ORIGIN, DIVERSITY AND HSP70 GENE FUNCTIONAL POLYMORPHISM OF THE HELMETED GUINEA FOWL IN KENYA

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Abstract

Despite the importance of helmeted Guinea fowls, little is known about their origin and genetic background. This study aimed to understand the genetic background of helmeted Guinea fowls in Kenya through the characterization of their morphological traits, analysis of the mitochondrial DNA D-loop and identification of polymorphisms in the HSP70 gene associated with drought/heat tolerance. Ninety (90) Guinea fowls randomly selected from four domestic populations (70 individuals) and a wild population (20 individuals) sampled from Bungoma, Busia and Laikipia counties in Kenya were scored for primary phenotypic characteristics (shank length, body length, wing length, helmet width, helmet height, head size, live body weight, wattle colour, skin colour and shank colour). DNA was also extracted from blood collected from the 90 individuals. The phenotypic data collected were analyzed and the descriptive statistics obtained compared as percentages, continuous bar graphs and tables using Excel version 2013 and R Core version 3.1.2. Two wattle colour variations were identified, red and blue. Skin colours were grey and white while shank colours were black, pink and grey. The wild population showed the highest mean values for the quantitative traits measured except live body weight and head size. The populations of Kenyan helmeted Guinea fowls showed a possible limited heterogeneity in the qualitative traits considered. There was a possible positive (0.333) and significant ($p<0.05$) correlation between body temperature of the birds and outside temperature. The first 700bp of the mtDNA D-loop and first 600bp of HSP70 were amplified, sequenced and edited. The 25 mitochondrial DNA haplotypes identified will be compared to those observed in other parts of Africa to determine origin and diversity. The SNPs in HSP70 discovered will also be compared with reference sequences from Genbank to identify polymorphisms associated with drought/heat tolerance. These findings present a genetic pool from which decisions on sustainable use and conservation of helmeted Guinea fowls could be made. This would help farmers, breeders and conservationists to genetically improve domestic helmeted Guinea fowls and also improve their survival in the wild.

Key words: agro-ecological zones, climatic change, morphological traits, *Numida meleagris,* population

1.0 Introduction

The helmeted Guinea fowl (*Numida meleagris*), is one of Africa's most widespread and abundant terrestrial game bird and can be found in a broad range of sub-Saharan, open country vegetation types (Crowe et al 2006;Walker et al 2004). The advent of Guinea fowls in the history of human activity is traced back to the Egyptian fifth dynasty about 2,400 B.C. when its figure was drawn in a mural(Nishibori et al 2004). Early domestication is supposed to have occurred in Southern Sudan and West Africa (Crawford 1990; Nishibori et al 2004). There were at least several independent domestications involving more than one subspecies. Present day domesticated Guinea fowls were probably all derived from the West African subspecies *Numida meleagris galeata* (Walker et al 2004) and then introduced repeatedly worldwide (Long 1981; Hastings Belshaw 1985; Donkin 1991).

Two types of helmeted Guinea fowls are found in Kenya. These include the red wattle and the blue wattle. The domesticated Guinea fowls are red wattled while the blue wattle helmeted Guinea fowls are the most numerous in the wild and are found in almost every ecological zone, from the coast to the shores of Lake

Victoria in Kenya. The widespread distribution of helmeted Guinea fowls in Kenya suggests they are adapted to the local environmental conditions such as drought and heat.

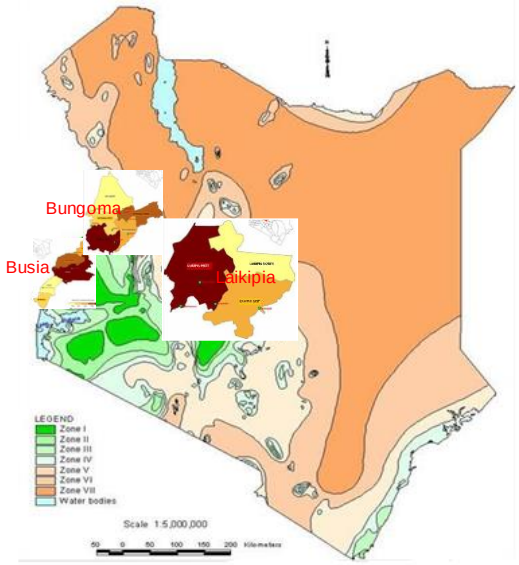
Guinea fowl production as a rural poultry enterprise has a lot of potential if kept and managed well (Agbolosu et al 2015). Guinea fowls are widely exploited in West Africa (Adeola et al., 2015). They are a ready source of animal protein (meat and eggs), income, welcoming of important guests, funerals, gifts, sacrifices, payments of dowries as well as 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 flavor is relished by the local farmers and can contribute substantially to supply the protein needs of the rural populations especially in Western Kenya.

