COMPARATIVE GENOMICS AND TRANSCRIPTOMICS OF HEAT STRESS IN CHICKEN AND GUINEAFOWL POPULATIONS FROM SELECTED COUNTRIES IN AFRICA, ASIA, AND EUROPE

GRACE MORAA KENNEDY

DOCTOR OF PHILOSOPHY

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Comparative Genomics and Transcriptomics of Heat Stress in Chicken and Guineafowl Populations from Selected Countries in Africa, Asia, and Europe

Grace Moraa Kennedy

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DECLARATION

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

Signature…….. Date…….

Grace Moraa Kennedy

This thesis has been submitted for examination with our approval as the University Supervisors

Signature…… Date……

Dr. Sheila Ommeh, PhD

JKUAT, Kenya

Signature……..… Date……

Dr. Jacqueline Lichoti, PhD

Ministry of Agriculture, Livestock, Fisheries and Co-operatives, Kenya

DEDICATION

This work is dedicated to my parents, Mr. Rogers Kennedy and Mrs. Jane Nyamusi, as well as my siblings, James Nyamamba and Deborah Mokeira, for their unwavering support, love, and sacrifices throughout my academic journey.

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ACRONYMS AND ABBREVIATIONS

BACs	Bacterial Artificial Chromosomes
BC	Before Christ
BWA	Burrows-Wheeler Alignment
CV	Cross-Validation error
DAVID	Database for Annotation, Visualization and Integrated Discovery
DCMS	De-correlated composite of multiple signals
DEGs	Differentially expressed genes
DNA	Deoxyribonucleic acid
EHH	Extended Haplotype Homozygosity
FAO	Food and Agriculture Organization
FDR	False Discovery Rate
F_{ST}	Fixation index
GATK	Genome Analysis Toolkit
GBS	Genotyping by Sequencing
GGRS	Genotyping by Genome Reducing and Sequencing
GO:	Gene Ontology
GRC	Genome Reference Consortium
GWAS	Genome-wide Association Studies
HGF	Helmeted guineafowl
HSF	Heat Shock Factors
HSP	Heat Shock Proteins
IACUC	The Institutional Animal Care and Use Committee
IBR	Institute for Biotechnology Research

iHH	Integrated Haplotype Homozygosity
iHS	Integrated Haplotype Score
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEGG	Kyoto Encyclopedia of Genes and Genomes
LD	Linkage Disequilibrium
MAPK	Mitogen-Activated Protein Kinase
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
rEHH	relative extended haplotype homozygosity
RJF	Red jungle fowl
RNA	Ribonucleic acid
ROH	Runs of Homozygosity
SFS	Site Frequency Spectrum
SnpEff	Genetic Variant Annotation and functional effect prediction toolbox
SNPs	Single Nucleotide Polymorphisms
SRA	Sequence Read Archive
XP-EHH	Cross population extended haplotype homozygosity

ABSTRACT

Poultry farming is a crucial component of the global agriculture industry, providing a significant source of protein and economic livelihoods to millions of people worldwide. However, climate change has led to an increase in extreme weather conditions, including rising temperatures, which can negatively impact poultry production. Heat stress, a physiological response to high temperatures, has been identified as a major challenge affecting poultry health and performance, leading to reduced growth rates, decreased egg production, and increased mortality rates. Understanding the genetic and molecular mechanisms underlying heat stress response in different poultry breeds is essential for developing effective strategies to mitigate its impact on poultry farming.

This study aimed to investigate the comparative genomics and transcriptomics of heat stress in chicken and guineafowls from selected countries in Africa, Asia, and Europe. Chicken (*Gallus gallus domesticus*) and guineafowls (*Numida meleagris*) are two important poultry breeds widely raised for their meat and egg production, as well as for their cultural significance in various regions of the world. By comparing the genetic and molecular responses to heat stress in these two poultry species from different geographic locations, this study sought to identify candidate genes, and molecular pathways that are associated with heat stress tolerance. This study utilized the "omics" techniques (genomics and transcriptomics), to investigate the signatures of selection for heat stress in chicken, and guineafowls. Three methods: Fixation index (F_{ST}), Integrate Haplotype Score (iHS), and Cross-population Extended Haplotype Homozygosity (XP-EHH) were used for the detection of signatures of selection for heat stress. Blood samples from 20 chicken were obtained for whole genome sequencing from Lamu, Kilifi, Mombasa, and Kwale. An additional 14 chicken whole genome sequences were downloaded from the NCBI SRA archive for comparative genomics. For the guineafowls, 16 blood samples from Laikipia, Lamu, Kilifi, and Taita Taveta were obtained for whole genome sequence analysis. An additional 56 guineafowl whole genome sequences were downloaded from the NCBI SRA archive. Through the signatures of selection methods, several candidate genes such as CNBP, COL1A1, YWHAE, PPARG, SREBF1, ATP6V0 and LRRC8A ATP2A2, TBXAS1, PER2, BOK, and RAF1 were selected in the chicken genomes. These candidate genes were subjected to annotation and several pathways including response to oxidative stress, localization, metabolic process, cellular process, developmental process, response to stimuli, signaling, and homeostatic process, that play a role in heat stress were identified. In the guineafowl genomes, some of the significant candidate genes that were selected included the MAPK1, SLC27A4, ATP5MF, PPARG, SREBF1, ATP6V0 and LRRC8A BRAF, CRYGN, and ANGPT2. These genes were annotated and found to play a role in several significant pathways like localization, metabolic process, positive regulation of the biological process, cellular process, signaling, and response to stimuli. Some pathways that were significant for both the chicken and guineafowl genomes included the metabolic process, localization, signaling, cellular process, and response to stimuli. Some of the candidate genes that were selected for both chicken and guineafowls include the PPARG, SREBF1, ATP6V0 and LRRC8A. RNA sequencing was used to profile the kidney tissue of 13 chicken and 13 guineafowls from Lamu and Mombasa Counties. At a false discovery rate (FDR) < 0.05, 278 DEGs were identified in chicken kidney tissue samples from these regions. Among these, 129 were upregulated (e.g., CD36, ANGPTL4, PLIN1, GLRA3, GABRA4) and 149 were downregulated (e.g., GABRA2, C3, GHRHR, GHSR, GRIA3, ACSL6, VTN). In guineafowl kidney tissue

samples, a total of 349 DEGs were identified, among these, 190 were upregulated (e.g., SDR16C5, RETSAT, ALDH1A1, BCO1, ACSM3, AKR1D1, MDH2, CYP24A1) and 159 were downregulated (e.g., PAH, MAPK12, GRM1, RAG1, CARNS1, CNDP1, FTCD, HTR2A, MYLK2, AOX1). Gene Ontology (GO) enrichment analysis revealed that in the chicken samples, these genes were significantly enriched for metabolic and developmental processes, whereas in guineafowls, they were prominently enriched for metabolic biological processes. Similarly, when conducting KEGG pathway analysis on the DEGs from chicken kidney tissue samples, it was observed that pathways related to neuroactive ligand-receptor interaction, the PPAR signaling pathway, the Adipocytokine signaling pathway, and ECM-receptor interaction were differentially enriched. In guineafowls, the KEGG pathway analysis of DEGs indicated differential enrichment in metabolic pathways, neuroactive ligand-receptor interaction, the calcium signaling pathway, and the FoxO signaling pathway. The study's discoveries have offered fresh perspectives on the intricate relationship between genetic and environmental elements in poultry's response to heat stress. The identification and selection of genes associated with heat tolerance enhances our comprehension of the molecular mechanisms implicated in heat stress among poultry. Consequently, this provides a promising and sustainable approach for breeding heat-tolerant poultry. This approach effectively addresses the challenges posed by heat stress, particularly in the context of climate change, and ultimately contributes to ensuring food security.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Chicken (*Gallus gallus domesticus*) are believed to have been domesticated in the Indus Valley between 2500-2100 B.C (Kanakachari, 2023; Peters et al., 2016; Zeuner, 1963). The red jungle fowl is believed to be the wild progenitor of domestic chicken. Recent research by Wang et al. (2020) indicated that domestic chicken originally descended from the red jungle fowl (RJF) subspecies *Gallus gallus spadiceus*, whose current distribution is primarily in Southwestern China, northern Thailand, and Myanmar. Their study analyzed 863 whole-genome sequences from a global sample of chicken and, representatives of all four species of wild junglefowl, and each of the five subspecies of RJF (Wang et al., 2020).

Domestication of helmeted guineafowl (HGF) (*Numida meleagris*) has been traced to the African continent in Mali and Sudan about 2,000 years before the present (BP) (Crawford, 1990; Gifford-Gonzalez & Hanotte, 2011; Larson & Fuller, 2014). Domestic guineafowls have been domesticated from wild helmeted guineafowls. Additionally, there exist other guineafowl species, including the crested and vulturine, that are hunted from their natural wild habitats (Shen et al., 2021; Murunga et al., 2018).

Chicken and guineafowls are kept for their meat, eggs, and feathers, for cultural activities, and also used as animal models for the study of various diseases and biological processes (Li et al., 2021). Though in most parts of the world, guineafowl is hunted as bushmeat, its production has increased to roughly 1.4% of the world's poultry population as a result of its superior nutritional value and economic potential. In terms of output, chicken lead with 92.3%, followed by ducks and turkeys at 4.4% and 1.8% respectively (Food and Agriculture Organization Corporate Statistical, accessed 2018) (Food and Agriculture Organization, 2007).

Since their introduction at their points of domestication, chicken, and guineafowls have dispersed to different countries across Africa, Asia, and Europe and have adapted to live in diverse environmental conditions (Gheyas et al., 2022). For instance, Africa has diverse weather conditions with the northern regions such as the Sahara Desert

experiencing extremely hot and dry conditions with temperatures often exceeding 40°C during the day and dropping significantly at night. The central regions, including the tropical rainforests, are characterized by high temperatures, high humidity, and heavy rainfall throughout the year. Sudan, located in central Africa, experiences temperatures ranging from 35°C to 40°C, humidity levels fluctuating between 30% and 80%, and annual rainfall that varies from 100mm to 800mm (Zittis et al., 2022).

Kenya, located in East Africa, exhibits a diverse climate, with temperatures ranging from -10°C in highlands to 40°C in arid areas. The annual rainfall ranges from 500mm to 2000mm, and humidity levels between 40% and 90%. The Kenyan coast, along the Indian Ocean, has a tropical maritime climate with warm temperatures (25°C to 32°C), high humidity, and distinct wet and dry seasons. Ethiopia, which is also located in East Africa, experiences temperatures between 15°C to 30°C, humidity levels of 30% to 70%, and annual rainfall ranging from 200mm to 2000mm.

The southern regions have a Mediterranean-type of climate with hot summers and mild winters, along with moderate precipitation. South Africa, found in the southern part of Africa experiences temperatures from 10℃ to 30℃, humidity levels of 40%-80%, and annual rainfall from 200mm to 1200mm (Https://public.wmo.int/en; Https://www.ipcc.ch/). Countries found in West Africa like Nigeria, has temperatures ranging from 25℃ to 40℃, humidity levels between 30% and 90%, and an annual rainfall of between 300mm to 3000mm. Burkina Faso experiences temperatures between 30℃ to 40℃, humidity levels from 30% to 70%, and an annual rainfall of 250mm to 1000mm. Benin, is characterized by humidity levels from 40% to 90%, temperatures of between 20℃ to 40℃, and annual rainfall of 600mm to 2000mm.

In Asia, the northern regions experience extremely cold and harsh winters with temperatures plummeting well below freezing, with short and mild summers. Central regions like China have varied climates ranging from humid subtropical to continental, with hot summers and cold winters. Temperatures in China can rise up to 40℃ in summer, and drop to -10℃ to 0℃ in winter (Soflaei et al., 2017). Western regions like Iran experiences temperatures from 10℃ to 30℃, annual humidity of 40%-80% and rainfall from 100-1000mm. The southern regions have a tropical climate with high temperatures, high humidity, and heavy rainfall during the monsoon season (Https://www.ecmwf.int/; Https://www.ipcc.ch/).

Europe has a temperate climate overall, with a mix of maritime and continental influences. The northern regions have cold winters with temperatures dropping below freezing and cool summers. The western regions have a maritime climate with mild winters and mild summers, often accompanied by frequent rainfall. France, located in Western Europe has mild winters (0℃-10℃) and warm summers (20℃-30℃), an annual precipitation of 500mm to 1000mm, and humidity levels averaging 60%-80% (Thompson et al., 2009). The central regions have a continental climate with cold winters and warm summers.

The southern regions have a Mediterranean climate with hot and dry summers and mild, wet winters. Italy, found in South Europe experiences scorching, dry summers with daytime temperatures ranging from 30℃ to 40℃, while winters are generally mild and wet, seldom dropping below 0℃, with humidity levels between 40% and 75% and annual rainfall of 500mm to1000mm. Hungary, in East Europe, experiences humidity levels ranging from 40% to 70%, with cool winters (0℃-5℃) and warm summers (25℃-30℃), along with an annual rainfall of 500mm-800mm. (Https://www.ecmwf.int/; Https://www.ipcc.ch/; Https://climate-adapt.eea.europa.eu/). It is important to note that these general weather conditions can vary depending on the specific locations, elevation and other factors. Climate change has also been shown to have an impact on weather patterns across the world, leading to shifts in temperature, precipitation, and other weather parameters.

Climate change, particularly the increasing global temperatures and heat stress events, has a significant impact on poultry production. Poultry, such as chicken, guineafowl turkeys, and ducks, are particularly vulnerable to heat stress due to their high metabolic rate, lack of sweat glands, and limited ability to dissipate heat (Oguntunji et al., 2019; Saeed et al., 2019; Kennedy et al., 2022).

One of the main impacts of climate change on poultry is increased heat stress, which occurs when the birds are exposed to temperatures beyond their thermal comfort zone (Saeed et al., 2019). Heat stress can lead to reduced feed intake, decreased egg production, poor egg quality, impaired growth, and increased mortality rates (Goel, 2020; Saeed et al., 2019; Vandana et al., 2021). Poultry may exhibit panting, wing spreading, decreased activity, and increased water consumption as they try to regulate their body temperature in response to heat stress (Vandana et al., 2021).

Heat stress also weakens the immune system of poultry, making them more susceptible to diseases such as bacterial infections and viral diseases (Hirakawa et al., 2020). This can result in increased mortality rates and the need for increased use of antibiotics, which can have implications for both animal and human health due to the risk of antibiotic resistance (Saeed et al., 2019; Vandana et al., 2021).

To mitigate the impacts of heat stress due to climate change on poultry production, farmers may need to implement various adaptation measures, such as providing access to shade, increasing ventilation in poultry houses, optimizing nutrition and feeding strategies, and improving water management (Goel, 2020; Kennedy et al., 2022). Genetic selection for thermotolerance in poultry may also be considered (Saeed et al., 2019; Vandana et al., 2021).

Genetic selection for thermotolerance in poultry demands a thorough understanding of the underlying genetics of poultry's response to heat stress (Balakrishnan et al., 2023;Perini et al., 2020). The levels of thermo-tolerance vary between various poultry breeds, making genetic modification a potential method to relieve heat stress in poultry (Melesse et al., 2011; Vandana et al., 2021). Genetic tools and technologies like the recent "omics" technologies have been used to study the genetic basis of various processes like heat stress tolerance across multiple poultry breeds (Cho et al., 2021; Wang et al., 2017; Weimann et al., 2016). Over the years, improvements in the high throughput sequencing platforms have lowered the cost of acquiring genetic data, thus leading to a large amount of genetic information on poultry that is easily accessible (Perini et al., 2020).

