GENETIC BACKGROUND AND HSP70 GENE POLYMORPHISMS FOR HEAT TOLERANCE IN INDIGENOUS CHICKENS OF KENYA

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Abstract

It is postulated that chickens were first domesticated in Asia around 8000 years ago and spread to the rest of the world. This study investigated the origins, phenotypes as well as polymorphisms at a candidate gene implicated for heat-tolerance in indigenous chickens from four agro climatic zones of Kenya (Lamu archipelago, the Turkana basin, Victoria basin and the Mount Elgon Catchment). Phenotypic traits for heat tolerance were collected using the ODK collect suite and recorded. DNA was extracted from 296 indigenous chickens. Mitochondrial DNA d-loop region and the heat shock protein 70(HSP70) gene were amplified using an optimized PCR protocol. Analysis of the HSP70 revealed the presence of three heterozygous and three homozygous sequences. The ancestral red jungle fowl was used as the reference sequence.

DnaSP generated 28 mtDNA haplotypes. Our samples clustered into four of the five reference haplogroups downloaded from GenBank. Most of our samples clustered into Haplogroup E. Haplogroup E. This study characterized the phenotypes of indigenous chickens, characterized the origins and HSP70 gene functional polymorphisms in the various climatic regions of Kenya

Key words: arid and semi-arid lands, climate change, drought, heat stress, poultry

1.0 Introduction

Chickens are from Kingdom *Animalia*, Phylum *Chordate*, Class *Aves*, Order *Galliformes*, Family *Phasianidae*, Subfamily *Phasianinae*, and Genus *Gallus*. There are four species of chickens namely: *Gallus gallus* (red jungle fowl), *Gallus lafayettei* (Ceylon jungle fowl), *Gallus sonnerati* (Grey jungle fowl), and *Gallus varius* (green jungle fowl). Domesticated chickens belong to Subspecies *Gallus gallus domesticus* (Eriksson et al., 2008). Chickens were first domesticated in Asia as early as 3200BC and since then they have dispersed to the rest of the world (Mwacharo et al., 2011). Africa and Asia are connected by a terrestrial route and ever since historical times, they have experienced important seafaring and maritime exchange of people, crops and livestock like indigenous chickens by the maritime traders (Boivin and Fuller, 2009). It's postulated that in Africa, chickens were first introduced via Egypt where they were dispersed southwards into East Africa following the Nile river basin (Macdonald et al., 1992). In Kenya they were introduced at around in 800 AD (Horton and Mudida, 1993). Since their introduction in Kenya, indigenous chickens have spread throughout the country and have adapted into various agro-climatic zones. There are six phenotypes of indigenous chickens in Kenya that were described by Kingori et al. (2010) and they are the, frizzled-feathers, naked-neck, barred-feathers, feathered-shanks, bearded and dwarf . Another study by Moraa et al. (2015) described an additional six phenotypes: The rumpless, kuchi, mixed, plain feathered, crested and mottled phenotypes. These ecotypes are spread throughout the seven agro-climatic zones in Kenya (Nyaga, 2007). However, the local genetic pool was affected in the 1970's by the cockerel and pullet exchange program spearheaded by the national poultry breeding program in several counties. This program led to genetic dilution of pure indigenous breeds in Kenya in the affected (Nyaga, 2007). Most of the areas that were not affected by this programme are in the Arid and semi-arid regions. These regions are considered very dry and are characterized by high ambient temperatures. There is a possibility that indigenous chickens in these areas still have adaptive traits for heat tolerance. Indigenous chickens in these regions, just like other organisms, have a group of highly conserved proteins that are produced when exposed to high temperatures. These proteins are known as the Heat Shock Protein 70 (HSP70) and they protect the cells of the body against the toxic effects of heating thereby enabling their survival in heat stressed environments (Mazzi et al., 2003)

2.0 Materials and Methods

This study was conducted in Lake Turkana basin, Lamu archipelago, Lake Victoria basin and Mt. Elgon catchment. These were the preferred areas since Mt. Elgon catchment and Lake Turkana basin experience extreme climatic conditions whereas Lake Victoria basin and Lamu archipelago experience intermediate weather conditions. Besides, these areas were not affected by the cockerel and pullet exchange program. These areas are indicated in figure 1 below.