There is need to understand the origin and genetic diversity of the helmeted Guinea fowls of Kenya and how they have adapted to local environmental conditions such as drought and heat. Mitochondrial DNA is widely used as a marker to study origin and diversity of species, while polymorphisms in the HSP70 gene have been postulated to be associated with drought/heat tolerance traits in birds. Tolerance or susceptibility of birds to stressful environment could also be linked to their phenotypic traits (Egahi et al 2010; Agbolosu et al 2015). Analysis of mtDNA is therefore expected to help understand the origin and genetic background of the helmeted Guinea fowl. Characterization of morphological traits and study of functional polymorphisms in the HSP70 gene is equally expected to help in understanding the drought/heat tolerance traits in these birds and how they have adapted to the local environmental conditions.

Studies have previously been carried on phenotypic and morphological traits of indigenous Guinea fowls in Ghana (Mogre 2010; Agbolosu et al 2015). Work on mitochondrial DNA variation of Nigerian domestic helmeted Guinea fowls has recently been done by Adeola et al (2015). However, neither the diversity of Guinea fowls nor polymorphisms in a candidate gene associated with drought/heat tolerance in the helmeted Guinea fowls has been studied in Kenya. This study therefore aimed to identify the primary phenotypic variations of helmeted Guinea fowl populations in Kenya such as shank length, body length, wing length, helmet width, helmet height, head size, live body weight, wattle colour, skin colour and shank. It also aimed to study the origin and genetic diversity of helmeted Guinea fowls and polymorphisms in their HSP70 gene associated with drought and heat tolerance. Information generated from the study will support conservation efforts for these birds and help develop breeding programs aimed towards increasing Guinea fowl production in the country. This will go a long way in ensuring future food security and wildlife conservation.

2.0 Materials and Method

2.1 Study area



The study was carried out from September 2014 to January 2015 in five regions of Kenya with varying agro-climatic features. The five regions comprising five populations are Teso North, Bungoma South, Bungoma West, Mt. Elgon and Laikipia (where wild Guina fowls were sampled). Five populations comprising four populations of domesticated helmeted Guinea fowls (Teso North, Bungoma South, Bungoma West and Mt. Elgon) and the wild population from private sanctuaries in Laikipia were sampled. Teso North, Bungoma South, Bungoma West and

Mt. Elgon are found in Western Kenya and 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 while Laikipia is located in the Rift Valley region of Kenya and lies 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). Western Kenya receives a yearly rainfall of 950-1,500mm and Laikipia receives on average an annual rainfall of 300-600mm. The vegetation type is mostly forest-mosaic and savannah. Wild Guinea fowls are free scavenging mobile birds found in the wild while domestic populations are kept in homesteads mostly by small scale low income rural farmers under free range systems where they scavenge for food around these homesteads during the day.

2.2 Ethical Clearance

This study received ethical clearance from the Kenya Wildlife Service under permit number KWS/BRM/5001 to sample wild Guinea fowls and a "no objection for the research" from the director of Veterinary Services, Ministry of Agriculture, Livestock and Fisheries in Kenya under permit number RES/POL/VOL.XXVII/162 to sample domestic Guinea fowls.

2.3 Study Design and Sample Collection

Five regions were selected within Western Kenya and Laikipia County of Kenya. These are Bungoma South, Teso North, Bungoma West, Mt. Elgon and Laikipia. These regions represented five populations; namely Bungoma South (13 individuals), Teso North (18 individuals), Bungoma West (18 individuals), Mt. Elgon (21 individuals) and the wild population (20 individuals) respectively making a total of 90 adult individuals. Sample size was chosen based on the recommendations of Hale et al. (2012) for population genetic analyses. 70 individuals comprising the populations of Bungoma South, Teso North, Bungoma West and Mt. Elgon were domestic while 20 individuals were wild.

