domestications involving more than one subspecies. Majority of present day domesticated helmeted Guinea fowls are believed to derived from the West African subspecies *Numida meleagris galeata* (Crawford, 1990).

It is likely that separate Guinea fowl domestications have occurred in many separate regions over time. According to Crowe and Crowe (1985), wild populations of *Numida meleagris* readily become commensals of man, increasing in numbers and distribution because of the water, roosting, and feed resources resulting from human activity. However, unlike the situation for other poultry species, there is little indication in the historical records that Guinea fowls were utilized other than as a food resource (Crawford, 1990). Hastings Belshaw (1985) briefly mentioned their role in religion and folklore and use of their feathers in decoration. Eggs were probably of first importance and edible meat was secondary. Information on the history of domestication of Guinea fowls within Africa is scanty and, except for Egypt, depends on oral history. Crawford (1990) reported early domestication in Southern Sudan and West Africa, but the dates are not certain. This process of domestication probably continues even now. Guinea fowls were depicted in a mural from the Egyptian fifth dynasty about 2400 B.C. but there was no evidence that they were domesticated then (Crawford, 1990; Nishibori et al., 2004). They also appear in archaeological remains at Famak dated about 1900 B.C. and at Thebes (1570-1300 B.C.). It is postulated that they were artificially hatched and reared in large numbers concurrently with chickens during that period (Hastings Belshaw, 1985) but firm evidence is lacking; chickens are known to have been in Egypt at that time, but they were absent from the archaeological records in subsequent centuries, not appearing again until about 600 B.C. under Greek and Persian influence (Crawford, 1990).

The Portuguese of the late 16th century are generally credited with rediscovering Guinea fowls on the west coast of Africa, from where the bird acquired its common name. The term *poule de Guinée* may have been used first in 1555 by Belon (Mongin and Plouzeau, 1984). Portuguese took these Guinea fowl to Europe, the Americas, and other places. Diffusion through Europe was probably concurrent or perhaps slightly in advance of turkey introductions, resulting in the confusion of names and identity of the two species which is reflected in their scientific nomenclature (Crawford, 1990). Nearly all modern

Guinea fowl are likely to have originated from Portuguese introduction of the west African subspecies *Numida meleagris galeata* (Crawford, 1990); (Nishibori et al., 2004). There are indications that new commercial hybrids may involve crosses of several subspecies (Hastings Belshaw, 1985; Crawford, 1990) but documentation is not available. Domesticated Guinea fowls in Malagasy and those exported from there to other places may be descended from *Numida meleagris mitrata* (Crawford, 1990). Crawford (1990) further stated that those of eastern Africa are likely to be domesticates of *Numida meleagris meleagris* and *Numida meleagris somaliensis* subspecies, and those of the Mediterranean area may still bear traces of both East and West African subspecies.

2.4 Agro-climatic zones in Kenya

Kenya is divided into seven agro- climatic zones based on their importance to agricultural production, weather patterns and altitude according to FAO agro-ecological zoning guidelines (FAO, 1996) and the representative zones shown below (Table 2.1 and Figure 2.1).

Table 2.1: Agro-climatic zones of Kenya showing seven distinct ecological zones

Zone	Elevation (m)	Rainfall amount (mm)	Main characteristics	Representative regional examples
I	>2,700	>1,000	-Source of rain, rivers And streams -Confined to mountains and their surroundings	Mt. Kenya Mt. Elgon
II	1,980-2,700	1,000	-Occurs as forest or grasslands	Surroundings of Mt. Kenya,Mau region, Aberdares and Mt. Elgon
III	900-1,800	950-1,500	-Numerous but shorter trees -Significant for agriculture -Most resettled by humans	Former Nyanza, Western Central provinces, and parts of Rift Valley (Nandi, Nakuru, Bomet, Eldoret, Kitale) and a small strip at the coast
IV	900-1,800	500-1,000	-Mostly acacia trees and shrubs	Naivasha, parts of Laikipia and Machakos counties and vast parts of central and southern Coast
V	<900	300-600	-Low trees (mostly acacia) and shrub	Northern Baringo, Laikipia, Turkana, lower Makueni, vast parts of north eastern counties
VI		200-400	-Semi desert, driest parts of Kenya -Dominated by acacia, shrubs and scattered taller trees	Marsabit, Laikipia, Samburu Turkana, Mandera and Wajir
VI			Salt desert, very sparse salt bushes, source of mineral lick for livestock	Chalbi desert

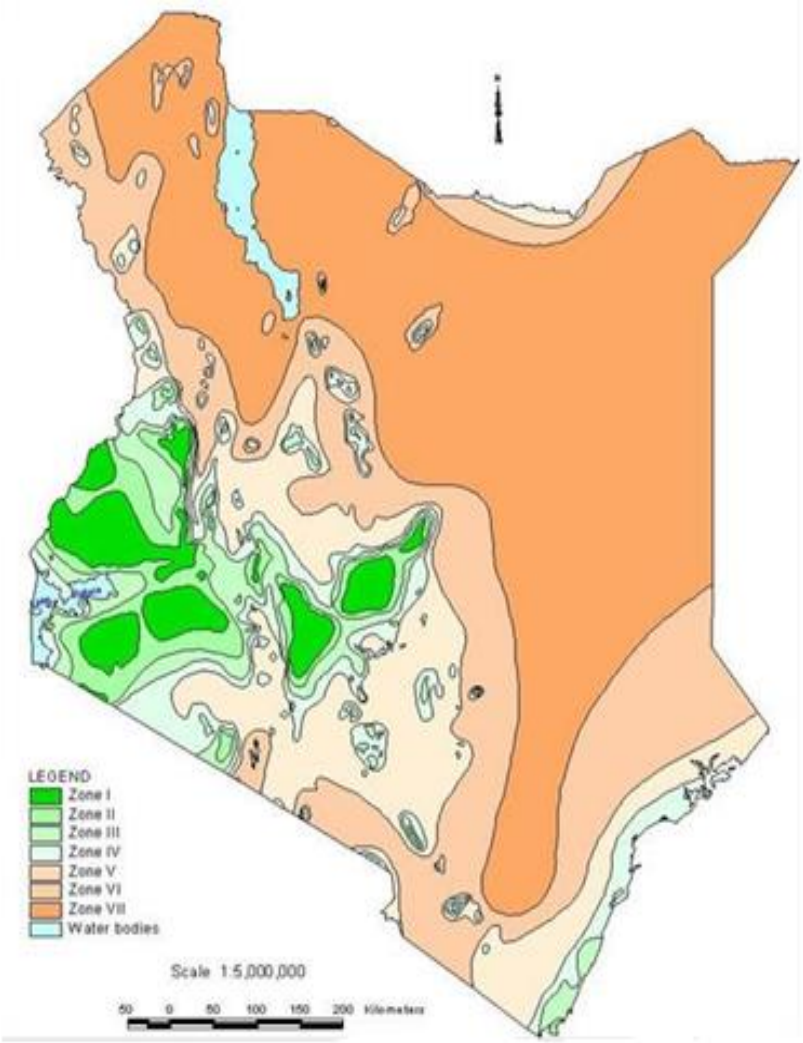


Figure 2.2: The agro-climatic zones of Kenya showing seven distinct ecological zones (Sombroek, Braun, & Van der Pouw, 1980)

It is worth noting that most wild helmeted Guinea fowls are found in Zone IV, V and VI which include ASAL areas of Kenya like Laikipia and Turkana while most domesticated helmeted Guinea fowls are found in Zone III covering parts of Western Kenya including Busia and Bungoma counties which are of interest to this study.

2.5 Description of primary phenotypic traits of helmeted Guinea fowls in Kenya

Phenotypic markers are cheap and easy to apply but unlike genetic markers, they are subject to environmental influences which result in variations in morphological traits. A substantial amount of phenotypic diversity for various traits in Kenya's helmeted guinea fowl genetic resources is expected because of diverse agro-climates. Helmeted guinea fowls in Kenya are distributed across many agro-ecological zones (National Farmers' Information Service, 2014). Their widespread distribution indicates their adaptive potential to the local environmental conditions such as heat stress. Tolerance or susceptibility of birds to stressful environment could be linked to their phenotypic traits (Agbolosu *et al*., 2015). Characterization of phenotypic traits in Guinea fowls is therefore expected to help in understanding how they have adapted to the local environmental conditions.

Studies on local Guinea fowl populations in Ghana revealed heterogeneity in the phenotypic traits considered (Agbolosu et al., 2015). In another study on the morphostructural characteristics of three varieties of helmeted Guinea fowl in Nigeria, Fajemilehin (2010) inferred that the small body size and body measurements could be the features required by Guinea fowls to survive in the wild. Phenotypic traits relevant for adaptation of indigenous chickens to hot environments have also been assessed in Kenya (Moraa et al., 2015). However, in Kenya, the phenotypic diversity of Guinea fowls or its importance in prevention of heat stress is not well understood. This study therefore aimed to identify the primary phenotypic variations of helmeted Guinea fowl populations in Kenya based on their phenotypic descriptors such as shank length, body length, wing length, helmet width, helmet height, head size, live body weight, wattle colour, skin colour and shank colour.

2.6 Assessment of genetic diversity using different molecular markers

Genetic variation is crucial to all organisms living on Earth. The greater the adaptability of a population to varying environmental conditions, the larger the gene pool of this population (Semik & Krawczyk, 2011). Targeted and long term selection, especially within small population, may considerably reduce the gene pool, which could result in lower adaptability (Semik & Krawczyk, 2011). It is therefore necessary to monitor changes in the genetic structure of animals. One of the ways to do this is by estimating the genetic distance of these populations based on molecular marker polymorphism. Polymorphic proteins and blood groups were the first markers used in genetic study of animals. Several new techniques have been developed for in-depth genome analysis and evaluation of genetic variation in different species. These include Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), micro-satellites, Single Nucleotide Polymorphism (SNP) and mitochondrial DNA analysis.

A molecular marker is a particular segment of DNA that contains nucleotide variations as a result of genome evolution. 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 development or defense status of a cell. Unlike phenotypic traits, molecular markers are not confounded by the environmental effects and they provide useful information about genetic diversity.

Earlier studies based on DNA-DNA hybridization demonstrated that birds named Guinea fowl were classified into six species and four genera, exclusively forming the family *Numididae* (Sibley & Ahlquist, 1990; Sibley & Monroe Jr, 1990).

Using cross-species microsatellite markers from the Japanese quail and chicken to estimate the genetic diversity across diverse populations of helmeted Guinea fowls,

Kayang et al. (2010) showed that the indigenous West African populations are more genetically diverse but less differentiated compared to the non-indigenous populations in Japan. Weimann et al. (2016) also used microsatellite markers to distinguish between farm-kept and wild Guinea fowl populations in Sudan. From their work, Weimann et al. (2016) further showed that it is not possible to find great differences between local breeds or ecotypes.

RAPD markers were also used to reveal a low level of genetic variation within and among Lavender, Pearl (wild type), and white helmeted Guinea fowl varieties in India, and this was attributed to a small founder population and many years of multiplication without selective breeding (Sharma et al., 1998). Similarly, helmeted Guinea fowl stocks in Japan were constituted from small founder populations and therefore, due to population size, genetic drift, inbreeding and selection, a reduction in genetic diversity occurred (Sharma et al., 1998).

2.7 Assessment of genetic diversity using mitochondrial DNA marker

The mitochondrial DNA (mtDNA) is a circular molecule that is 16,726 base pairs in size in Guinea fowls (Nishibori et al., 2004) and has a maternal mode of inheritance and therefore does not undergo recombination (Giles et al., 1980). mtDNA is relatively easy, rapid and inexpensive to sequence and research work on rapidly evolving loci provides sufficient variation to draw inferences on the structure of populations (Brown et al., 1982; Clayton, 1984; Saccone et al., 1991; Khaliq et al., 2011). The control region, also referred to D-loop often mutates faster than the rest of the mtDNA (Baker & Marshall, 1997; Khaliq et al., 2011) and appears to be highly variable in birds (Wenink et al, 1994; Khaliq et al., 2011). Analysis of polymorphism in the D-loop region has proved to be useful in preliminary studies on genetic variation, structure and phylogeography in birds (Merilä et al., 1997; Godoy et al., 2004; Roques et al., 2004; Cadahía et al, 2007; Kirchman and Franklin, 2007; Khaliq et al., 2011).

One of the first attempts to look into the problem of genealogical origin of Guinea fowls was undertaken by Kimball et al., (1997), who examined the phylogenetic position of three species of peafowl in the family *Phasianidae* in relation with the helmeted Guinea fowl in the family *Numididae*, using mtDNA D-loop and cytochrome b sequences. In their examination, Kimball et al. (1997) showed that the three peafowl species formed a monophyletic clade, and that peafowl were genetically separated from *Numida meleagris* in the phylogenetic tree. A similar study was carried out on Guinea fowls based on avian ovomucoid intron G sequences (Armstrong et al., 2001) which also indicated that the three peafowl species formed a monophyletic clade. Additionally, work on mitochondrial DNA variation of domesticated helmeted Guinea fowls in Nigeria revealed a lack of genetic differentiation within most Nigerian domesticated helmeted Guinea fowl which could likely be due to intensive genetic admixture (Adeola et al., 2015).

The Guinea fowl mitochondrial DNA is represented as a genetic map in Figure 3 below.

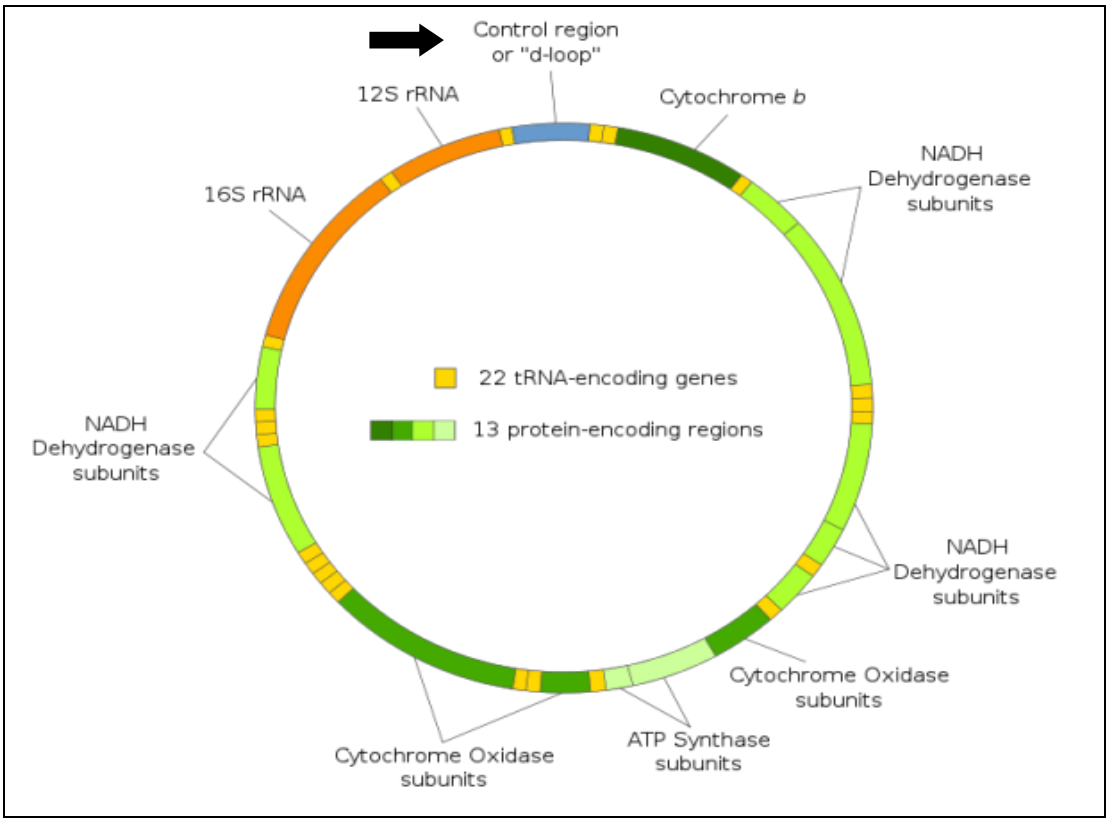


Figure 2.3: Guinea fowl mitochondrial DNA map. It shows the position of the D-loop region that is used to study genetic variations in many animals, including Guinea fowls (Shanel, 2008).

2.8 Analysis of HSP70 polymorphisms

Heat stress in birds is one of the main concerns in poultry farming since it causes high mortality and low productivity especially during the hottest seasons (Mazzi et al., 2003; Gaviol et al., 2008). In response to thermal stress in the tissues of living animals, cells synthesize proteins of low molecular weight that have specific functions in cell growth and in reversing or preventing damage caused by stress (Gaviol et al., 2008). These proteins, whose synthesis is increased when the cell is exposed to a stressful condition, are called heat shock proteins or HSPs (Gaviol et al., 2008).

The response of various organisms to thermal shock is one of the most conserved genetic systems known. Though stress proteins are not among the most abundant, they include one of the most conserved protein families found in different organisms (Parsell & Lindquist, 1993; Gaviol et al., 2008).

The acquisition of thermal tolerance is thought to be related to increased levels of heat shock protein 70 (HSP70) protein. Heat shock protein 70 (HSP70) gene is a family of molecular chaperones that plays many important roles in a highly elaborate quality control mechanism for many proteins, including directing the correct folding of newly synthesized proteins to their 3-D conformations, protecting proteins from several degenerative stresses such as heat shock and starvation, and destroying irreversibly denatured proteins (Hartl, 1996; Iwamoto *et al*., 2005). The exposure of individuals to hyperthermia leads to quick and transient responses at transcriptional and translational levels, which were considered to be the mechanism responsible for cell survival during the stress period (Burdon, 1986). Among the HSPs, HSP70 shows the highest levels under stressful conditions (Gaviol *et al.*, 2008). The HSP70 is therefore a useful molecular marker for studying environmental stress in poultry.

The 70-kDa HSP assists in the folding of other proteins by binding to nascent peptide chains on ribosomes, protecting the hydrophobic surface that would normally be exposed to solvent, therefore preventing aberrant folding or aggregation, until the whole peptide chain is synthesized and proper folding occurs (Gaviol et al., 2008)

The complete cDNA sequences of three members of the heat shock protein 70 family (HSPA2, HSPA5 and HSPA8) from Guinea fowl (Iwamoto et al., 2005) and Japanese quail (Iwamoto et al., 2008) have been identified and analyzed. The Guinea fowl HSP70 cDNA (*Nm*HSPA2, *Nm*HSPA8 and *Nm*HSPA5) are available in DDBJ/EMBL/GenBank under accession numbers AB096696, AB167744 and AB167743 respectively (Iwamoto et al., 2005). Studies on heat shock protein 70 genes in chicken revealed that only the expression of HSP70 (*Nm*HSPA2 in Guinea fowl) is

promoted by heat shock (Morimoto et al., 1986; Rosa et al., 1998). Other findings on HSP70 in Japanese quail from Brazil revealed alterations in the DNA sequences with the appearance of a possible polymorphism (Gaviol et al., 2008). Gaviol et al. (2008) suggested that there was need to study this polymorphism to determine if it had any association with heat resistance.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

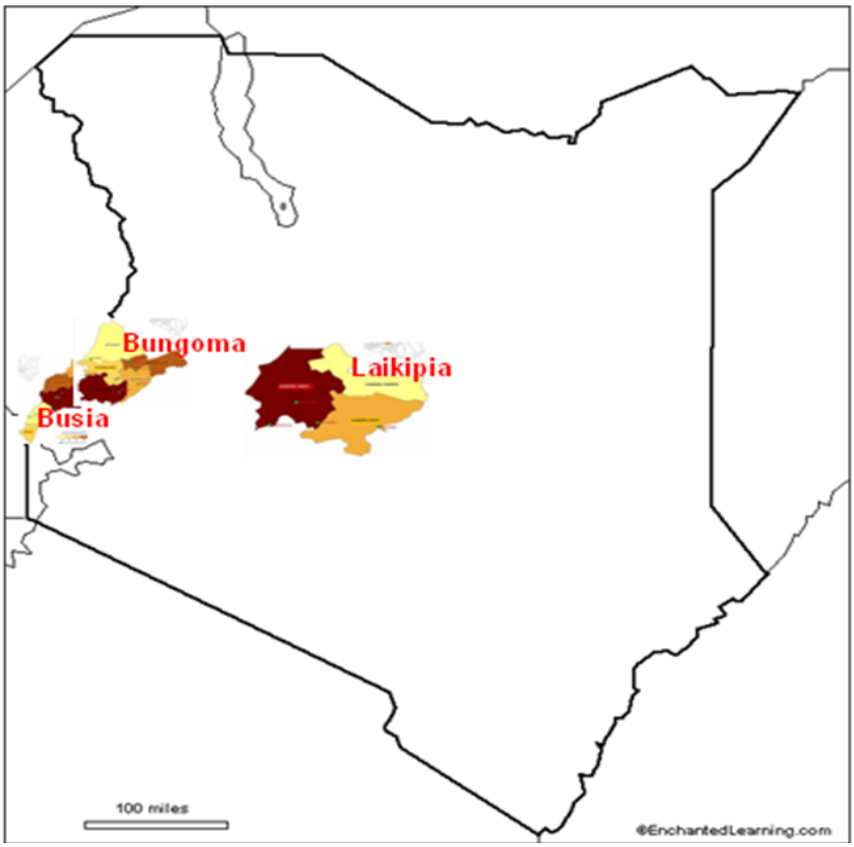


Figure 3.1: Map of Kenya showing the main sampling sites for the helmeted Guinea fowls (Source: http://www.nhantlarning.com)

The field studies were carried out from September 2014 to January 2015 in Busia (Teso North) and Bungoma (Bungoma South, Bungoma West and Mt. Elgon) counties in Western Kenya and Laikipia County in the Rift Valley region of Kenya. Western Kenya is a major source of domestic Guinea fowls which are reared by a number of rural households while wild helmeted Guinea fowls are common in Laikipia. Wild Guinea

fowls are free scavenging mobile birds found in the wild while domesticated populations are kept in homesteads mostly by small scale rural farmers under free range systems where they scavenge for food around these homesteads during the day. The summary of the sampled locations and samples per population are shown in Table 3.1.