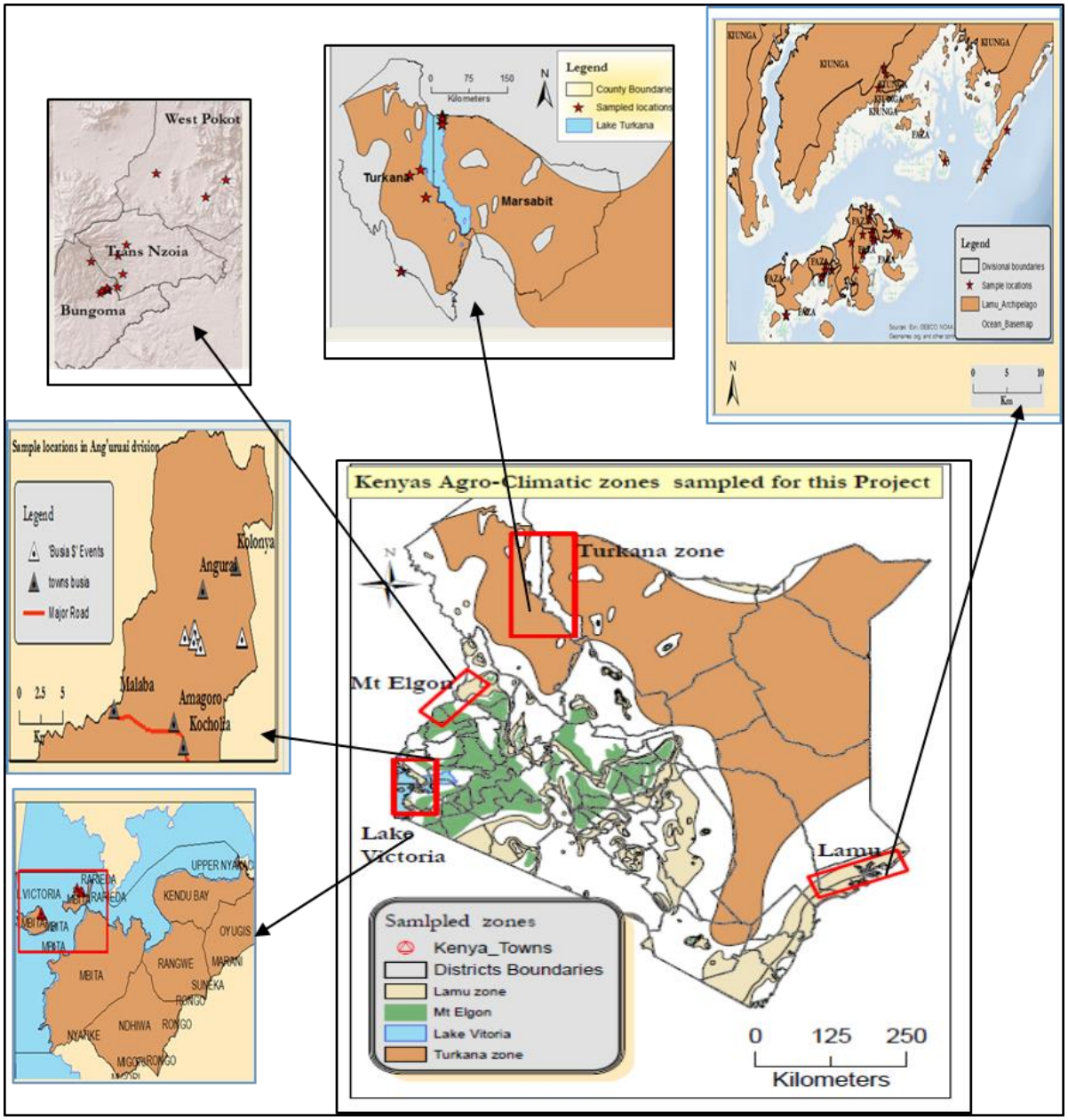

Figure 1: Study areas

2.1 Clearance

Permit of no objection to conduct this study was obtained from the department of Veterinary Services, State Department of Livestock, Ministry of Agriculture, Livestock and Fisheries, Kenya.

2.2 Study Design

We collected our samples at the farms by the use of a rural participatory approach whereby we explained to the farmers about our research in order to obtain consent from them. A pre-tested questionnaire on open data kit (ODK) on phones was used to obtain the phenotypic data of the indigenous chickens. We collected blood samples from 296 genetically unrelated indigenous chickens from 10 populations each population having 20-30 individuals as recommended by (Hale et al., 2012). These were collected from Lake Turkana

basin, Lake Victoria basin, Lamu archipelago and Mt. Elgon catchment. Two mature birds were sampled per flock as previously described by (Mwacharo et al., 2011).

2.3 DNA Extraction

All blood samples were spotted on FTA® Classic cards (Whatman Biosciences) and stored at room temperature prior to DNA extraction. Genomic DNA was extracted from all the samples using an in-house protocol.

2.4 PCR Amplification, Purification and Sequencing

The first 360-bp of the HSP70 gene was amplified via PCR using primers HSPF (5'AACCGCACCACACCCAGCTATG-3') ;HSPR (5'CTGGGAGTCGTTGAAGTAAGCG-3') (Akaboot et al., 2012). This position corresponds to position 52784283 to 52784642 of the Galgal4 on chromosome 5. PCR amplifications were carried out in a 10µlreaction volumes containing 3.8µl of double distilled water, 1µl of template genomic DNA, 5µl Dream Taq Green Master Mix (2X), 0.2µl Primer (forward + reverse, 20pmol/µl). Thermo-cycling conditions were: 94^0 C (3min), 5 cycles of 94^0C (15 seconds), 60^0C (30 seconds) and 72^0C (30 seconds) and a final extension step at 72^0C (10 minutes). The PCR products were run on 1% agarose gel using 1X TBE buffer (89Mm Tris, 89mM boric acid, 2Mm Na_2EDTA) in a voltage of 100V for 25 minutes. The gels were stained with GelRed™ Nucleic acid gel stain and visualised under UV light (BTS-20 model, UVLtec Ltd., UK). One kb DNA Ladder was used to identify the approximate size of the molecule run on a gel. All PCR products that were positive upon visualization on agarose gel were sequenced at Macrogen in Europe. The products were sequenced in both the forward and reverse directions using Sanger ABI 3730 method.