The availability of numerous poultry genome sequences has made it easy to categorize genetic markers linked with heat stress tolerance (Gong et al., 2023). This has shed light on the various mechanisms involved in heat stress regulation and the detection of valuable biomarkers that can improve the various poultry breeding programs (Wang et al., 2017). Currently, functional genomics research may give fresh insights into how heat stress affects adaptive capabilities by identifying candidate genes that are selected for heat stress, genes that are upregulated or downregulated during heat stress, and the molecular pathways that are involved in heat stress (Cedraz et al., 2017; Wang et al., 2015; Srikanth et al. 2020; Rao et al., 2021). Incorporating functional genomics and transcriptomics data into molecular markers allows researchers/breeders to discover

potential candidate genes that play a role in the pathways responsible for heat stress tolerance (Grover & Sharma, 2016; Ouborg et al., 2010).

Comparative genomics is a powerful tool for establishing relationships between gene function and location in various livestock species. Moreover, it allows insight into large-scale genomic rearrangements, conservation and functional elements, and tracing of evolutionary phylogenies through the examination of closely related species (Cho et al., 2022). The most powerful strategies combine in-silico and experimental approaches like sequence comparison (Griffin et al., 2008). The availability of complete sequence of the chicken and guineafowl genomes and their genetic variation maps has promoted the exploration and research of the genetic mechanisms using whole-genome-based strategies (Warren et al., 2017; Qanbari et al., 2019; Rubin et al., 2010; Tian et al., 2020). By comparing the chicken and guineafowl genomes, researchers can identify genes or genomic regions that show signs of positive selection, indicating that they have been favored by natural selection in certain chicken and guineafowl populations or species.

Conversely, considering their short reproductive and growth periods and also widespread distribution, chicken, and guineafowls can be used as ideal models to study genetic adaptations to the environments, especially now that the effects of climate change are being felt and the global demand for animal protein is on the rise (Tian et al., 2020). Despite the many efforts that have been made, the genetic basis of adaptation of chicken and guineafowls to high ambient temperatures remains limited.

In this study, genomic data of chicken and guineafowls from Kenya, plus additional downloaded genome sequences of chicken and guineafowls from selected countries in Africa, Asia, and Europe, were used for comparative genomics to elucidate the signatures of selection for heat stress. The signatures of selection tests were used to identify the candidate genes that are implicated in heat stress, and the role of these genes in several pathways implicated in heat stress. Also, transcriptomics data of chicken and guineafowls from Kenya were used to identify the differentially expressed genes and the role of these genes in various molecular pathways involved in heat stress regulation.

1.2 Problem Statement

Climate change has resulted in increasing temperatures and more frequent heatwaves, which are affecting animal welfare and productivity. Heat stress is a major concern for poultry producers, and it is estimated to cause significant economic losses annually. Extreme weather conditions lead to production losses; reduced growth rate, decreased egg production, and increased morbidity and mortality in poultry (Leal Filho et al., 2021; Nawab et al., 2018; Nawaz et al., 2021).

Secondly, the differences in heat tolerance between chicken and guineafowls are not fully understood, and the genetic factors responsible for these differences have not been identified. Also, scanty information exists on the genetic mechanisms underlying heat stress response and tolerance in poultry. Scanty genomic/transcriptomic information and limited elucidation of their pathways there-in, hampers validation studies aimed at adopting molecular breeding techniques (Gu et al., 2020). This limits the development of effective genetic strategies to mitigate the impact of heat stress.

Lastly, the ongoing consequences of heat stress on poultry health and production are expected to persist and may even be inherited by the next generation during gestation. This is because the primary focus of most poultry selection revolves around production traits, often with insufficient consideration for heat tolerance and climate adaptation, particularly given the prevailing trends of global warming. Additionally, traditional breeding methods, which are predominantly employed, tend to be slow and may not prove to be highly effective when it comes to selecting for complex traits like heat tolerance (Nawab et al., 2018).

1.3 Justification

Heat stress presents a significant global challenge to poultry production, resulting in reduced animal welfare, decreased productivity, and substantial economic losses within the agricultural sector. It is crucial to comprehend the genetic foundations of the heat stress response in the poultry to formulate strategies that can mitigate its effects, improve poultry resilience, and promote sustainable poultry farming (Nawaz et al., 2021).

Comparative genomics and transcriptomics of heat stress in chicken and guineafowls can identify key genes and molecular pathways involved in stress responses (Liu et al., 2023). The present study used comparative genomics and transcriptomics to identify candidate genes, and molecular pathways that play a role in heat stress tolerance.
The comparison of these two species, which have different heat tolerance levels, can provide valuable insights into the mechanisms of heat stress response and tolerance (Li et al., 2022). Such information is useful for breeding stress-tolerant poultry and improving their performance under heat stress.

Genetic selection for heat tolerance in poultry represents the most sustainable solution to mitigate the adverse effects of heat stress especially in the tropical and subtropical regions of the world. Acknowledging the potential risk to food security, it is imperative to foster collaboration between scientists and various industries, including poultry breeding. This cooperative approach is crucial for effectively tackling the issue. One potential strategy within these efforts involves the incorporation of heat-tolerant traits into poultry. For instance, candidate genes associated with heat stress resistance can be utilized in the breeding of heat-tolerant poultry. These candidate genes encompass traits like Naked neck (Na), Frizzle (F, with candidate genes KRT6A and KRT75L4), and Dwarf (Dw, with the candidate gene GHR) (Nawaz et al., 2021).

1.4 Objectives of the Study

1.4.1 General Objective

To analyze the comparative genomics and transcriptomics of heat stress in chicken and guineafowl populations from selected countries in Africa, Asia, and Europe

1.4.2 Specific Objectives

1. To determine the genomic signatures of selection for heat stress in chicken genomes from selected countries in Africa and Asia
2. To determine the genomic signatures of selection for heat stress in guineafowl genomes from selected countries in Africa, Asia, and Europe
3. To analyze specific candidate genes associated with heat stress tolerance in chicken and guineafowls from selected countries in Africa, Asia, and Europe
4. To analyze the transcriptomic landscape of heat stress in chicken and guineafowls from Kenya

1.5 Null Hypotheses

1. There are no genomic signatures of selection for heat stress in chicken genomes from selected countries in Africa and Asia
2. There are no genomic signatures of selection for heat stress in guineafowl genomes from selected countries in Africa, Asia, and Europe
3. There are no specific heat stress genes in chicken and guineafowls from selected countries in Africa, Asia, and Europe
4. There is no transcriptomic landscape in chicken and guineafowl populations from Kenya

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of the Chicken Genome

Chicken are widely used as a model organisms in biomedical and agricultural research to study various diseases and adaptation mechanisms leading to the identification of candidate genes affecting such traits (Ichikawa et al., 2022; Smith et al., 2022). Chicken have 39 pairs of chromosomes, including 30 pairs of microchromosomes, eight pairs of macrochromosomes, and one pair of sex chromosomes (Z and W) (Lin et al., 2023). Unlike mammals, male birds are homozygous (ZZ) while females are heterozygous (ZW) (Das et al., 2023; Lin et al., 2023). The chicken genome sequence of the RJF was the first livestock species to be sequenced, with the first draft released in 2004 (Pan et al., 2023; Suminda et al., 2022). This provided substantial advances for avian genetics, enabling a range of new "omics" analyses and technologies to be applied (Dai & Shen, 2022; International Chicken Genome Sequencing Consortium, 2004).

Subsequent versions of the chicken genome assembly, such as Gallus_gallus 2.1; GCA_000002315.1, and Gallus_gallus-4.0; GCA_000002315.2 were released with improved assembly base presentation, order, and orientation and increased contig, and scaffold sizes (Pan et al., 2023; Smith et al., 2022; Warren et al., 2017; Zhang & Backström, 2014). However, issues such as duplication errors, unassigned chromosomal sequences, and gaps in the ordered and oriented chromosome sequences persisted in these assemblies, limiting their use for genetic mapping and scanning for natural or artificial selection (Qanbari et al., 2015; Reyer et al., 2015; Warren et al., 2017). The Gallus_gallus-5.0 assembly, released in December 2015, addressed some of these issues and included additions of new sequences and annotated genes (Zhang, 2015; Warren et al., 2017).

The Genome Reference Consortium (GRC) has been maintaining updates to the chicken reference genome assembly since the release of Gallus_gallus-5.0. The GRCg6a assembly, with improved quality metrics, was released as the latest version, and the current chicken genome assembly is bGalGal1.mat.broiler.GRCg7b, submitted by the Vertebrate Genomes Project in January 2021, consists of 677 contigs assembled

into 214 scaffolds with an estimated size of 1,053,332,251 base pairs (Table 2.1) (Huang et al., 2023; Rhie et al., 2021)

Since the release of the first draft of chicken genome assembly, there have been tremendous improvements leading to updated versions of the previous genomes. These and other developments will help future efforts to reannotate the current collection of avian genomes as well as many others that are now under work (Rhie et al., 2021; Suminda et al., 2022). However, many genes in chicken and other species still have no known functions and can only be identified by expensive manual curation and experimentation. Since the initial draft assemblies have been proven to be insufficient for the more thorough detection of allelic contributions to particularly complex traits, higher-quality genome assemblies are becoming increasingly necessary to realize the full potential of next-generation sequencing investigations (Warren et al., 2017). There is an ongoing desire to increase the quality of the chicken reference genome given the already significant advancements in avian trait mapping and the accessibility of genomic resources (Huang et al., 2023; Pan et al., 2023; Smith et al., 2022).

Table 2.1: Genome Assembly Statistics for the Chicken

Statistics	**Description**
Assembly	bGalGal1.mat.broiler. GRCg7b, INSDC Assembly GCA_016699485.1
Base pairs	1,053,332,251
Gene build last updated/patched	Jan 2022
Database version	108.7
Gene counts	
Coding genes	17,007
Non-coding genes	13,040
Small non-coding genes	1089
Long non-coding genes	11,946
Pseudogenes	61
Other	
Short Variants	22,693,906

2.2 Overview of the Guineafowl Genome

Although HGF is important, few genetic studies have been done on it, especially when compared to chicken. Currently, much effort has been focused on the exploration of the genetic variation of poultry species, which serves as an important first step to reveal the uniqueness and identify valuable genetic resources. It was not until 2019 that the first draft of the HGF genome assembly (*NumMel1.0*), which was based on the Galgal5 chicken genome as a reference was published (Vignal et al., 2019). The assembly was on chromosome level, consisting of 16,014 contigs assembled into 2,739 scaffolds. From these sequences, 31 chromosomes were built. The N50 length for the contigs is 234.9Mb while the scaffold N50 is 7.8Mb. The assembly sequence size is 1.04 Gb with only 3.8% assigned to chromosomes (Table 2.2). The NumMel1.0 assembly metrices were comparable to previous assemblies of the Galliformes (Vignal et al., 2019).

The chicken and guineafowl karyotypes are typical of avian genomes with a few large chromosomes (macrochromosomes) and a much larger set of smaller chromosomes (microchromosomes) that are comparable to previous assemblies of Galliformes. Unlike in chicken, the exact number of microchromosomes in guineafowls has not been determined to date, but all the chicken chromosomes having assigned sequence in Galgal5 have some sequence similarity to guineafowl sequence (Shibusawa et al., 2002). In total, the NCBI Eukaryotic genome annotation pipeline identified and annotated 16,101 protein-coding genes and 43,227 protein models in the *N.meleagris* genome, which is in line with other assembled and annotated Galliformes, and suggests the gene representation is sufficient (Vignal et al., 2019).

Table 2.2: Genome Assembly Statistics for the Helmeted Guineafowl

Statistics	Description
Assembly	NumMel1.0, INSDC Assembly GCA_002078875.2
Base pairs	1,043,264,150
Gene build last updated/patched	Dec 2018
Database version	108.1
Gene counts	
Coding genes	15,661
Non-coding genes	7,507
Small non-coding genes	494
Long on coding genes	6,886
Pseudogenes	89
Gene transcripts	40,117
Genscan gene predictions	36,491

2.3 Poultry Ecotypes Implicated for Heat Stress Tolerance

In the process of adaptation to the local conditions, poultry may have sufficiently evolved into distinguishable ecotypes which aid in their adaption to the harsh environments where they live. There are several ecotypes, mostly related to feather types, that have been implicated for the alleviation of heat stress (Kennedy et al., 2022). The plumage color, coverage and density determine the amount of heat dissipation from the poultry's body to the environment. It has been shown that dense plumage delays the process of heat elimination from the skin surface (Vandana et al., 2021).

To enhance poultry breeds successfully, traits related to productivity, production, and adaptability must be considered. Notably, genes like naked neck, frizzle feathered, and dwarf in chickens demonstrate enhanced fitness in heat stress conditions (Fathi et al., 2022; Kennedy et al., 2022; Rimoldi et al., 2015). Decisions regarding the use of these traits in improvement programs should rely on genetic distinctness and performance data. The naked neck gene, denoted as Na, is a dominant autosomal gene. In heterozygous birds (Na/na^{+}), a small tuft of feathers appears on the ventral neck, while homozygous birds (Na/Na) lack these feathers or have only a few. The exposed skin turns reddish, particularly in males nearing sexual maturity. The significance of the

naked neck gene in tropical regions lies in its association with heat tolerance (Fathi et al., 2022; Amrutkar et al., 2014; Asadollahpour et al., 2022).

The reduced feather coverage (30-40%) in naked neck birds facilitates better heat dissipation and thermoregulation, resulting in improved heat tolerance in hot climates, especially at temperatures around 30°C or higher (Perini et al., 2020). Homozygous Na/Na or heterozygous Na/na^+ naked neck birds exhibit better weight gain than normal na^+/na^+ birds in high temperatures. The heterozygous genotype is also linked to improved carcass yield, laying rate, egg weight, eggshell strength, and egg mass (Bekele et al., 2010; Perini et al., 2020). Additionally, this gene has favorable effects in high temperatures, including higher breast weight, superior growth rate, improved feed conversion ratio, reduced impact of high temperatures on fertility, less weight loss during heat stress, increased levels of heat shock protein (Hsp70), lower incidence of pathologies such as cloacal cysts, ascites, prolapse, Marek's disease, coccidiosis, osteodystrophy, and Salmonellosis, as well as resistance to sudden death and ascites syndrome (Bekele et al., 2010). Furthermore, combining the naked neck allele with other tropically relevant alleles, such as frizzling, results in a beneficial additive effect on various productive parameters.

Frizzling is caused by a single autosomal gene (*F*) with incomplete dominance, influenced by a recessive modifier gene (mf) (Wasti et al., 2020). In homozygous frizzled birds without modification, all feathers are highly curved and prone to breaking, leaving the birds looking mostly featherless. The modifier gene reduces the extreme curvature in homozygotes, making them appear less fluffy (Fathi et al., 2014). Unmodified heterozygotes have mildly curved feather shafts and barbs, resembling wild-type birds to some extent. The *F* gene's action occurs in the feather follicle and is not due to a metabolic disorder. This gene has a positive impact on production, increasing egg numbers and egg mass while reducing mortality in hot conditions.