Although Guinea fowls exhibit almost no sexual dimorphism (Crawford 1990), the size and shape of the head, helmet and wattle was used to distinguish sexes as recommended by Ayorinde (2004). Males are usually slightly larger than females and have more pronounced helmets and wattles. Wild birds were caught by blinding using Maglite torches at their roost sites and by use of foot traps. The domestic birds were usually baited by their owners and caught. Care was taken to handle these birds as humanely as possible.

This study only considered domestic populations in Western Kenya and the wild population in Laikipia. Western Kenya was chosen because it is the focal point of Guinea fowl diversity and migration from West Africa through Central Africa. The study also focused on Western Kenya because it is a major source of domestic Guinea fowls which are reared by most low income rural households.

Sampling was done through a rural participatory approach. Farmers were chosen based on willingness to participate in the survey. Informal and formal field surveys were conducted through observation, oral interviews and questionnaires in the selected regions to explore available knowledge about the types, distribution and utility of helmeted Guinea fowl varieties. Interviews were conducted on the Kenya Wildlife Service (KWS) warders and farmers with the assistance of local agricultural extension officers. More information about domesticated Guinea fowls was provided by the local agricultural extension officers. In the survey, information on the phenotypic characteristics of Guinea fowl phenotypes was recorded. Moreover, visual appraisal of the appearance of the Guinea fowls and their typical features of environmental adaptations were collected using a structured questionnaire for morphological description (Batty and Francis 1979). Morphologically distinct Guinea fowls were identified using phenotypic traits such as wattle colour, skin colour, shank colour, tarsus length, body length, live body weight, wing length, head size, helmet width and helmet height following the standard descriptor as recommended by Food and Agriculture Organization (2012) and the Guinea fowl colour chart (GFIA, 2009). Body measurements were done using a flexible measuring tape graduated in centimeters and a venier caliper graduated in millimeters.

2.4 PCR Amplification

The 700 base pairs of the mtDNA D-loop region of *Numida meleagris* were amplified via PCR using the forward primer AVIF2 5'-AGGACTACGGCTTGAAAAGC-3' (20) and reverse primer CR1b 5'-CCATACACGCAAACCGTCTC-3' (20). PCR amplifications were carried out in 25 µM reaction volumes containing 20 ng genomic DNA, 1 XPCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl, 0.1% Triton X-100), 2.5 mM of each dNTP, 10 pM of each primer and 1 unit of Taq DNA polymerase (Promega, Madison WI, USA). Thermo cycling conditions were as

follows: 94°C (3min), 30 cycles of 94°C (30 sec), 55°C (30 sec) and 72°C (30 sec) and a final extension step at 72°C (7 min).

The *Gallus gallus* HSP70 ortholog in *Numida meleagris*, that is NmHSPA2, was amplified via PCR using the forward primer HSP70-F 5'-ATCATCGCCAATGACCAGGG-3' (20) and reverse primer HSP70-R 5'-CATTCTTCTCTCCAGCCCGG-3' (20). PCR amplifications were performed in 10ml reaction volumes containing 1 ml 50 ng genomic DNA, 1ml 10 XPCR buffer, 1 ml 3 mM primers, 1ml 1 mM of each dNTP (/Promega, USA), 0.8 Ml 25^2m M $MgCl_2$, and 0.1 Ml 5 U Taq DNA polymerase. The PCR was run under the following conditions: 5 minutes at 94°C, followed by 30 cycles of 30s at 94°C, for 30-45s and 45-90 at 72°C, with a final extension of 5 minutes at 72°C.

2.5 PCR Purification and Sequencing

PCR products were purified using the Wizard SV Gel and PCR Clean-Up Kit (Promega, Madison WI, (USA). Purified products were sequenced directly using the Big Dye Terminator v3.1 (Applied Bio systems, USA) on an ABI prism 3730 Avant DNA analyzer. The relevant PCR primers were used for the sequencing reactions

2.6 Data Analysis

Data obtained from phenotypic descriptors for drought and heat tolerance were analyzed using descriptive statistics in R Core version R.3.1.2 statistical software and Excel version 2013.