Results: **Indigenous Chicken Phenotypes**

Figure2: Sampled phenotypes A: Naked neck, B: Kuchi, C: Plain feathered, D: Mottled, E: Crested: F: Barred feathers G: Frizzled H: Feathered shanks I: Mixed, J: Rumpless, K: Bantam

A total of 11 phenotypes were observed that varied across the various climatic conditions in various locations of the country. Theses phenotypes are shown in figure 2 above. These phenotypes varied in feather coverage, pattern, colour, density and phenotypes. The plain feathered phenotypes and the dwarf phenotypes were observed in Lake Turkana basin which is a very dry land that experiences harsh climatic conditions. Chickens with dense plumage, the crested and feathered shank phenotypes were present in Mt. Elgon catchment which is a highland and is characterized by low temperatures.

2.5 Amplification of Mitochondrial DNA (mt-DNA) d-loop Region

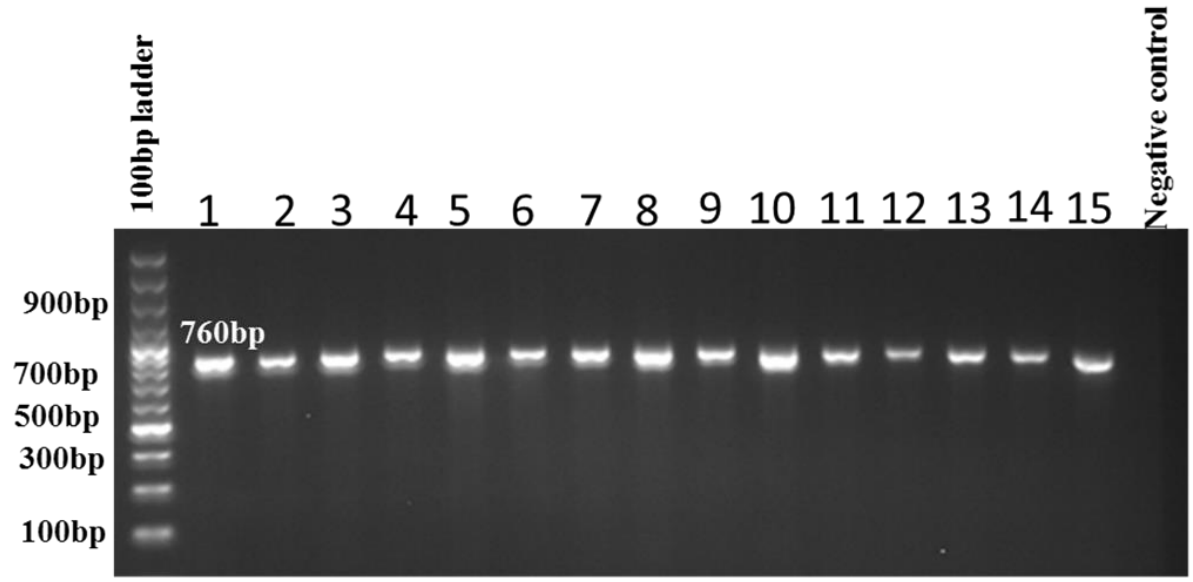

Figure 3: PCR amplification gel image of d-loop region of mtDNA in indigenous chickens of Kenya. Key: 1-14-various chicken samples, 15-positive control

After DNA extraction amplification was done on all the 296 samples. Figure 3 displays a 2% agarose gel electrophoresis with representative results that were obtained with primers targeting the d-loop region of mtDNA in indigenous chickens of Kenya. The amplicons size was 760 base pairs. This showed positive amplification. All the 296 indigenous chicken samples showed positive amplification. Water was used as a negative control. The samples were thereafter purified and sequenced.