Table 3.1: Summary of sampling sites

Sampling sites	Population	Number of samples
Bungoma County	Bungoma South	13
	Bungoma West	18
	Mt. Elgon	21
Busia County	Teso North	18
Laikipia County	Wild	20
Total		90

All the surveyed birds were adults (46 males and 44 females).

3.1.1 Western Kenya

Most farmers interviewed in urban and peri-urban regions of Nairobi, Mombasa and Central Kenya pointed out that they got their Guinea fowls from Western Kenya, while those interviewed in Western Kenya indicated that they sourced their Guinea fowls either from neighbours or from the neighbouring country of Uganda. Western Kenya therefore seems to be the focal point of Guinea fowl diversity and migration from West Africa through Central Africa. Western Kenya is also a major source of domestic Guinea fowls which are reared by most low income rural households. There was therefore no need to collect samples from Rift Valley, Nairobi, Central or Coastal regions of Kenya since they all indicated the source of their Guinea fowls to be from Western Kenya.

The sampling sites in Western Kenya lie between latitudes 0° 27´ N and 0° 47´ N of the equator and longitudes 34° 16´ E and 34° 39´ E of the Greenwich Meridian. Western Kenya is classified under Zones I, II and III of the agro-climatic zones of Kenya. These three zones are considered wet and are characterized by an annual rainfall amount above 950 mm per annum and an annual average temperature of 17°C minimum and 29°C maximum (Jaetzold and Schmidt, 1983). The climate is marked by one dry season (during November to March) and two rainy seasons (April to July and September to October). The vegetation type is mostly forest-mosaic.

3.1.2 Laikipia County

Sampling sites in Laikipia are located between latitudes 0° 2´ S and 0° 31´ N of the equator and longitudes 36° 52´ E and 37° 8´ E of the Greenwich Meridian. The climate is marked by one dry season (November to March) and two rainy seasons (April to July and September to October). Laikipia receives on average an annual rainfall of 300-600mm. The vegetation type is mostly savannah.

3.2 Study design

This was a stratified random cross-sectional study involving field surveys, laboratory assays and *in silico* methods. Field surveys were conducted in remote villages and animal sanctuaries in Western Kenya and Laikipia County, respectively. A rural participatory approach was used with interviews being conducted at the farmers' houses with the assistance of local agricultural extension officers. Visual appraisal of the appearance of the Guinea fowls and their typical features for environmental adaptations were collected using a pretested questionnaire on open data kit (ODK) on phones (Appendix 7) to obtain morphological and physiological data of the helmeted Guinea fowl. A total of 90 adult Guinea fowls from both sexes were characterized from five groups in Western Kenya and Laikipia. The number of birds sampled per population was based on published recommendations by Hale et al. (2012) for population genetic

studies.

3.3 Ethical approval

This study received ethical approval from the Kenya Wildlife Service under permit number KWS/BRM/5001 to sample wild Guinea fowls and a “no objection for the research” under permit number RES/POL/VOL.XXVII/162 to sample domestic Guinea fowls. The Guinea fowls used were handled as humanely as possible, with critical care before, during and after the data collection. They were then released back to the wild or to their owners after sampling.

3.4 Data collection

The phenotypic traits studied include shank length, body length, wing length, helmet width, helmet height, head size, live body weight, wattle colour, skin colour and shank colour. Body measurements were done using a flexible measuring tape graduated in centimetres and a venier caliper graduated in millimetres. Although Guinea fowls exhibit almost no sexual dimorphism (Crawford 1990), the size and shape of the head, helmet and wattle were used to distinguish sexes as recommended by Ayorinde (2004). Males are usually slightly larger than females and have more pronounced helmets and wattles. Body temperature as a physiological trait, environmental temperature and the co-ordinates of the sampling sites obtained using global positioning system (GPS) device were also recorded. Samples used for the study were obtained frpm wild Guinea fowls caught by blinding using Maglite torches at their roost sites and by use of foot traps, and domestic birds baited by their owners. Blood was drawn from the wing vein of the 90 genetically unrelated adult helmeted Guinea fowls and stored on FTA classic cards (Whatman Biosciences) which were then air-dried in readiness for molecular studies. The birds were under normal (unstressed) condition.

3.5 Molecular laboratory experiments

3.5.1 DNA extraction for mtDNA and HSP70

Genomic DNA was extracted from air-dried blood preserved on FTA® classic cards (Whatman Biosciences) according to the manufacturers' protocol (Gutiérrez-Corchero et al., 2002). Five 1.2mm discs were punched from each sample preserved on FTA® classic card using a micro-punch (Harris) and then placed in clean 1.5ml Eppendorf tubes. 1ml of 100mM Tris with 0.1% sodium dodecyl sulphate (SDS) (BDH, Poole, England) 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 the supernatant discarded. Then 500µl of 1.5M guanidine thiocyanate was added and gently vortexed frequently for 10 minutes and the supernatant discarded. The next step involved 500µl of triple distilled water being added and gently vortexed several times for 10 minutes. This step was repeated three times before the water was discarded. 50µl of triple distilled water was added and placed in a water bath at 90°C for 20 minutes. This was left to cool at room temperature for 30 minutes. The supernatant that contained DNA was spun and transferred into clean Eppendorf tubes. This generated 50-70µl of DNA sample. 1µl was used for PCR reaction and the rest stored at -20°C. DNA concentration was determined by a nanodrop 1000 spectrophotometer and the integrity checked using 260/280nm ratio. DNA was then diluted to a working stock of 50ng/µl for polymerase chain reaction (PCR) amplification of mtDNA and HSP70 genes.

3.5.2 Amplification of mtDNA and HSP70 genes and product resolution by gel electrophoresis

Polymerase chain reactions (PCR) were performed in a final volume of 10µl containing 3.8µl of double distilled water; 1µl of template genomic DNA, 5µl of Thermoscientific™ DreamTaq™ Green Master Mix (2X), 0.2µl of 20pM/µl primer (forward and reverse primer). mtDNA D-loop of *Numida meleagris* was amplified

using the forward primer AVIF2 5′-AGGACTACGGCTTGAAAAGC-3′ and reverse primer CR1b 5′-CCATACACGCAAACCGTCTC-3′ *(Mwacharo* et al., 2011). The first 600 bp of the promoter region of *Gallus gallus* HSP70 ortholog in *Numida meleagris*, that is *Nm*HSPA2, was amplified via PCR using the forward primer HSP70-F 5'-ATCATCGCCAATGACCAGGG-3' (20) and reverse primer HSP70-R 5'-CATTCTTCTCTCCAGCCCGG-3' (20). Amplification was carried out in a Veriti 9901 96 Well Fast gradient Systems thermo-cycler. Thermo-cycling conditions were as follows: One cycle of initial denaturation at 94°C (3min), 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds annealing, 72°C for 30 seconds for primer extension and a final extension step at 72°C (7 min).

For electrophoretic analysis, 2% agarose gel in 1X Tris Boric Ethylene diamine tetraacetic acid (TBE) buffer was prepared by adding 2g of agarose to 100 ml 1X TBE. The solution was then heated in a microwave at short intervals of 15-30 sec with occasional shaking until it boiled and became clear indicating that agarose is well developed. This was left to cool to about 55°C. 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).

PCR products were purified using the Wizard SV Gel and PCR Clean-Up Kit (Promega, Madison WI, USA) to remove the excess primers, $MgCl_2$, DNA template, dNTPs, and *Taq* DNA polymerase. Purified products were sequenced in 20µl volumes at Macrogen Inc., South Korea using Sanger ABI 3730 method (Sanger & Coulson, 1975) described in detail in the next section.

3.5.3: Mechanism of DNA sequencing by Sanger's dideoxy terminator method

The amplified DNA was sequenced at Macrogen Inc. in South Korea. The process is done in several steps. The first step is to prepare the template DNA. The next step is the cycle sequencing ration using Sanger's dideoxy terminator method containing the amplified DNA, DNA polymerase, primers, four dNTPs (dCTP, dTTP, dATP and dGTP) four dideoxy terminator nucleotides fluorescently labeled with four different dyes and enzyme buffering containing Mg^{2+} and K^{+}. Since only one primer is used for sequencing, this single primer binds to the complimentary DNA strand and extends itself in a linear fashion. The extension continues until by chance a particular ddNTP is incorporated depending on the complimentary base. Due to the latter's dideoxy-configuration, the polymerase cannot add any other base to this fragment hence 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 manipulation of the reaction components ensures that the fragments of every possible length starting from *n*+1 to say 1,000bp are generated *n* being the number of bases in the primer. Since only one primer is used, it means that only one strand can be sequenced in one reaction and a primer cannot read itself. The next step involves the post-sequencing reaction clean-up that is necessary to remove excess dNTPs, tagged ddNTPs and salts from the reaction products. This purification is done using ABI's Big Dye Terminator kit. The samples are then transferred to the sequencer.

The fragments are separated by capillary electrophoresis on the ABI-capillary 3730 XL sequencer. The samples are eletrokinetically injected into the array of capillaries, the negatively charged fragments migrating towards the anode by size, the smallest ones moving furthest. 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 collected from all the 96 capillaries at once, spectrally separated and focused onto a CCD camera. Very sophisticated optical and

electronic devices produce a colour that is read out and translated with the help of a sequence analysis software into a sequence. The data obtained is edited and blasted in NCBI Genbank for identification, and then aligned against a reference sequence.

3.6 Data analysis

3.6.1 Phenotypic data

Phenotypic data were analyzed using Excel spread sheet software package version 2013 (Liengme, 2015) to compute frequencies of occurrence of each trait. ANOVA tests in R Core statistical software version 3.1.2 (Ihaka & Gentleman, 1996) was used to determine mean measurements of various quantitative traits in each population. To determine the relationship between body temperature and environmental temperature in each population, a conditioning plot in R was used. Results are presented in the form of continuous bar graphs, tables and as percentages.

3.6.2 Molecular data analysis

3.6.2.1 Editing and alignment of mtDNA and HSP70 trace files

The raw mtDNA and HSP70 sequences generated were edited manually using Chromas Lite version 2.1.1 (Technelysium Pty Ltd, 2012). The reverse compliment of the reverse trace file sequence was used to correct the forward trace file sequence. The final or consensus trace file sequence was exported in FASTA format onto a notepad file which contained the consensus sequences of all the samples analyzed. The consensus sequences were then aligned using ClustalX version 2.1 (Thompson et al., 1997) against a reference sequence from Genbank accession number AP005595 (Crowe et al., 2006) for mtDNA and accession number AB096696 (Iwamoto et al., 2005) for HSP70. Subsequent analyses were restricted to the first 351-353 bp of mtDNA incorporating the first hyper variable segment (HVS1) and a 508bp promoter region of HSP70. The primer

sequences were trimmed out and consensus sequences generated. These consensus sequences were used for further analyses.

3.6.2.2 Mitochondrial DNA haplotype analysis

Mitochondrial DNA haplotypes were manually constructed and confirmed with DnSP v5.10 (Librado & Rozas, 2009) and Arlequin v3.5 (Excoffier et al, 2005) based on genetic similarities or variations. Sequences that appeared to be closely related genetically formed a haplotype. Closely related haplotypes likewise formed a haplogroup. Distribution of the frequencies of the haplotypes in various populations were displayed by the help of pie charts constructed using Excel spreadsheet 2013 (Liengme, 2015).

3.6.2.3 Phylogenetic analysis of mtDNA and HSP70 haplotypes

mtDNA and HSP70 haplotypes were first aligned using MUSCLE version 3.8.31(Edgar, 2004). Phylogenetic trees involving the mtDNA and HSP70 haplotypes discovered in the five helmeted Guinea fowl populations were constructed using the maximum likelihood algorithm as implemented in MEGAv6.06 following 1000 bootstrap replications (Tamura et al., 2013). The model used for mtDNA was TN93+G with a gamma shape parameter of 0.0947 and that used for HSP70 was K2+G with a gamma shape parameter of 0.1264 to model the nucleotide substitution pattern. The affinity of the Kenyan helmeted Guinea fowls to the Nigerian domesticated Guinea fowls was revealed by the maximum likelihood tree incorporating 241 mtDNA sequences downloaded from GenBank that were grouped into 22 haplotypes (Adeola et al., 2015) with the vulturine Guinea fowl included as the outgroup. Similarly, the affinity of the Kenyan Guinea fowls to other avian species was revealed by the maximum likelihood tree incorporating HSP70 sequences of nine avian species downloaded from GenBank. The resultant trees were viewed and edited by Dendroscope v3.2.10 (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 in SplitsTree version 4.13.1 (Huson & Bryant, 2006). The phylogenetic network diagram produced from this analysis was used to validate the haplotypes.

3.6.2.4 Phylogenetic analysis of mtDNA network profiles of major clades

To determine the possible relationships among the mtDNA haplotypes and compare the populations under study to populations in other parts of Africa, a median joining network was constructed using Network v5.0.0.0 software package (Bandelt et al., 1999). DnaSP v5.10 (Librado & Rozas, 2009) was used to generate the Roehl file which is the input file used for network analysis. In addition, network analysis included 341 mtDNA reference sequences representing the Nigerian, Kenyan and Chinese domesticated helmeted Guinea fowls (Adeola et al., 2015). The list of sequences used and the corresponding Genbank accession numbers are provided in Appendix 1 and 2. The sequences from Genbank were aligned to mtDNA haplotypes observed in this study using MUSCLE version 3.8.31 software program (Edgar, 2004). Extra nucleotide bases in the Genbank sequences that were outside the 353bp region sequenced in this study were excluded from analysis.

3.6.2.5 mtDNA population demographic structure

Population dynamics were inferred on the basis of mismatch distribution patterns (Harpending, 1994) for all the mtDNA haplotypes and the reference sequences. Departures of the observed mismatch distributions from the simulated model of expansion were tested with χ^2 test of goodness of fit and Harpending's raggedness index "*r*" (Harpending, 1994) in ARLEQUIN version 3.5.2.2 (Excoffier et al., 2005) following 10100 coalescent simulations.

3.6.2.6 mtDNA and HSP70 population genetic variation and structure

Haplotype diversity (h), which is th**e** probability that two haplotypes sampled within a population are different (Nei, 1973), nucleotide diversity and nucleotide differences for each population were calculated using ARLEQUIN v3.5.2.2 software (Excoffier et al., 2005). The analysis of molecular variance (AMOVA) were computed with the algorithms suggested by Excoffier et al., (1992) as implemented in the ARLEQUIN software. MtDNA molecular components were estimated between and within (i) all the five populations, (ii) the wild and domesticated helmeted Guinea fowls and (iii) Teso South and Mt. Elgon on one hand and the Bungoma West and Bungoma South on the other hand. The groupings used for HSP70 AMOVA were as follows: among populations, among individuals within populations and within individuals in (i) the wild and domesticated helmeted Guinea fowls and (ii) three groups; Teso South and Mt. Elgon, Bungoma West and Bungoma South, and the wild population. Significance testing was performed using 10100 coalescent simulations. A Mantel test was used to assess the association by distance model using GenAIEx v6.501 software (Peakall & Smouse, 2006) which is an add-on in Microsoft Excel and used to plot the regression graph between the genetic and geographic distances.

CHAPTER FOUR

RESULTS

4.1 Phenotypic characterization of helmeted Guinea fowls in Kenya

4.1.1 Observed features in Guinea fowls

Figures 4.1a and 4.1b below show the photographs of domesticated and wild helmeted Guinea fowls.

a b

Figure 4.1: Photographs of sampled phenotypes of helmeted Guinea fowls; a= red wattled, b= blue wattled

The crown of the head of helmeted Guinea fowl carries a bony helmet with a horny sheath, and a pair of wattles hangs from the gape. The legs are long and lack a spur. Plumage is monotypic. The background colour is black, with white spots.

All the domesticated helmeted Guinea fowls were observed to have a naked cere and rounded red wattles (fitting the description of *Numida meleagris meleagris*, *Numida*

meleagris somaliensis and *Numida meleagris galeata*) while all the wild helmeted Guinea fowls had blue wattles.

4.1.2 Distribution of wattle, skin and shank colours in helmeted Guinea fowls

The frequencies of occurrence of wattle, skin and shank colours in the helmeted Guinea fowls in Kenya are shown in Figure 4.2 below**.**

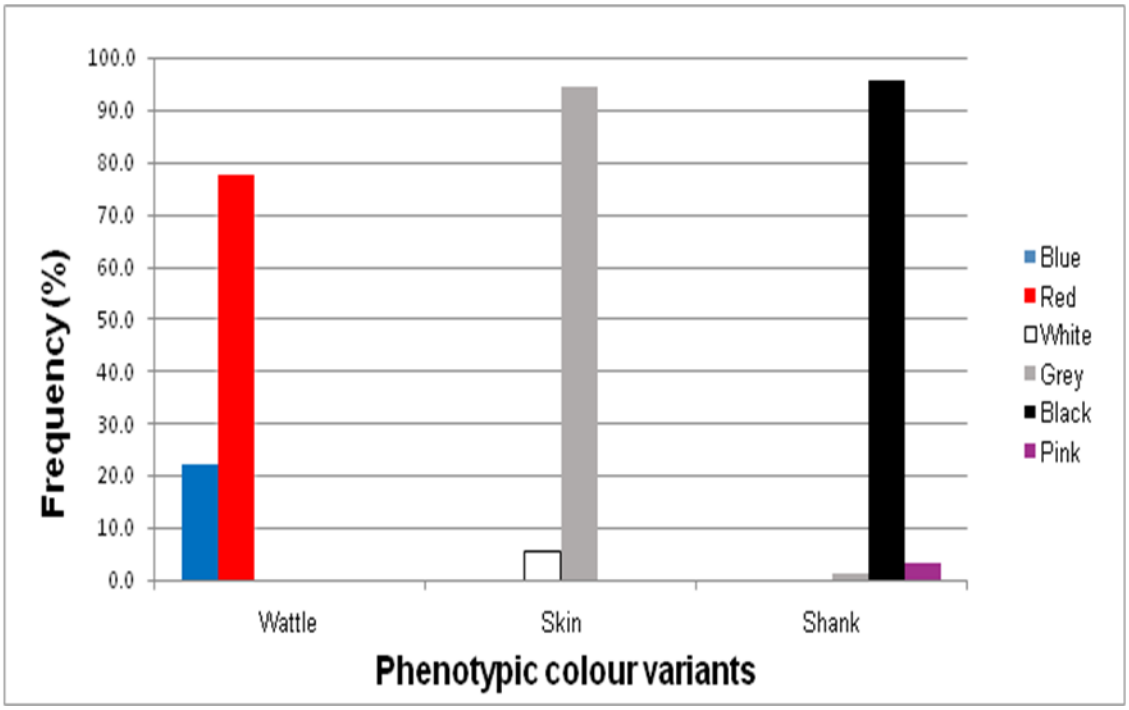


Figure 4.2: Proportion of wattle, skin and shank colours in helmeted Guinea fowls of Kenya generated using Excel spread sheet software package version 2013 (Liengme, 2015). The results show that these traits in helmeted Guinea fowls are variable.

4.1.2.1 Wattle colour

Two wattle colour types (red and blue) were observed among the local helmeted Guinea fowls. The most dominant wattle colour type was red. It is also noted that all domesticated helmeted Guinea fowls representing the populations in Bungoma South,

Teso North, Bungoma West and Mt. Elgon have red wattles. All the wild type individuals had blue wattles.

4.1.2.2 Skin colour

Results from this study showed that the skin colour distribution was mostly grey with only a few individuals having white skin. All five individuals with white skin were sampled from Bungoma West.

4.1.2.3 Shank colour

Most helmeted Guinea fowls in Kenya have black shanks, with a few exhibiting pink and black shanks.

Observation of qualitative traits in Kenyan helmeted Guinea fowls showed no significant difference in primary qualitative traits measured except wattle colour.

4.1.3 Mean measurements of body parameters of male and female Guinea fowls

The mean measurements of body parameters in male and female domesticated and wild helmeted Guinea fowls in Kenya is compared below (Table 4.1)

Table 4.1: Mean measurements of body parameters of male and female Guinea fowls

Body parameter	Male	Female	Pr (>F)
Body length	448.70±44.82	432.27±44.82	0.11
Shank length	92.35±4.30	89.07±3.93	0.00029***
Live weight	1476.09±194.58	1409.09±213.30	0.12
Wing length	253.15±39.24	252.82±27.01	0.96
Head size	74.35±6.03	72.27±6.34	0.12
Helmet width	16.22±4.81	15.18±4.34	0.29
Helmet height	32.89±6.95	28.86±6.07	0.0044**
Number of individuals	46	44	

±= standard deviation (sd), *= significant @ 0.05, **= significant @ 0.01 and ***= significant @ 0.001. All surveyed birds were mature adults

Results show that male Guinea fowls generally have higher mean measurements for all body parameters measured when compared to their female counterparts.

4.1.3 Shank length, body length and body weight

The mean shank length, body length and live body weight of the sampled adult Guinea fowls are compared in each region and shown in Table 4.2 below.

Table 4.2: Mean shank length, body length and live body weight of the helmeted Guinea fowl in Kenya. Body length was measured from tail to the base of the neck.

Region	**Shank length** (in mm)	**Body length** (in mm)	**Body weight** (in g)	n
Bungoma South	89.6±4.3	436±25	1538±214	13
Teso North	90.8±3.9	421±18	1278±239	18
Bungoma West	91.1±5.0	452±33	1467±146	18
Mt. Elgon	88.8±3.1	426±28	1510±155	21
Laikipia	93.1±4.7	467±84	1440±190	20
Pr (>F)	0.0263*	0.0172*	0.00119**	

n= number of birds, ±= standard deviation (sd), *= significant @ 0.05, **= significant @ 0.01 and ***= significant @ 0.001. All surveyed birds were mature adults (46 males and 44 females).