Dwarfism can be categorized into two types: sex-linked dwarfism (dw, dwM, dwB) and autosomal dwarfism (adw). Sex-linked dwarfism (dw) is a recessive gene linked closely to gold-silver and slow rapid feathering genes (Gan et al., 2015; Kingori et al., 2010; Wang et al., 2017). It causes significant dwarfing, reducing male size by 43% and female size by 26-32%, with slightly smaller eggs and reduced egg numbers. Bantam Dwarfism dwB is another sex-linked recessive gene, with a milder effect, reducing

female size by 5-11% and male size by 5-14% (Gan et al., 2015; Wang et al., 2017). MacDonald Dwarfism dwM is a related gene with a distinct effect, reducing female size by 13.5% and shank length by 9%. Autosomal Dwarfism (adw) is an autosomal gene that reduces body size by 30%, with birds remaining easily distinguishable at 6-8 weeks of age (Wang et al., 2017). These genes have known effects on productivity, but their relevance in rural poultry flocks, especially in the tropics, remains unexplored.

The color of feathers in chickens is a crucial factor affecting their response to heat stress (HS) (Moraa et al., 2015). Recent research found that darker chicks had lower gene expression related to stress pathways (cellular stress (*SOD2* and *HSPA8*) and DNA damage repair (*ALKBH3*)) compared to paler chicks (Perini et al., 2020). This is because plumage reduced solar heat gain by 5% in both light and dark feathers. However, the reduction in heat load differed, with a 41% decrease for light plumage and a 25% decrease for dark plumage. Additionally, the density of contour feathers was strongly linked to heat tolerance, serving as a potential marker for heat tolerance in chickens (Perini et al., 2020; Moraa et al., 2015).

There is therefore the need to revisit the local poultry resource and assess the contribution of these genes. This will assist on the genetic improvement on adaptability and productivity and the conservation of the identified desirable genes.

2.4 Effects of Climate Change and Heat Stress on Poultry Production and Physiology

Climate change refers to the spatial and temporal variations in the average environmental climatic parameters such as temperature, solar radiation, humidity, and precipitation over a long time (Duchenne-Moutien & Neetoo, 2021). According to the fifth assessment report of the Intergovernmental Panel on Climate Change (IPCC), the Earth's surface would warm by 0.3°C to 4.8°C by 2100 (Malla et al., 2022; Https://www.ipcc.ch/). This change is bound to affect livestock since it will affect their biodiversity, physiology, welfare, production, and reproduction traits as well as the feed crops and forage (Grace et al., 2022; Perini et al., 2020).

Climate change has led to temperature increases over time leading to heat stress (Figure 2.2), (https://data.giss.nasa.gov/gistemp/maps/), and unpredicted rainfall patterns and this harms livestock welfare (Duchenne-Moutien & Neetoo, 2021; Angel et al., 2018).

Heat stress has been documented as one of the worst environmental stressor for poultry production worldwide, leading to annual economic losses in the poultry industry (Kennedy et al., 2022; Vandana et al., 2021). During heat stress, the physiological adaptations can be manifested at various levels like behavioral and molecular changes at the genomic, transcriptomic, proteomic, and metabolomic levels (Jastrebski et al., 2017; Perini et al., 2020; McFarlane & Curtis, 1989; Kennedy et al., 2022; Perini et al., 2020; May & Lott, 1992).

Some of the changes that are seen in poultry to survive heat stress include seeking shade, drinking more water, less feed intake, panting, flapping of feathers, and there will be an increase in blood flow to the skin (Kennedy et al., 2022). At a molecular level, the primary response to acute heat stress is an increase in the expression of heat shock proteins (HSP) genes, which are molecular chaperones responsible for stabilizing protein structures at high temperatures (Feder & Hofmann, 1999; Grace et al., 2022). Changes in protein translation and the expression of genes influencing the cell cycle, DNA replication, and DNA repair are other responses to acute heat stress in poultry (te Pas et al., 2022). Chronic heat stress can trigger other molecular reactions that make it possible for the poultry to adapt to the ongoing heat stress challenge due to climate change.

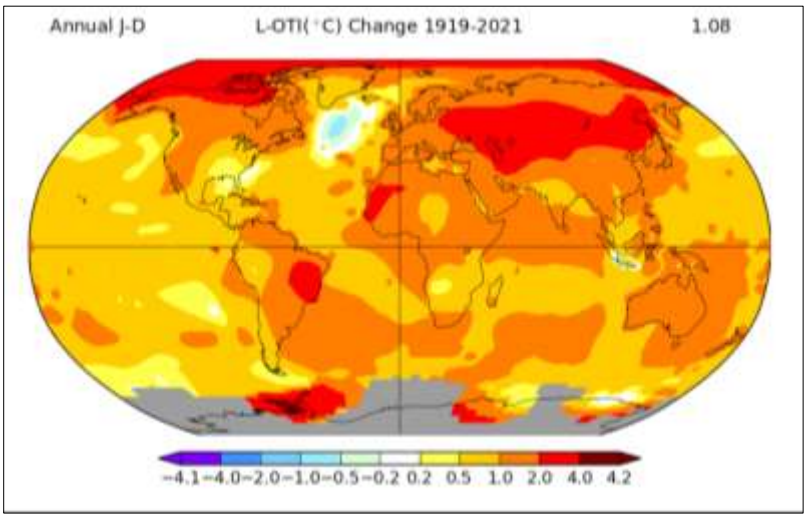

Figure 2.2: Global Temperature Rise: 2021 Compared to 1919

2.5 The “Omics” Technologies and Heat Stress

The term "omics" in biology refers to a variety of scientific disciplines that characterize and quantify groups of biological molecules derived from DNA, RNA, proteins, and metabolites, that translate into the structure, function, and dynamics of an organism (Dehau et al., 2022). The study of heat stress in poultry has been greatly advanced by the emergence of “omics” technologies, which encompass a range of high-throughput analytical approaches that enable comprehensive and systematic investigation of various biological molecules and processes at the molecular, cellular, and organismal levels (Zampiga et al., 2018). Omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, have provided valuable insights into the molecular mechanisms underlying the response of poultry to heat stress, shedding light on the complex and dynamic interplay of molecular events involved in this physiological response (Ahmad et al., 2023).

Genomics, which involves the study of an organism's entire set of genes, has been instrumental in identifying key genes and genetic pathways associated with heat stress response in poultry (Jensen, 2005; Rothschild & Plastow, 2014). Genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping have been used to identify genetic markers and genomic regions that are associated with heat tolerance traits in poultry (Gholami et al., 2015; Luo et al., 2022; Reyer et al., 2015). These studies have revealed important genes involved in heat stress response, such as heat shock proteins (HSPs), antioxidant enzymes, and genes related to immune function and metabolism (Sun et al., 2021). Additionally, gene expression profiling using microarray and RNA sequencing (RNA-seq) technologies has provided insights into the changes in gene expression patterns in response to heat stress, revealing key regulatory pathways involved in the cellular response to heat stress in poultry (Kim et al., 2022).

Transcriptomics, which focuses on the study of an organism's complete set of RNA molecules, has allowed for a comprehensive understanding of the dynamic changes in gene expression in response to heat stress in poultry (Wang et al., 2009). Transcriptomic studies have revealed alterations in the expression of genes related to heat shock response, oxidative stress, immune response, metabolism, and cellular repair mechanisms. Additionally, alternative splicing events and non-coding RNAs, such as microRNAs, have been implicated in the regulation of heat stress response in poultry,

further expanding our understanding of the complexity of gene expression regulation in this context (Yépez et al., 2021; Wang et al., 2009).

2.6 Genomic Response to Heat Stress in Poultry

Genomic sequencing technologies have been applied to characterize many genetic traits such as heat stress resistance, production and reproduction potential, and disease resistance among other traits in various poultry populations (Mukhopadhyay & Kaur, 2023). A study by Gu et al. (2020) analyzed the whole genome sequence data in Niya chicken and identified putative genes that might be related to the adaptation to the hot arid and harsh environment. Their study identified some candidate genes involved in different molecular processes and pathways that were involved in the adaptation of Niya chicken to hot environments. Whole genome sequencing and comparative genomics analysis in yak identified the expansion of gene families related to sensory perception and energy metabolism and some positively selected genes related to hypoxia and nutrition metabolism. Several selective regions with genes responsible for the circulatory system and blood vessel development, central nervous system development, and apoptosis were identified (Chai et al., 2020).

A study by Tian et al., (2020) identified genes that were under selection and that were responsible for chicken adaptations to different climates in Sri Lankan and Saudi Arabian chicken. Walugembe et al. (2019) also identified positively selected genes responsible for the survival of chicken in the hot climate of Sri Lanka, Ethiopia, and Brazil. Similar studies have been done on guineafowls (Shen et al., 2021), cattle (Freitas et al., 2021; Weldenegodguad et al., 2019; Yurchenko et al., 2018), goats (Brito et al., 2017; Guo et al., 2018), ducks (Zhang et al., 2018), sheep (Abied et al., 2020; Liu et al., 2016; Wang et al., 2019).

The detection of genomic differences in chicken and guineafowl species can shed light on the genetic basis of adaptation to diverse environments and provide insights into functionally important genetic variants (Tian et al., 2020). These genomic differences can be used to provide the basis for genetic improvements for better production and performance to be useful in genetic breeding programs. Resilient genotypes identified from such studies can then be selected or integrated in improved productive breeds for superior performance in chicken and guineafowls under their local climate.

2.7 Transcriptomic Response to Heat Stress in Poultry

Transcriptomics is an approach used to understand genetic control by quantifying the expression of transcripts, including mRNA, microRNAs, long non-coding RNAs, and circular RNAs (Dai & Shen, 2022; Khodadadian et al., 2020; Wang et al., 2009). RNA sequencing, particularly next-generation sequencing (NGS), has made it possible to identify and quantify RNA transcripts in an unbiased and high throughput manner. Different RNA sequencing techniques have been developed for specific tasks, such as 3' end sequencing, alternative splicing, gene fusion analysis, targeted RNA sequencing, and single-cell RNA sequencing (Gondane & Itkonen, 2023; Shi et al., 2023).

RNA sequencing is preferred for assaying transcriptional levels as it assays the expression of all genes that are transcribed without the need to pre-select genes for analysis (Dai & Shen, 2022). RNA sequencing can reveal genes and pathways that are responsible for a trait of interest. Transcriptomics studies have been conducted in various livestock species, including cattle, quails, and chicken, to investigate the response to heat stress and identify differentially expressed genes (DEGs) and pathways related to oxidative stress, metabolism, and signal transduction.

A study by Li et al. (2022), did a comparative transcriptomics study on the hypothalamic-pituitary gonad axis of mammals and poultry. They identified two important pathways responsible for the neuroactive ligand-receptor interaction and the calcium signaling pathway that play an important role in animal reproduction. Their study also identified some differentially expressed genes in the hypothalamus, pituitary, and ovaries. Kim et al. (2022) did a study on differential gene expression in chicken exposed to thermal stress and they identified genes that were differentially regulated and were responsible for reactive oxygen species production, glucose metabolism, cell nutrient intake, and circadian rhythm.

Several studies on transcriptomics in cattle (Chen et al., 2022), quails (Caetano-Anolles et al., 2015; Wang et al., 2021), and chicken (Kim et al., 2021; Monson et al., 2018, 2019; Mutryn et al., 2015; Park et al., 2018; Perini et al., 2020; Srikanth et al., 2019; Sun et al., 2015; Van Goor et al., 2017; Zhang et al., 2020) have identified several DEGs as well as revealed several pathways about heat stress that are related to oxidative stress, metabolism, and signal transduction. Taken together, a variety of tissues from

various livestock breeds including poultry have been investigated for response to heat stress. The kidney tissue has also been used to study the transcriptomics of poultry exposed to heat stress (Wu et al., 2014; Xu et al., 2022). In this study, it was the preferred organ for analysis because of its role in homeostasis and extensive use in heat stress studies

Integration of multi-omics data, including transcriptomics, has the potential to provide more precise insights into biological mechanisms, but challenges remain in the analysis and interpretation of these integrative approaches (Dehau et al., 2022). Accurate genome annotation is crucial for accurate gene expression estimation using RNA-Seq, and further research is needed to utilize omics technologies for understanding genomes and contributing to theoretical population genetics and the study of adaptation and economic traits in agricultural animals (Cho et al., 2022).

2.8 Genomic Signatures of Selection Tests

Signatures of selection are regions of the genome that contain beneficial mutations that have been subjected to natural or artificial selection, leaving behind specific patterns of DNA (Gondane & Itkonen, 2023; Qanbari & Simianer., 2014; Jensen et al., 2016). The factors that influence the domestication of livestock, including genetic drift, inbreeding, and natural/artificial selection, have resulted in the emergence of diverse livestock species adapted to varying environmental conditions (Gheyas et al., 2022; Groeneveld et al., 2010). In poultry, natural selection plays a crucial role in shaping genetic variation and improving survival and reproductive fitness (Saravanan et al., 2020).

Artificial selection is carried out using two methods: methodological and unconscious, with the former being more goal-oriented (Saravanan et al., 2020). The use of these selection strategies has led to changes in specific genomic loci, resulting in signatures of selection that control breed characteristics such as adaptation, disease resistance, reproduction, production performance, behavior, and morphology (Gheyas et al., 2022). Selective sweeps, where a new beneficial mutation increases in frequency and reduces variability in associated neutral sites, can occur during artificial selection as shown in Figure 2.3 (Fay & Wu, 2000; Saravanan et al., 2020; Stephan, 2019).

Selective sweeps can be categorized as hard or soft, complete or partial, depending on the origin, type, and frequency of the mutation. Soft sweeps, which act on standing genetic variation, are more difficult to detect and do not drastically reduce genetic variation (Pritchard et al., 2010; Hermisson & Pennings, 2017; Hermisson & Pennings, 2005). Various methods, such as linkage disequilibrium, site frequency spectrum, reduced local variability, and haplotype characteristics, can be used to detect signatures of selection depending on the tools used and the time scale of selection (Qanbari & Simianer, 2014; Stephan, 2019; Cadzow et al., 2014; Sabeti et al., 2006). Studying signatures of selection can provide insights into evolutionary pressures and genes involved in recent adaptation in livestock species. Advances in genotyping data and statistical methods have improved the ability to detect signatures of selection in livestock genomes.