2.6 Sequence Chromatograms of mtDNA Indigenous Chicken Samples

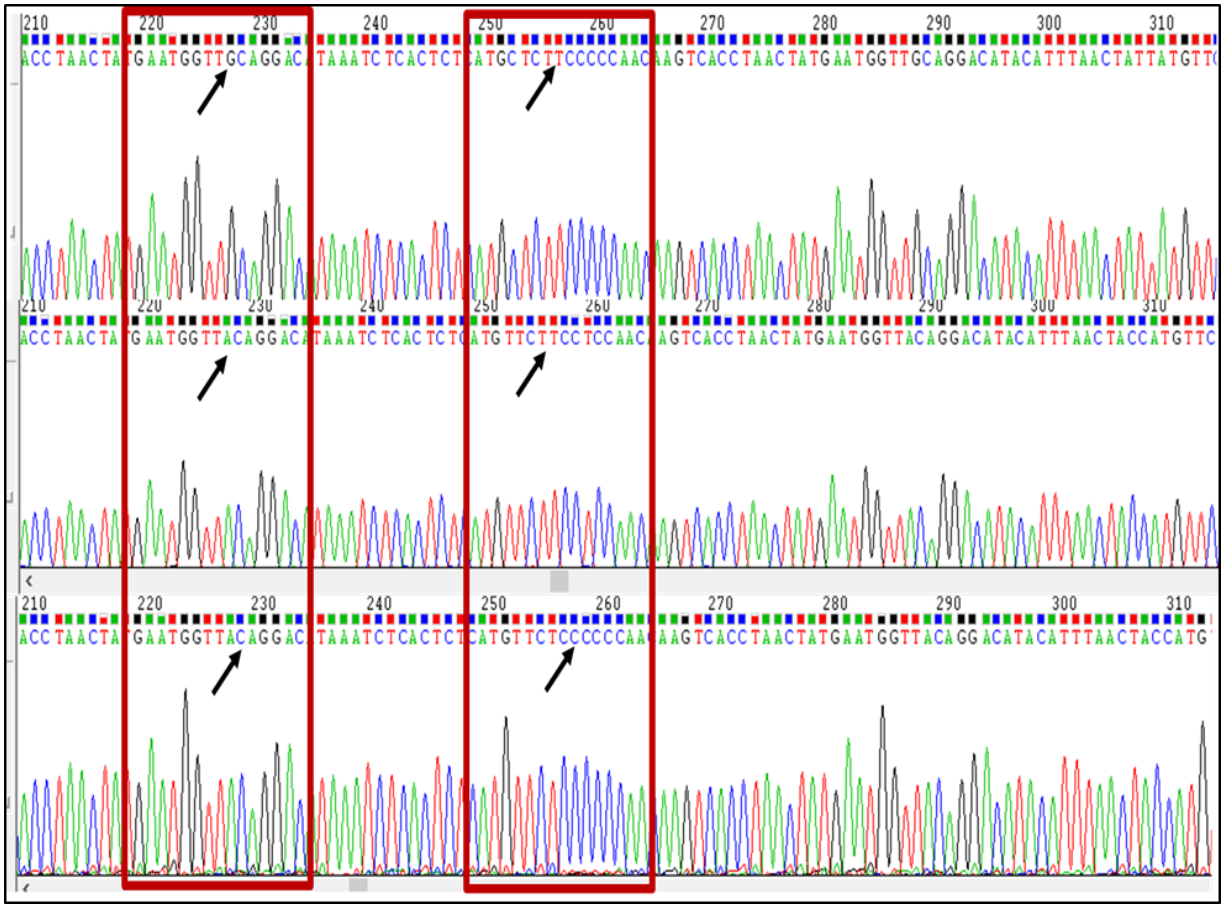

Figure 4: Sequence chromatogram of various mtDNA samples of indigenous chickens. The arrows show some regions of polymorphism

After sequencing, the chromatograms were edited manually by the use of Chromas Lite 2.1 (Avin, 2012). Figure 4 above shows polymorphism result at various locations. This has been indicated by the arrows. The first location was position 227 where there is a transition where a purine base adenine is replaced by another purine guanine. Position 256 shows a transition between pyrimidine base thymine with another thymine base cytosine. The arrows show regions of nucleotide differences. The various samples varied at different positions. A total of 28 variable sites were observed in all the sequences.

2.7 Multiple Sequence Alignment of mtDNA Indigenous Samples with Reference Sequences from Genbank

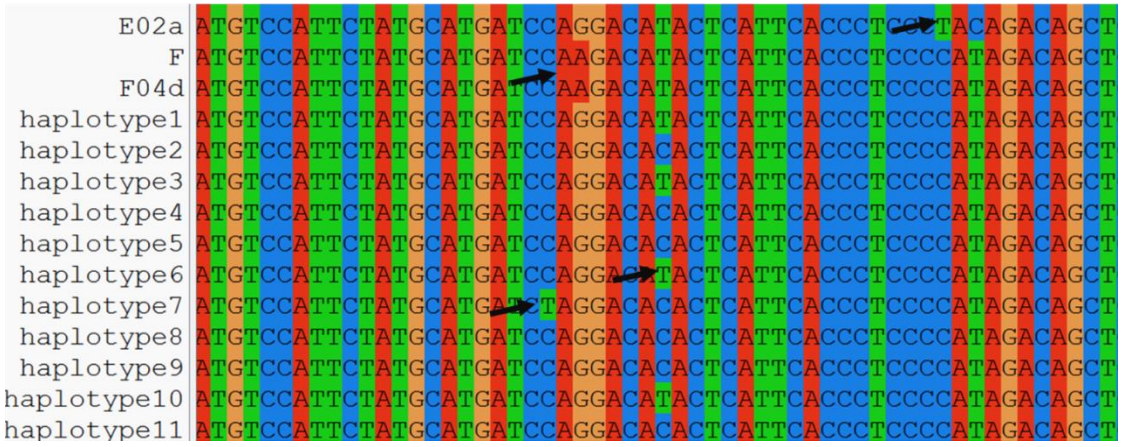

Figure 5: A multiple sequence alignment of some of the 28 haplotypes and 9 clades used as reference downloaded from Gen Bank.

A multiple sequence alignment was done with Clustal X2 software (Thompson et al., 1997). The 28 haplotypes and reference sequences by (Dana et al., 2011) were aligned using this software. This was done to show variable regions in the haplotypes and the reference sequences. Variable sites are clearly indicated by the arrows and this is clearly indicated in figure 5 above. This confirms the transitions in the purine and pyrimidine bases at different positions as was previously shown in figure 4 by the chromatograms.