The gene sequences of the heat shock protein 70 and mtDNA D-loop region were retrieved from Genbank databases. SNP discovery was then done manually. The sequences generated were edited manually using Chromas Lite version 2.1.1 (Hall, 1999) and subsequently joined to reconstruct a fragment of 351-353 bp for mtDNA D-loop region and 507 bp for HSP70. For both HSP70 and mtDNA, the sequences were aligned using ClustalX version 2.1 (Thompson *et al*., 1997) against a reference sequence Gen Bank accession number AP005595 (Crowe *et al*., 2006) for mtDNA and AB096696 (Iwamoto *et al*., 2005) for HSP70. Subsequent analyses for mtDNA were restricted to the regions incorporating the first hyper-variable segment (HVS1).

3.0 Results

3.1 Phenotypic Measurements

The results showed that the colours of the wattle, skin and shank of both the domesticated and wild adult Guinea fowls were variable (Figure 1(a) and (b)). Two wattle colour types (red and blue) were observed among the local Guinea fowls. All individuals in the domestic populations of Bungoma South, Teso North, Bungoma West and Mt. Elgon had red wattles. All the wild individuals were observed to be blue wattled.

a) *b)*

Figure 1 (a) and (b):Pictures showing wattle, skin and shank colours in helmeted Guinea fowls of Kenya

The skin colour distribution was observed to be mostly grey with a few individuals observed to be of white skin. Shank colours observed were mostly black with a few pink and grey.

The mean shank length, body length, live body weight, wing length, head size, helmet width and helmet height of the sampled adult Guinea fowls were also compared by population. It was observed that the wild population registered high mean values for all quantitative traits measured except live body weight and head size.

There was a positive (0.333) and significant ($p<0.05$) correlation between body temperature of the birds and the outside temperature, indicating that body temperature generally increased as the outside temperature increases.

3.2 Mitochondrial DNA Analysis

The gel image of the amplified D-loop region of the mitochondrial DNA of the helmeted Guinea fowl is shown in Figure 2. Mitochondrial DNA is widely used as a molecular marker to deduce genetic background of organisms.

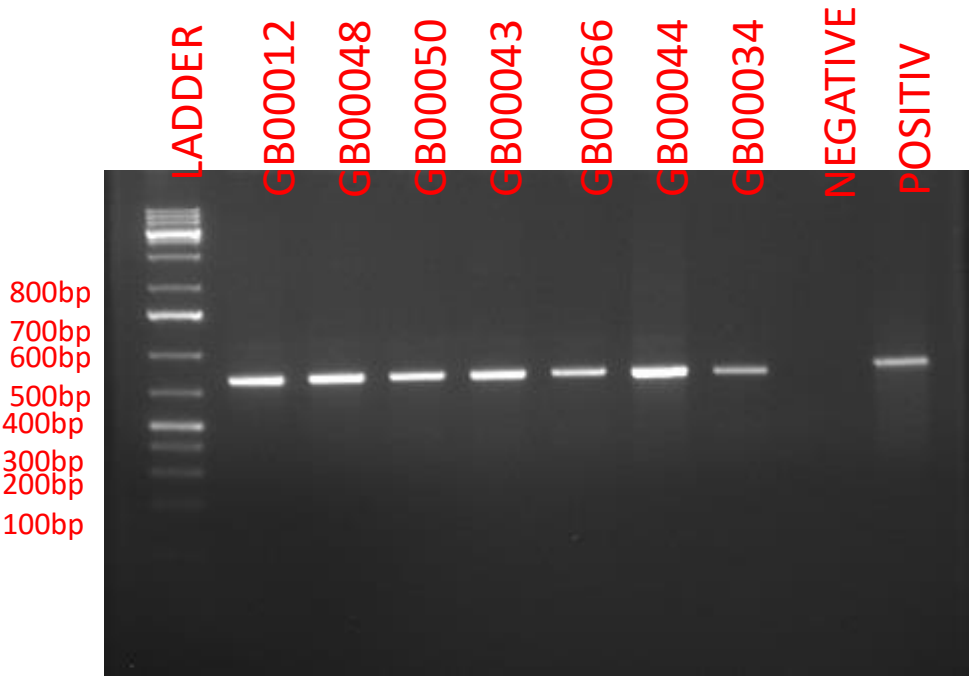


Figure 2: Gel image of the mtDNA of selected helmeted Guinea fowls in Kenya

The chromatograms and multiple sequence alignment of a variable region showing polymorphic sites with insertions and/or deletions are shown in Figure 3 and 4 respectively.