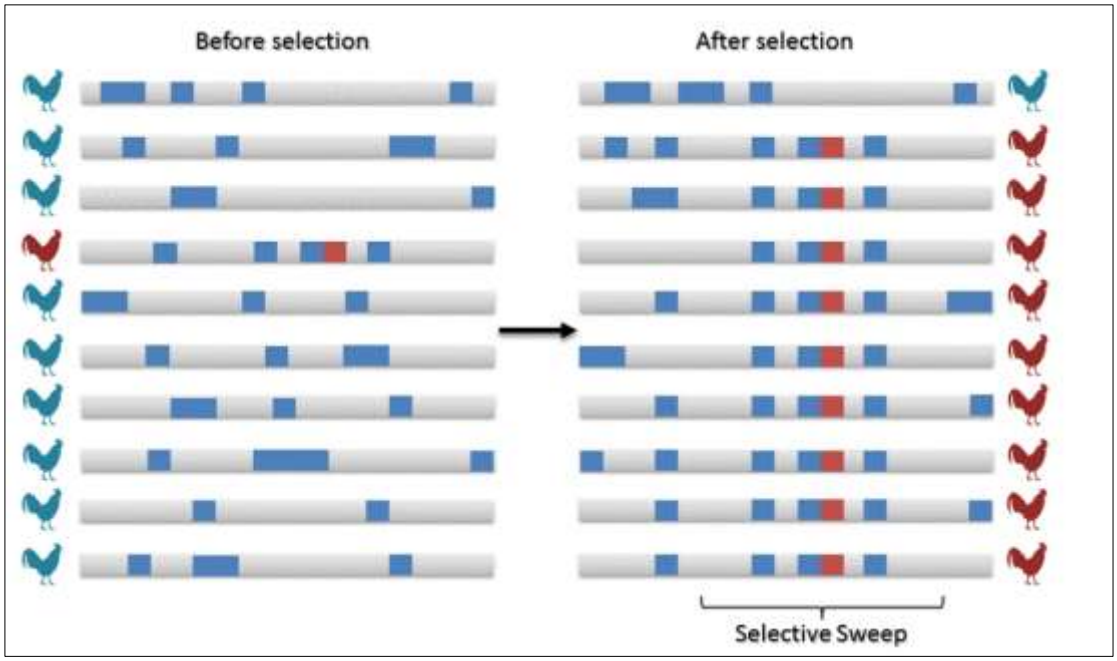

Figure 2.3: Illustration of a Selective Sweep Process

Key: Figure 2.3 illustrates how natural selection results in the increase of a beneficial mutation's prevalence in a population. It displays polymorphisms along a chromosome including the selected allele before and after selection. The grey part represents the ancestral alleles and the derived/non-ancestral alleles are shown in blue. As the positively selected allele (red) becomes more frequent, nearby linked alleles on the chromosome also rise to high frequency due to the association causing a "selective sweep".

2.8.1 Overview of the Methods Used for the Detection of Genomic Signatures of Selection

Methods for detecting microevolutionary selective events at the population level, also known as selection signatures, have evolved with advancements in high-throughput sequencing, SNP genotyping technologies, and population genomics (Oleksyk et al., 2010; Qanbari & Simianer, 2014). These methods involve genome-wide scans for signatures of selection within and between populations of different species (Figure 2.4). The hitchhiking theory can result in informative signatures such as reduced local variability, deviated allele frequency spectrum, and specific linkage disequilibrium patterns (Kanaka et al., 2023; Panigrahi et al., 2023).

Statistical approaches, including intra-population and inter-population statistics, are used to detect these signatures. Intra-population statistics focus on three neutrality theories: site frequency spectrum (SFS), linkage disequilibrium (LD), and reduced local variability (Rubin et al., 2010; McQuillan et al., 2008; Tajima, 1989). Inter-population statistics are grouped into single-site and haplotype-based differentiation, which depend on the level of differentiation between populations caused by the frequency of locus-specific alleles (Zhao et al., 2015).

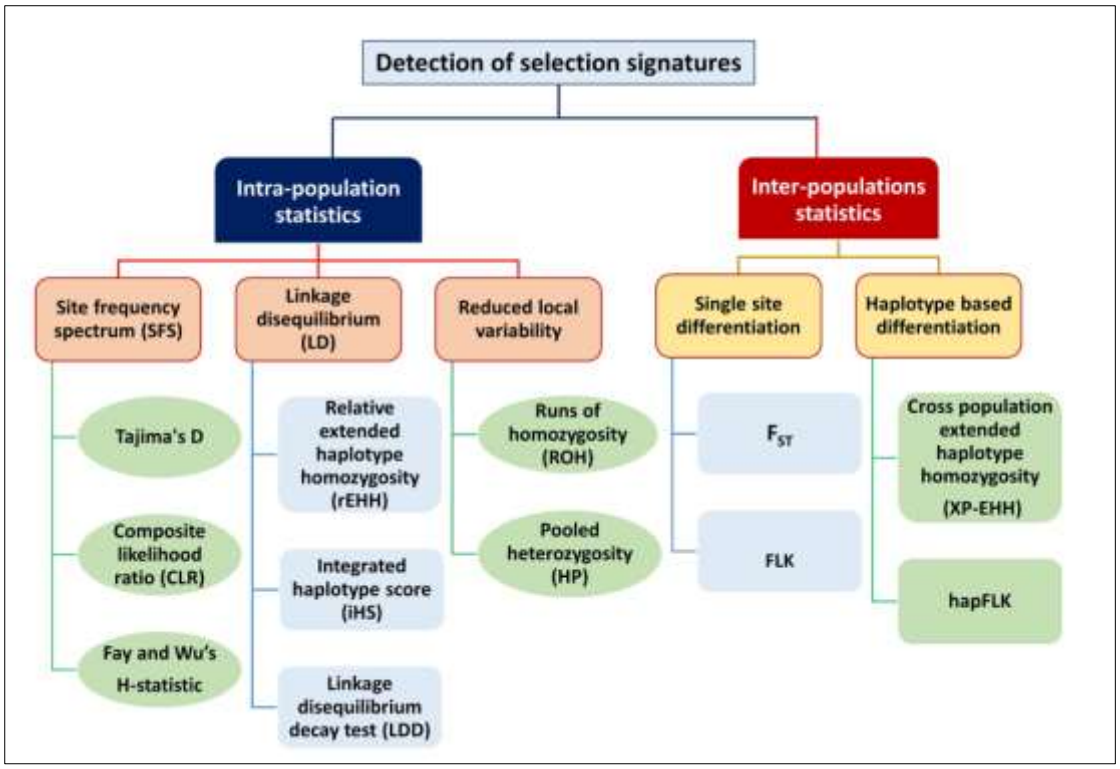

Figure 2.4: Various Methods for the Detection of Genomic Signatures of Selection

Key: Figure 2.4 shows the categories of the various methods used to detect the genomic signatures of selection based on the intra-population statistics (site frequency spectrum, linkage disequilibrium, and reduced local variability) and inter-population statistics (single site differentiation and haplotype-based differentiation) (Saravanan et al., 2020)

2.8.1.1 Haplotype-based Differentiation Methods

The haplotype-based methods, hapFLK, and the cross-population extended haplotype homozygosity (XP-EHH), utilize haplotype information in multiple populations to reduce SNP ascertainment bias (Panigrahi et al., 2023; Fariello et al., 2013; Sabeti et al., 2007). XP-EHH calculates integrated haplotype homozygosity (iHH) values for each population by integrating extended haplotype homozygosity (EHH) data, which requires phased genotype data. The hapFLK statistic considers different effective population numbers and population hierarchies, increasing its power for detecting selection (Fariello et al., 2013). Unlike XP-EHH, hapFLK can be applied to unphased SNP genotype data (Saravanan et al., 2020).

XP-EHH has been used to identify regions and genes associated with the adaptation of livestock to specific environmental conditions (Xu et al., 2022; Wang et al., 2015). In cattle, genes such as APOB, SPATA17, and TNN13K were identified for production and adaptation (Singh et al., 2020). In chicken, genes such as ATP6AP1L, HEXB, SLC33A1, ADCY1, and HIF1AN, were selected for adaptation to tropical and frigid environments using the XP-EHH method (Shi et al., 2022; Tian et al., 2020). To increase detection power and reduce false positives, multiple methods are commonly used in studies of selection signatures in livestock species (Xu et al., 2022; Singh et al., 2020; Shen et al., 2021). The current study used the XP-EHH method to scan for selection signatures in chicken and guineafowl populations.

2.8.1.2 Single Site Population Differentiation

The single-site population differentiation-based methods are popular for detecting signatures of selection among different populations due to their simplicity and ability to handle large genotype datasets (Cadzow et al., 2014; Chen et al., 2015; Saravanan et al., 2020). These methods, such as F_{ST} (Chen et al., 2015), and FLK (Bonhomme et al., 2010), detect local increases or decreases in population differentiation. F_{ST}, whose value ranges from 0 indicating no differentiation to 1 indicating the fixed difference between populations, is a commonly used statistic that measures the correlation between randomly drawn alleles drawn from a single population relative to the most recent ancestral population. High F_{ST} values indicate positive selection, while low values indicate negative selection (Zhao et al., 2015).

There are several F_{ST} estimators available, with Weir and Cockerham's F_{ST} being the most commonly used (Chen et al., 2015; Holsinger & Weir, 2009; Gianola et al., 2010). F_{ST} has the advantage of being SNP-specific and capable of identifying specific genetic variants under selection. It is recommended to evaluate consecutive SNPs with an average F_{ST} score rather than individual SNPs. F_{ST} has been used to detect signatures of selection and candidate genes such as FAM110B, TNN13K, CACNA2D1, and LRP1B involved in adaptation and production among livestock like in chicken (Tian et al., 2020; Xu et al., 2022; Guo et al., 2022), in guineafowls (Shen et al., 2021), in yaks (Chai et al., 2020), and cattle (Shen et al., 2021; Maiorano et al., 2018). The current study used the F_{ST} method to scan for selection signatures in chicken and guineafowl populations.

2.8.1.3 Linkage Disequilibrium-based Methods

Linkage disequilibrium (LD) is the nonrandom association between two or more loci. LD-based models focus on long homozygous regions with high-frequency haplotypes resulting from selective sweeps, which are the rapid increase in the frequency of a haplotype carrying a beneficial mutation (Sabeti et al., 2002). Extended haplotype homozygosity (EHH) is a method proposed by Sabeti et al. (2002) based on LD, which calculates the likelihood that a pair of chromosomes contain homozygous core haplotypes. The relative extended haplotype homozygosity (rEHH) is then calculated to compare the EHH values of two core haplotypes, with high rEHH values indicating positive selection.

Voight et al.(2006) developed an integrated haplotype score (iHS), an extension of the EHH test, which incorporates recombination distance into the statistics. iHS measures how haplotypes around a single nucleotide polymorphism (SNP) are unusual compared to the entire genome (Voight et al., 2006). When compared to haplotypes associated with ancestral alleles, extreme negative iHS values (iHS <-2) indicate extended haplotypes on derived allelic backgrounds, while extreme positive values (iHS > 2) indicate widespread population distribution of ancestral alleles (Weigand & Leese, 2018). The iHS method requires haplotype phasing, recombination map, genomic position, and ancestral and derived allelic information for each core SNP, and is ideal for detecting signatures of selection when selected alleles are at intermediate frequencies.

The iHS method has been used to detect regions under selection and genes involved in diverse biological processes in chicken (Vallejo-Trujillo et al., 2022). In indigenous chicken from the southern parts of Italy, the *ST7* gene was selected for thermotolerance using the iHS method (Mastrangelo et al., 2023). In cattle, Singh et al. (2020) identified the SPATA17, FAM110B, and TNN13K genes that are associated with adaptation and production. In sheep, Saravanan et al. (2021) identified some genes such as TRPM8 which is involved in cold adaptation, and JADE2, PPP3CA, and TSHR which are involved in meat quality traits. The iHS approach is less impacted by demographic factors, resulting in a lower likelihood of false-positive results compared to other methods such as rEHH (Voight et al., 2006). The current study used the iHS method to scan for selection signatures in chicken and guineafowl populations.

2.8.1.4 Site Frequency Spectrum Methods

These methods analyze the distribution of genetic variants (alleles) within a population based on their frequencies (Tournebize et al., 2019). The key idea is to identify deviations from the expected neutral model of genetic variation, which can indicate regions of the genome that have been under selective pressure. SFS methods involve constructing a frequency spectrum that counts the number of alleles at different frequency bins in a population (Rajawat et al., 2022).

SFS methods start with the collection of genetic data, typically DNA sequences or genotype information from a sample of individuals within a population. The data are used to construct an allele frequency spectrum, which represents the distribution of allele frequencies in the population (Weigand & Leese, 2018). It categorizes alleles based on their frequencies, from rare to common. SFS methods rely on models of genetic variation under neutrality, which predict the expected allele frequency distribution in the absence of selective pressure. Deviations from these neutral expectations can suggest the action of selection.

SFS methods include various statistical tests and summary statistics to identify genomic regions with unusual patterns in the allele frequency spectrum. Commonly used tests include Tajima's D, Fay and Wu's H, and Nielsen's composite likelihood ratio (CLR) test (Saravanan et al., 2020). Significant deviations from neutral expectations may indicate different forms of selection, such as positive selection (increased frequency of advantageous alleles), balancing selection (maintaining genetic diversity), or background selection (removal of deleterious mutations). SFS methods assume simplified demographic models and may not account for complex population histories or other factors that influence allele frequencies. False positives and false negatives can occur, so validation and corroborating evidence are often necessary. SFS methods have been applied to a wide range of organisms and have been used to detect selection in various contexts, including adaptation to local environments, recent selective sweeps, and identification of candidate genes under selection (Saravanan et al., 2020; Weigand & Leese, 2018).

2.8.1.5 Reduced Local Variability Methods

Reduced local variability methods are used in population genetics and genomics to identify genomic regions with reduced genetic diversity or variability compared to the rest of the genome (Saravanan et al., 2020). Runs of homozygosity (ROH), which is one of the main methods under reduced local variability, is characterized by contiguous lengths of homozygous genotypes that occur within an individual when two haplotypes share a recent common ancestor, i.e. identical by descent (IBD) (McQuillan et al., 2008; Saravanan et al., 2020). ROHs are widely used to assess genomic inbreeding levels, population structure, and demography history in livestock populations (Lavanchy & Goudet, 2023). Based on the hitchhiking theory, a selective sweep should have stretches of homozygous loci which exhibit higher homozygosity than the average of the genome (Almeida et al., 2019). Hence, ROH can be used to identify signatures of selection as the individuals that have undergone selective process will exhibit long runs of homozygosity around the target locus

The second main method is the pooled heterozygosity (HP), which uses allele counts to calculate heterozygosity (Guo et al., 2016). This statistic estimates the deviation of expected local heterozygosity depression in chromosomal windows from the average heterozygosity of the genome (Saravanan et al., 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location and Description of Study Sites

Chicken samples were collected from the coastal counties of Kilifi, Mombasa, Kwale, and Lamu in Kenya, while guineafowl samples were obtained from Laikipia County and the coastal counties of Taita Taveta, Kilifi, and Lamu, also in Kenya. These regions lie within zones III and IV of the agro-climatic zones of Kenya Figure 3.1.