2.8 Phylogeny of mtDNA Haplotypes with GenBank Reference Sequences

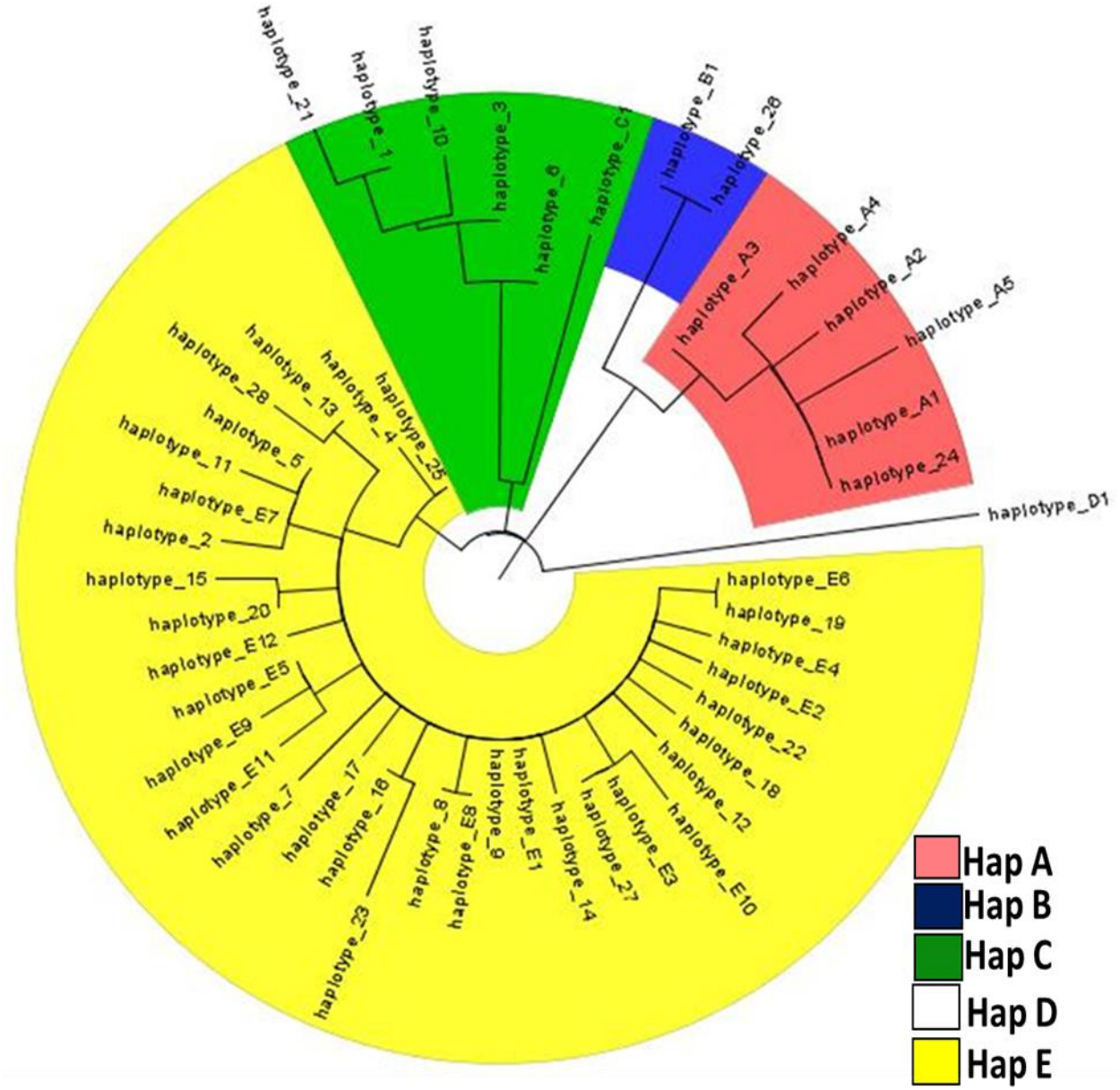

Figure 6: A phylogeny of the mtDNA in indigenous chickens with reference sequences by (Dana et al., 2011)

Phylogeny of the mtDNA was done by Mega software v6 (Tamura et al., 2011). A maximum likelihood tree was generated with mtDNA haplotypes and the reference sequences by (Dana et al., 2011). Tamura Nei model with a gamma shape parameter of 0.08 and a bootstrap value of 1000 was used. The haplotypes clustered in four of the five haplogroups. Haplogroup E had most haplotypes while none of the samples clustered in haplogroup D. This information is clearly displayed in figure 6.

2.9 Amplification of HSP70 gene in indigenous chickens of Kenya

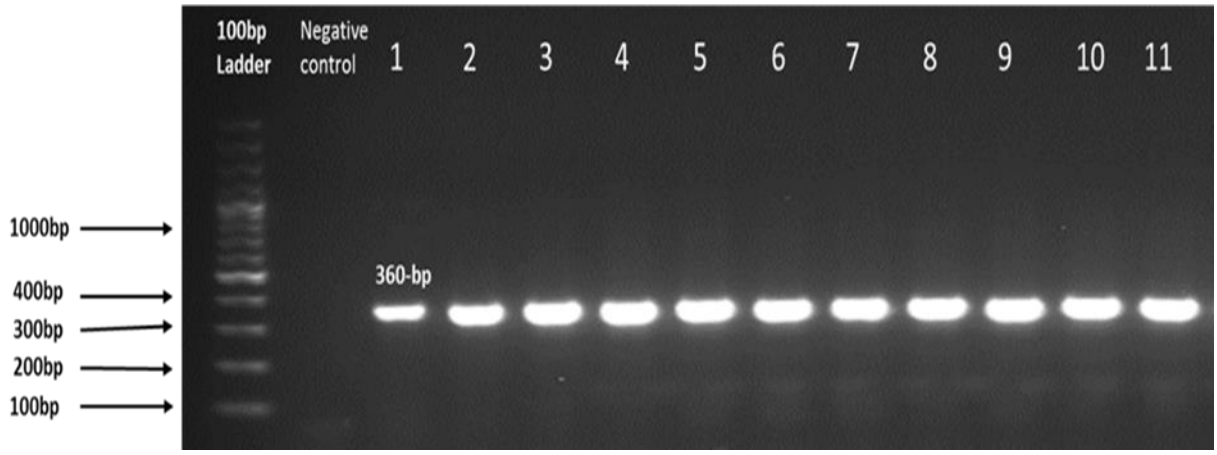

Figure 7: PCR amplification gel image of HSP70 in indigenous chickens of Kenya. Key: 1-10- various chicken samples, 11-positive control.