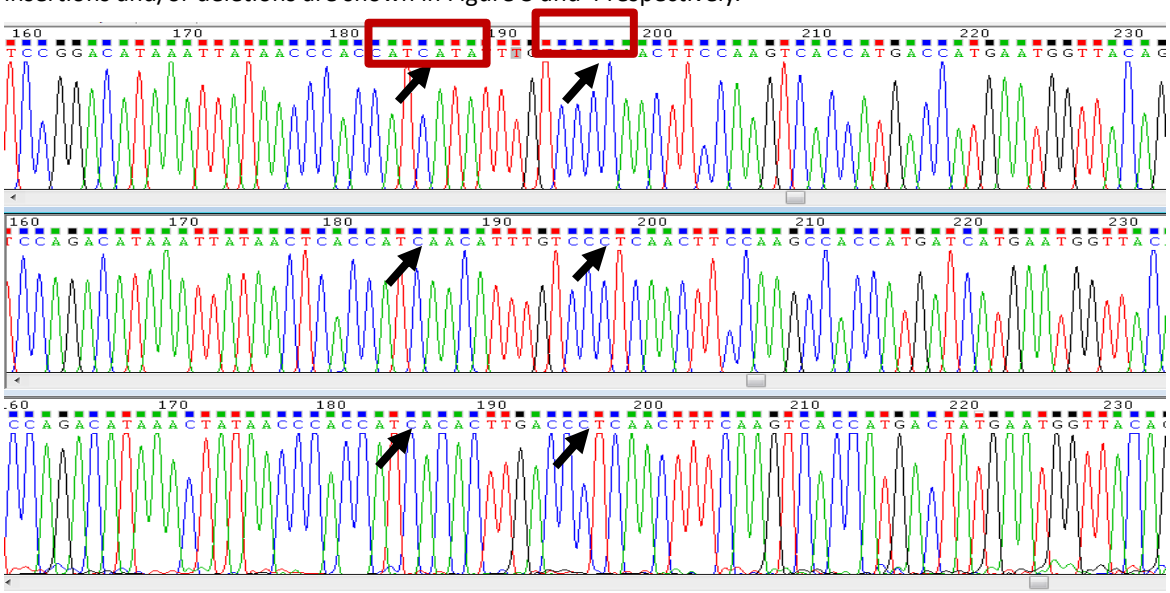


Figure 3: Chromatogram of the mtDNA variable region of selected helmeted Guinea fowls in Kenya

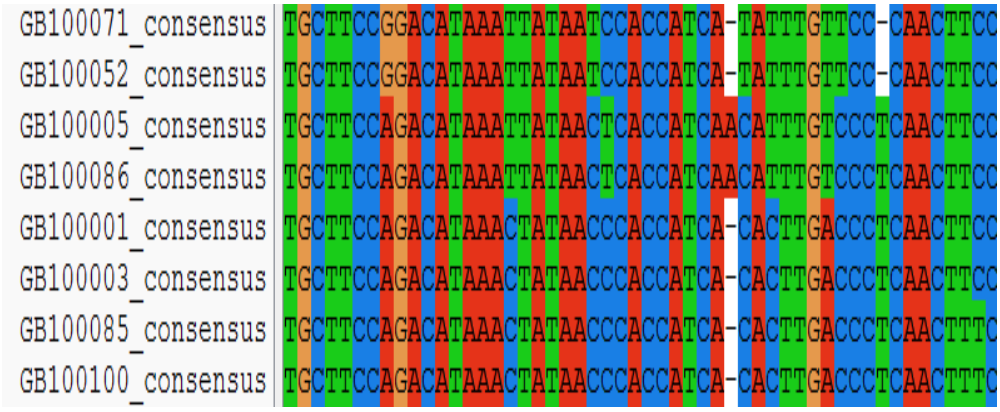


Figure 4: Multiple sequence alignment of the mtDNA variable region of selected helmeted Guinea fowls in Kenya

It was observed that the mitochondrial DNA sequence of all the wild Guinea fowls sampled was one base pair longer than their domestic counterparts. This is a result of an insertion/deletion (INDEL) of a thymine nucleotide. Within the wild individuals, two individuals were also observed to be a nucleotide longer than the rest, a result of another INDEL. The second INDEL is an adenine

3.3 HSP70 Analysis

The gel image of the amplified region of the heat shock protein 70 (HSP70) gene of selected helmeted Guinea fowls in Kenya is shown in Figure 5. Polymorphisms in HSP70 have been postulated to be associated with drought/heat tolerance traits in birds.