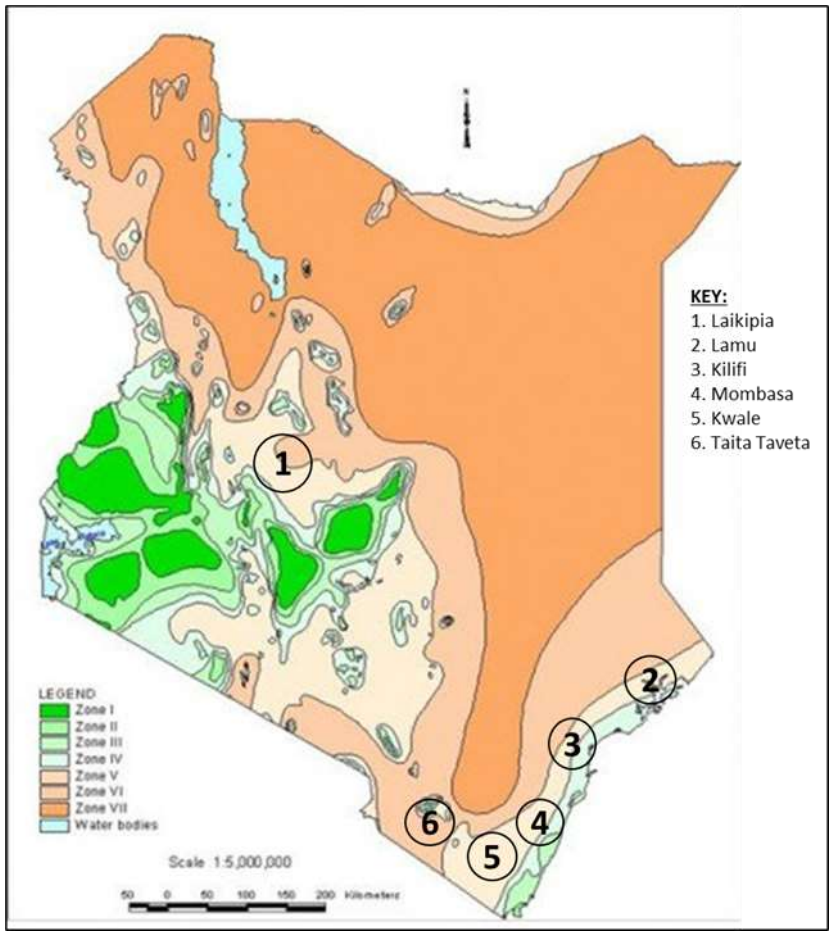

Figure 3.1: Map of Agro-Climatic Zones of Kenya

The coastal regions lie within zones III and IV of the agro-climatic zones of Kenya, which extend from sub-humid to arid zones (*AEZs FAO System Infonet Biovision Home*) (Figure 3.1). The average annual rainfall ranges between 500-1500 millimeters (mm), this is quite unreliable, especially for crop and livestock farming. The mean annual temperature ranges between 24ºC and 33ºC (Kogo et al., 2021; Kenya Meteorological

Department, 2019). Livestock production, especially poultry, is important in the region since it plays a significant role in the hospitality industry as a result of domestic and international tourism (Waaijenberg, 1994; Mshenga et al., 2010).

Laikipia County is located in Zone IV of the agro-climatic zones of Kenya (*AEZs FAO System Infonet Biovision Home*) (Figure 3.1). It occurs mostly at elevations between 900-1800m. The weather in Laikipia County is generally semi-arid, with an annual average rainfall of about 500-1000 mm (Kogo et al., 2021). The rainfall is sporadic occurring mostly between March and May and between October and December. The average temperature ranges between 15ºC and 30ºC, with the hottest months being January and February (Kenya Meteorological Department, 2019). Despite the challenging weather conditions, guineafowls thrive well in the wild environment in Laikipia and poultry farming is a significant economic activity in the County (Food and Agriculture Organization, 2007; Waaijenberg, 1994).

3.2 Study Clearance and Permits of Compliance

This study was approved by the Directorate of Veterinary Services, National Veterinary Laboratories, State Department of Livestock, Ministry of Agriculture, Livestock, Fisheries, and Co-operatives under permit number RES/POL/VOL.XXVII/162. For wild guineafowl sampling, the study was approved by the Kenya Wildlife Services under permit number KWS/BRM/5001. The Institutional Animal Care and Use Committee (IACUC) at JKUAT approved all the poultry handling procedures used in this study. Before sampling permission was sought from the County governments, farmers, and various poultry traders.

3.3 Sampling Design

This was a stratified purposive cross-sectional study. With the help of local veterinary extension officers, field surveys were conducted in remote villages in farms and markets with chicken and guineafowls. Prior informed consent was sought from the farmers and a rural participatory approach was used where interviews were conducted at the farmers' houses with the assistance of local agricultural extension officers. From each trader or farmer, mature chicken and guineafowls were purchased for blood and

tissue collection. A non-probability purposive sampling technique was used to collect study samples.

To allow for meaningful genomic analyses, better statistical power, and a more comprehensive understanding of gene expression patterns and biological variability within and between the chicken and guineafowls, a total of 20 adult indigenous chicken (Lamu-5, Kilifi-5, Mombasa-5, and Kwale-5) were obtained. For the guineafowls a total of 16 adult guineafowls (Laikipia-9, Lamu-5, Kilifi-1, and Taita Taveta-1), were obtained. On the other hand, 13 chicken and 13 guineafowl kidney tissue samples were obtained from Lamu County, and 13 chicken and 13 guineafowl kidney tissue samples were obtained from Mombasa County for RNA sequencing.

3.3.1 Additional Samples for Comparative Genomics

In addition to the 20 chicken samples from Mombasa, Lamu, Kilifi, and Kwale Counties, some additional 14 whole genome sequences of chicken samples from the SRA database from NCBI were downloaded for comparative genomics study. These included chicken from Ethiopia (Horro-5, Jarso-4), and China (Yunnan-4, Hainan-1). Details about the chicken SRA accession numbers can be found in Appendix I. In total, 34 whole-genome chicken sequences were used for the downstream analyses.

In addition to the 16 guineafowl samples from Laikipia, Lamu, Kilifi, and Taita Taveta Counties, some additional 56 whole genome sequences of guineafowl samples were downloaded from the SRA database from the NCBI for comparative genomics study. These included guineafowls from Africa: Sudan (10), Nigeria (10), Burkina Faso (5), Benin (1), and South Africa (1), Asia: China (6), and Iran (6), Europe: Hungary(7), France (5), and Italy(5) (Shen et al., 2021; Vignal et al., 2019). Details about their accession numbers can be found in Appendix II. In total, 72 whole genome guineafowl sequences were used for the downstream analyses.

3.4 Sampling Procedure

3.4.1 Blood Sample Collection Procedure for Genomics Work

Blood was collected from the wing vein of each of the 20 sampled chicken and each of the 16 sampled guineafowls. Briefly, the chicken/guineafowl was held horizontally on their back. One hand was used to hold the chicken/guineafowl legs and the other hand was placed under the back to support the chicken/guineafowl. The wing vein was pulled outwards towards the person bleeding. The feathers that obscure the vein were plucked off. The area was then disinfected by swabbing with 70% alcohol.

A 20-gauge needle was inserted in the tendon and directed into the wing vein in the direction of blood flow. Once the tip of the needle was in the vein, the plunger of the syringe was pulled gently. This triggered blood flow into the syringe. Once enough blood was collected (2ml), the needle was removed into a needle disposal container. A new needle and syringe were used for each chicken/guineafowl. After the needle was removed, pressure was applied to the vein for a few seconds to discourage further bleeding. Blood was carefully transferred from the vacutainer syringe to the tube and gently inverted 2-3 times to thoroughly mix the anticoagulant with the blood. The tubes were then firmly capped and taped to prevent leakage. These were placed in dry ice and transported to Sino-Africa Joint Research Center laboratories domiciled in Jomo Kenyatta University of Agriculture and Technology for further processing.

3.4.2 Tissue Sample Collection Procedure for Transcriptomics Work

Kidney tissue samples of 13 chicken from Lamu, along with 13 kidney tissue samples of chicken from Mombasa, were harvested. Similarly, 13 kidney tissue samples of guineafowls from Lamu, as well as 13 kidney tissue samples of guineafowls from Mombasa, were also harvested. Briefly, the chicken and guineafowls were killed by the rapid decapitation method to ensure that they are not subjected to prolonged pain and suffering. They were then dissected and fresh tissues collected and placed in RNAlater® (Thermo Scientific, Walton, Massachusetts, USA). The collected tissue samples were placed in liquid nitrogen and transported to the Sino-Africa Joint Research Center laboratories at Jomo Kenyatta University of Agriculture and Technology for RNA extraction.

3.5 Sample Processing

3.5.1 DNA Extraction for Whole Genome Sequencing for Chicken and Guineafowls

200µl of each of the 20 sampled chicken and 16 sampled guineafowls was taken from the collected 2ml blood and placed into 1.5ml Eppendorf tubes for each sample. Then, 400µl of STE (Sodium Chloride-Tris-EDTA; 30mM Tris-HCl (pH 8), 200mM EDTA, disodium salt (pH 8), 50mM NaCl) + 50µl 10% SDS added. This was followed by the addition of 20µl of Proteinase k (20mg/ul). The contents were vortexed and incubated at 56°C -60°C for 6-8 hours. Phenol (670µl), was added and then vortexed for 6-8 hours. Centrifugation was then done at 9,000 rpm for 10 minutes and the upper layer was transferred into a new tube. Phenol and Chloroform (Trichloromethane) at a 1:1 ratio of starting volume (i.e., 335µl:335µl) were added and vortexed for 6-8 hours. This was centrifuged at 9,000 rpm for 10 minutes and the upper layer transferred into a new tube.

Chloroform (Trichloromethane; 24:1), an equal amount to starting volume, i.e., 670µl was added and vortexed for 6-8 hours. This was centrifuged at 9,000 rpm for 10 minutes then the upper layer was transferred into a new tube. Isopropyl alcohol (1000µl) was added and this was incubated at -20°C for 8 hours. This was then centrifuged at 10,000 rpm for 10 minutes. The Isopropyl alcohol was poured out and addition of 1000µl of 70% Ethanol was added to wash the pellet. This was then centrifuged at 10,000 rpm for 10 minutes. Ethanol was poured out, and the Ethanol washing step was repeated.

The DNA was dried at 56°C-60°C for 6-8 hours. TE (50µl) was added to dissolve the DNA, this was mixed and centrifuged briefly for 5 seconds. This was incubated at room temperature for 6 hours before performing quality and quantity checks. DNA integrity especially for long fragments, was assessed by gel electrophoresis to help verify that the DNA was not degraded. The purity of DNA samples was done by measuring the A260/A280 ratio. Samples with ratios close to 1.8, which indicates minimal contamination with proteins or other impurities were retained. The DNA was transferred into cold storage (-20°C).

3.5.1.1 DNA Library Preparation

Library preparation for total DNA was carried out using the TruSeq® DNA Library Preparation Kit v2 (Illumina, USA) following the manufacturer's instructions. In summary, 2 micrograms of genomic DNA (gDNA) were purified and fragmented. The DNA fragments were then rendered blunt-ended through a combination of fill-in reactions and exonuclease activity. Subsequently, an 'A' base was appended to the blunt ends of each DNA strand in preparation for the ligation of sequencing adapters.

These adapters were designed with a 'T' base overhang at their 3' ends, which perfectly complemented the A-tailed fragmented DNA. Moreover, the adapters included all the necessary sequences for sequencing primer hybridization, obviating the need for additional PCR steps to introduce index tags and index primer sites. After denaturation, the prepared libraries were combined for subsequent sequencing. The DNA was then sent out for whole genome sequencing using Illumina HiSeq 2500 sequencing platform at BGI Hong Kong

3.5.2 RNA Extraction and Purification

One hundred milligrams (100mg) of each of the frozen 26 kidney tissue samples of the chicken and 26 kidney tissue samples of the guineafowls were crushed into powder form using a sterile prechilled mortar and pestle, and occasionally adding liquid nitrogen into the mortar to prevent thawing. Each crushed tissue sample was aliquoted into two and placed in two sterile 200µl cryotubes. RNA extraction was done using one of the aliquots into which 1000µl (1ml) of Trizol reagent was added. The other aliquot was stored at -80°C. Trizol LS reagent (Invitrogen, Carlsbad, CA, USA) was used for RNA extraction following the manufacturer’s instructions. Briefly, 1000µl of Trizol LS reagent (Invitrogen, Carlsbad, CA, USA) was added to the processed samples and vortexed for 20 seconds, and incubated at room temperature for five minutes. To the mixture, 200µl of chloroform was added and vortexed vigorously for 15 seconds, followed by incubation for 2-3 minutes at room temperature. This was then centrifuged at 12,000 × g at 4°C for 15 minutes in a pre-chilled centrifuge.

The topmost layer of the centrifuged sample (around 500µl) was pipetted into a fresh RNase-free tube and 500µl of Isopropanol-2 added. The tube was inverted by hand to mix and then incubated for 10 minutes at room temperature and centrifuged at 12,000 × g for 10 minutes at 4°C. The supernatant was then discarded and the pellet was washed using 1ml of 75% Ethanol by inverting the tube gently. The sample was vortexed briefly and centrifuged for 5 minutes at 7500 × g at 4ºC. The wash was discarded and the pellet air dried for 10 minutes. The RNA pellet was re-suspended in RNase-free water.

The quantity of the extracted RNA was determined using a Nano Drop® ND-1000 spectrophotometer (Thermo Scientific, Walton, Massachusetts, USA) and Qubit fluorimeter (Invitrogen, Carlsbad, CA, USA) using Quant-IT RiboGreen RNA Assay Kit (Invitrogen, Carlsbad, CA, USA) and the integrity of RNA was visualized by electrophoresis in a 1.2% formaldehyde agarose gel stained with Gel Red and also by the use of a Bioanalyzer. RNA extracts were selected for library preparation when the 260/280 purity index was equal to or greater than 2 and the integral RNA in electrophoresis and Bioanalyzer measurements was greater than (RIN >8). The concentration and purity of the extracted RNA were determined using a Qubit fluorimeter (Thermo Scientific, Walton, Massachusetts, USA) before storage at -80°C until use.

3.5.2.1 RNA Library Preparation

Library preparation was done on total RNA using TruSeq RNA Sample Preparation Kit v2 (Illumina, USA) according to the manufacturer's instructions. Briefly, RNA was prepared by purification and fragmentation of 2ug of total RNA using oligo (dT) magnetic beads and used as a template for cDNA synthesis by random hexamer priming followed by end repair, A-tailing, and ligation of Illumina adaptors. DNA particles with adaptors were then amplified using PCR After various steps, cleanup of the template was done using Agencourt Ampure RNAClean XP (Beckman Coulter, Pasadena, CA, USA). These clean-up steps included: post fragmentation clean up, post-cDNA synthesis; post-end repair, A-Tailing and adapter ligation, and post-PCR library clean-ups. Amplified libraries were analyzed for size distribution using the Agilent Tapestation 2200 DNA kit. Libraries were quantified using Quant-IT Qubit dsDNA

High sensitivity Assay (Invitrogen, Carlsbad, CA, USA). The libraries were sequenced on the HiSeq 4000 platform (Illumina, CA).

3.6 Data Analysis

3.6.1 Whole-genome Sequence Quality Control Processing and Variant Calling

The dataset which comprised of 20 sequenced chicken FASTQ files, along with the additional downloaded 14 chicken genomes, plus 16 sequenced guineafowl FASTQ files along with the 56 downloaded guineafowl genomes for comparative genomics analysis were preprocessed using Trimmomatic v0.36 (Bolger et al., 2014) to filter out low-quality reads and adapter sequences of raw data, based on default parameters. The chicken high-quality paired-end reads were mapped to the chicken reference genome (GRCg6a) from the NCBI database and guineafowl high-quality paired-end reads were mapped to the guineafowl reference genome (NumMel1.0) from the NCBI database.