The above results show that the wild Guinea fowls sampled in Laikipia have marginally higher mean shank and body lengths compared to domesticated populations. However, the mean live body weight is proportionately lower when compared to its longer shank and body.

4.1.4 Wing length, head size, helmet width and helmet height

Table 4.3 below presents the mean wing length, head size, helmet width and helmet height respectively in the local Guinea fowl populations in Kenya.

Table 4.3: Mean wing length, head size, helmet width and helmet height (in mm) of the helmeted Guinea fowl populations in Kenya

Region	Wing length	Head size	Helmet width	Helmet height	n
Bungoma South	262±13	76.9±1.8	16.9±2.9	28.5±7.4	13
Teso North	246±31	74.7±5.5	17.1±3.5	29.3±5.8	18
Bungoma West	229±38	76.2±3.1	11.1±1.6	29.8±7.2	18
Mt. Elgon	259±24	75.7±4.2	16.1±3.8	34.7±5.5	21
Wild	269±39	64.8±5.0	17.4±6.3	31.1±7.2	20
Pr (>F)	0.00229 **	3.37e-14 ***	2.89e-05 ***	0.0445 *	

n= number of birds, ±= standard deviation (sd), *= significant @ 0.05, **= significant @ 0.01 and ***= significant @ 0.001. All the surveyed birds were mature adults (46 males and 44 females).

It was observed that the mean wing length, helmet width and helmet height of the wild helmeted Guinea fowls were relatively larger than those of the domesticated populations except for the Mt. Elgon group that registered longer mean helmet height. However, the wild helmeted Guinea fowls had the lowest mean head size. Guinea fowls from Bungoma West had the smallest mean wing length and helmet width. The wild population generally had larger mean values for all quantitative traits measured except the head size and live body weight.

4.1.5 Relationship between body temperature and environmental temperature

To determine whether environmental temperature across the five sampled regions was uniform, an XY conditioning plot was constructed using R Core version 3.1.2 statistical software (Figure 4.3)

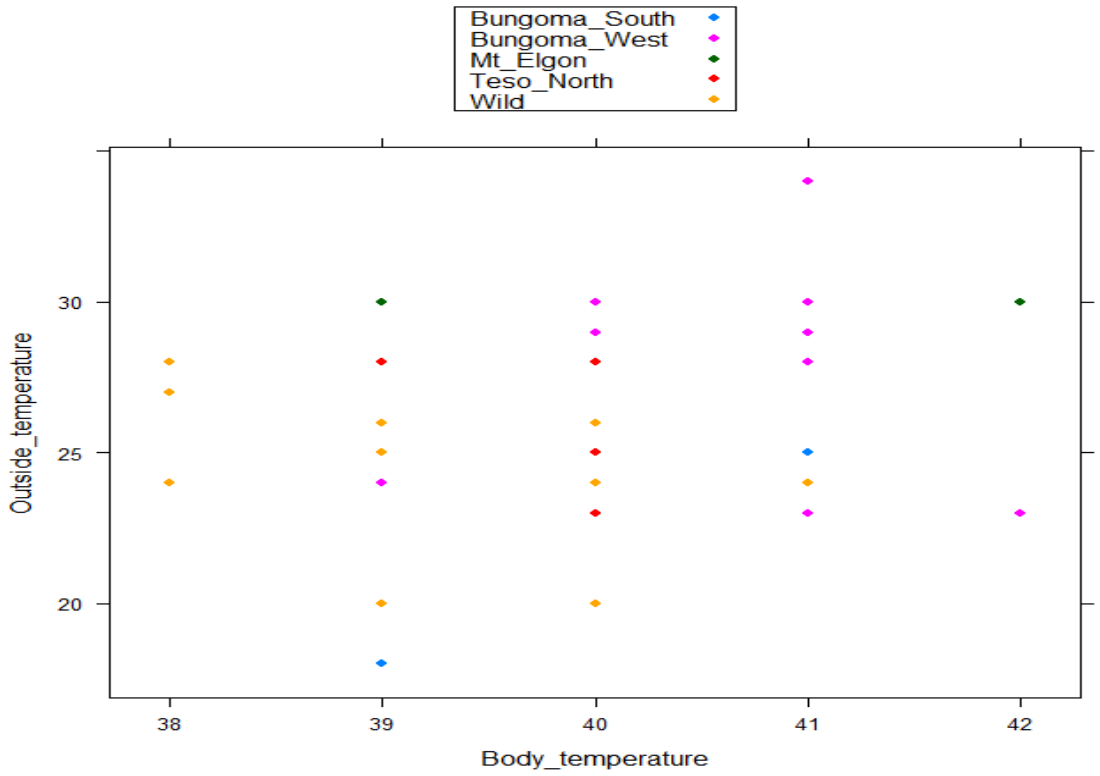


Figure 4.3: XY conditioning plot illustrating the relationship between body temperature and environmental temperature constructed using R Core version 3.1.2 statistical software.

Guinea fowls from Bungoma West have highest body temperatures while the Bungoma South Guinea fowls have lowest body temperatures. Bungoma South is generally forested and colder hence this result agrees with the observed conditions of this region.

4.2 mtDNA D-loop as a marker for deducing genetic diversity

4.2.1 Gel pictures showing PCR amplification of mtDNA D-loop

The amplicon size of 700bp showed positive amplification for all the 96 helmeted Guinea fowl samples. The images of amplified region of the mitochondrial DNA D-

loop of selected domesticated and wild helmeted Guinea fowls in Kenya are shown below in Figures 4.4 and 4.5 respectively.

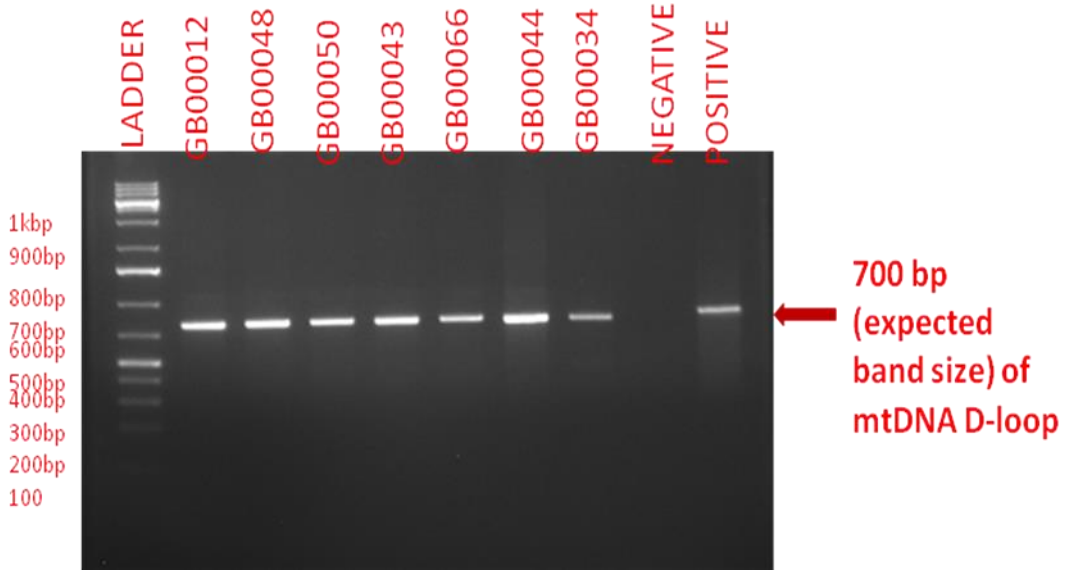


Figure 4.4: Gel picture showing amplification of mtDNA in selected domesticated helmeted Guinea fowls in Kenya. The image displays a 2% agarose gel electrophoresis showing a 700bp fragment.

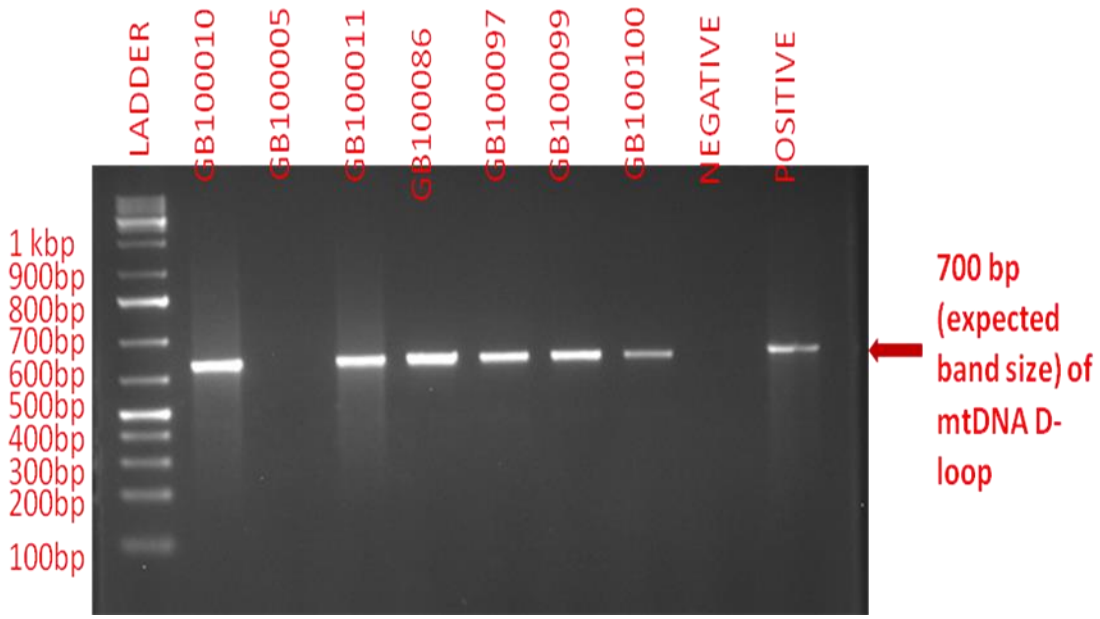


Figure 4.5: Gel picture showing amplification of mtDNA in selected wild helmeted Guinea fowls in Kenya. The image displays a 2% agarose gel electrophoresis showing a 700bp fragment.

The expected amplicon size was about 700bp and all the helmeted Guinea fowl samples showed positive amplification. Chicken samples from the International Livestock Research Institute (ILRI) were used as positive control while water was used as a negative control. The PCR products were thereafter purified and sequenced.

4.2.2 mtDNA chromatograms showing variable regions

A 351-353bp mitochondrial DNA D-loop sequence was obtained from samples of 90 *Numida meleagris.* Mitochondrial DNA variable regions are shown below in chromatograms in Figures 4.6.

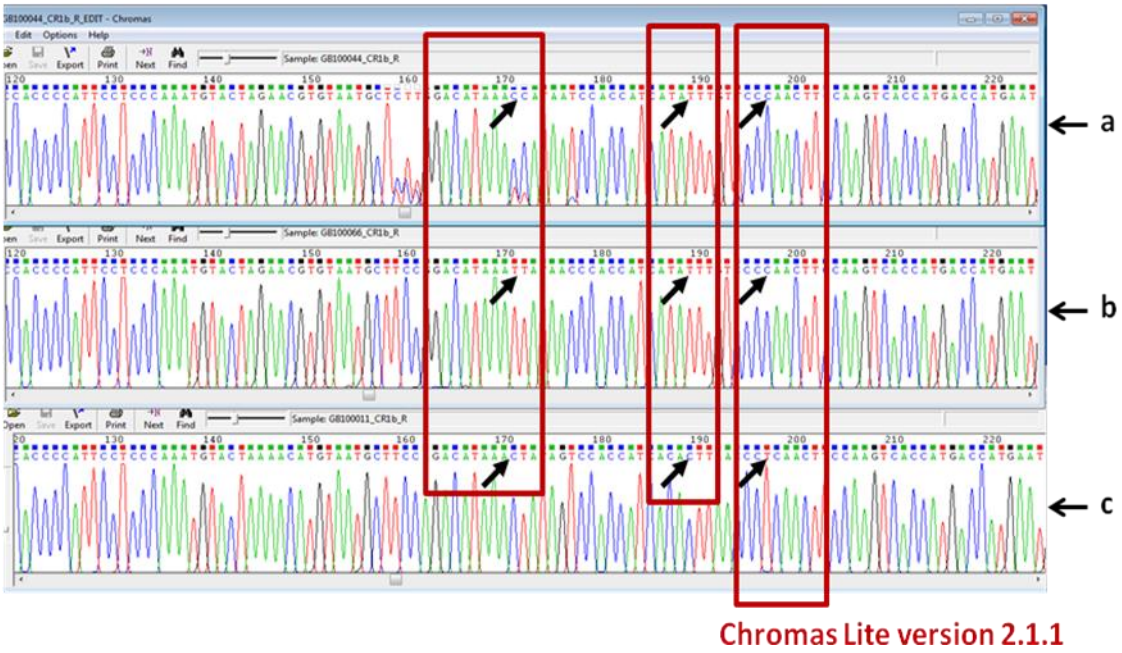


Figure 4.6: Chromatograms showing variations (SNPs and INDELs) in the mtDNA D-loop of the helmeted Guinea fowls in Kenya (a and b are chromatograms of domesticated individuals and c is a chromatogram of a wild Guinea fowl). The figure shows part of the control region of three edited chromatograms from three different samples of domesticated and wild helmeted Guinea fowls in Kenya. The colour scheme represents the nucleotide base type. The blue signals represent cytosine (C), black signals represent guanine (G), red signals represent thymine (T), and the green signals represent adenine (A).

Single nucleotide polymorphisms (SNPs) are evident in the above chromatograms (position 187 and 189 of the edited portion of the mtDNA D-loop). An insertion/deletion (INDEL) in all individuals in the wild population (between position 196 and 197 of the edited portion of the mtDNA D-loop) is also observed in the above chromatograms. A second INDEL in two wild type individuals, (sample GB100005 and GB100086 between position 186 and 187 of the edited portion of the mtDNA D-loop), is also observed. These SNPs are novel and their roles have not been previous studied.

4.2.3 Multiple sequence alignment of mtDNA with reference sequences

A multiple sequence alignment for mtDNA sequences of selected Kenyan helmeted Guinea fowls shows DNA variations (Figure 4.7.).

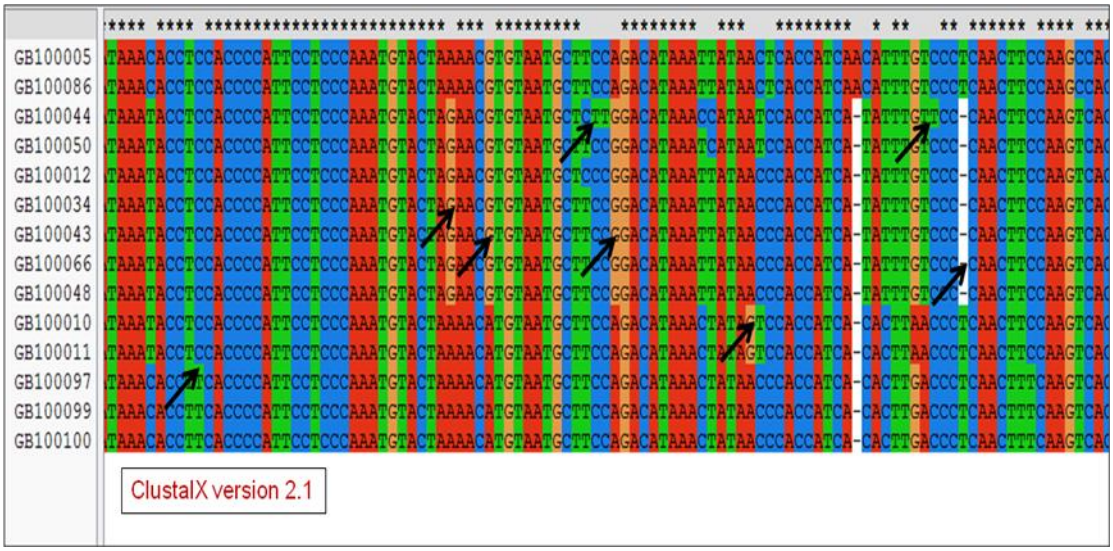


Figure 4.7: A multiple sequence alignment of mtDNA sequences of selected Kenyan helmeted Guinea fowls showing variations (ClustalX version 2.1 was used to perform multiple sequence alignment). The colour scheme represents the nucleotide base type. The blue regions represent cytosine (C), orange regions represent guanine (G), red regions represent adenine (A), and the green regions represent thymine (T). Arrows represent the variable regions (single nucleotide polymorphisms).

A multiple sequence alignment for the mtDNA haplotypes of the Kenyan helmeted Guinea fowl with reference sequences initially obtained from Nigeria (Adeola et al., 2015) and deposited in GenBank of NCBI is shown below (Figure 4.8).

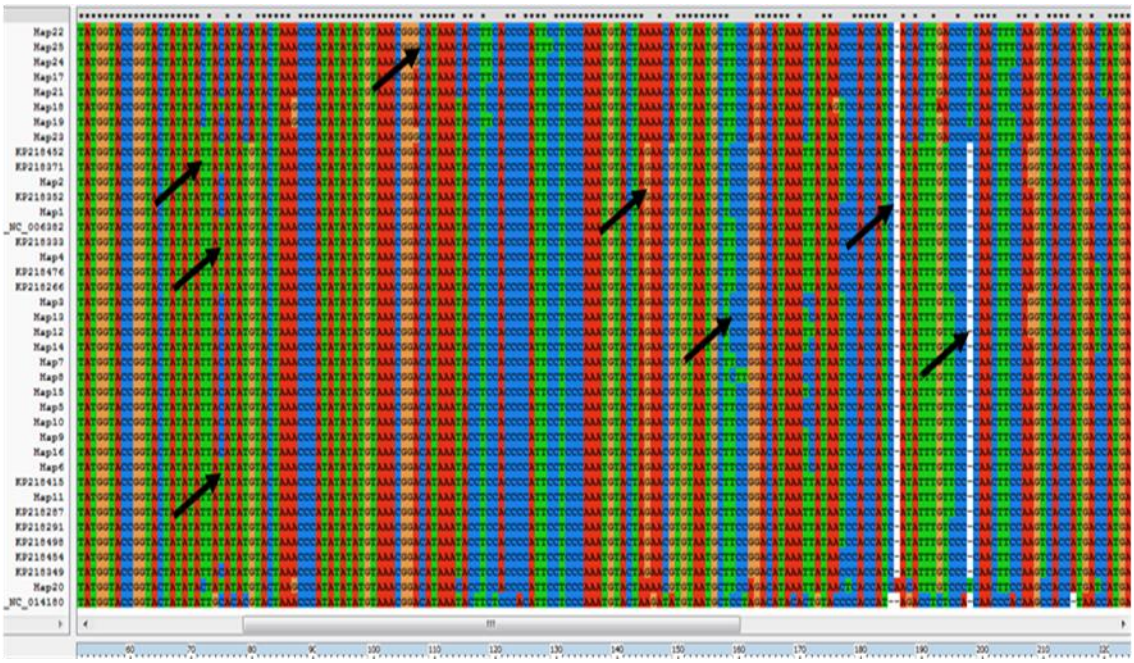


Figure 4.8: A multiple sequence alignment of mtDNA haplotypes representing Kenyan helmeted Guinea fowls versus Nigerian, Kenyan and Chinese domesticated helmeted Guinea fowls (Adeola et al., 2015). The colour scheme represents specific nucleotide bases (C, G, A and T). The blue regions represent cytosine (C), orange regions represent guanine (G), the red regions represent adenine (A), and the green regions represent thymine (T).

Single nucleotide polymorphisms (both transitions and transversions and INDELs) were observed. For example, an INDEL in all individuals in the wild population (between position 196 and 197 of the edited portion of the mtDNA D-loop) and a second INDEL in two wild individuals (between position 186 and 187 of the edited portion of the mtDNA D-loop) is present. The two individuals with two insertions are GB100005 and GB100086 were briefly described in the previous section.

These sequences were compared with those obtained from GenBank accession numbers KP218263-KP218503 (Adeola et al., 2015) and AP005595 (Nishibori et al., 2004). The

vulturine Guinea fowl, accession number NC_014180 (Shen et al., 2010) was used as the outgroup because it is closely related to the helmeted Guinea fowls.

4.2.4 Distribution of mtDNA haplotypes in helmeted Guinea fowls in Kenya

Twenty five unique haplotypes (Hap1-Hap25) defined by 41 polymorphic sites were identified. The frequencies and distribution of these haplotypes in the various regions are shown in Figure 4.9, Table 4.4 and Table 4.5 below. The pie chart diagrams were drawn using Excel and these were placed onto a map of Western Kenya to show geographical distribution.