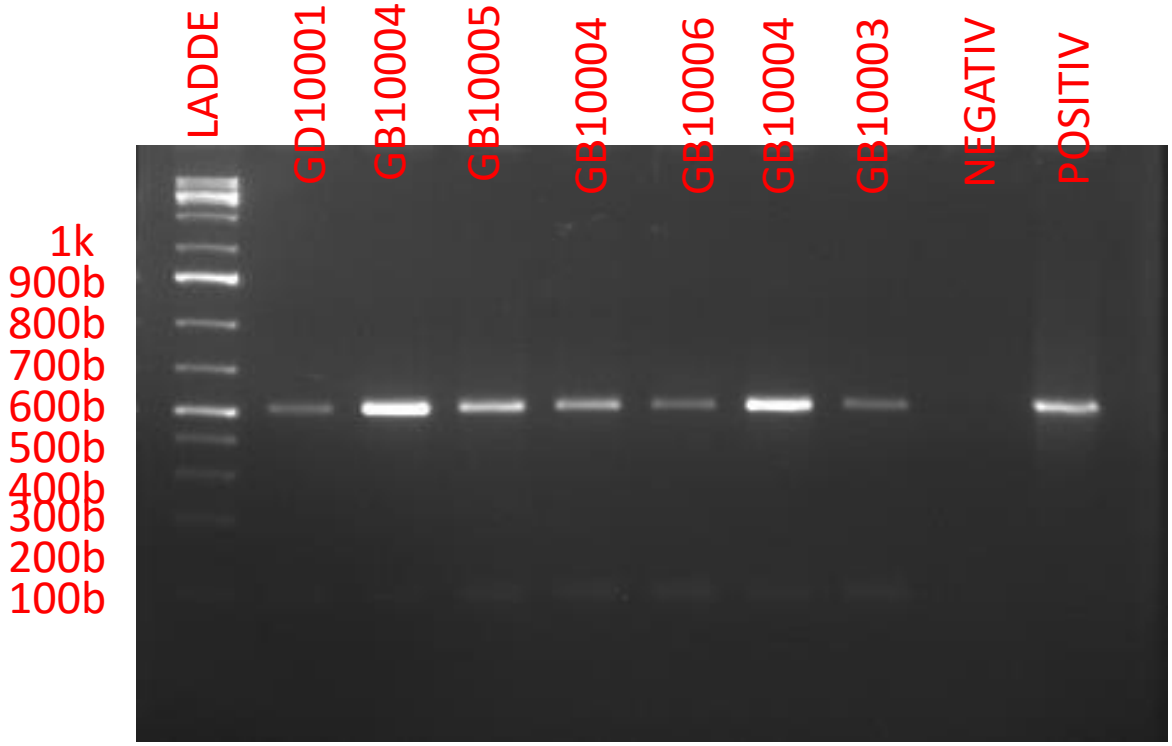


Figure 5: Gel image of the mtDNA of selected helmeted Guinea fowls in Kenya

Figure 6 below shows HSP70 chromatograms of selected helmeted Guinea fowls indicating the variable sites identified. Figure 7 are chromatograms showing mutations in the HSP70 gene of selected helmeted Guinea fowls.