Each individual was aligned using Burrows-Wheeler Aligner (BWA) with the option "BWA-MEM" algorithm with conventional parameters (Li and Durbin, 2010). Following the recommendations of the Broad Institute Genome Analysis Toolkit v.4.1.3.0 (GATK) (McKenna et al., 2010) Best Practices for the pre-processing workflow preceding variant discovery (*Https://Informatics.Fas.Harvard.Edu/Whole-Genome-Resquencing-for-Population-Genomics-Fastq-to-Vcf.Html*) (Figure 3.2). Picard tools v.1.56 (Li and Durbin, 2010) was used to sort and merge the alignment files by coordinates, index them, calculate the alignment matrices, and to MarkDuplicates. Calling of variants for each sample was performed using the default parameters of the GATK "HaplotypeCaller". Joint genotyping (GenotypeGVCFs) was done to identify variants simultaneously in all the guineafowl and chicken samples. Single Nucleotide Polymorphisms (SNPs) and insertion/deletions (InDels) in the guineafowl and chicken genomes were filtered into two different files using GATK VariantFiltration. Hard filtering was performed to reduce false positive variants.

To exclude SNP calling errors caused by incorrect mapping, only high-quality SNPs (Filtered by the VariantFiltration of GATK with options -filter "QD < 2.0" --filter-name "QD2" -filter "QUAL < 30.0" --filter-name "QUAL30" -filter "SOR > 4.0" --filter-name "SOR3" -filter "FS > 60.0" --filter-name "FS60" -filter "MQ < 40.0" --filter-name "MQ40" -filter "MQRankSum < -12.5" --filter-name "MQRankSum-12.5" -filter "ReadPosRankSum < -8.0" --filter-name "ReadPosRankSum-8" were retained for

subsequent analyses (Liu et al., 2022). The Indels were filtered with the following criteria; -filter-name "QD_filter" -filter "QD < 2.0" -filter-name "FS_filter "FS > 200.0" -filter-name "SOR_filter" -filter "SOR > 10.0". To estimate SNP statistics like the number of total and average bi-allelic autosomal SNPs and the ratio of the heterozygous and homozygous SNPs BCFtools v.1.10.2 was used. The SNP file was used for all the downstream analyses.

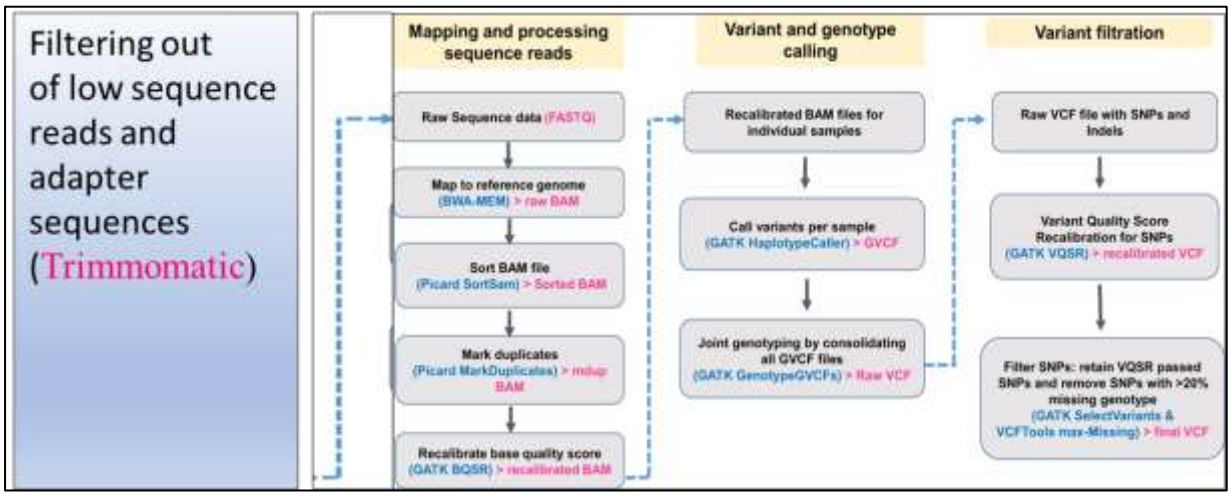

Figure 3.2: Overview of the GATK Variant Calling Steps

3.6.1.1 Annotation of Genomic Variants in Chicken and Guineafowls

Annotation of the chicken SNP file using the GRCg6a database and the guineafowl SNP file using the NumMel1.0 database was performed using the variant annotation and effect prediction tool (SnpEff) (Cingolani, 2022).

3.7.1 Population Structure Analysis

Population structure was inferred by Principal component analysis (PCA), and ADMIXTURE analysis for the chicken populations and the guineafowl populations. To minimize SNP redundancy, the two datasets were pruned using PLINK v1.90 (Purcell et al., 2007) with options "-indep-pairwise 50 10 0.2" (Anderson et al., 2010). Based on pruned SNPs, PCA was performed with smartpca in EIGENSOFT v7.2.0 (Patterson et al., 2006). Then, by using the first (PC1) and second (PC2) principal components, the figures were plotted with R v4.0.5 (Lüdecke et al., 2021) using an in-house script. The unsupervised hierarchical clustering was performed for all guineafowl and chicken populations using ADMIXTURE v1.3.0 (Alexander et al., 2009).

This analysis usually includes a cross-validation procedure that allows the identification of several populations (K) which fits best the model based on cross-validation (CV) error. To identify the best value of K clusters, an ADMIXTURE with cross-validation for values of K from 2 to 5 for chicken and 2 to 6 for guineafowls was calculated.

3.7.2 Selective Sweep Analysis

To identify the candidate regions under positive selection in the various guineafowl and chicken populations, the fixation statistics (F_{ST}) were calculated as previously described (Li et al., 2013). The F_{ST} value was estimated based on the differences in allelic frequencies between the guineafowls and chicken populations (Porto-Neto et al., 2013). This was done using VCFtools v0.1.13 (Holsinger & Weir, 2009), in a 100-kb sliding window with a 50-kb step size (Li et al., 2013; Qin et al., 2020). The top 0.1% F_{ST} values were empirically selected as potential candidate regions under selection (Qin et al., 2020). Since F_{ST} is an inter-population statistic, analyses were done for the following 3 groupings in chicken: Kenya vs. Ethiopia, Kenya vs. China, and Ethiopia vs. China. For the guineafowl populations, the analyses were done in the following 3 categories: Africa vs. Europe, Africa vs. Asia and Europe vs. Asia.

Two complementary EHH-derived methods, the Cross Population Extended Haplotype Homozygosity (XP-EHH) (Sabeti et al., 2007), and the Integrated Haplotype Score (iHS) (Voight et al., 2006), were also used to detect signatures of selection. The guineafowl and chicken SNPs were phased using SHAPEIT 4.2.1 (Browning et al., 2021). The iHS and the XP-EHH scores were calculated by rehh v3.2.2 (Gautier & Vitalis, 2012) with default parameters.

To detect positive selection in guineafowl and chicken populations, the average XP-EHH and iHS scores were computed for 100-kb regions with a 50-kb overlap. Regions with absolute XP-EHH and iHS scores of 4 or higher were considered putative candidate regions. Since XP-EHH is an inter-population statistic, similar to FST, analyses were conducted in three categories for chicken samples: Kenya vs. Ethiopia, Kenya vs. China, and Ethiopia vs. China. For the guineafowl populations, the analyses were carried out in three categories: Africa vs. Europe, Africa vs. Asia, and Europe vs. Asia. On the other hand, iHS is an intra-population statistic, and as such, analyses were performed for each individual population in chickens (Kenya, China, and Ethiopia) and guineafowls (Africa, Asia, and Europe).

3.7.2.1 Functional Annotation for Candidate Genes

To uncover the biological functions of candidate genes, the Gene Ontology (GO) Biological processes, Reactome gene sets, CORUM, Canonical Pathways, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis (Kanehisa et al., 2019) with Metascape website (http://metascape.org) was performed (Yingyao Zhou et al., 2019). The single list analysis method was used in the annotations using the chicken and guineafowl reference genomes to assign genes to the corresponding terms. Enrichment tests were performed using the Benjamini-Hochberg *p*-value correction algorithm as described in Metascape (Zhou et al., 2019).

3.7.3 RNA Sequence Quality Control Processing and Read Counts

Trimmomatic v0.36 (Bolger et al., 2014; Sewe et al., 2022) was used to filter out low-quality reads and adapter sequences of raw FASTQ data of the 26 chicken kidney tissue samples and 26 guineafowl kidney tissue samples, based on default parameters. The chicken high-quality trimmed paired-end reads were mapped to the chicken reference genome (GRCg6a) from the NCBI database and guineafowl high-quality trimmed paired-end reads were mapped to the guineafowl reference genome (NumMel1.0) from the NCBI database using the Tophat aligner v2.1.1 with default parameters (Trapnell et al., 2012). featureCounts v1.6.2 was used to calculate the read counts assigned to the genes (Liao et al., 2014).

3.7.3.1 Identification and Analysis of Differentially Expressed Genes

The read counts of genes, for the kidney tissue of the chicken and guineafowls were loaded into R v4.3.1. The gene expression matrix was constructed via DESeqDataSetFromMatrix() function wrapped in DESeq2 package v1.32.0 (Wen, 2017). Principal component analysis (PCA) of the sample was performed based on the rlog-transformed counts matrix using rlog() function followed by plotPCA() function. Outliers were removed based on the visualization of PCA results. The DESeq() function in the DESeq2 package was used to identify differentially expressed genes (DEGs). Genes were considered as candidates of DEGs by the following thresholds: $|\log2FoldChange| > 1$, and $padj < 0.05$. The heat map of DEGs was visualized by pheatmap v1.0.12 package and the volcano plot of DEGs was visualized by in-house script, which was mainly achieved by ggplot2 v3.3.5 software.

3.7.3.2 Pathway Analysis of Differentially Expressed Genes

The DAVID database was used in the GO functional enrichment analysis of the DEGs. All genes were placed on the background list and the DEGs were included in the candidate list. The hypergeometric distribution was used to calculate *p* values before multiple testing and Benjamini-Hochberg correction (Benjamini & Hochberg, 1995). GO items with a false discovery rate ≤ 0.05 were regarded as significantly enriched. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used in the functional annotation and classification of genes associated with differential peaks (Kanehisa et al., 2019).

CHAPTER FOUR

RESULTS

4.1 Genomic Signatures of Selection for Heat Stress in Chicken Genomes

4.1.1 Annotation of the Chicken SNPs

The mapping of the chicken genome samples to the GRCg6a.99 resulted in an average of 98.1% coverage and a 10.2X depth. SnpEff v 5.0 was used for the annotation of the chicken SNPs to obtain the variants rate details for the chicken genome. The number of variants obtained after filtration was 13,151,574 for the chicken genome samples.

Out of the variants obtained, 10,249,664 (77.94%) are known variants. The chicken genome's total length is 1,065,365,425bp. The variant rate was also found to be 1 variant for every 72 bases (Table 4.1). The intronic region had the highest number of SNPs at 57.9%, followed by the intergenic regions at 19.5%. Moreover, the exon regions contained approximately 1.8% of the total variants. As expected, there were some SNPs within downstream, upstream, 3' untranslated region, and 5' untranslated region which may regulate gene expression (Table 4.2). Chromosome 1 had the highest number of variants at 2,707,315 followed by chromosome 2 at 2,033,765. Chromosome 32 had the least number of variants at 3509.

Table 4.1: Summary of the Chicken Variant Rates Details

Variant	Details
Genome	GRCg6a.99
Number of variants after filter	13,151,574
Number of known variants	10,249,664 (77.94%)
Number of effects	26,899,580
Genome total length	1,065,365,425
Genome effective length	954,643,754
Variant rate	1 variant every 72 bases

Table 4.2: Summary of the Number of Effects by Region in Chicken Genomes

Region	Count	Percentage
Downstream	2,610,042	9.70%
Exon	486,138	1.81%
Intergenic	5,265,761	19.58%
Intron	15,562,544	57.85%
Upstream	2,727,602	10.14%
3'UTR	146,451	0.54%
5'UTR	49,451	0.18%

4.1.2 Population Structure Analysis in Chicken

4.1.2.1 Principal Component Analysis for the Chicken Genome

Components one (PC1) (10.3%) and two (PC2) (5.95%) of the chicken PCA analysis jointly account for 16.25% of the total variance as shown in Figure 4.1. In general, the PC2 separates the chicken populations into 4 clusters; cluster 1 comprised of chicken from China (Yunnan and Hainan) and some from Kenya (Lamu and Kilifi), cluster 2 comprised of chicken from Kenya (Mombasa, Kwale, and Kilifi), cluster 3 comprised of chicken from Ethiopia-Jarso while cluster four comprised of chicken from Ethiopia-Horro. Chicken from Ethiopia were separated by PC1 from chicken from Kenya and China (Figure. 4.1).

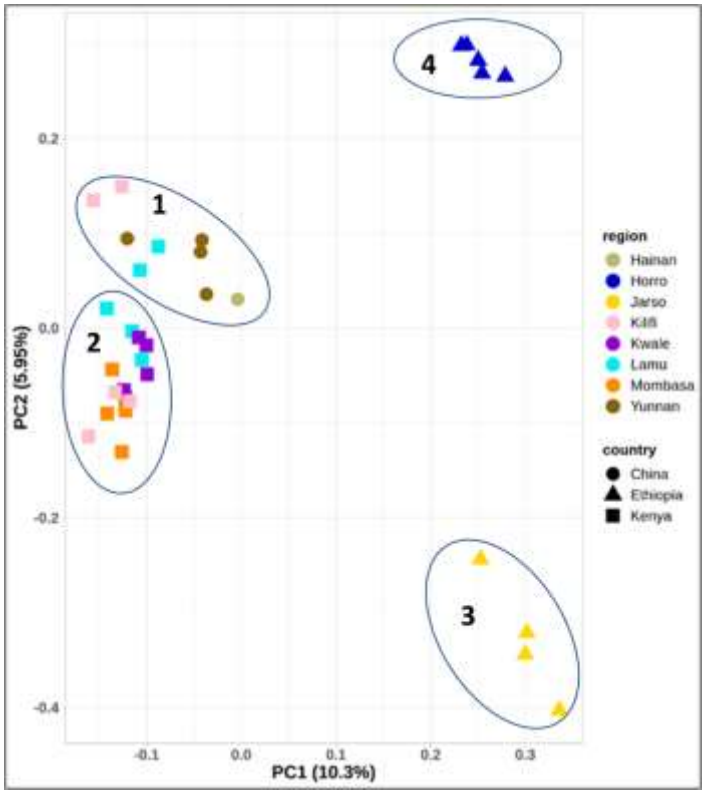

Figure 4.1: Chicken Population Structure as Revealed by PCA.

4.1.2.2 ADMIXTURE Analysis for the Chicken Genome

The ADMIXTURE test was also used to test for genetic clustering in chicken populations. The hypothetical ancestral populations *(K)* varied from 2 to 5. A cluster with *K*=4 was regarded as the most appropriate model because the average likelihood was the highest. The results clearly show no admixture across all *K* values in some samples from Ethiopia-Jarso, Kenya: Mombasa and Kilifi. At *K*=4, there was no admixture in 1 sample from Ethiopia-Horro, 2 samples from Kenya-Lamu, 3 samples from Kenya-Mombasa and 2 samples from China-Yunnan (Figure 4.2).