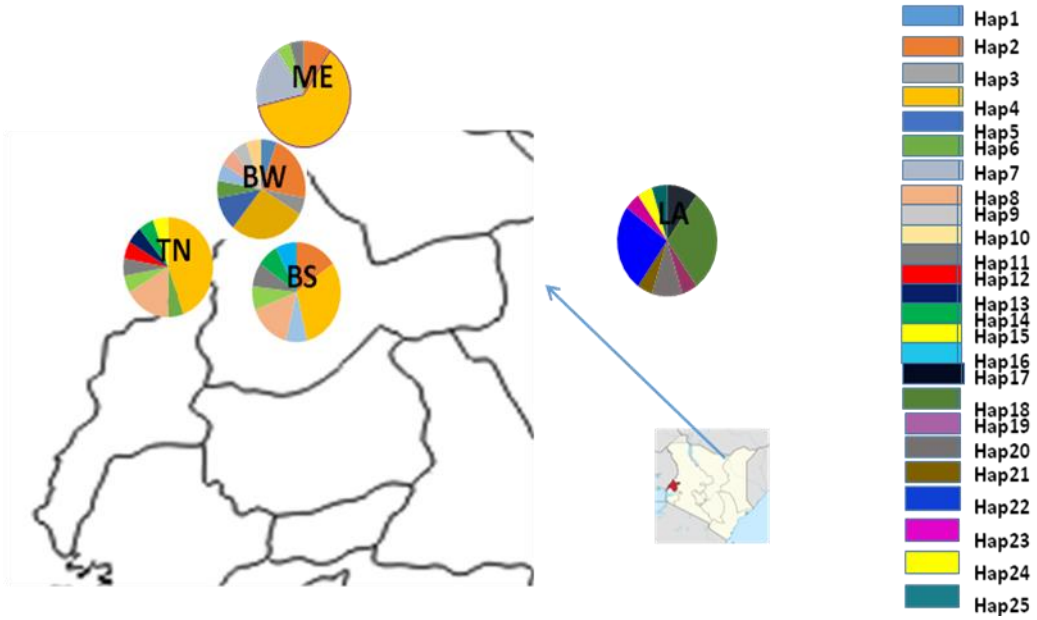
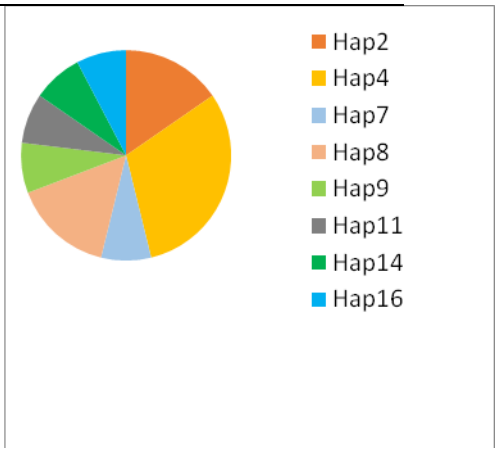
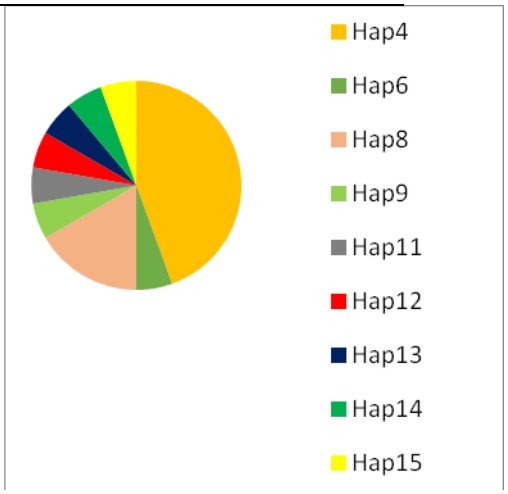
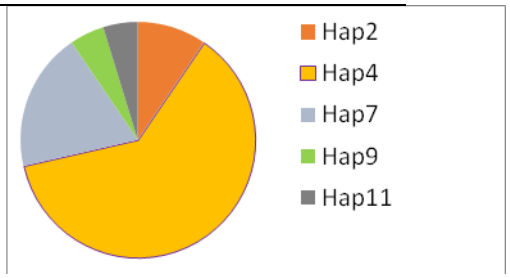
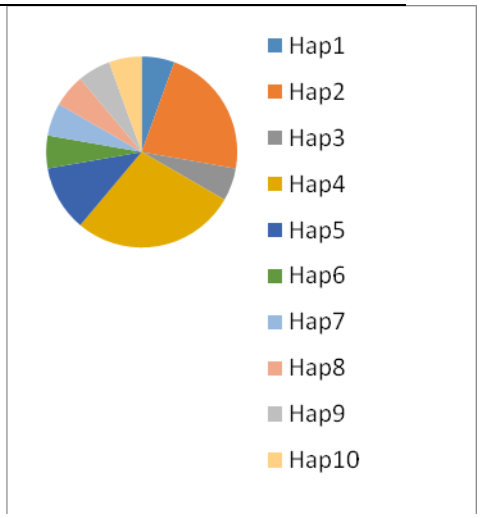
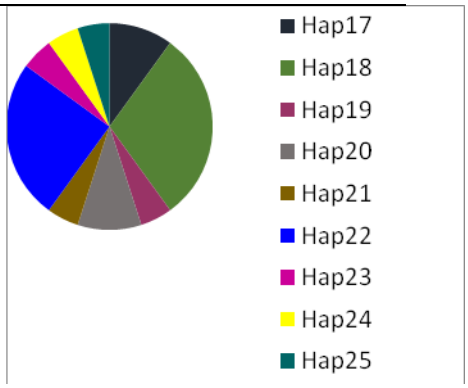


Figure 4.9: Pie charts showing the distribution of mtDNA haplotypes in the helmeted Guinea fowls in Kenya. Different colours indicate specific haplotypes. Initials indicate the populations sampled; BW represents Bungoma West, TN Teso North, ME Mt. Elgon and LA the wild Guinea fowls which were sampled in Laikipia county in Kenya.

Table 4.4: Summary of mtDNA haplotype distribution in Kenyan helmeted Guinea fowls

Region	Number of samples	Haplotype	Haplotype frequency	Pie chart
Bungoma South	13	Hap2	2	
		Hap4	4	
		Hap7	1	
		Hap8	2	
		Hap9	1	
		Hap11	1	
		Hap14	1	
		Hap16	1	
Teso North	18	Hap4	8	
		Hap6	1	
		Hap8	3	
		Hap9	1	
		Hap11	1	
		Hap12	1	
		Hap13	1	
		Hap14	1	
		Hap15	1	

Mt. Elgon	21	Hap2	2	
		Hap4	13	
		Hap7	4	
		Hap9	1	
		Hap11	1	
Bungoma West	18	Hap1	1	
		Hap2	4	
		Hap3	1	
		Hap4	5	
		Hap6	1	
		Hap7	1	
		Hap8	1	
		Hap9	1	
		Hap10	1	
Laikipia (wild)	20	Hap17	2	
		Hap18	6	
		Hap19	1	
		Hap20	2	
		Hap21	1	
		Hap22	5	
		Hap23	1	
		Hap24	1	
		Hap25	1	

From the pie charts, it is evident that the most common haplotype shared by all the domesticated Guinea fowls is Hap4 followed by Hap2 which occurs in three sampled regions comprising of domesticated Guinea fowls. Hap18, Hap19, Hap20, Hap21, Hap22, Hap23, Hap24 and Hap25 are uniquely found in the wild helmeted Guinea fowls. The most common haplotypes were Hap4, Hap2 and Hap8 in Bungoma South, Hap4 and Hap8 in Teso North, Hap4 and Hap7 in Mt. Elgon, Hap4 and Hap2 in Bungoma West, and Hap18 and Hap22 in the wild Guinea fowls.

The frequencies of mtDNA haplotypes in different populations of helmeted Guinea fowls in Kenya was computed using DnSP version 5 (Librado & Rozas, 2009) and ARLEQUIN version 3.5 (Excoffier & Lischer, 2010) and is shown below in Table 4.5.

Table 4.5: Distribution of Kenyan helmeted Guinea fowl mtDNA haplotypes in different regions. The numbers represent the frequency of occurrence of a haplotype in a given sampled region.

Haplotype	Bungma South	Teso North	Bungoma West	Mt. Elgon	Laikipia (wild)
Hap1			1		
Hap2	2		4	2	
Hap3			1		
Hap4	4	8	5	13	
Hap5			2		
Hap6		1	1		
Hap7	1		1	4	
Hap8	2	3	1		
Hap9	1	1	1	1	
Hap10			1		
Hap11	1	1		1	
Hap12		1			
Hap13		1			
Hap14	1	1			
Hap15		1			
Hap16	1				
Hap17					2
Hap18					6
Hap19					1
Hap20					2
Hap21					1
Hap22					5
Hap23					1
Hap24					1
Hap25					1
n	13	18	18	21	20

n= the total number of individuals sampled

A major haplotype, Hap4, occurs at a frequency of 33.3% and is widely distributed in all the four domestic populations (Bungoma South, 30.8%; Teso North, 44.4%; Bungoma West, 27.8% and Mt. Elgon, 61.9%). The second major haplotype (Hap2),

which occurs at a frequency of 8.9′% across all populations, is common in three domesticated populations (15.4% in Bungoma South, 22.2% in Bungoma West and 9.5% in Mt. Elgon). Other frequent haplotypes include Hap7, Hap8, Hap18 and Hap22. Hap7 which occurs at a frequency of 6.7% across all populations is found in 1 individual in Bungoma South, 1 individual in Bungoma West and 4 individuals in Mt. Elgon. Hap8 also occurs at an overall frequency of 6.7% and is found in 2 individuals in Bungoma South, 3 individuals in Teso North and 1 individual in Bungoma West. Hap18 and Hap22 occur at an overall frequency of 6.7% and 5.6%, respectively and are found in the wild individuals only. The most common haplotypes in the various populations were Hap2 and Hap4 in the Bungoma West, Hap4 and Hap8 in Teso North, Hap2, Hap4 and Hap8 in Bungoma South, Hap 4 and Hap7 in Mt. Elgon and Hap18 and Hap22 in the wild population. Hap1, Hap3, Hap10, Hap12, Hap13, Hap15, Hap16, Hap19, Hap21, Hap23, Hap24 and Hap25 only occurred in one individual.

When the ninety samples in this study were pooled together with the 241 Nigerian, Kenyan and Chinese samples from GenBank (Adeola et al., 2015), most of the Nigerian samples are also observed to cluster in Hap4 and Hap2.

4.2.5 Phylogenetic analysis of mtDNA haplotypes

4.2.5.1 mtDNA maximum likelihood tree

The phylogenetic relationship of the various helmeted Guinea fowl haplotypes in Kenya and other parts of Africa was inferred in Figure 4.10 using Maximum Likelihood algorithm as implemented in MEGA version 6.06 (Tamura et al., 2013) following 1000 bootstrap replications. The tree was rooted using the vulturine Guinea fowl.

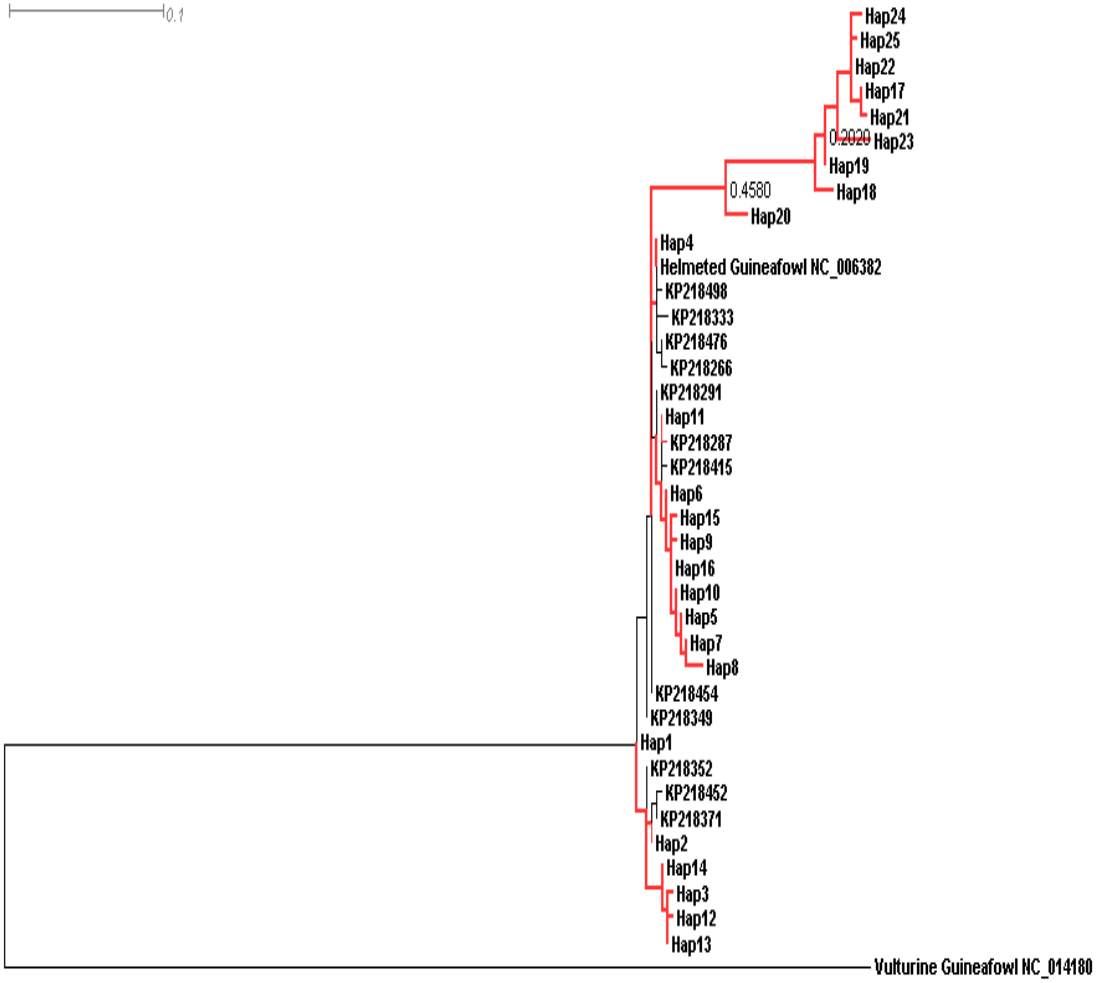


Figure 4.10: Phylogenetic relationship of helmeted Guinea fowl haplotypes representing Kenyan, Nigerian and Chinese sequences which were pooled together and clustered into haplotypes based on genetic similarities identified. The list of published Nigerian, Kenyan and Chinese sequences used and their corresponding GenBank accession numbers are provided in Appendix 1. The algorithm used was the Maximum Likelihood method as implemented in MEGA v6.06 following 1000 bootstrap replications. Model used was TN93+G, gamma shape parameter was 0.0947. The vulturine Guinea fowl was included as an outgroup.

Phylogenetic analysis of the 25 mtDNA haplotypes with reference sequences of Nigerian, Kenyan and Chinese domesticated Guinea fowls (Adeola et al., 2015) shows that most of the domesticated helmeted Guinea fowls cluster into two clades which represent the two major haplogroups identified that represent the domesticated helmeted Guinea fowls. The Nigerian, Kenyan and Chinese reference sequences of domesticated helmeted Guinea fowls also cluster into the two major clades representing domesticated Guinea fowls. There is a close relationship between the Nigerian and most Kenyan domesticated helmeted Guinea fowls based on phylogenetic analysis. The wild helmeted Guinea fowls are observed to cluster in their own distinct clades.

4.2.5.2 Splits decomposition network

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 in SplitsTree version 4.13.1 (Huson & Bryant, 2006) as shown in Figure 4.11 below. The phylogenetic network diagram produced from this analysis was used to validate the haplotypes.

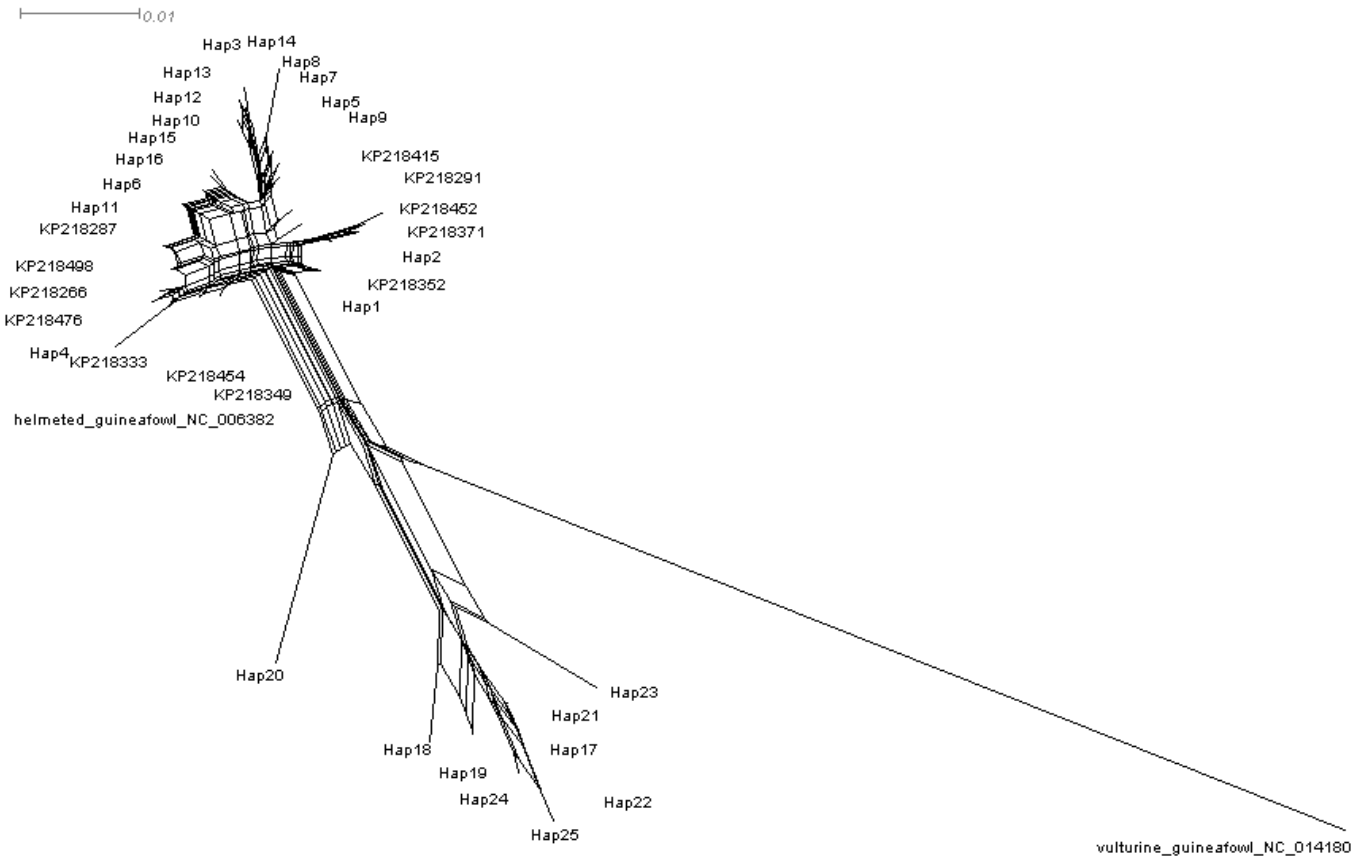


Figure 4.11: Splits decomposition network of the helmeted Guinea fowls constructed using Splits tree v4.13.1 (Huson & Scornavacca, 2012). The vulturine Guinea fowl was included as an outgroup.

The splits decomposition network again reveals a genetic relationship between Kenyan and Nigerian domesticated helmeted Guinea fowls.

4.2.5.3 Median joining network of mtDNA haplotypes

Figure 4.12 shows a median-joining network of the 90 helmeted Guinea fowl samples constructed using NETWORK v5.0.0.0 (Bandelt et al., 1999). Most of the domesticated helmeted Guinea fowl individuals are grouped into two major haplogroups named HgA and HgB in a previous study (Adeola et al., 2015), lineages of which are clustered around Hap2 and Hap4 within different steps of mutations. All the published sequences of Nigerian, Kenyan and Chinese domesticated helmeted Guinea fowls (Adeola et al., 2015) also group into haplogroups HgA and HgB. A few domesticated helmeted Guinea fowls grouped in two other haplogroups, named HgC (Hap1) and HgD (Hap8) in this study. The 20 wild helmeted Guinea fowls are grouped into five distinct haplogroups named HgE, HgF, HgG, HgH and HgI in the current study, retaining the nomenclature used by Adeola et al. (2015) to name Guinea fowl haplogroups. A very clearly distinct haplogroup HgI (Hap21) comprising of two wild individuals (samples GB100005 and GB100086) was identified. This haplogroup has a second INDEL between position 186 and 187 of the edited portion of the mtDNA D-loop unlike all the other wild helmeted Guinea fowls with only one INDEL between position 196 and 197 of the edited portion of the mtDNA D-loop. The median joining network seems to suggest that haplogroup HgI has a closer genetic relationship with domesticated helmeted Guinea fowls than with other wild helmeted Guinea. Haplogroup HgB was connected to HgA via 7 median vectors and 6 mutation steps. The median vectors may represent either un-sampled haplotypes, haplotypes never introduced into Kenya, or introduced into Kenya but becoming extinct shortly upon arrival or later (Mwacharo et al., 2011). Haplogroups HgA and HgB exhibit a star-like pattern indicating rapid population expansion, with HgA being the most common haplogroup.