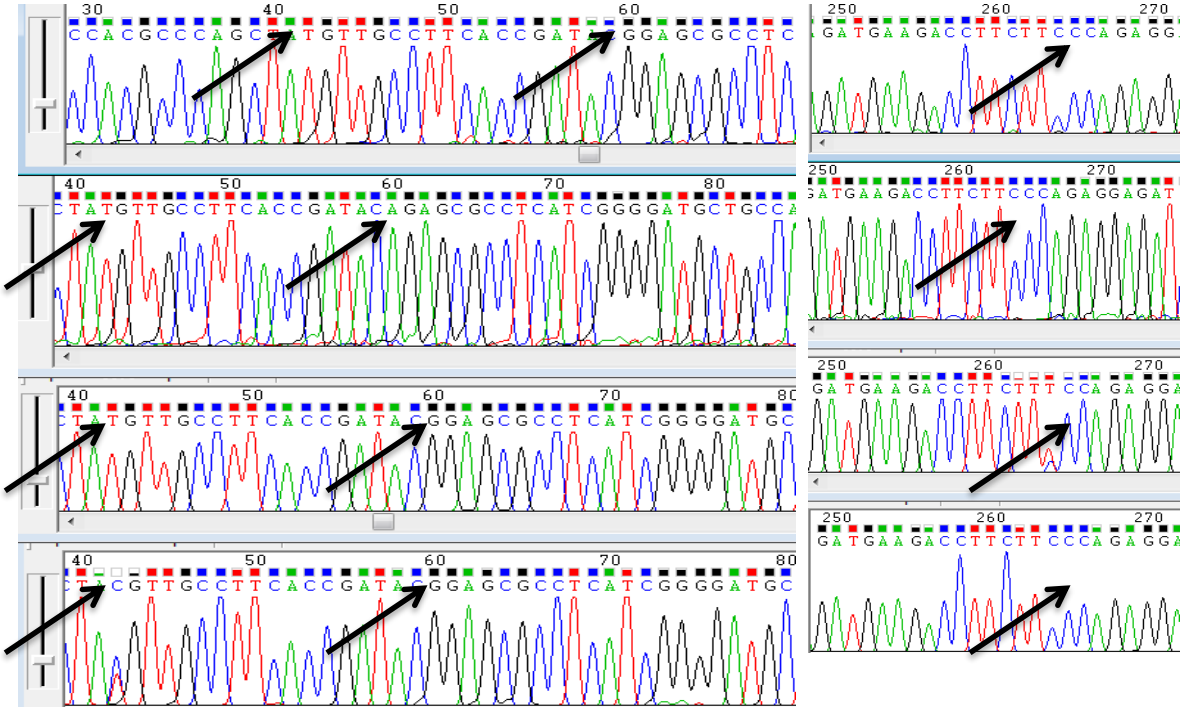


Figure 6: Chromatogram of the HSP70 variable region in helmeted Guinea fowls in Kenya

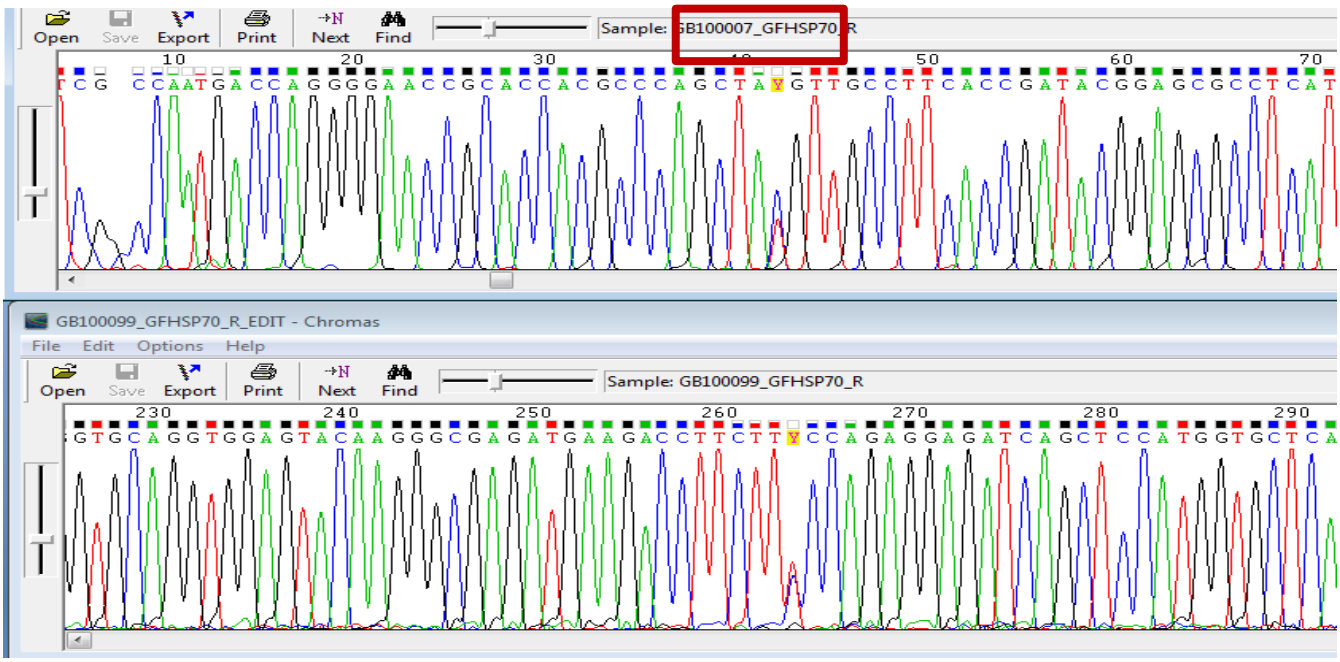


Figure 7: Chromatogram of the HSP70 mutations in helmeted Guinea fowls in Kenya

Figure 8 also shows mutations in the HSP70 gene as indicated by a multiple sequence alignment of sequences of selected helmeted Guinea fowls.