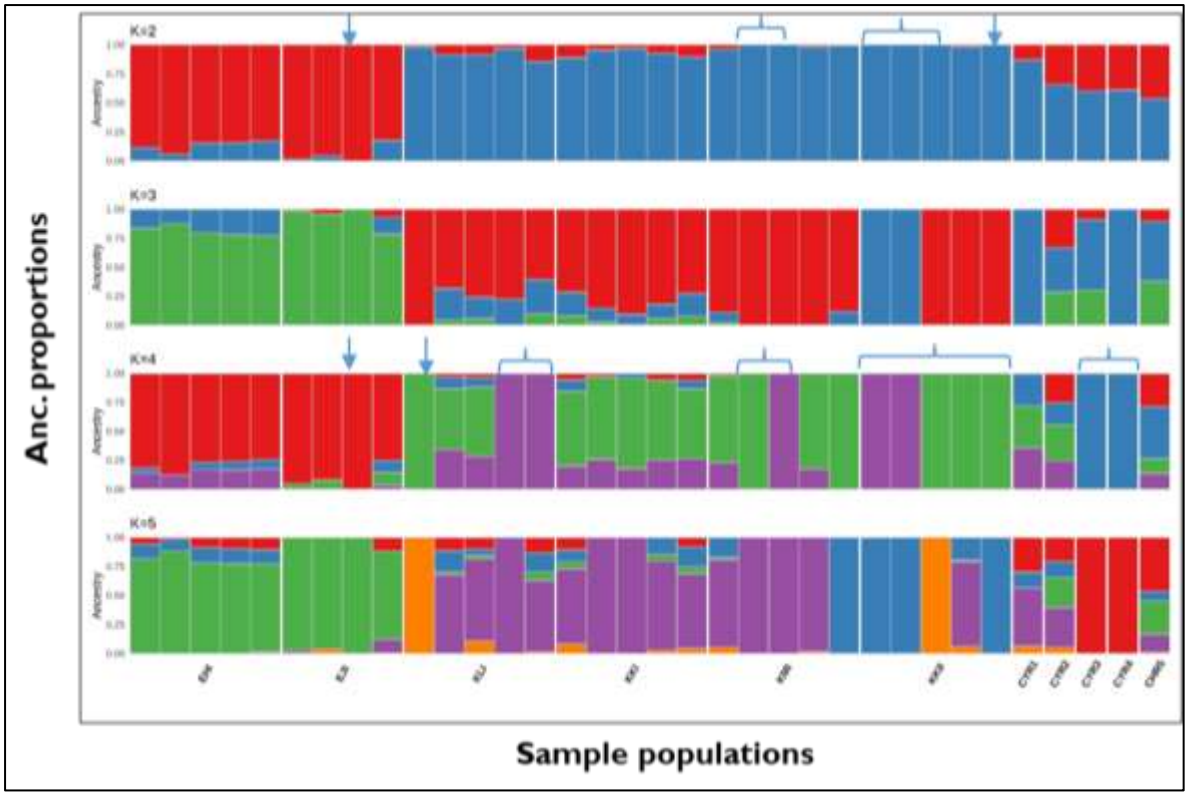

Figure 4.2: Chicken Population Structure Revealed by Admixture Analysis.
Key: EHI: Ethiopia-Horro, EJI: Ethiopia-Jarso, KLI: Kenya-Lamu, KKI: Kenya-Kwale, KMI: Kenya-Mombasa, KKII: Kenya-Kilifi, CYR1-CYR4: China-Yunnan, CHR5: China-Hainan.

4.1.3 Genomic Signatures of Selection Analysis in Chicken

4.1.3.1 Genomic Signatures of Selection Common in Chicken Populations from Kenya and China

A total of 195 genes and 294 genes were detected by the F_{ST} and XP-EHH analyses respectively, representing regions putatively under positive selection for chicken populations from Kenya and China. Strong selection sweep regions were detected on chromosomes 1-7,13,17, and 20 by the F_{ST} method (Figure 4.3A) and on chromosomes 1-4, 6, 10, and 14 by the XP-EHH method (Figure 4.3B).

A total of 896 GO terms were enriched. The top enriched terms were involved in the positive regulation of the biological process, signaling, localization, response to stimulus, biological regulation, negative regulation of the biological process,

developmental process, metabolic process, cellular process, and multicellular organismal process (Table 4.3). Other enriched terms were involved in the homeostatic process, growth, locomotion, viral process, immune system process, the biological process involved in interspecies interaction between organisms, reproductive process, and pigmentation (Figure 4.3C)

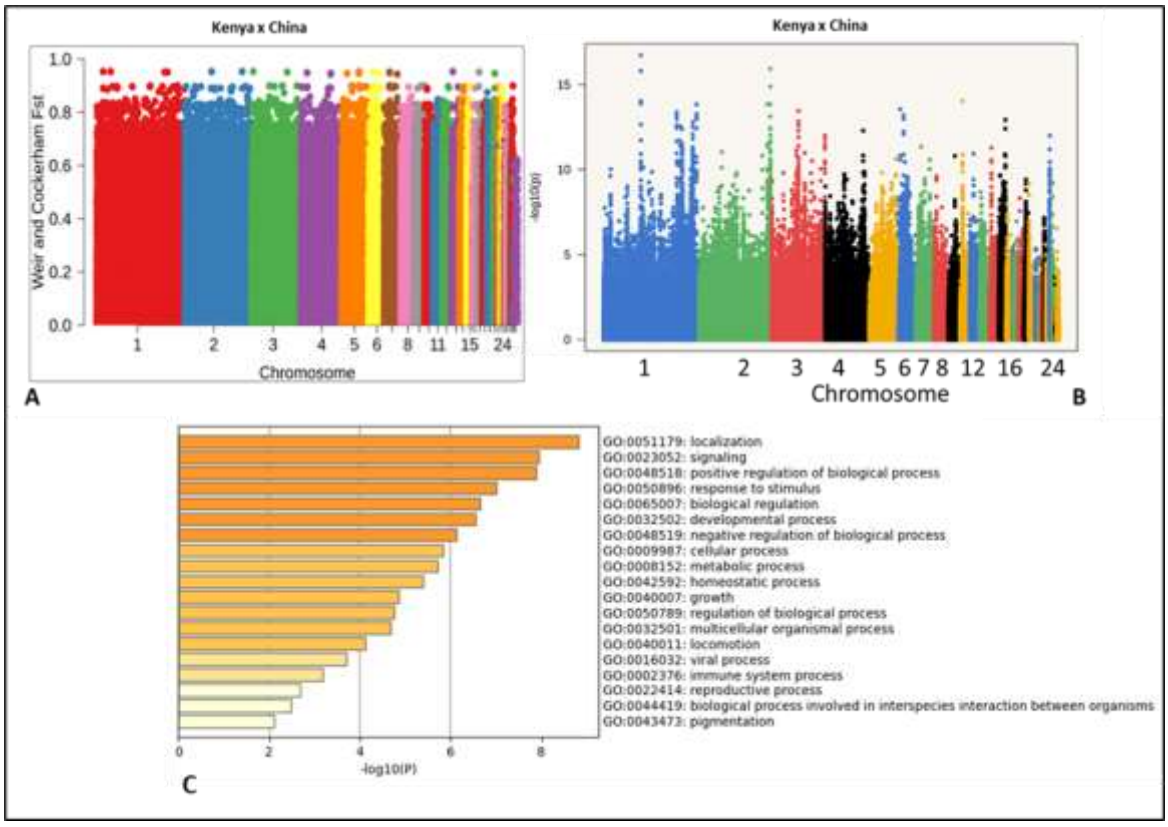

Figure 4.3: Distribution of F_{ST}, XP-EHH Scores, and GO Enrichment Analysis Amongst Chicken Population from Kenya and China.

Table 4.3: A Subset of GO Enrichment Pathways of F_{ST} and XP-EHH Analysis in Chicken Genomes from Kenya and China

Pathway	GO term	GO ID
Localization	Inorganic ion transmembrane transport	GO:0098660
	Endocytosis	GO:0006897
	Vesicle-mediated transport	R-HSA-5653656
Signaling	Second-messenger-mediated signaling	GO:0019932
Positive regulation of biological process	Transport of small molecules	R-HSA-382551
Response to stimulus	Response to wounding	GO:0009611
	Cellular response to organonitrogen compound	GO:0071417
	Response to ischemia	GO:0002931
	Regulation of response to DNA damage	GO:2001020
Biological regulation	Negative regulation of catalytic activity	GO:0002931
	Regulation of anatomical structure size	GO:0090066
Developmental process	Skeletal system development	GO:0001501
Negative regulation of biological process	Eye development	GO:0001654
	Negative regulation of cellular component organization	GO:0051129
Cellular process	Mitochondrion organization	GO:0007005
Metabolic process	Carbohydrate derivative biosynthetic process	GO:1901137
Multicellular organismal process	Muscle contraction	R-HAS-397014

4.1.3.1.1 Annotated Genes on Enriched Pathways in Chicken Populations from Kenya and China

Several genes were annotated for the various pathways in chicken populations from Kenya and China. The ATP2B4, ARCN1, and ASCL1 in the developmental process, NPPA, PPARG, YWHAE, PER2, and ADIPOQ in the negative regulation of biological process, LRRC8A, ATP2A2, and TBXAS1 in the homeostatic process were some of the annotated genes (Table 4.4).

Table 4.4: Annotated Genes in Enriched Pathways in Chicken Genomes from Kenya and China

Pathway	GO ID	Genes
Localization	GO:0051179	CANX,DRD2,CD81,SNAP25,FXYD2,P2RX7
Signaling	GO:0023052	CD74,IGF2,COL1A1,CSF1R,CD3E,FLT4
Positive regulation of biological process	GO:0048518	CD3E,CSF1R,DRD2,FLT4,INS,MAPT,NTRK3
Response to stimuli	GO:0050896	ADA,BNIP2,COL1A1,ENDOG,KCNMA1,ASCL2
Biological regulation	GO:0065007	ANXA2,ATP2A2,NRP1,ADIPOQ,PER2,MKKS
Developmental process	GO:0032502	ARCN1,ASCL1,ATP2B4,BOK,CD3E,CRK,FPGS, GBX2,GLI1,HDAC1,HPCA,PAFAH1B2,PAX6
Negative regulation of biological processes	GO:0048519	ATP2B4,ENG,IGFBP3,MAPT,NPPA,PAX6,PPAR G,PKN1,SET,YWHAB,YWHAE,PER2,ADIPOQ
Homeostatic process	GO:0042592	LRRC8A,ATP2A2,ATP6V0C,TBXAS1,PPT1, TMEM199
Multicellular organismal process	GO:0042592	SLC12A7,LRRC8A,PAX6,PPARG,RAF1, ADIPOQ,SLC4A1,SLC9A3,ATP2A2,YWHAE, ATP6V0A2,TRPM8,TBXAS1,SLC2A10,ATP2B4

4.1.3.2 Genomic Signatures of Selection Common in Chicken Populations from Kenya and Ethiopia

Several genes were detected by the F_{ST} and XP-EHH analyses, representing regions putatively under positive selection for the chicken populations from Kenya and Ethiopia. Strong selection sweep regions were detected on chromosomes 1-7, 9, 10, 13, and 14 by the F_{ST} method (Figure 4.4A) and on chromosomes 1, 6, 14, 16, 19, and 25 by the XP-EHH method (Figure 4.4B). A total of 87 GO terms were enriched. The top enriched terms were involved in localization, reproductive process, cellular process, positive regulation of biological process, response to stimulus, regulation of biological process, signaling, homeostatic process, metabolic process, multicellular organismal process, negative regulation of biological process, and developmental process (Figure 4.4C, Table 4.5).

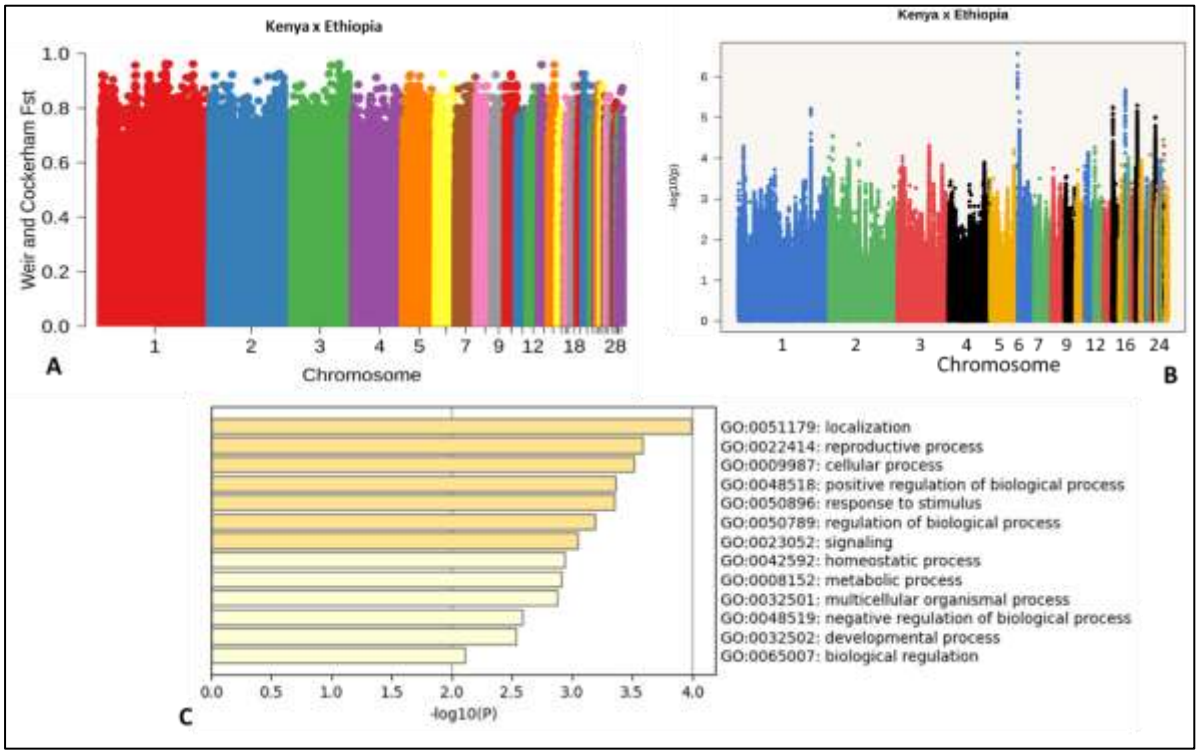

Figure 4.4: Distribution of F_{ST}, XP-EHH Scores, and GO Enrichment Analysis Comparing Amongst Chicken Populations from Kenya and Ethiopia.

Table 4.5: A Subset of GO Enrichment Pathways of F_{ST} and XP-EHH Analysis in Chicken Genomes from Kenya and Ethiopia.