The 296 HSP70 samples were amplified with the relevant primers. The gel image in figure 7 displays a 2% agarose gel electrophoresis showing representative results. Water was used as a negative control. The primers targeted a region of 360 base pairs which is clearly indicated by the 100 base pair molecular weight DNA marker. All the 296 samples amplified. They were then purified and sequenced.

2.10 Sequence Chromatograms of HSP70 Homozygotes in Indigenous Chickens of Kenya

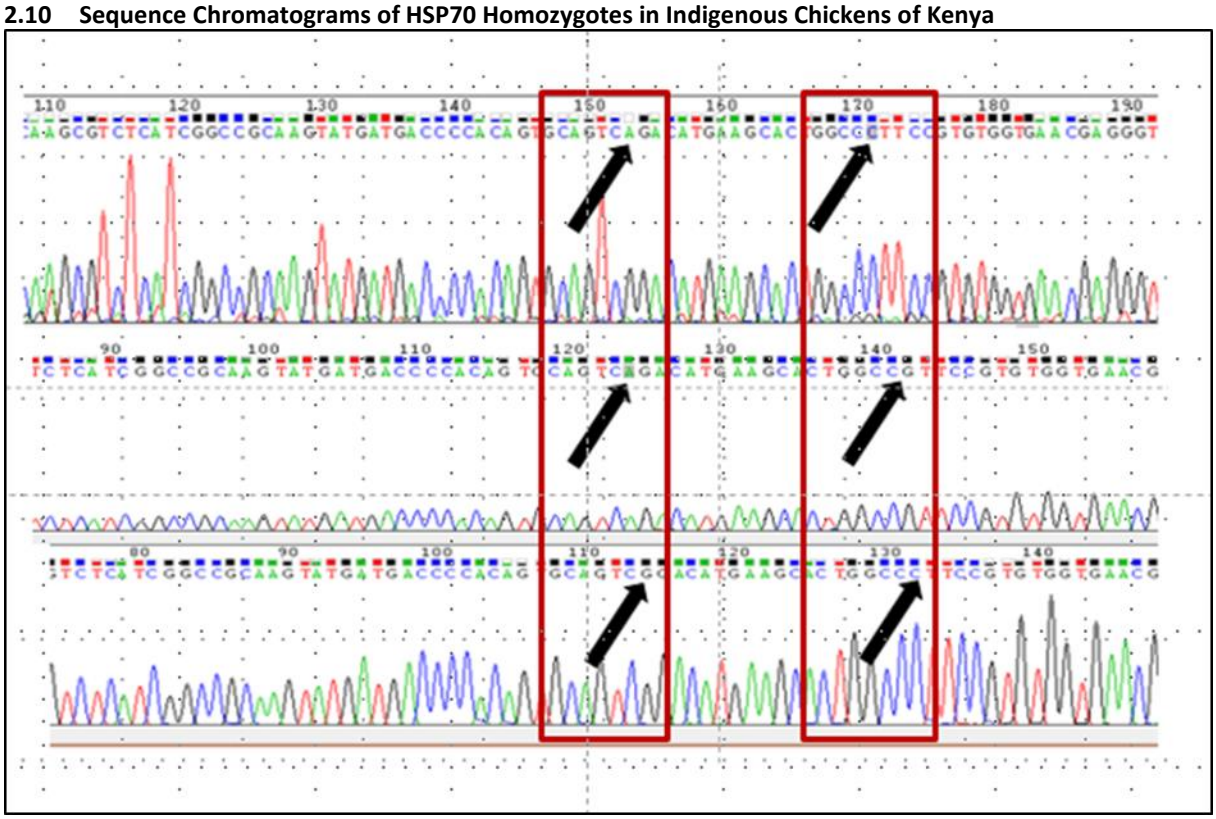

Figure 8: Chromatogram of HSP70 sequence the locations of the two variable positions of the homozygote sequence

After sequencing, the chromatograms were edited manually by the use of chromas lite 2.1 (Avin, 2012). Polymorphisms within the chromatogram sequence was evident at position 153 and 171 as shown in figure 8.

Three homozygous sequences were observed at two locations in various samples. There were transitions and transversions at the two locations. The cytosine base was replace by an adenine or a guanine base in the transversion while in the transition adenine was replaced by a guanine.