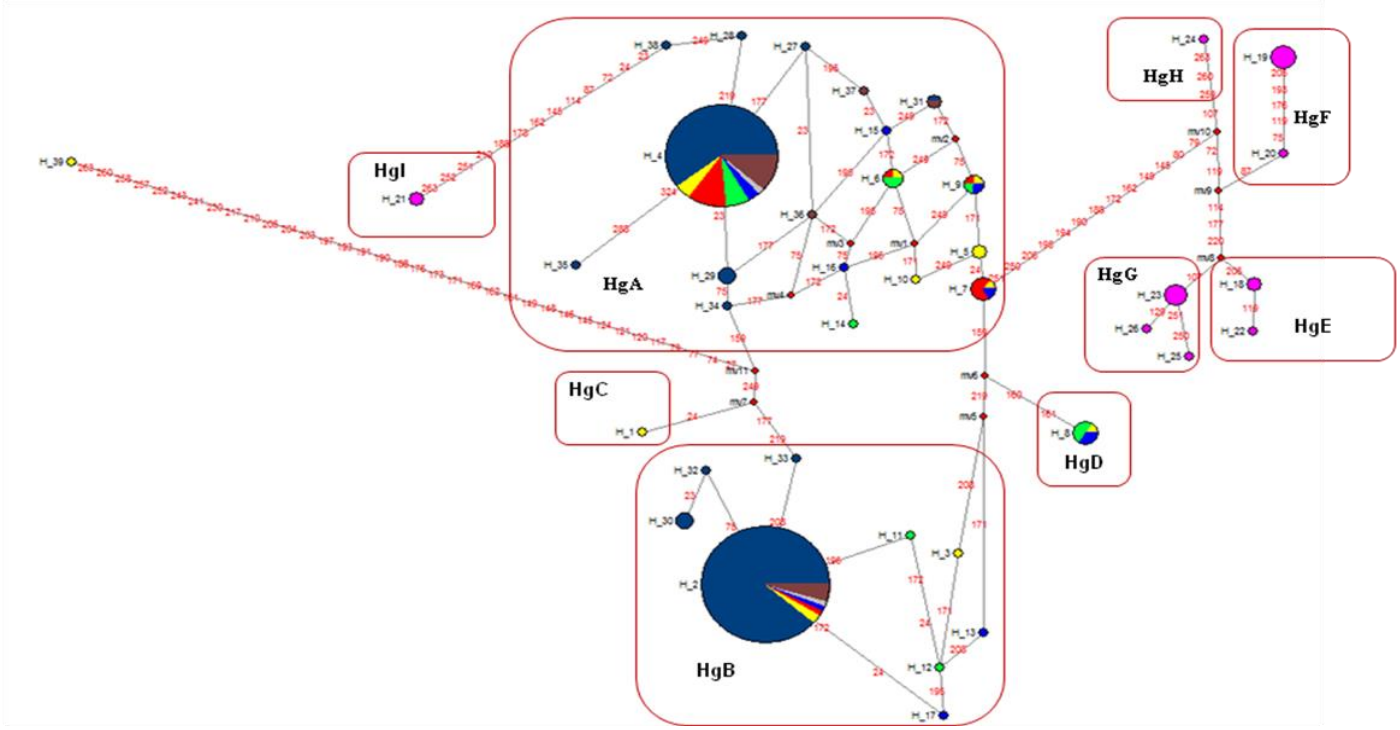


Figure 4.12: Median joining network of 90 helmeted Guinea fowls in Kenya and 241 reference sequences of Guinea fowls in Nigeria, Kenya and China (Adeola et al., 2015) constructed using NETWORK v5.0.0.0 (Bandelt et al., 1999). Pie charts showing haplotypes and colours indicate the populations sampled; yellow, Bungoma West; green, Teso North; red, Mt. Elgon; blue, Bungoma South; pink, wild; deep blue, Nigerian reference sequences; grey, Kenyan reference sequences; brown, Chinese reference sequences. Sizes of circles are proportional to frequencies and *m* is the number of mutation steps. *mv* is the median vector used to connect indirectly related haplotypes. The vulturine Guinea fowl was *used* as the outgroup.

4.2.6 mtDNA diversity indices

Tables 4.6 and 4.7 below show several diversity indices for the five sampled regions for the helmeted Guinea fowls in Kenya..

Table 4.6: Diversity indices of mtDNA gene of helmeted Guinea fowl in Kenya

Region	n	Number of haplotypes	Number of polymorphic sites	H_O	H_E
Bungoma South	13	8	13	0.016±0.084	0.434±0.101
Teso North	18	9	13	0.016±0.083	0.421±0.122
Bungoma West	18	10	13	0.015±0.081	0.404±0.143
Mt. Elgon	21	5	11	0.012±0.074	0.395±0.154
Laikipia (wild)	20	9	29	0.024±0.092	0.289±0.163

H_O = observed heterozygosity; H_E = Expected heterozygosity, n= number of samples

All the five populations were polymorphic, with the number of haplotypes ranging from 5 (Mt. Elgon) to 10 (Bungoma West). Observed and expected heterozygosities were generally low ranging from 0.012±0.074 (Mt. Elgon) to 0.024±0.092 (Wild) and 0.29±0.16 (Wild) to 0.43±0.10 (Bungoma South) respectively.

Table 4.7: Diversity indices of mtDNA gene in the helmeted Guinea fowls in Kenya

Region	n	h	k	π
Bungoma South	13	0.90±0.067	5.64±2.89	0.016±0.0093
Teso North	18	0.80±0.090	5.48±2.76	0.016±0.0088
Bungoma West	18	0.89±0.053	5.26±2.66	0.015±0085 .
Mt. Elgon	21	0.64±0.079	4.34±2.23	0.012±0.0071
Laikipia (wild)	20	0.86±0.054	8.39±4.054	0.024±0.013

h = haplotype diversity; k = mean number of pairwise differences; π =nucleotide diversity

Haplotype diversities (h) varied from 0.638±0.079 (Mt. Elgon) to 0.897±0.067 (Bungoma West) while nucleotide diversities (π) range from 0.0124±0.00707 (Mt. Elgon) to 0.0238±0.0128 (Laikipia). The lowest haplotype diversities were thus observed in Mt. Elgon and Teso North. The other regions show higher haplotype diversity values. The nucleotide diversity values are generally low.

4.2.7 Helmeted Guinea fowl population dynamics revealed by mtDNA variations

To understand the historical population dynamics of the studied helmeted Guinea fowls across Kenya, the distribution of the observed pairwise nucleotide differences (mismatch distribution) and the expected values for no recombination were computed under the model of growing-declining populations (Rogers & Harpending, 1992), using DnSPv5 (Librado and Rozas, 2009) as shown in Figure 4.13.

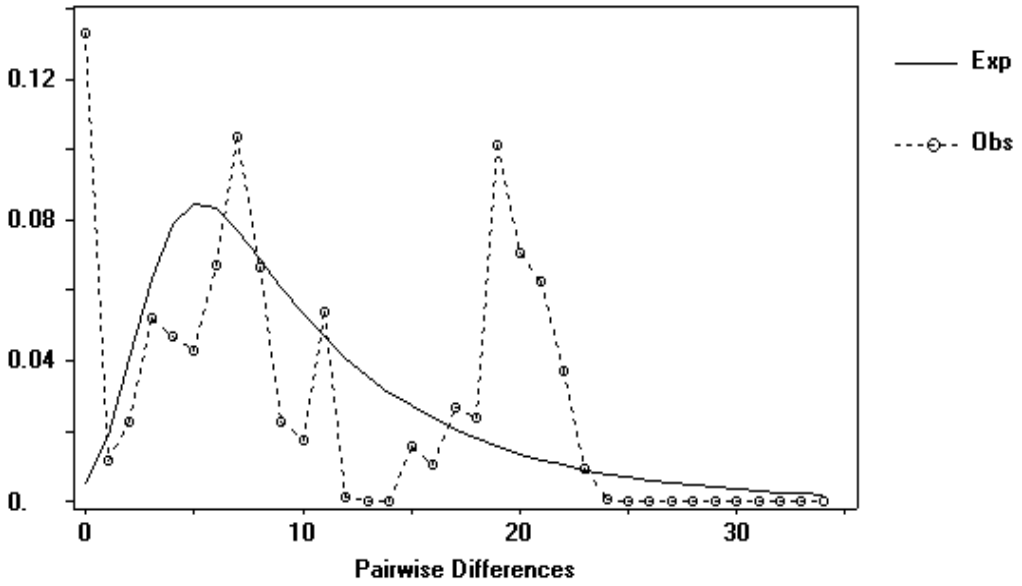


Figure 4.13: Observed and expected distributions of mtDNA pair-wise nucleotide differences (mismatches) under the model of growing-declining populations in the helmeted Guinea fowls in Kenya. The mismatch distribution pattern is multimodal.

Table 4.8 shows a summary of statistics about the demographic history of helmeted Guinea fowl populations in Kenya (simulated sum of squares differences or SSD, Tajima's D and Fu's F_s).

Table 4.8: Summary of statistics about the demographic history of helmeted Guinea fowl populations in Kenya

Region	SSD (P-value)	D (P-value)	F_s (P-value)
Bungoma South	0.019 (0.49)	1.43 (0.94)	-0.39 (0.45)
Teso North	0.041 (0.24)	1.68 (0.96)	-0.13 (0.46)
Bungoma West	0.053 (0.031)*	1.46 (0.94)	-1.11 (0.32)
Mt. Elgon	0.15 (0.075)	0.99 (0.86)	3.05 (0.92)
Laikipia (wild)	0.04 (0.45)	0.15 (0.65)	1.71 (0.81)

SSD= sum of squared differences; D= Tajima's statistics; F_s= Fu's statistics; *P<0.05

Results show that for all the regionss except Bungoma West, the SSD values differed significantly from the observed (P>0.05). Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) values are equally not significant (P>0.05).

Demographic and spatial expansion of the mtDNA haplotypes in the various regions is shown in Table 4.9 below.

Table 4.9: Demographic and spatial expansion of the mtDNA haplotypes in the helmeted Guinea fowls in Kenya

	Demographic expansion		Spatial expansion	
Region	**Raggedness index**	**Raggedness p value**	**Raggedness index**	**Raggedness p value**
Bungoma South	0.056	0.39	0.056	0.68
Teso North	0.099	0.19	0.099	0.57
Bungoma West	0.12	0.021	0.12	0.26
Mt. Elgon	0.29	0.011	0.29	0.64
Wild	0.075	0.19	0.075	0.39

Harpending's demographic expansion raggedness index "r" (Harpending, 1994) of the mtDNA haplotypes is significant for Bungoma West (P=0.021) and Mt. Elgon (P=0.011) supporting a model of demographic expansion for these regions. However, the spatial expansion raggedness index of the mtDNA haplotypes was not significant (P>0.05) across all the five regions sampled.

4.2.8 Maternal genetic structure revealed by mtDNA D-loop variations

To infer the maternal genetic structure of helmeted Guinea fowls across Kenya, analysis of molecular variance (AMOVA) was performed (Tables 4.10, 4.11 and 4.12). The AMOVA results were computed with the algorithms suggested by Excoffier *et al*. (1992) as implemented in the ARLEQUIN software. Molecular components were estimated between and within (i) all the five regions, (ii) the wild (Laikipia) and domesticated helmeted Guinea fowls and (iii) three groups; Teso South and Mt. Elgon, Bungoma West and Bungoma South, and Laikipia. Significance testing was performed using 10100 coalescent simulations.

Table 4.10: Results of AMOVA analysis on five helmeted Guinea fowl regions sampled in Kenya

Source of variation	df	Sum of squares	Variance components	Percentage variation	F_{ST}	P-value
Among regions	4	230	3.05	51.54	0.52	0.00
Within regions	85	244	2.87	48.46		

df= degrees of freedom

48.46% of the mtDNA D-loop variations are distributed within regions and 51.54% between regions supporting a more recent arrival.

Table 4.11: Results from the AMOVA on wild (Laikipia) and domesticated helmeted Guinea fowls in Kenya

Source of Variation	df	Sum of squares	Variance components	Percentage variation	F_{ST}	P-value
Among regions	1	220	6.99	70.74	0.71	0.00
Within regions	88	254	2.89	29.26		

df= degrees of freedom

29.26% of the mtDNA D-loop variations are distributed within regions and 70.74% between regions also indicating a more recent arrival of the domesticated helmeted Guinea fowls in Kenya.

Table 4.12: Results of AMOVA analysis on Teso South and Mt. Elgon, Bungoma West and Bungoma South, and the Laikipia (wild)

Source of Variation	df	Sum of squares	Variance components	Percentage variation	F_{ST}	P-value
Among regions	2	226	2.79	57.02	0.57	0.00
Within regions	87	249	2.86	42.98		

df= degrees of freedom

42.98% of the mtDNA D-loop variations are distributed within regions and 57.02% between regions again pointing to a more recent arrival of the domesticated helmeted Guinea fowls in Kenya.

4.2.9 Association by distance model revealed by Mantel test

A Mantel test was used to assess the non-random association between genetic differentiation (F_{ST}) and geographic distances between sampled regions by plotting the regression graph of the genetic and geographic distances using GenAIEx v6.501

software (Peakall & Smouse, 2006) which is an add-on in Microsoft Excel (Figure 4.14).

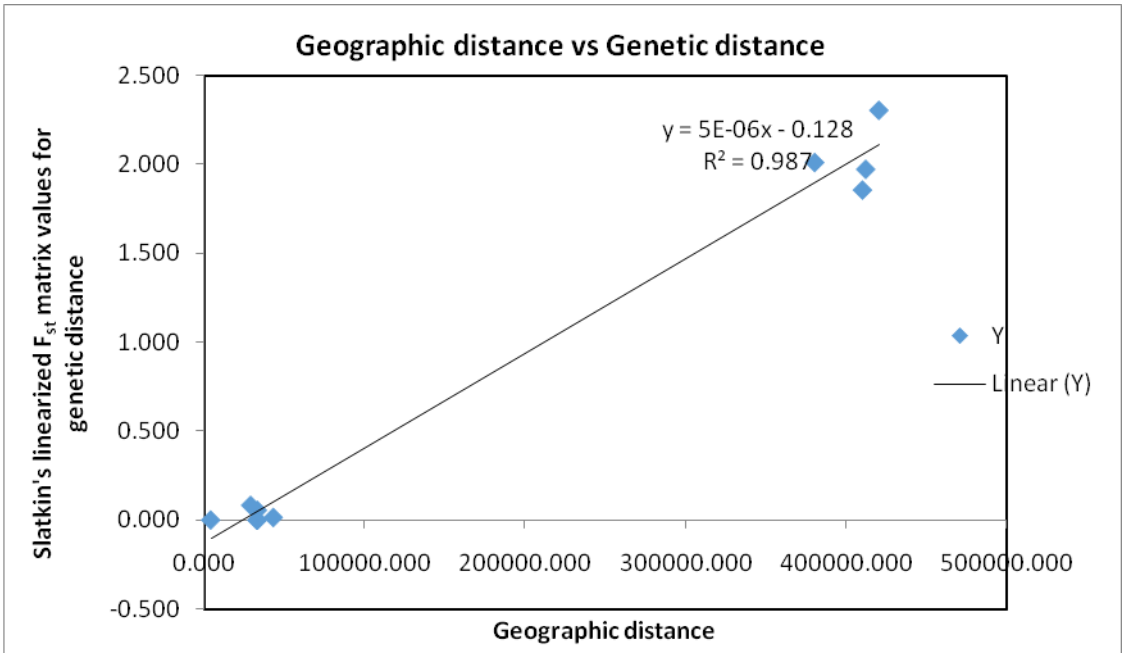


Figure 4.14: A regression graph showing the relationship between geographic and genetic distance matrices of helmeted Guinea fowls in Kenya

A strong and significant positive correlation (r = 0.9936, P>0.05) is observed between genetic variations and the geographic location in helmeted Guinea fowls in Kenya.

4.3 Polymorphisms in HSP70 gene in helmeted Guinea fowls of Kenya

The 90 HSP70 samples were amplified with the relevant primers and visualized under UV light.

4.3.1 Gel pictures

The gel images show amplified regions of the HSP70 gene of selected domesticated helmeted Guinea fowls in Kenya (Figures 4.15 and 4.16). The gel was stained with gel

red and stained for an image using Adobe Photoshop. All the 90 samples were amplified. The positive control was a sample of chicken HSP70 orthologous to the region of interest in the present study and was obtained from the International Livestock Research Institute (ILRI), while water served as the negative control. The samples optimized are representative of all the five populations of domesticated and wild helmeted Guinea fowls in Kenya.

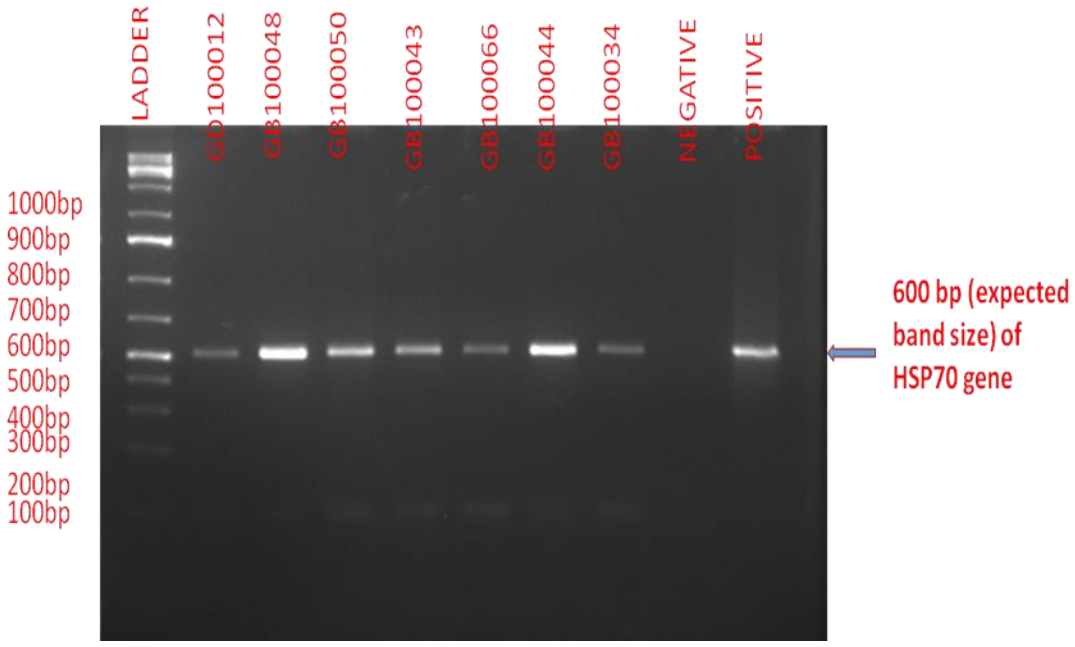


Figure 4.15: A gel pictures showing HSP70 gene amplification in selected domesticated helmeted Guinea fowls in Kenya. The image displays a 2% agarose gel electrophoresis showing a 600bp fragment.

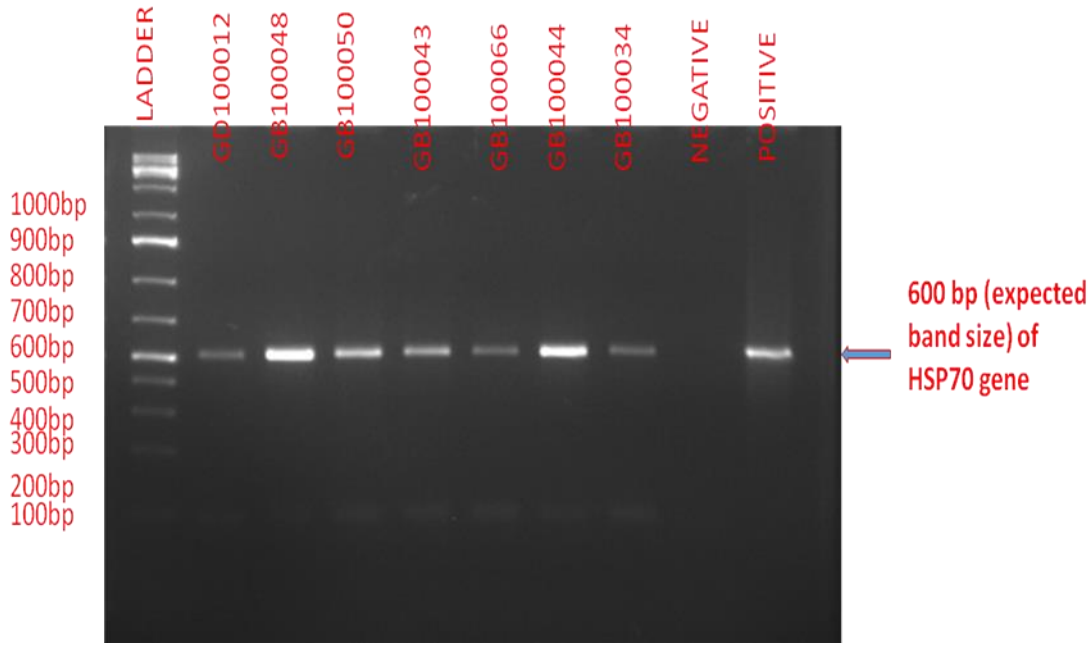


Figure 4.16: A gel picture showing HSP70 gene amplification in selected wild helmeted Guinea fowls in Kenya. The image displays a 2% agarose gel electrophoresis showing a 600bp fragment.

The primers targeted a region of about 600 base pairs which is clearly shown by the 100 bp DNA marker.

4.3.2 HSP70 chromatograms showing variable sites and haplotypes

Partial 508 bp HSP70 sequences from samples of 87 *Numida meleagris* were obtained. Three samples, GB100013, GB100055 and GB100059 yielded sequences of very poor quality which could not be used for subsequent analyses. HSP70 variable regions and mutations are shown in chromatograms in Figures 4.17 below.

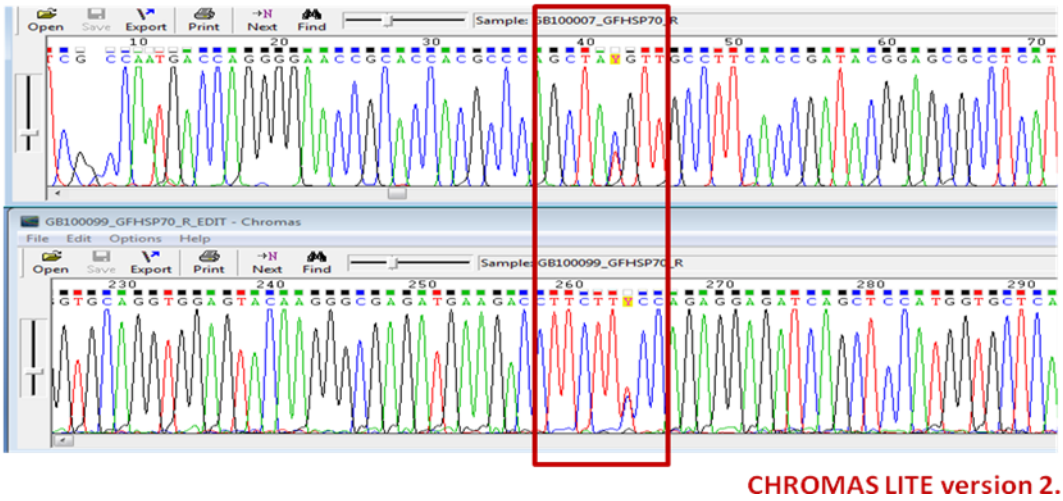


Figure 4.17: A chromatogram showing HSP70 variable regions and point mutations in selected helmeted Guinea fowls in Kenya. The figure shows three chromatograms from three different samples from domesticated and wild helmeted Guinea fowls in Kenya. Y is a code in Chromas Lite representing cytosine/thymine transition. The colour scheme represents the nucleotide base type. The blue signals represent cytosine (C), black signals represent guanine (G), red signals represent thymine (T), and the green signals represent adenine (A).