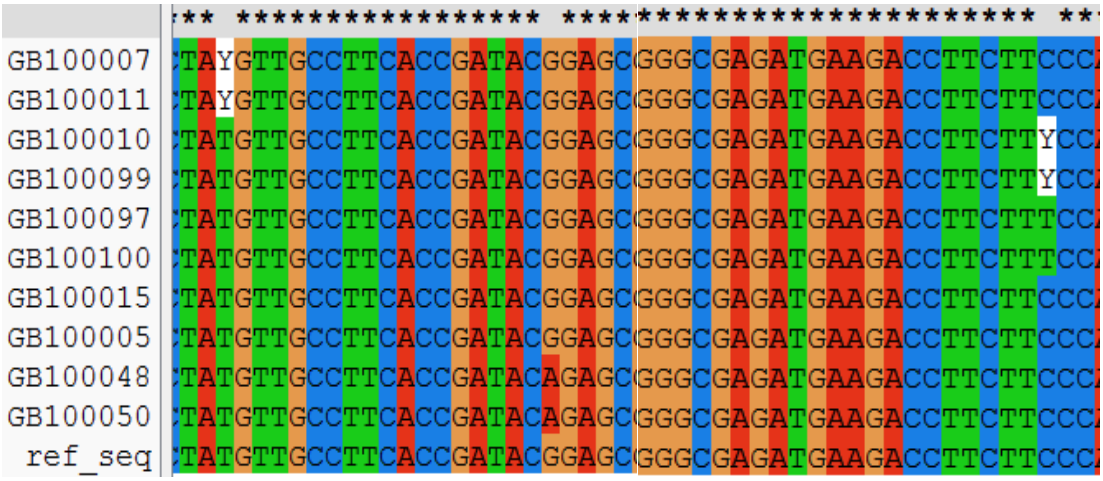


Figure 8: Multiple sequence alignment of HSP70 sequences of selected helmeted Guinea fowls in Kenya

The domestic populations of Bungoma South, Bungoma West and Mt. Elgon had only no variable region. Teso North population had one variable region while the wild population had two variable regions.

4.0 Discussion

From the results of observation of qualitative traits, we infer that domestic helmeted Guinea fowls are all red wattled while wild Guinea fowls are blue wattled. Most helmeted Guinea fowls also generally have black shanks and grey skin colour. Mogre (2010) and Agbolosu et al (2015) observed that orange and black shank colours cut across all Guinea fowl colour varieties with some cases of a mixture of orange and black also encountered. Colour plays a role in the absorption and reflection of ultraviolet radiation. Birds with black phenotypic characteristics may therefore be more susceptible to stress under intense solar radiation (Agbolosu et al 2015). Birds with white phenotypic characteristics on the other hand may be more tolerant to stress under same conditions. Wild Guinea fowls generally showed higher values for all quantitative traits measured except head size and live body weight. This could be an adaptation to to maneuver easily in the wild.

The many single nucleotide polymorphisms (SNPs) observed in the mtDNA of wild helmeted Guinea fowls is a pointer to the higher genetic diversity of these birds unlike their domesticated counterparts.

There are observed variable sites in the HSP70 gene of the wild population. All the domestic populations except the Teso North population however, had no variable site. The wild population is therefore more genetically diverse.

5.0 Conclusion and Recommendations

The helmeted Guinea fowl populations in Kenya showed limited heterogeneity in the primary qualitative traits considered with the wild population generally registering higher values for all quantitative traits measured except head size and live body weight. Mitochondrial DNA analysis showed higher genetic diversity in wild helmeted Guinea fowls with domestic Guinea fowls being less diverse. Some SNPs in the HSP70 gene were discovered in the wild population.

This study will serve as a source of information for genetic improvement to increase productivity and also help in conservation of these birds. Wild Guinea fowls can also be exploited under the national breeding program.

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