2.11 Sequence Chromatograms of HSP70 Heterozygotes Sequences in Indigenous Chickens of Kenya

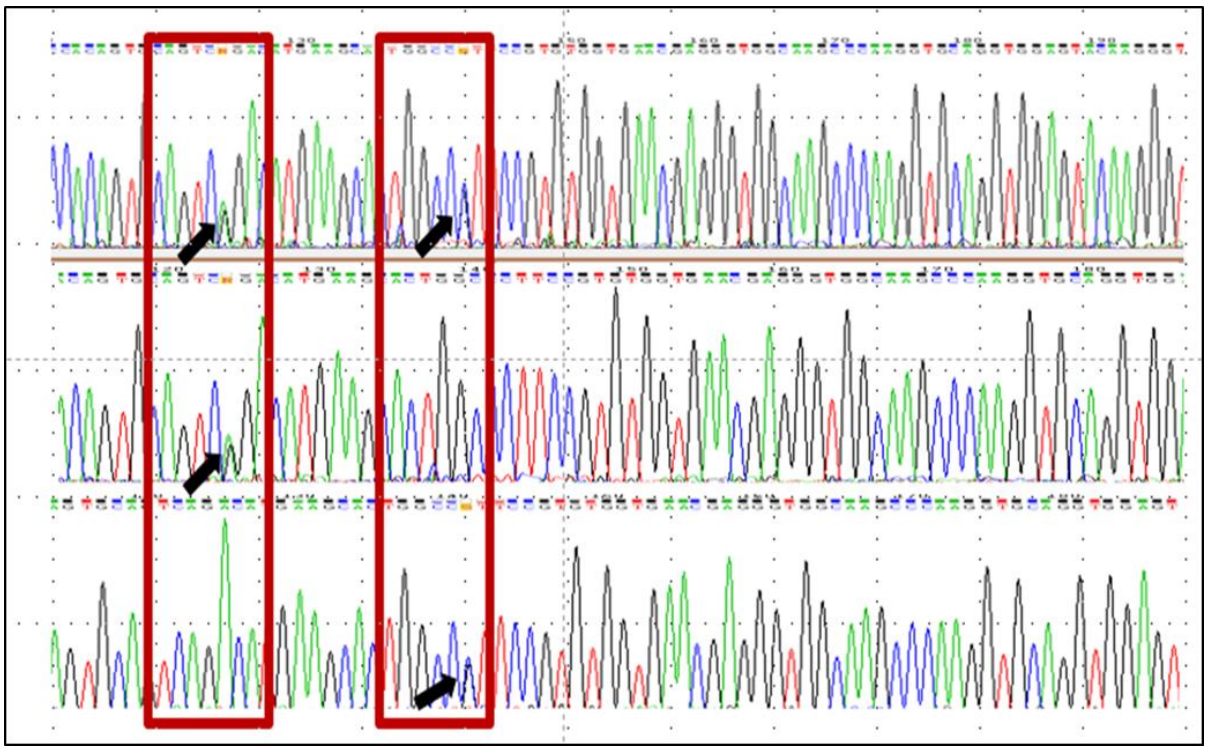

Figure 9: Chromatogram of HSP70 sequence the locations of the two variable positions of the heterozygous sequence

Polymorphisms within the chromatogram sequence was evident as shown in figure 9. Three heterozygous sequences were observed in the various indigenous chicken population samples. The heterozygosity was observed at position 153 and 171. The three heterozygous sequences were: RC, RS and AS. R and S are codes in chromas meant to replace adenine or guanine for R and cytosine or guanine for S.

2.12 Multiple Sequence Alignment of HSP70 and the Red Jungle Fowl

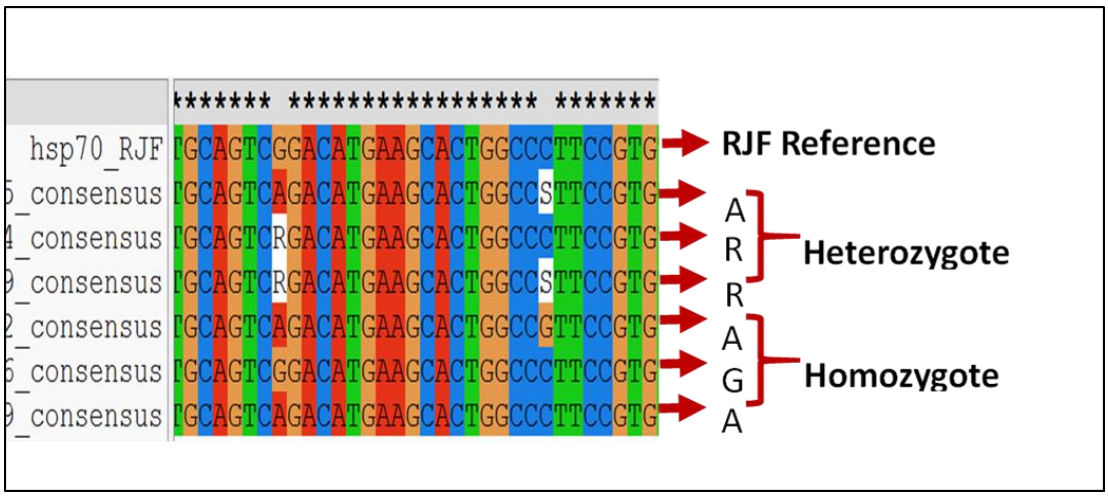

Figure 91: Multiple sequence alignment of the three HSP70 Heterozygotes and three HSP70 Homozygotes

A multiple sequence alignment was done by the use of Clustal X2 (Thompson et al., 1997). Figure 9 above shows a multiple sequence alignment of the heterozygous and homozygous sequences with the ancestral red jungle fowl. This clearly shows the exact locations of variations in the various samples.