Single nucleotide polymorphisms (SNPs) and mutations are evident in the above chromatograms (positions 42, 60 and 264 of the edited portion of the HSP70). Two heterozygous sites are observed in a few wild helmeted Guinea fowls. The heterozygosity was observed at position 42 and 264 of the edited portion of the promoter region of Guinea fowl HSP70 gene. The two heterozygous sites had C/T point mutations. These SNPs are novel and have not been validated in previous studies.

HSP70 haplotypes are shown below in a chromatogram (Figure 4.18). Four haplotypes were observed; TGC, TAC, TGT and CGC with three polymorphic sites (all transitions).

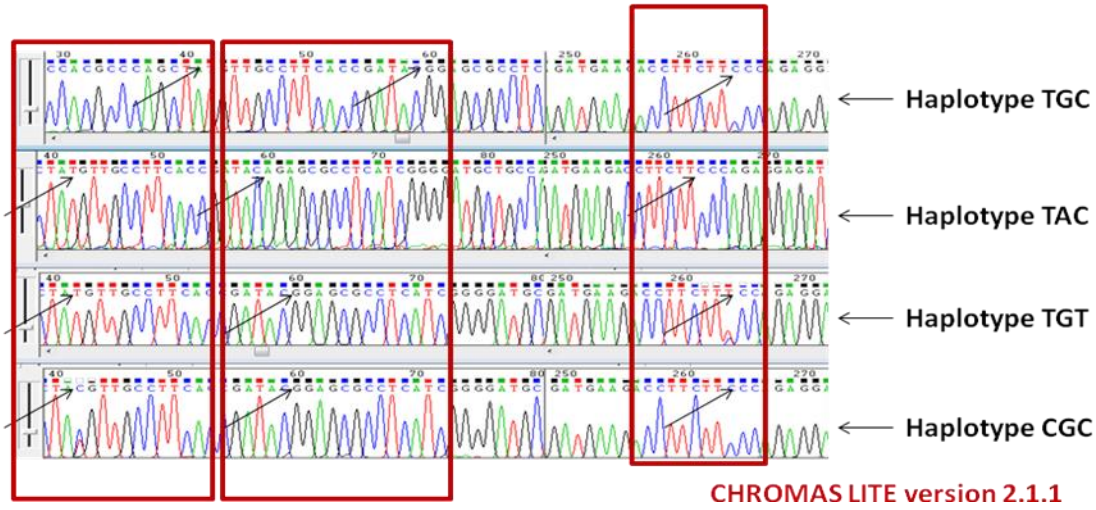


Figure 4.18: A chromatogram showing HSP70 haplotypes of helmeted Guinea fowls in Kenya. The figure shows four chromatograms from four different samples of domesticated and wild helmeted Guinea fowls in Kenya. Y is a code in Chromas Lite representing cytosine/thymine transition. The colour scheme represents the nucleotide base type. The blue signals represent cytosine (C), black signals represent guanine (G), red signals represent thymine (T), and the green signals represent adenine (A).

The polymorphic sites of the observed haplotypes are described in Table 4.13. No insertions or deletions (INDELS) were found.

Table 4.13: Description of the polymorphic sites of the HSP70 haplotypes in the helmeted Guinea fowls in Kenya

	Position (in bp)		
Haplotype	**42**	**60**	**264**
TGC	T	G	C
TAC	T	A	C
TGT	T	G	T
CGC	C	G	C

A: adenine; G: guanine; C: cytosine; T: thymine

Positions 42, 60 and 264 of the edited portion of the control region of Guinea fowl HSP70 gene had T/C, G/A an C/T point mutations respectively.

4.3.3 HSP70 variations and haplotypes revealed by multiple sequence alignment

A multiple sequence alignment of the HSP70 sequences showing areas of variation and haplotypes is shown below in Figure 4.19.

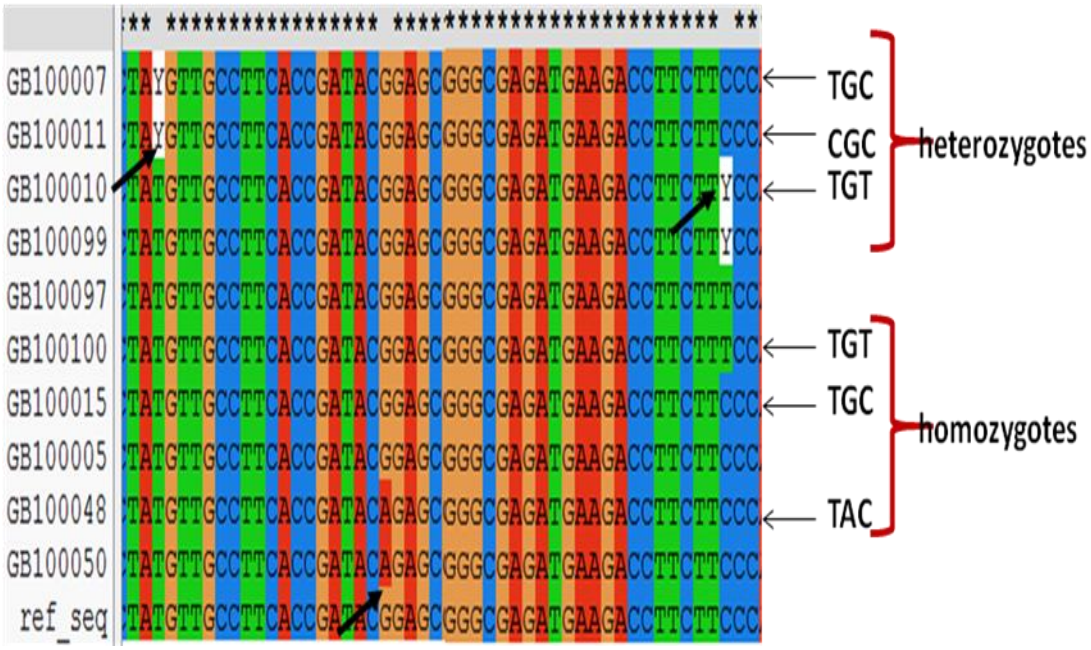


Figure 4.19: A multiple sequence alignment showing HSP70 gene variations and haplotypes of helmeted Guinea fowls in Kenya. Ref_seq is the published reference sequence of the helmeted Guinea fowl, *Numida meleagris* (Iwamoto et al., 2008). Y is a code in Chromas Lite representing cytosine/thymine transition. The colour scheme represents the nucleotide base type. The blue regions represent cytosine (C), orange regions represent guanine (G), red regions represent adenine (A), and the green regions represent thymine (T). Arrows represent the variable regions (single nucleotide polymorphisms).

Most of the sequences observed were homozygotes, with a few heterozygotes. Four haplotypes were observed; TGC, TAC, TGT and CGC with three polymorphic sites (all transitions). No insertions or deletions (INDELS) were found.

4.3.4 HSP70 haplotype distribution in helmeted Guinea fowls in Kenya

The distribution of HSP70 haplotypes in helmeted Guinea fowl in Kenya is shown below in pie charts (Figure 4.20).

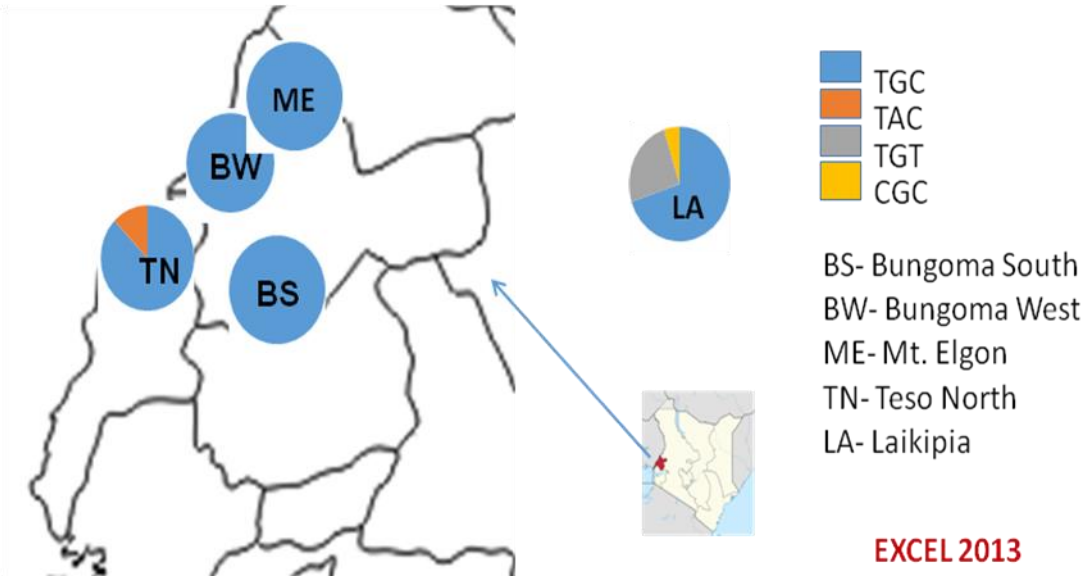


Figure 4.20: Pie charts showing the distribution of HSP70 haplotypes in Kenya's helmeted Guinea fowls. Different colours indicate specific haplotypes. Initials indicate the regions sampled and it is shown in the legend.

The most frequent haplotype, TGC, is shared in all the five helmeted Guinea fowl populations. The second major haplotype (TGT) is found only in 25% of the wild population. Haplotype TAC is found only in 2 individuals in Teso North while haplotype CGC is found in only 1 individual in the wild population.

The relative frequencies of the observed haplotypes in the studied regions are shown below in Table 4.14.

Table 4.14: Relative frequencies of HSP70 haplotypes in helmeted Guinea fowls in Kenya

Haplotype	Bungoma South	Teso North	Bungoma West	Mt.Elgon	Laikipia (wild)
TGC	1.00	0.875	1.00	1.00	0.70
TAC		0.125			
TGT					0.25
CGC					0.05

A: adenine; G: guanine; C: cytosine; T: thymine

The most frequent haplotype, TGC, occurs at a frequency of 90.8% and is shared in all the five helmeted Guinea fowl populations (Bungoma South, 100%; Teso North, 87.5%; Bungoma West, 100%, Mt. Elgon, 100% and Wild, 70%). The second major haplotype (TGT), which occurs at a frequency of 5.75′% across all populations, is found only in 25% of the wild population. Haplotype TAC occurs at an overall frequency of 2.3% across all populations and is found in 2 individuals in Teso North. Haplotype CGC occurs at an overall frequency of 1.15% and is found in only 1 individual in the wild population.

4.3.5 Phylogenetic analysis of HSP70 haplotypes in relation to other avian species

4.3.5.1 Maximum likelihood tree of Guinea fowl HSP70 haplotypes

Phylogenetic analysis of the four HSP70 haplotypes with other avian HSP70 sequences downloaded from Genbank shows that all the haplotypes clustered together (Figure 4.21). It is also observed that haplotype TAC seems to be more genetically distant from the other haplotypes. The helmeted Guinea fowl HSP70 phylogenetic tree reveals a strong relationship with HSP70 sequences of other *Galliformes*..

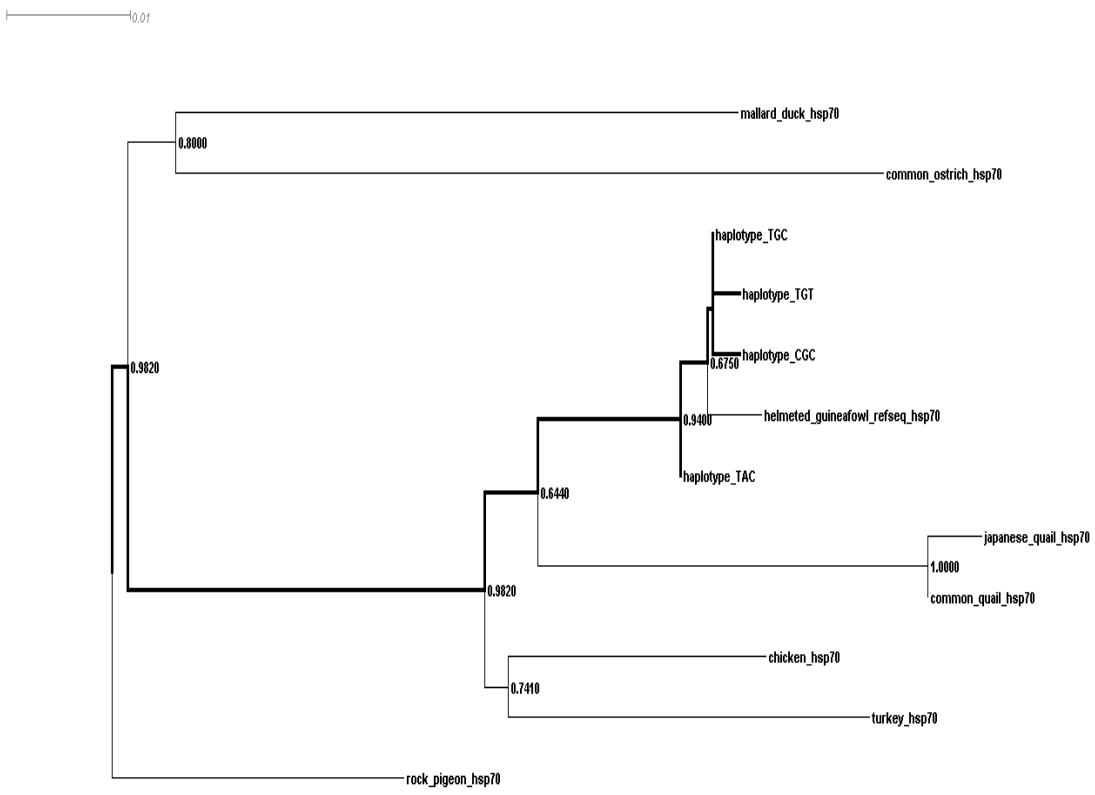


Figure 4.21: HSP70 phylogeny of the helmeted Guinea fowls constructed using Maximum Likelihood as implemented in MEGA v6.06 (Tamura et al., 2013) with 1000 bootstrap replications. The model used was K2+G, gamma shape parameter is 0.1264. The rock pigeon was used as the outgroup.

4.3.5.2 The splits decomposition network of Guinea fowl HSP70 haplotypes

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 and Bryant, 2006). The phylogenetic network diagrams produced from this analysis were used to validate the haplotypes. The splits decomposition network of the

HSP70 haplotypes in Guinea fowls and related avian species is shown below in Figure 4.22.

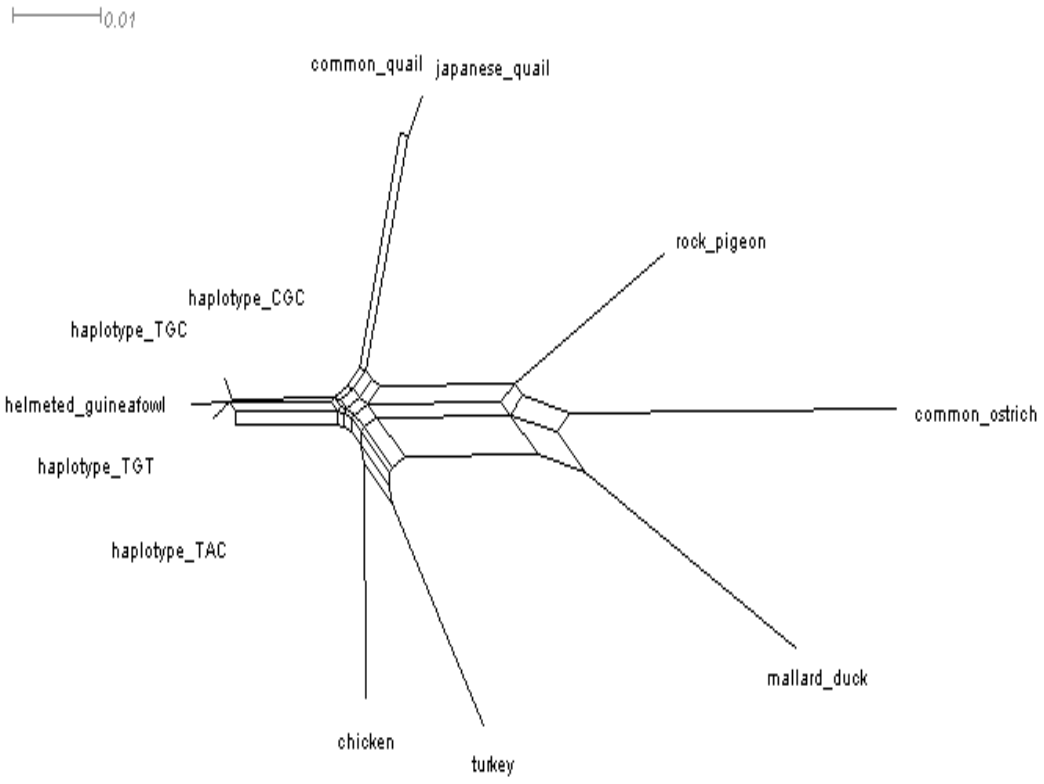


Figure 4.22: Splits decomposition network of the four helmeted Guinea fowl HSP70 haplotypes with other avian HSP70 sequences. The network was generated using splitstree version 4.13.1 (Huson and Bryant, 2006)

All the haplotypes clustered together. Haplotype TAC is however, observed to be genetically distant in relation to the other haplotypes. The other avian species are relatively distant from the four Guinea fowl HSP70 haplotypes.

4.3.6 HSP70 diversity indices of the helmeted Guineafowl s

Several diversity indices were calculated in the five sampled regions as shown in Tables 4.15 and 4.16 below.

Table 4.15: Diversity indices of HSP70 gene in helmeted Guinea fowls in Kenya

Region	n	Number of homozygotes	Number of heterozygotes	H_O	H_E
Bungoma South	26	26	0	0.00±0.00	0.00±0.00
Teso North	32	32	0	0.00±0.00	0.22±0.00
Bungoma West	34	34	0	0.00±0.00	0.00±0.00
Mt. Elgon	42	42	0	0.00±0.00	0.00±0.00
Laikipia (wild)	40	32	8	0.20±0.00	0.0010±0.018

n= number of haploid individuals sampled; H_O = observed heterozygosity; H_E = expected heterozygosity

All the domesticated Guinea fowls sampled were monomorphic. The wild Guinea fowls, however, were genetically diverse with a degree of polymorphism (four heterozygous individuals). Observed and expected heterozygosities were low as shown in the results above.

Table 4.16: Diversity indices of HSP70 gene in helmeted Guinea fowls in Kenya

Region	n	Number of polymorphic sites	h	k	π
Bungoma South	26	0	0.00±0.00	0.00±0.00	0.00±0.00
Teso North	32	1	0.22±0.062	0.22±0.27	0.00048±0.00063
Bungoma West	34	0	0.00±0.00	0.00±0.00	0.00±0.00
Mt. Elgon	42	0	0.00±0.00	0.00±0.00	0.00±0.00
Laikipia (wild)	40	2	0.45±0.05	0.48±0.42	0.0010±0.00099

n= number of haploid individuals sampled; h = gene diversity; k = mean number of pairwise differences; π =nucleotide diversity

All individuals sampled had low values of the diversity indices. The number of polymorphic sites range from 1 (Teso North) and 2 (Laikipia). Haplotype diversities (h) varied from 0.000±0.000 (Bungoma South, Bungoma West and Mt. Elgon) to 0.451±0.051 (Laikipia). Just like haplotype diversities the nucleotide diversity (π) values are generally low.

4.3.7 Demographic and spatial expansion of HSP70 in helmeted Guinea fowls

The demographic and spatial expansion of HSP70 gene in various regions sampled in Kenya for helmeted Guinea fowls is shown below in Table 4.17.

Table 4.17: Demographic and spatial expansion of HSP70 gene in the helmeted Guinea fowls in Kenya

	Demographic expansion		Spatial expansion	
Region	**Raggedness Index**	**Raggedness p-value**	**Raggedness index**	**Raggedness p-value**
Bungoma South	0.00	0.00	0.00	0.00
Teso North	0.36	0.092	0.36	0.46
Bungoma West	0.00	0.00	0.00	0.00
Mt. Elgon	0.00	0.00	0.00	0.00
Laikipia (wild)	0.18	0.091	0.18	0.12

Harpending's raggedness index "r" (Harpending, 1994) of the HSP70 haplotypes is not significant (P>0.05) for Guinea fowls sampled in Teso North and Laikipia. These are the only Guinea fowls that showed genetic variations in their HSP70 gene. No information about the demographic or spatial expansion could be inferred from the Harpending's raggedness indices since the values were not statistically significant for Guinea fowls that showed genetic variations.

4.3.8 Genetic structure revealed by HSP70 variations

To infer the population genetic structure of HSP70 haplotypes of helmeted Guinea fowls across Kenya, analysis of molecular variance (AMOVA) was performed as shown in Tables 4.18 and 4.19. The AMOVA results were computed with the algorithms suggested by Excoffier *et al*. (1992) as implemented in the ARLEQUIN software. Molecular components were estimated among regions, among individuals within regions and within individuals in (i) the wild and domesticated helmeted Guinea fowls and (ii) three groups; Teso South and Mt. Elgon, Bungoma West and

Bungoma South, and the wild Guinea fowls sampled in Laikipia. Significance testing was performed using 10100 coalescent simulations.