2.13 Multiple Sequence Alignment of HSP70 and the Red Jungle Fowl

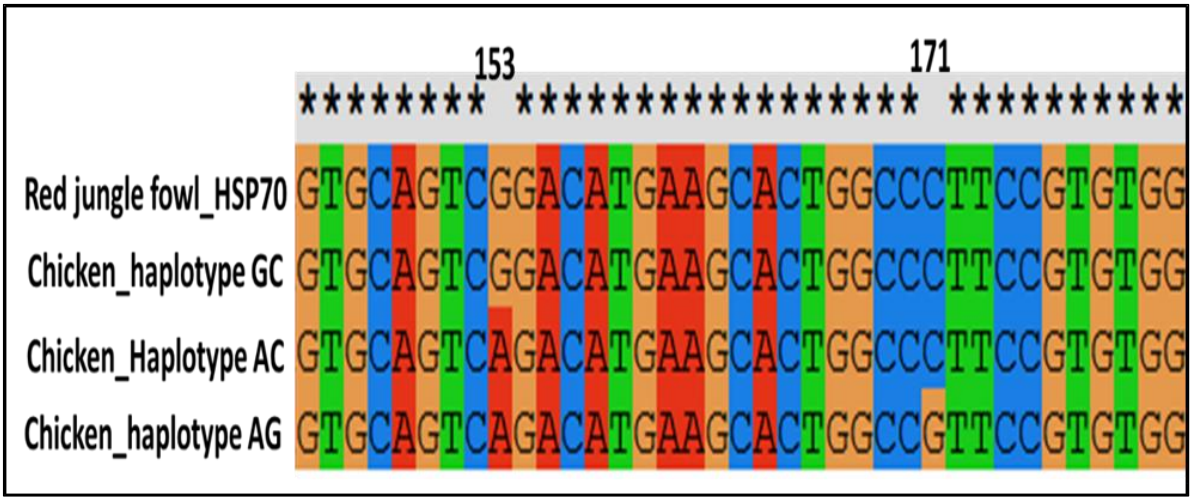

Figure 102: Multiple sequence alignment of the three HSP70 chicken haplotypes and the red jungle fowl HSP70 sequence

The multiple sequence alignment was done by the use of Clustal X 2 software (Thompson et al., 1997). The red jungle fowl was used as a reference sequence. This also showed clearly the three homozygous sequences in various HSP70 samples. This also shows the locations of variations at position153 and 171 in the various samples.

3.0 Discussion

This study clearly shows the great diversity that exists in indigenous chickens of Kenya. This was evidenced by the various phenotypes that were seen in the various parts of the country. Some phenotypes were more frequent in warmer regions while others were frequent in the cooler regions of the country. Kingori et al. (2010) identified six phenotypes in indigenous chickens of Kenya while another study in Kenya by Moraa et al. (2015) identified an additional six phenotypes. Various studies in Africa have also characterized indigenous chickens. Some of these studies reported low frequencies of some phenotypes in several parts of sampled countries. Low frequencies of naked neck phenotypes have been reported in Tanzania (Guni and Katule, 2013), Ethiopia (Dana et al., 2010) and in Botswana (Badubi et al., 2006). Low frequencies of the frizzled and rumpless were also reported in Tanzania (Guni and Katule, 2013). This clearly shows that some phenotypes are common to certain areas due to climatic conditions while other phenotypes were less common in other locations.

The 28 mtDNA haplotypes that were discovered clustered in four of the five haplogroups by (Dana et al., 2011). Most of these haplotypes clustered in haplogroup E. None of our samples clustered in haplogroup D. These haplogroups have been previously observed in various African chickens. A study done in South Africa by (Muchadeyi et al., 2008; Razafindraibe et al., 2008) identified haplogroups A and D in indigenous chickens. Haplogroup E has been reported in Ethiopia and Sudan (Mwacharo et al., 2011). These haplogroups have various origins. Haplogroup D is believed to have originated from the Middle East. Haplogroup A has been shown to have originated from South and Southwest China and surrounding regions. (Mwacharo et al., 2011). These results clearly indicates that indigenous chickens in Kenya have various domestication locations thus supporting the theory of multiple domestication of Indigenous chickens in Asia.

Heat shock protein 70 is a protein that is produced by organisms when exposed to harsh climatic conditions like extreme heat (Mazzi et al., 2003). Polymorphisms in this gene were clearly shown in HSP70 gene.

Polymorphisms in chicken HSP70 gene in different breeds has been found to be related to different resistance to heat stress (Zhang et al., 2002). These polymorphisms resulted in three heterozygous and the three homozygous sequences in the HSP70. This could be an adaptation to enable chickens survive in various climatic conditions especially in regions with heat stress.

4.0 Conclusion

This study clearly demonstrated that there are various mtDNA haplotypes that exist in Kenya. These haplotypes have also been shown to have originated from various regions in South and Southeast Asia. Thus it is evident that mtDNA can indeed be used as a marker to detect the origins and diversity of indigenous chickens.

Just like in mtDNA, there are unique HSP70 heterozygotes and homozygotes that exist in Kenya. These heterozygotes and homozygotes have been shown to dominate in various regions of the country. They could also have a common point of origin. Thus it is clear that HSP70 can be used as a marker in molecular breeding for drought/heat tolerance.

5.0 Recommendations

This study characterized indigenous chickens in some parts of the country thus further studies to characterize indigenous chickens should be carried out in the other regions of the Country.

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