Table 4.18: Result of AMOVA analysis of the wild and domesticated helmeted Guinea fowls in Kenya

Source of Variation	**df**	**Sum of squares**	**Variance components**	**Percentage variation**	**P-value**
Among regions	1	4.06	0.032	29.26	0.00
Among individuals within regions	172	22.56	0.054	49.64	
Within individuals	174	4.00	0.023	21.10	

df= degrees of freedom

21.10% of the HSP70 variations are distributed within individuals, 49.64% among individuals within regions and 29.26% among regions hence most variations were among individuals within regions. The fixation indices F_{IS}, F_{ST} and F_{IT} were 0.70, 0.30 and 0.79 respectively. The positive F_{IS} value indicates a heterozygote deficiency which suggests inbreeding within the sampled regions.

Table 4.19: Result of AMOVA analysis of the three groups; Teso South and Mt. Elgon, Bungoma West and Bungoma South, and the wild Guinea fowls sampled in Laikipia

Source of variation	df	Sum of squares	Variance components	Percentage variation	P-value
Among regions	2	4.25	0.018	18.73	0.00
Among individuals within regions	171	22.37	0.054	56.98	
Within individuals	174	4.00	0.023	24.30	

df= degrees of freedom

24.30% of the HSP70 variations are distributed within individuals, 56.98% among individuals within regions and 18.73% among regions again showing that most variations were among individuals within regions. The fixation indices F_{IS}, F_{ST} and F_{IT} were 0.7011, 0.1873 and 0.7570 respectively. Again, the F_{IS} value was positive, indicating a heterozygote deficiency which suggests inbreeding within the sampled regions.

4.3.9 Association by distance model revealed by Mantel test

A Mantel test was used to assess the non-random association between genetic differentiation (F_{ST}) and geographic distances between populations by plotting the regression graph of the genetic and geographic distances using GenAIEx v6.501 software (Peakall & Smouse, 2006) which is an add-on in Microsoft Excel (Figure 4.23).

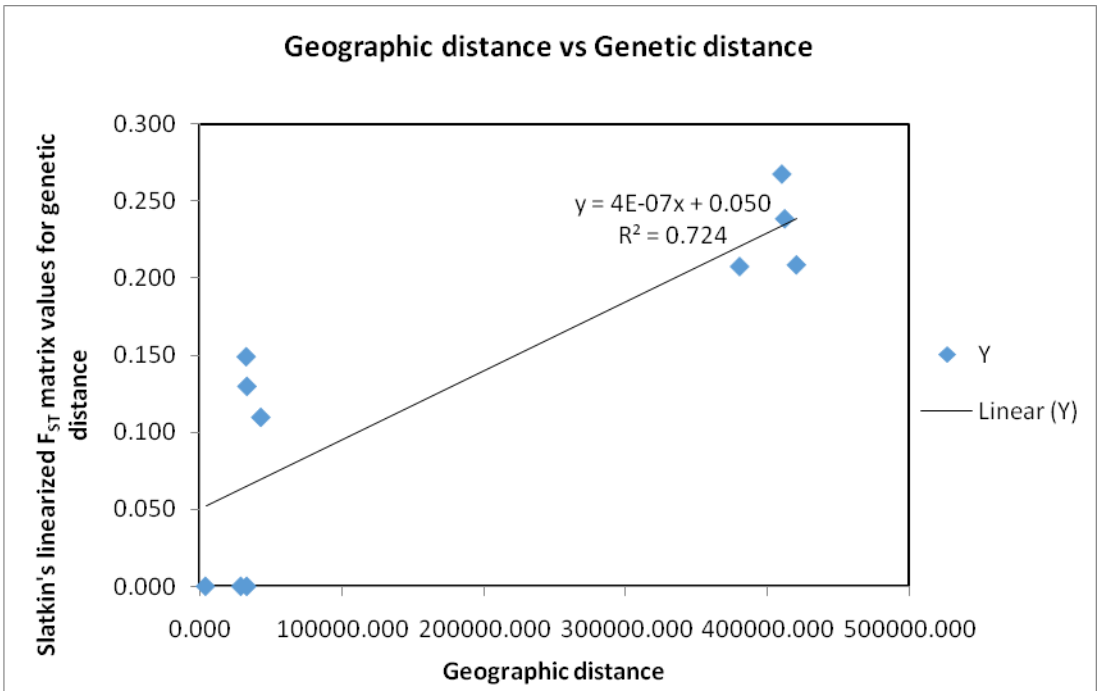


Figure 4.23: A regression graph showing the relationship between geographic and HSP70 genetic distance matrices of helmeted Guinea fowls in Kenya

A strong and significant positive correlation (r = 0.85, P>0.05) is observed between genetic variations and the geographic location in helmeted Guinea fowls in Kenya.

CHAPTER FIVE

DISCUSSION

5.1 Phenotypic characterization of helmeted Guinea fowls in Kenya

5.1.1 Observed features

Crowe (1985) suggested that the East African *N.m. meleagris* and *N.m. somaliensis* subspecies are medium sized with long bristles on the cere and rounded red wattles. True to expectation, results from this study showed the plumage of helmeted Guinea fowls to be monotypic with the background colour observed to be black intermeshed with white spots. Two phenotypes were identified based on wattle colour; the red wattled and blue wattled Guinea fowls. All the domesticated helmeted Guinea fowls were red wattled while all the wild helmeted Guinea fowls were blue wattled. According to Crawford (1990), domesticated helmeted Guinea fowls of Eastern Africa are likely to be domesticates of *Numida meleagris galeata*, *Numida meleagris meleagris* and *Numida meleagris somaliensis* subspecies which fit the description of the Kenyan domesticated helmeted Guinea fowls.

5.1.2 Skin colour and shank colour

Skin colour observed in Kenyan helmeted Guinea fowls is mostly grey with a few individuals of white skin. It was also observed that most helmeted Guinea fowls have black shanks, with a few having pink and black shanks. Colour distribution is explained by the findings of Ayorinde (2004) who reported that the skin of the white Guinea fowl is light yellow to white depending on the amount of xanthophylls, while the skin of the other varieties is either grey or black due to a high melanin concentration. The mostly black shank colour with a few pink and grey in some way also agrees with the study of Mogre (2010) and Agbolosu et al. (2015) who showed that orange and black shank

colours cut across all Guinea fowl colour varieties with some cases of a mixture of orange and black.

Helmeted Guinea fowls are generally known to be hardy and quite adapted to their local environment (Agbolosu et al., 2015). Blood supply to the helmet, wattles and cere in Guinea fowls is thought to play a role in thermoregulation (Crawford, 1990). However, more studies need to be done to determine the degree of tolerance or susceptibility of these birds to stressful environment due to their phenotypic pattern (Egahi et al., 2010; Agbolosu et al., 2015).

The observed lack of significant difference between Kenyan helmeted Guinea fowls in the primary qualitative traits measured could be an indication of low level of variation especially in the domesticated helmeted Guinea fowls. While carrying out this study, it was noted that the current husbandry practices for domesticated Guinea fowls in Kenya involves mostly rearing a few Guinea fowls from related stock in small scale under free range system. This has generally led to inbreeding among these Guinea fowls thus accounting for their low diversity. Molecular work was hence necessary to compliment and corroborate these findings. These results also show significant difference in wattle colours between domestic and wild helmeted Guinea fowls in Kenya with the wild Guinea fowls that are most common in the ASAL regions of Laikipia having blue wattles, while the domesticated Guinea fowls have red wattles. The less bright blue colour could be an adaptation to achieve some degree of camouflage in the wild. Further phenotypic work on Kenyan wild Guinea fowls is proposed especially on a larger sample from other regions not sampled in order to understand the phenotypic traits necessary to mitigate against the effects of adverse environmental conditions brought about as a result of climate change, such as heat stress.

5.1.3 Guinea fowl body measurements

Results generally showed that other than the wing length, body weight and head size, the other phenotypic had no marked difference between domestic and wild helmeted Guinea fowls of Kenya. The smaller head size and body weight of the wild population despite their higher shank and body lengths is of interest and calls for more studies to understand if it has any significance in ecological conservation. It could be inferred that perhaps the small head size and body weight are some of the features required by Guinea fowls to survive in the wild (Fajemilehin, 2010). The wing length of the wild helmeted Guinea fowls was also found to be higher than that of domesticated helmeted Guinea fowls. This is expected considering that wild Guinea fowls fly frequently either in search of food or while escaping from predators, unlike the domesticated Guinea fowls that only fly occasionally. The large wings could therefore be an adaptation to help them successfully survive in the wild.

Comparison of male and female helmeted Guinea fowl body parameters revealed that male Guinea fowls had higher values for all body measurements taken. Ayorinde (2004) reported that the most prominent feature of the head of both male and female helmeted Guinea fowl is the median, caudal-dausal bony process or helmet of the frontal bones. The mean helmet height of males was 3.28 cm and the females was 2.9cm. The results clearly agree with the findings of Ayorinde (2004) who had proposed that the helmet of Guinea fowls is slightly longer in males (3.7 cm) than in females (2.0 cm). Ayorinde (2004) further stated that although the size and shape of the head, helmet and wattle can be used to distinguish sexes within a flock by an experienced person, there is need to pursue more aggressively the search for more morphological features for sexual differentiation. Males are slightly larger than females though they exhibit almost no sexual dimorphism. Results of live body weights of male and female helmeted Guinea fowls again showed that males (1476.09±194.58) were marginally heavier than females (1409.09±213.30). Again, this compares favourably

with the findings of Long (1981) who had reported that adult body weight of helmeted Guinea fowls ranges from 0.7-2.0kg.

5.1.4 Relationship between body temperature and environmental temperature

The observed lower body temperature of Guinea fowls found in Bungoma South when compared to the environmental temperature could be attributed to the fact that Bungoma South is generally forested and colder hence this result agrees with the observed conditions of this ecological zone.

5.2 mtDNA D-loop as a marker for deducing genetic diversity

The amplified and sequenced first hyper-variable region of the mtDNA D-loop in this study is commonly used for assessing genetic diversity and phylogeographic structure in *Galliformes* (Muchadeyi et al., 2008; Mwacharo et al., 2011; Khaliq et al., 2011; Adeola et al., 2015). Assessment of helmeted Guinea fowl mtDNA D-loop region shows that most Kenyan and Nigerian domesticated helmeted Guinea fowls clustered in two major haplotypes (Hap 2 and Hap 4) showing a clear genetic relationship between Kenyan and Nigerian domesticated helmeted Guinea fowls. This points to a possible common origin of most Kenyan and Nigerian domesticated helmeted Guinea fowls, most likely derived from the West African subspecies *Numida meleagris galeata.*

5.2.1 Mitochondrial DNA D-loop sequence variability and haplotype distribution pattern

Single nucleotide polymorphisms (SNPs) were discovered in the chromatograms and multiple sequence alignments of helmeted Guinea fowl mtDNA sequences.. An insertion/deletion (INDEL) in all individuals in the wild population (between position 196 and 197 of the edited DNA sequence of the mtDNA D-loop) and a second insertion in two wild individuals (between position 186 and 187 of the edited DNA sequence of the mtDNA D-loop) was also observed. Furrther, 25 unique haplotypes defined by 41

polymorphic sites were also identified. In agreement with previous studies on Nigerian helmeted Guinea fowls (Adeola et al., 2015), two major haplotypes, Hap4, occurring at a frequency of 33.3% and widely distributed in all the four regions with domesticated Guinea fowls, and Hap2 that occurred at a frequency of 8.9′% across all sampled regions and common in three regions with domesticated helmeted Guinea fowls, were observed. The 18 haplotypes in the four regions with domesticated individuals compare favourably with the 19 haplotypes identified in Nigerian domesticated helmeted Guinea fowls (Adeola et al., 2015). Most of the Nigerian, Kenyan and Chinese domesticated helmeted Guinea fowls also belong to haplotypes Hap2 and Hap4 that strongly suggest possibility of a common origin of both the Kenyan domesticated helmeted Guinea fowls and West African domesticated helmeted Guinea fowls which are derived from the West African *Numida meleagris galeata* (Crawford, 1990). It was demonstrated that the 8 haplotypes identified in the wild helmeted Guinea fowls were not shared by the domesticated helmeted Guinea fowls. Their unique haplotypes could be a consequence of unique demographic histories that have shaped haplotype profiles of wild helmeted Guinea fowls (Adeola et al., 2015) that belong to a distinct subspecies that is different from the domesticated helmeted Guinea fowls. Using microsatellite markers to compare genetic variation between red jungle fowl and commercial chicken lines (Tadano et al,. 2014) and genetic variation between wild and domesticated helmeted Guinea fowl (Weimann et al., 2016) it was shown that the wild populations genetically differed from the domesticated populations.

The extent of haplotype sharing indicates the absence of a population structure in Kenya's domesticated helmeted Guinea fowls. Muchadeyi *et al*. (2008) and Mtileni *et al* (2011) proposed that large effective population sizes as well as continuous gene flow may be the forces responsible for the lack of population differentiation among the local chicken genotypes in their studies. Similarly, Weimann *et al*. (2016) attributed the lack of a clear population differentiation between the domesticated helmeted Guinea fowl populations to large population sizes and continuous gene flow.

5.2.2 Phylogenetic analysis of mtDNA haplotypes

The close relationship between the Nigerian (Adeola et al., 2015) and Kenyan domesticated helmeted Guinea fowls based on phylogenetic analysis further indicates that Kenyan domesticated helmeted Guinea fowls probably share a common origin with the West African domesticated helmeted Guinea fowls. Crawford (1990) had earlier suggested that nearly all modern domesticated Guinea fowls are likely to have been derived from the introduction of the West African subspecies *Numida meleagris galeata*. Crawford (1990) also proposed that domesticated helmeted Guinea fowls of eastern Africa could likely be domesticates of *Numida meleagris meleagris* and *Numida meleagris somaliensis*. The 8 wild helmeted Guinea fowl haplotypes clustered into closely related clades that represent distinct haplogroups. The robustness of the sequence clusters detected using the Maximum Likelihood tree was tested by the analysis of the phylogenetic networks from uncorrected p-distances using the phylogenetic splits decomposition network implemented in SplitsTree version 4.13.1 (Huson & Bryant, 2006). The splits decomposition network again revealed a genetic relationship between Kenyan and Nigerian domesticated helmeted Guinea fowls providing additional support to our findings that suggest that Kenyan domesticated helmeted Guinea fowls share a common origin with West African domesticated helmeted Guinea fowls.

5.2.3 mtDNA diversity indices

Results from the mtDNA data show that Guinea fowls from all the five regions sampled were polymorphic, with the number of haplotypes ranging from 5 in Mt. Elgon to 10 in Bungoma West. The mean observed and expected heterozygosities were generally lower across all populations. Based on microsatellite analysis, Kayang et al. (2010) found that the mean observed and expected heterozygosities were greater in the West African populations than in the Japanese populations. Previous findings showed that the indigenous West African helmeted Guinea fowl populations are more genetically diverse

but less differentiated compared to non-indigenous populations in Japan (Kayang et al., 2010).

Haplotype diversities were also high, varying from 0.638±0.079 in Mt. Elgon to 0.897±0.067 in Bungoma West. The high levels of haplotype diversities observed in this study could be attributed to large population sizes (Avise, 1998). These results are similar to those observed in the Nigerian helmeted Guinea fowls (Adeola et al., 2015) where the lowest haplotype diversity was 0.529±0.095 and the highest haplotype diversity was 0.821±0.082. Nucleotide diversity values were generally low, therefore, the observed haplotypes were closely related (Khaliq et al., 2011). The lowest haplotype diversities were observed in the Mt. Elgon (0.638±0.079) and Teso North (0.797±0.090) populations. This low diversity observed in these two populations could be attributed to recent domestication (<5,000 years ago; Appleby et al., 1992) from a small founder population. Insufficient time may have passéd since domestication to allow for the accumulation of mutations. Additionally, the rearing system in most of the households keeping these poultry species encourages inbreeding since they usually start with two related birds, a male and female that are mostly siblings.

5.2.4 Helmeted Guinea fowl population dynamics revealed by mtDNA D-loop variations

Analysis of demographic history can be based on mismatch distribution (Rogers & Harpending, 1992). Unimodal distributions are expected in growing-declining populations while populations at demographic equilibrium give multimodal mismatch distributions (Khaliq et al., 2011). Tajima's D and Fu's F_s (neutrality indices) are also useful for detecting demographic changes (Tajima, 1989; Simonsen et al, 1995; Fu, 1997). In especially neutral loci like the control region (D-loop), significant negative D and F_s values would indicate recent population expansion, while significant positive values would point to genetic drift (Nyakaana et al., 2008; Khaliq et al., 2011). Fu (1997) demonstrated that F_s is a more powerful test than D for demographic changes.

But R_2 test has been found to be better than both D and F_s for smaller samples like the ones used for this study (Ramos-Onsins & Rozas, 2002). Coalescent simulation as implemented in DnSP v5 is the most adequate method for estimating significance for D, F_s and R_2 in small sizes of samples (Librado & Rozas, 2009)

Findings from this study show that all the sampled regions except Bungoma West have insignificant SSD values (P>0.05). Tajima's D (Tajima, 1989) and Fu's F_s (Fu, 1997) values are not significant (P>0.05). Harpending's demographic expansion raggedness index "r" (Harpending, 1994) of the mtDNA haplotypes was significant for Bungoma West (P=0.021) and Mt. Elgon (P=0.011). However, the spatial expansion raggedness index of the mtDNA haplotypes was not significant (P>0.05) for all the five regions with helmeted Guinea fowls in Kenya. Like in previous studies that supported a model of demographic expansion over all East African chicken populations (Mwacharo et al., 2011), these results support a model of demographic expansion of the Bungoma West and Mt. Elgon Guinea fowls. It is also worth noting that Guinea fowls found in Mt Elgon had the lowest number of haplotypes and haplotype diversity in comparison to the other Guinea fowls. Previous studies had shown low genetic diversity in domesticated Guinea fowl outside their area of origin, and this was attributed to a small founder population (Sharma et al., 1998; Adeola et al., 2015) and many years of inbreeding. However, the raggedness index, Tajima's D and Fu's F_s statistics do not support demographic and spatial expansion for mtDNA haplotypes across the other populations as previously suggested by Mwacharo et a*l*. (2011).

5.2.5 Maternal genetic structure revealed by mtDNA D-loop variations

Considering the five sampled regions as a hierarchical cluster, 51.54% of the genetic variation was observed among populations. This value however, increases to 70.74% when wild helmeted Guinea fowls as a group are compared against the domesticated helmeted Guinea fowls and decreases to 57.02% when three groups, that is, Teso South and Mt. Elgon, Bungoma West and Bungoma South, and the wild Guinea fowls are

considered. Results from the three hierarchical categories therefore show that among regions distribution of variation is higher than within region variation in the mitochondrial DNA D-loop region of helmeted Guinea fowls in Kenya.

5.2.6 Median joining network of mtDNA haplotypes

From the results, most of the domesticated helmeted Guinea fowl individuals are grouped into two major haplogroups named HgA and HgB in a previous study (Adeola et al., 2015) clustered around Hap2 and Hap4. All the published sequences of Nigerian, Kenyan and Chinese domesticated helmeted Guinea fowls (Adeola et al., 2015) also group into haplogroups HgA and HgB, indicating a most probable common origin of both West African and Kenyan domesticated helmeted Guinea fowls. A few domesticated helmeted Guinea fowls grouped in two other haplogroups. This could probably be of a different origin, hypothesized to be Eastern African in this study. The 20 wild helmeted Guinea fowls are grouped into five distinct haplogroups. A very clearly distinct haplogroup HgI comprising of two wild individuals was identified. The median joining network seems to suggest that haplogroup HgI has a closer genetic relationship with domesticated helmeted Guinea fowls than with other wild helmeted Guinea fowls. This may be a result of gene flow between the wild and domesticated helmeted Guinea fowls. The median vectors may represent either un-sampled haplotypes, haplotypes never introduced into Kenya, or introduced into Kenya but becoming extinct shortly upon arrival or later (Mwacharo et al., 2011). The star-like pattern exhibited in haplogroups HgA and HgB is an evidence of rapid population expansion (Adeola et al., 2015). The extent of haplotype sharing in the network between domesticated populations indicates the absence of population structure in Kenyan domesticated Guinea fowls. It is interesting to note that a similar pattern of lack of phylogeographic structure in poultry, such as domesticated helmeted Guinea fowl in Ghana (Kayang et al., 2010), chicken from East Africa (Mwacharo et al., 2011) and Nigeria (Adebambo et al., 2010) and domesticated helmeted Guinea fowls in Nigeria (Adeola et al., 2015) has been observed. This could likely be due to intensive genetic

intermixing between populations due to human migration and trading (Adebambo et al., 2010; Adeola et al., 2015). Hence the lack of genetic differentiation in Kenyan domesticated helmeted Guinea fowl may likewise be due to intensive genetic admixture. Adeola et al. (2015) however noted that short DNA sequences with inadequate sample size may result in insufficient genetic information to clearly infer the population structure. The wild helmeted Guinea fowls, which are a different subspecies, clustered separately and showed a distinct population structure with their haplotypes not shared by domesticated helmeted Guinea fowls. This may be due to unique demographic histories that have shaped their haplotype profile (Adeola et al., 2015).

5.2.7 Association by distance revealed by Mantel test

To test whether genetic differentiation was directly proportional to geographic proximity, a Mantel test involving pair-wise F_{ST} values against geographic distance between populations was performed. From the results, a significant ($P>0.05$) and strong positive correlation was observed between genetic variation and the geographic location in helmeted Guinea fowls in Kenya as previously described by Mwacharo et al. (2011). This contrasts the findings of Ommeh et al. (2010) that showed a slight negative correlation between allele frequencies and the geographic location in indigenous village chicken populations. Overall, the Mantel test reveals lack of a phylogeographic structure within Kenya's domesticated helmeted Guinea fowl mtDNA haplotypes.

5.3 Archaeological and linguistic insight on the origin of helmeted Guinea fowls

Previous analysis of Guinea fowl DNA indicates a possible *Numididae* divergence from the *Phasianidae* lineage some 38 million years ago (Martinez, 1994). Martinez, 1994 went on to suggest that Guinea fowls could have originated from the Savanna areas of Asia, having probably evolved from a francolin-like phasianid that colonized Africa around the middle to late Miocene with all the four Guinea fowl genera having clearly differentiated by the Pleistocene. Although Ayorinde (2004) agrees that Guinea fowls

could have evolved from a francolin-like Asiatic ancestor, he suggested that their evolution to modern forms solely occurred in Africa. Recent excavations of the footprint tuffs of the Laetolil beds at Laetoli in Northern Tanzania has revealed the presence of a large variety of footprints from the Pliocene Epoch between 3.5 and 3.8 million years ago (Leakey and Hay, 1979).The bird tracks found compare closely with tracks of the living helmeted Guinea fowls common in the Laetoli area today. Guinea fowl remains were also discovered at Shaqadud site in the Sudan around the 4th millennium bp and they do not seem to differ from modern wild specimens (Marshall, 2000; Peters, 1986; Peters, 1991). This clearly supports the position of Ayorinde (2004) on the evolution of Guinea fowls in Africa (especially Eastern Africa).

Studies show that appearance of Guinea fowls in the history of man's activities is traced to the fifth Egyptian dynasty about 2,400 B.C. when its figure was drawn in a mural (Nishibori et al., 2004) with early domestication believed to have occurred in Southern Sudan and West Africa (Crawford 1990; Nishibori et al., 2004). It is also suggested that present day domesticated helmeted Guinea fowls were probably all derived from the West African subspecies *Numida meleagris galeata* (Walker et al., 2004) which was then repeatedly introduced worldwide (Long 1981; Hastings Belshaw 1985; Donkin 1991). It is believed that separate domestications have occurred in many separate places over time. According to Crowe (1986), wild populations of *Numida meleagris* readily become commensals of man, increasing in numbers and distribution because of the water, roosting, and feed resources resulting from human activity. The process of domestication probably continues even now.

According to Shillington (2012), the languages of Kordofan, west of the middle Nile in Sudan, are linked to the Niger-Congo language family which includes all the Bantu speakers in Africa. This has prompted some linguists and historians to propose that Kordofan in Sudan may have been the original ancestral home of the Niger-Congo language group that then migrated westwards to West Africa. Other linguists however, feel it might have been the other way round, with Kordofanian being a remote offshoot

of Niger-Congo. Shillington (2012) also pointed out that by 3000 BCE, the Niger-Congo people had already domesticated Guinea fowls. Based on the Kordofan proposition, it can be hypothesized that in the course of their westward expansion into West Africa, the Niger-Congo peoples might have carried along the wild helmeted Guinea fowls that lived alongside them as commensals of each other and later domesticated them. From West Africa, the Bantu branch of the Niger-Congo expanded southwards and eastwards into Southern, Central and Eastern Africa. This study proposes that during this expansion, the domesticated helmeted Guinea fowls arrived into southern, central and eastern Africa with the migrating Bantu branch of the Niger-Congo people. Results from the mitochondrial DNA analysis also seem to point to a genetic relationship between West African domesticated helmeted Guinea fowls and most domesticated helmeted Guinea fowls found in Kenya.

Again, it is also imperative to note that the Lugbara, a Nilo-Saharan people of north-western Uganda, have traditionally reared Guinea fowls as one of their main economic activity although information on exactly when it was domesticated is scarce. Considering that the Nilo-Saharan peoples have their roots in Eastern Africa, it is possible that some helmeted Guinea fowl continuously lived in Eastern Africa since antiquity and has been utilized as an economic resource by its people.

Again, based on Western Bantu folklore, many Bantu communities of Uganda and Western Kenya claim that their origin is traced to Misri, which is a Bantu name for Egypt. These claims are however, not supported by any archaeological or linguistic evidence. On the basis of these claims though, it can be argued that these Western Bantus arrived into Uganda and Kenya with these birds (perhaps from Egypt or Sudan). The archaeo-linguistic evidence on the origin and domestication of helmeted Guinea fowls in Africa is summarized in Figure 5.1 below.

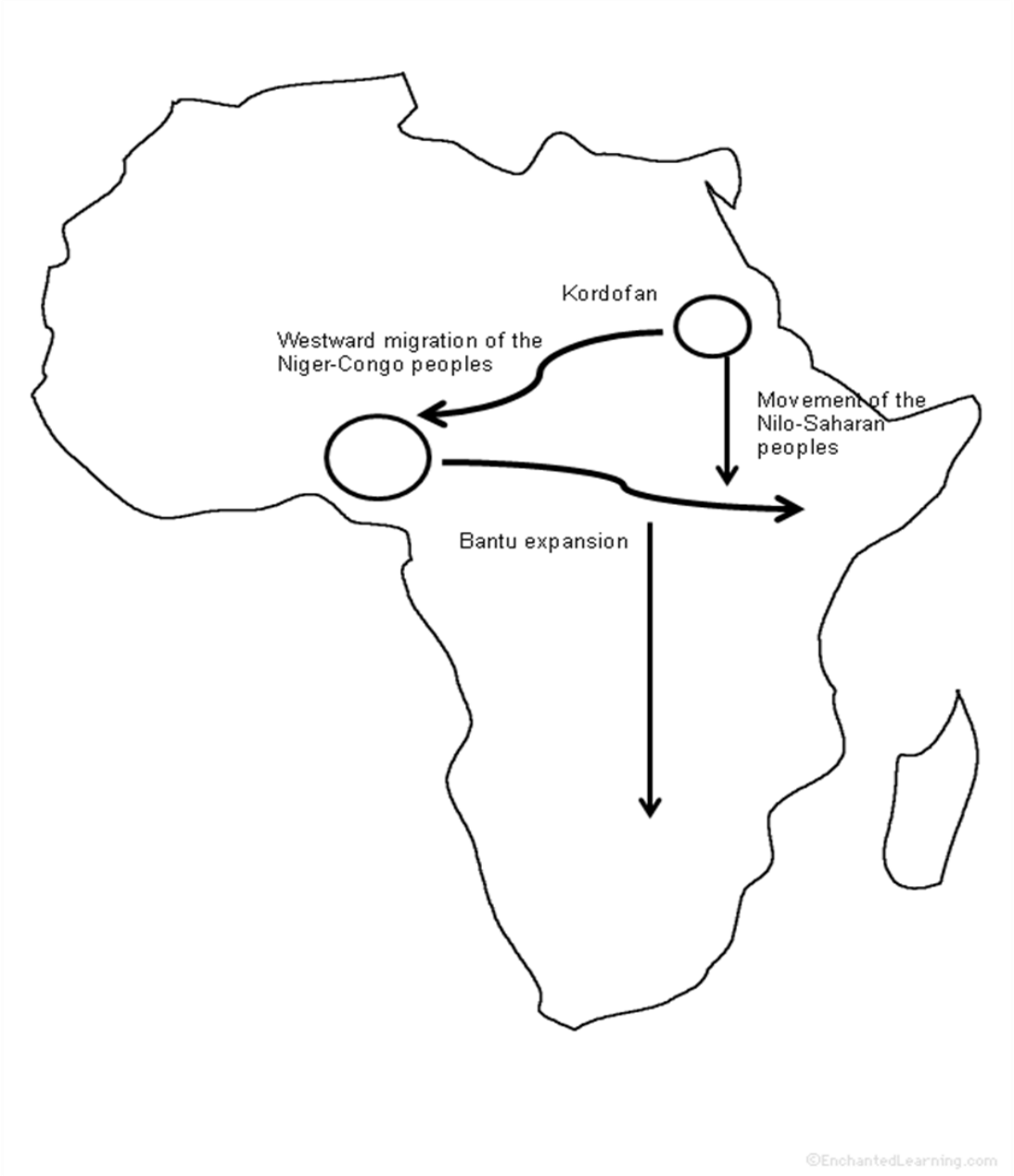


Figure 5.1: Possible migration routes of the domesticated helmeted Guinea fowls along with the movement of the Niger-Congo and Nilo-Saharan peoples into Kenya (Source: http://www.vinotique.com).

5.4 Polymorphisms in HSP70 gene in helmeted Guinea fowls

5.4.1 HSP70 gene variation and haplotype distribution of helmeted Guinea fowls

From the HSP70 gene data analysis, positions 42, 60 and 264 of the edited portion of the control region of Guinea fowl HSP70 gene was observed to have T/C, G/A and C/T point mutations respectively.

The most dominant HSP70 haplotype shared by all populations was the TGC haplotype. Unique mutations in the heat shock protein 70 gene in the wild helmeted Guinea fowl population (haplotypes TGT and CGC) were also observed that were not evident in the domesticated helmeted Guinea fowls. Haplotype TGT occurred at a higher frequency in the wild population, being found in 25% of this population. Again, an A/G transition (haplotype TAC) was observed in two domesticated individuals in the Teso North population that were not observed in all the other populations. Considering that TesoNorth sub-county in Western Kenya is occupied by the Iteso people who are Nilo-Saharans with roots in the Sudan region of Africa, it is possible that this haplotype has its origin within the Eastern African region and was carried into Kenya during earlier migrations. Phylogenetic analysis revealed that this haplotype seems to be more genetically distant to the other haplotypes. A theoretical relationship between *Gallus gallus* HSP70 genotype and heat shock resistance (heat tolerance) has been proposed (Maak *et al*., 2003). According to Morimoto *et al*. (1986) and Iwamoto *et al.* (2008), individual variations in heat shock responses may be related to DNA polymorphisms in the HSP70 gene in avian species. There is thus need to study these unique HSP70 haplotypes further to find out if they are associated in any way with specific environmental adaptations such as heat stress.

5.4.2 Phylogenetic analysis of HSP70 haplotypes

Phylogenetic analysis of the four HSP70 haplotypes with other avian HSP70 reference sequences showed that all the haplotypes clustered together. It was also observed that haplotype TAC seemed to be more genetically distant from the other haplotypes. The helmeted Guinea fowl HSP70 phylogenetic tree revealed a genetic relationship with HSP70 sequences of other *Galliformes.* A splits decomposition network of the HSP70 haplotypes in Guinea fowls and related avian species also revealed that all the haplotypes clustered together with haplotype TAC being observed to be genetically distant in relation to the other haplotypes. The other avian species were also relatively distant from the four Guinea fowl HSP70 haplotypes.

5.4.3 HSP70 gene diversity indices of helmeted Guineafowls

All the four domesticated Guinea fowl populations in Kenya were observed to be monomorphic. Some individuals in the wild population, however, were found to be polymorphic. This indicates that the wild helmeted Guinea fowls in Kenya were genetically more diverse than their domesticated counterparts.

It was also observed that all the populations of helmeted Guinea fowls in Kenya had low values of the molecular diversity indices. The number of polymorphic sites ranged from 1 (Teso North) to 2 (Wild). Haplotype diversities varied from 0.000±0.000 (Bungoma South, Bungoma West and Mt. Elgon) to 0.451±0.051 (Wild). The nucleotide diversity values were equally low.

5.4.4 Genetic structure revealed by HSP70 variations

When wild helmeted Guinea fowls as a group are compared against the domesticated helmeted Guinea fowls, 49.64% of the genetic variation was observed among individuals within population. This value increases to 56.98% when three groups; Teso South and Mt. Elgon, Bungoma West and Bungoma South, and the wild population are

considered. Results from the two hierarchical categories show that most variations occurred among individuals within population in the HSP70 gene of helmeted Guinea fowls in Kenya.

To test whether genetic differentiation was directly proportional to geographic proximity, a Mantel test involving pair-wise F_{ST} values against geographic distance between populations was performed. From the results, a significant ($P>0.05$) and strong positive correlation was observed between genetic variation and the geographic location in helmeted Guinea fowl populations in Kenya as previously described by Mwacharo *et al*. (2011). Again, the Mantel test reveals lack of a phylogeographic structure within Kenya's domesticated helmeted Guinea fowl HSP70 haplotypes just like the mtDNA data revealed.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study showed limited phenotypic diversity in helmeted Guinea fowls across Kenya. There was no significant difference in the primary phenotypic traits measured between Kenyan domesticated helmeted Guinea fowls in different regions. However, wild helmeted Guinea fowls appeared phenotypically different from domesticated helmeted Guinea fowls.

Two haplotypes dominated across all regions sampled for domesticated helmeted Guinea fowls; Hap2 and Hap4. Just like in Nigeria, most domesticated helmeted Guinea fowls in Kenya clustered into two mtDNA haplogroups; HgA and HgB, indicating a genetic relationship between Kenyan and West African Guinea fowls clustered in these two haplogroups. The wild helmeted Guinea fowls which belong to a different subspecies of helmeted Guinea fowls, are grouped into distinct. A very clearly distinct haplogroup HgI in the wild Guinea fowls was identified. The median joining network seems to suggest that haplogroup HgI has a closer genetic relationship with domesticated helmeted Guinea fowls than with other wild helmeted Guinea fowls. This may be a result of gene flow between wild and domesticated helmeted Guinea fowls. The lack of population structure in domesticated helmeted Guinea fowls could suggest intensive genetic intermixing between the domestic populations. The differentiation of the wild Guinea fowls may be due to a clearly distinct demographic history that shaped its genetic profile. Analysis of the Kenyan helmeted Guinea fowl population structure and history based on mitochondrial DNA variations complimented by archaeological and linguistic evidence clearly supports the hypothesis that majority of domesticated helmeted Guinea fowls are related to West African domesticated helmeted Guinea fowls.

All helmeted Guinea fowls in Kenya group into 4 HSP70 haplotypes with two of the halotypes unique to the wild Guinea fowl. Probably, some of these polymorphisms may be associated with certain environmental adaptations, such as heat tolerance. There is no significant ($P<0.05$) and positive correlation between genetic variations and the geographic location in helmeted Guinea fowl populations in Kenya, indicating lack of a phylogeographic structure within Kenya. This study will form the basis for more work on functional polymorphisms in HSP70 gene associated with heat tolerance in the helmeted Guinea fowls.

Overall, this study provides initial information on genetic variation across populations of the domesticated and wild helmeted Guinea fowls in Kenya. This information is expected to help support the conservation efforts for this important bird and also develop breeding programs aimed at mitigating the effects of climate change and improving food security.

6.2 Recommendations

- These preliminary results should pave way for more phenotypic work to be done across other parts of Kenya where Guinea fowls are found.
- The mtDNA results reveal unique haplotypes not shared across populations in either domesticated or wild populations of helmeted Guinea fowls in Kenya. More molecular work in many other parts of Kenya especially in arid and semi-arid lands is recommended to identify any other haplotypes not identified to help in designing approaches to conserve and utilize them in the breeding and conservation programs..
- Unique mutations in HSP70 gene in the wild helmeted Guinea fowls were observed that were not evident in the domesticated helmeted Guinea fowls. It is interesting to note that these haplotypes are only found in wild Guinea fowls which are in ASAL areas of Laikipia. More studies on HSP70 gene polymorphisms in helmeted Guinea fowls is therefore recommended to

determine if these polymorphisms may be associated with certain environmental adaptations, such as heat tolerance.

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APPENDICES

Appendix 1: Genbank accession numbers for mtDNA reference sequences of Nigerian, Kenyan and Chinese domesticated helmeted Guinea fowls and other published sequences

Sequence number	Accession number	Sequence	Accession
Isolate_783_KA4	KP218433	Isolate_750_KE1	KP218405
Isolate_781_KA5	KP218432	Isolate_75_LO1	KP218404
Isolate_780_KA5	KP218431	Isolate_749_KE1	KP218403
Isolate_78_LO1	KP218430	Isolate_747_KE5	KP218402
Isolate_746_KE6	KP218401	Isolate_665_KEN1	KP218358
Isolate 745_KE6	KP218400	Isolate_660_KEN1	KP218357
Isolate 744_KE4	KP218399	Isolate_66_AK3	KP218356
Isolate_743_KE1	KP218398	Isolate_659_KEN1	KP218355
Isolate_729_KE6	KP218382	Isolate_65_AK3	KP218354
Isolate_728_KE5	KP218381	Isolate_64_AK2	KP218353
Isolate_727_KE6	KP218380	Isolate 63_AK3	KP218352
Isolate_726_KE6	KP218379	Isolate_62_AK3	KP218351
Isolate_725_KE5	KP218378	Isolate_61_AK3	KP218350
Isolate_724_KE5	KP218377	Isolate_60_AK2	KP218349
Isolate_723_KE5	KP218376	Isolate_6_KAT1	KP218348
Isolate_721_KE4	KP218375	Isolate_59_AK2	KP218347
Isolate_720_KE4	KP218374	Isolate_58_AK2	KP218346
Isolate_72_AK4	KP218373	Isolate_57_AK2	KP218345
Isolate_719_KE3	KP218372	Isolate_56_AK2	KP218344
Isolate_724_KE5	KP218377	Isolate_60_AK2	KP218349
Isolate_723_KE5	KP218376	Isolate_6_KAT1	KP218348
Isolate_721_KE4	KP218375	Isolate_59_AK2	KP218347
Isolate_720_KE4	KP218374	Isolate_58_AK2	KP218346
Isolate_72_AK4	KP218373	Isolate_57_AK2	KP218345
Isolate_719_KE3	KP218372	Isolate_56_AK2	KP218344
Isolate_717_KE2	KP218371	Isolate_55_AK2	KP218343
Isolate_716_KE1	KP218370	Isolate_54_AK1	KP218342
Isolate_714_KE2	KP218369	Isolate_53_AK1	KP218341
Isolate_713_KE1	KP218368	Isolate_52_AK1	KP218340
Isolate_712_KE2	KP218367	Isolate_51_AK1	KP218339
Isolate_711_KE1	KP218366	Isolate_50_TA5	KP218338
Isolate_71_AK4	KP218365	Isolate_5_KAT	KP218337
Isolate_70_AK4	KP218364	Isolate 495_TA5	KP218336
Isolate_7_KAT1	KP218363	Isolate_493_TA5	KP218335
Isolate_69_AK2	KP218362	Isolate_492_TA5	KP218334
Isolate_717_KE2	KP218371	Isolate_55_AK2	KP218343
Sequence number	**Accession number**	**Sequence**	**Accession**

Isolate_713_KE1	KP218368	Isolate_52_AK1	KP218340
Isolate_712_KE2	KP218367	Isolate_51_AK1	KP218339
Isolate_711_KE1	KP218366	Isolate_50_TA5	KP218338
Isolate_71_AK4	KP218365	Isolate_5_KAT	KP218337
Isolate_70_AK4	KP218364	Isolate 495_TA5	KP218336
Isolate_7_KAT1	KP218363	Isolate_493_TA5	KP218335
Isolate_69_AK2	KP218362	Isolate_492_TA5	KP218334
Isolate_102_AK5	KP218267	isolate_734_KE2	KP218388
Isolate_101_AK5	KP218266	isolate_733_KE2	KP218387
Isolate_100_AK5	KP218265	isolate_732_KE2	KP218386
Isolate_10_KAT1	KP218264	isolate_731_KE2	KP218385
Isolate_1_KAT1	KP218263	isolate_730_KE5	KP218384
Isolate_742_KE3	KP218397	isolate_73_LO1	KP218383
Isolate 741_KE1	KP218396	Isolate_19546_CH	KP218287
Isolate_33_TA5	KP218315	Isolate_19545_CH	KP218286
Isolate_32_TA4	KP218314	Isolate_19544_CH	KP218285
Isolate_30_TA1	KP218313	Isolate_19543_CH	KP218284
Isolate_3_KAT1	KP218312	Isolate_19541_CH	KP218283
N.m. meleagris	NC_006382	*Acrylium vulturinum*	NC_014180

Appendix 2: Genbank accession numbers of HSP70 reference sequences

Organism	Accession number
Mallard duck	XM_005022658
Japanese quail	AB259847
Turkey	XM_010721161
Common quail	EU622852
Helmeted Guinea fowl	AB096696
Northern fulmar	XM_009576438
Common ostrich	XM_009675580
Rook pigeon	XM_005506375
Red throated loon	JJRM01051595

Appendix 3: Publication from this work

Panyako P M, Imboma T, Kariuki D W, Makanda M, Oyier P A, Malaki P, Ndiema E K, Obanda V, Agwanda B, Ngeiywa K J, Lichoti J and Ommeh S C (2016)**:** Phenotypic characterization of domesticated and wild helmeted Guinea fowl of Kenya. *Livestock Research for Rural Development*, 28(9), #158, 1-12..

Appendix 4: Questionnaire for the phenotypic characterization of domesticated helmeted Guinea fowl populations in Kenya

1. Farmers name…… Region …….
2. Location…… GPS……..
3. Enumerators name……. Date of interview……
4. Number of Guinea fowls…… Outside Temperature…….. …….
5. How long have Guinea fowls been kept in the household? .....
6. Source of foundation stock…….
7. Age of Guinea fowl……
8. Do you feel the need to improve Guinea fowl production? Yes ( ) No ( )
9. Traits to improve in the Guinea fowls……
10. What type of management system do you practice? Extensive ( ) semi-intensive ( ) intensive ( ) others, specify…….
11. Do you give supplementary food to your Guinea fowls? Yes ( ) No ( )
12. If you give feeds how frequently do you feed your birds daily? .....
13. How often do you cull your birds?.....
14. For what purpose do you cull your poultry? ( ) for consumption, ( ) sale, ( ) sacrifice, ( ) others, specify……

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

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

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

18. Morphometry

19. Age in months……

20. Sex; male ( ) female ( )

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

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

23. Wattle color; Red ( ), blue ( ), others ……

24. Guinea fowl phenotype; red wattled ( ), blue wattled ( ), others……

25. Plumage density; dense ( ) normal ( ) scares ( )
26. Body Temperature ……..

Other general issues

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

28. If yes to what size……

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

30. What traits do you wish to see improved in domesticated Guinea fowls? .....

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