Pathway	GO term	GO ID
Localization	Protein-containing complex localization	GO:0031503
	Inorganic anion transport	GO:0015698
Reproductive process	Binding of sperm to zona pellucida	GO:0007339
	Regulation of expression of SLITs and ROBOs	R-HSA-9010553
Cellular process	Chromosome organization	GO:0051276
	Cilium assembly	GO:0060271
Positive regulation of biological process	Positive regulation of cellular component biogenesis	GO:0044089
	Positive regulation of cell division	GO:0051781
Response to stimulus	Cellular response to chemical stress	GO:0062197
Signaling	Second-messenger-mediated signaling	GO:0019932
	Neuropeptide signaling pathway	GO:0007218
Metabolic process	Lipid biosynthetic process	GO:0008610
	Metabolism of amino acids and derivatives	R-HSA-71291
	Regulation of DNA biosynthetic process	GO:2000278
Multicellular organismal process	Muscle contraction	GO:0006936
Negative regulation of biological process	Negative regulation of endopeptidase activity	GO:0010951
Developmental process	Neural crest cell development	GO:0014032
Regulation of biological process	Regulation of cell division	GO:0051302

4.1.3.2.1 Annotated Genes on Enriched Pathways in Chicken Populations from Kenya and Ethiopia

Several genes were annotated for the various pathways in chicken populations from Kenya and Ethiopia. Some of these genes include the VDAC2, and ELOC in the reproductive pathway, NPPC, and CENPN in the cellular process, PLCG2, and TERF1 in the positive regulation of biological process, TRPA1, and ARL6IP5 in response to stimuli, AP2A2, and BIRC5 in localization (Table 4.6).

Table 4.6: Annotated Genes in Enriched Pathways in Chicken Genomes from Kenya and Ethiopia

Pathway	GO ID	Genes
Localization	GO:0051179	AP2A2,BIRC5,STAU2,TTC30B
Reproductive process	GO:0022414	VDAC2,ZP2,CCT4,PSMD1,RPL7 ,ELOC
Cellular process	GO:0009987	BIRC5,GTF2B,NPPC,TERF1,STAG2
Positive regulation of biological process	GO:0048518	PLCG2,TERF1,TENM1,IL1RAPL1, ATMIN,MPP7
Response to stimulus	GO:0050896	TRPA1,ARL6IP5,LRRC8D,LRRC8C,PJVK
Signaling	GO:0023052	HTR2B,NPPC,PLCG2,NMUR1,TENM1
Metabolic process	GO:0008152	HSD17B2,RDH10,CRYM,NPPC,TERF1
Multicellular organismal process	GO:0032501	CHRND,CHRNG,HTR2B,NMUR1, LMOD3
Negative regulation of biological process	GO:0048519	BIRC5,CST3,SPOCK1,BIRC8
Developmental process	GO:0032502	HTR2B,OVOL2,RDH10
Regulation of biological process	GO:0050789	BIRC5,HTR2B,PKN2,KAT14

4.1.3.3 Genomic Signatures of Selection Common in Chicken Populations from Ethiopia and China

A total of 56 genes and 245 genes were detected by the F_{ST} and XP-EHH analyses respectively, representing regions putatively under positive selection for the chicken population from Ethiopia and China. Strong selection sweep regions were detected on chromosomes 1-5, 7, 9, 10, 12-14, and 24 by the F_{ST} method (Figure 4.5A) and on chromosomes 1, 2, 4, and 7 by the XP-EHH method (Figure 4.5B). A total of 414 GO terms were enriched. Some of the enriched terms include the developmental process, negative regulation of biological process, homeostatic process, multicellular organismal process, signaling, regulation of biological process, positive regulation of biological process, biological regulation, localization, response to stimulus, metabolic process, and cellular process (Figure 4.5C, Table 4.7).

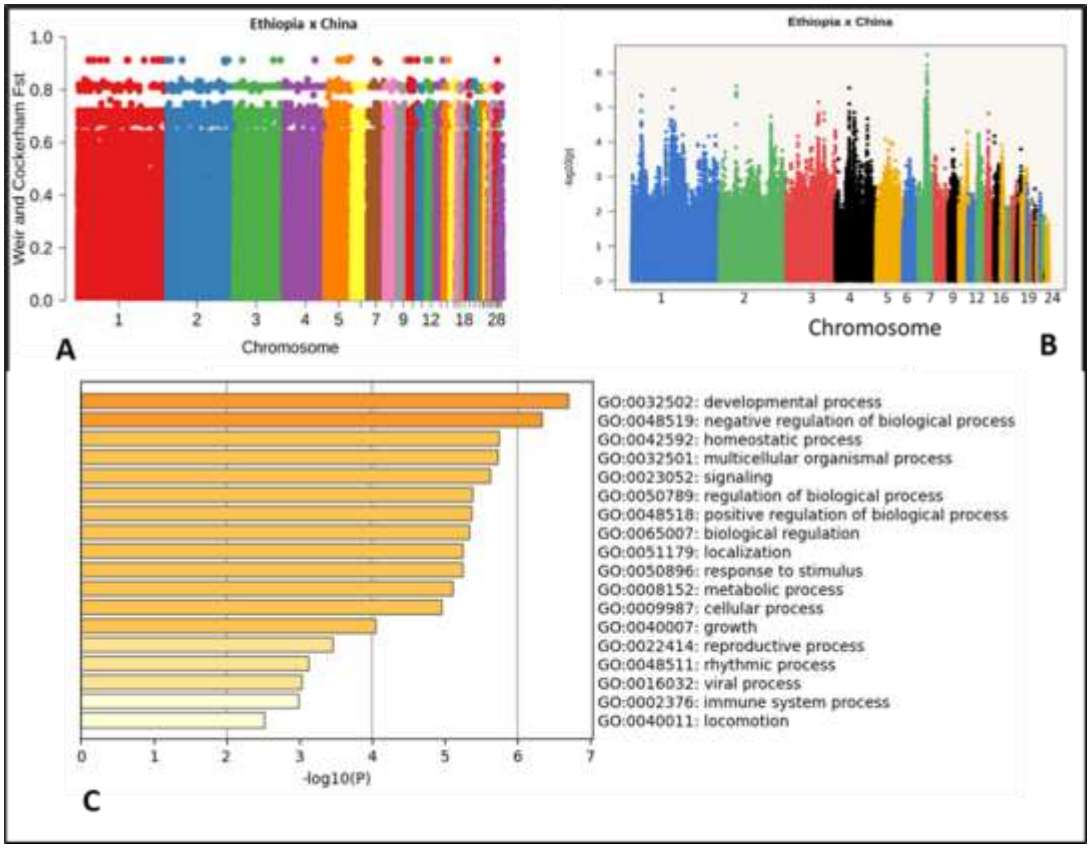

Figure 4.5: Distribution of F_{ST} and XP-EHH Scores, and the GO Enrichment Analysis Comparing Amongst Chicken Populations from Ethiopia and China.

Table 4.7: A Subset of GO Enrichment of F_{ST} and XP-EHH Analysis, and Annotated Genes in Chicken Genomes from Ethiopia and China

Pathway	GO Term	GO ID
Developmental process	Regulation of nervous system development	GO:0051960
	Brain development	GO:0007420
	Skin development	GO:0043588
Negative regulation of biological process	Negative regulation of protein modification process	GO:0031400
	Negative regulation of cellular component organization	GO:0051129
Homeostatic process	Cellular chemical homeostasis	GO:0055082
Multicellular organismal process	Regulation of system process	GO:0044057
	2q37 copy number variation syndrome	WP5224
Signaling	Second-messenger-mediated signaling	GO:0019932
	Regulation of neuron death	GO:1901214
	Sudden infant death syndrome (SIDS)	WP706
	Muscle contraction	R-HSA-397014
Positive regulation of biological process	Positive regulation of cell death	GO:0010942
Biological regulation	Regulation of anatomical structure size	GO:0090066
Localization	Regulation of establishment of protein localization	GO:0070201
	Inorganic ion transmembrane transport	GO:0098660
	Vesicle-mediated transport	R-HSA-5653656
Response to stimulus	Response to peptide	GO:1901652
Metabolic process	Transcription by RNA polymerase II	GO:0006366
Cellular process	Membrane organization	GO:0061024

4.1.3.3.1 Annotated Genes on Enriched Pathways in Chicken Populations from Ethiopia and China

Several genes were annotated for the various pathways in chicken populations from Ethiopia and China. Some of these genes include the ATP2B4, and YWHAE in the signaling pathway, PER2, and STK25 in the developmental process, NPPA, and PPARG in negative regulation of biological process, ATP2A2, and TBXAS1 in homeostatic process, SREBF1, and STK2 in multicellular organismal process, TRPM2, and HYOU1 in the regulation of biological process, TRPM2, and ADIPOQ in the positive regulation of biological process, LRRC8A, and ARPC3 in the biological regulation, ACTR10 and ARPC3 in localization, SREBF1, and PPARG in response to stimulus, PPARG, and SREBF1 in metabolic process, ATPA2, and ANXA2 in the cellular process (Table 4.8).

Table 4.8: Annotated Genes in Enriched Pathways in Chicken Genomes from Ethiopia and China

Pathway	Genes	GO ID
Developmental process	ATP2B4,HPCA,PAX6,YWHAE,MAPT,PER2, STK25,KRT5,KRT6A,SLC27A4,CASP14, SLC2A10	GO:0032502
Negative regulation of biological process	ATP2B4,IGFBP3,MAPT,NPPA,PAX6,PPARG, YWHAB,YWHAE,PER2,ADIPOQ,TPPP	GO:0048519
Homeostatic process	ATP2A2,ATP2B4,NPPC,RAF1,SLC4A1, SLC9A3,TBXAS1,YWHAE,SLC12A7, ATP6V0A2,LRRC8A	GO:0042592
Multicellular organismal process	ADRA2B,ATP2A2,ATP2B4,CAMK2B,IGF1, NPPA,PPARG,SCN2B,SCN4B,SCN5A, SREBF1,TBXAS1,YWHAE,PER2,ADIPOQ, ASCL1,BOK,STK25	GO:0032501
Signaling	ASCL1,HDAC1,SLC9A3,YWHAB,YWHAE, TPPP,ATP2A2,ATP2B4,CAMK2B,NPPA, NPPC,SCN2B,SCN4B,SCN5A,HPCA,TRPM2	GO0023052

Pathway	Genes	GO ID
Regulation of biological process	ASCL1,BOK,MAPT,MAP3K5,TRPM2, HYOU1,BACE1,TOX3,RILPL1,DRAXIN	GO:0050789
Positive regulation of biological process	ASCL1,ATP2A2,IGFBP3,MAPT,MAP3K5, PPARG,RET,TNFRSF1B,TRPM2,ADIPOQ, SSLC27A4	GO:0048518
Biological regulation	MAPT,NPPA,NPPC,TBXAS1,PER2,ARPC3, SLC12A7,LRRC8A	GO:0065007
Localization	ACTR10,YWHAB,YWHAE,ARPC3,HYOU1, ATP2A2,ATP2B4,CLCN6,SLC37A4,NPPA, SCN2B,SCN4B,SCN5A,SLC4A1,SLC9A3, SLC12A7,ATP6V0A2,LRRC8A,PPARG, SREBF1,TRPM2,PER2,ADIPOQ	GO:0051179
Response to stimulus	IGF1,IGFBP1,PDK2,SERPINF1,PPARG, RAF1,SREBF1,ADIPOQ,SLC27A4,KAT7, BACE1,INPP5K	GO:0050896
Metabolic process	CDK9,HNF4A,PAX6,PRRX1,PPARG,SREBF1	GO:0008152
Cellular process	ANXA2,ATP2A2,PPT1,SLC4A1,EXOC5, TMED2	GO:0009987

4.1.3.4 Genomic Signatures of Selection Common in Chicken Populations from Kenya

A total of 30 genes were detected by the iHS analyses, representing regions putatively under positive selection for the chicken populations from Kenya. Strong selection sweep regions were detected on chromosomes 1-5, and 23 (Figure 4.6A). A total of 8 GO terms were enriched. The top enriched terms were involved in developmental process, cellular process, and metabolic process (Figure 4.6B, Table 4.9).

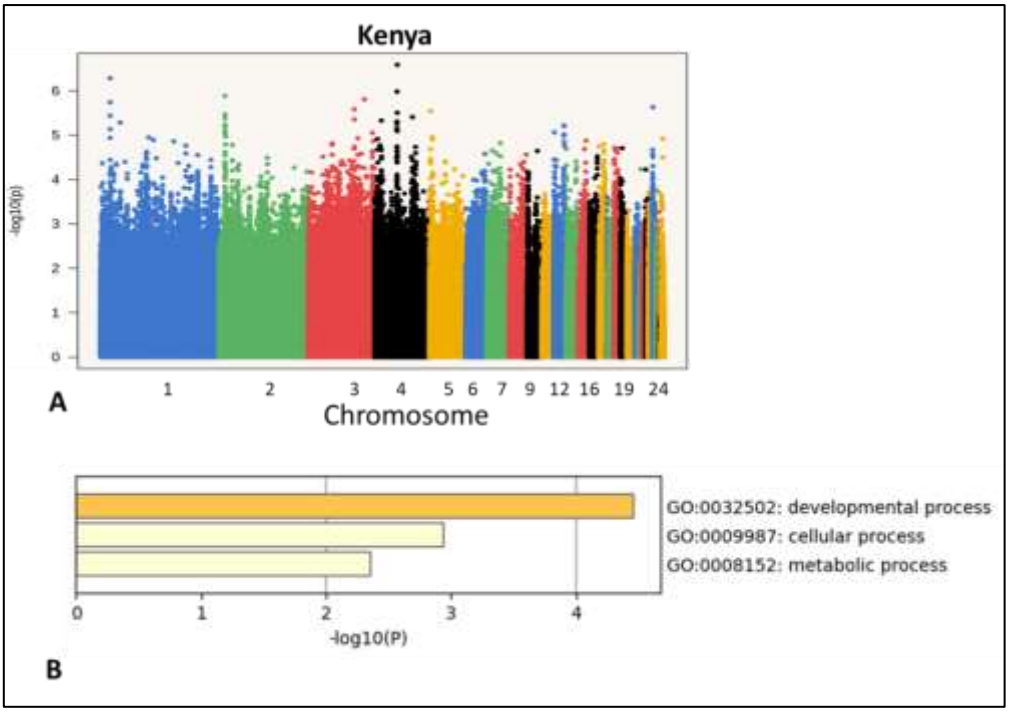

Figure 4.6: Distribution of iHS Scores and GO Enrichment Analysis Comparing Amongst Chicken Population from Kenya.

Table 4.9: A Subset of GO Enrichment of iHS Analysis in Chicken Genomes from Kenya

Pathway	GO term	GO ID
Developmental process	Forebrain development	GO:0030900
Metabolic process	Small molecule biosynthetic process	GO:0044283

4.1.3.4.1 Annotated Genes on Enriched Pathways in Chicken Genomes from Kenya

Several candidate genes were annotated in the pathways enriched in the genomes of chicken from Kenya. Some of these genes include the KCNC2, and RARB in the developmental process, SEC23A, and NGLY1 in the cellular process, MTR, and COQ6 in the metabolic process (Table 4.10)

Table 4.10: Annotated Genes in Enriched Pathways in Chicken Genomes from Kenya

Pathway	Genes	GO ID
Developmental process	KCNC2,RARB,SSTR1,TOP2B,LEF1	GO:0032502
Cellular process	SEC23A,NGLY1,ERO1B	hsa04141
Metabolic process	MTR,COQ6,OXSM,PNN,RPL34,GE MIN2,KRR1	GO:0008152

4.1.3.5 Genomic Signatures of Selection Common in Chicken Populations from Ethiopia

A total of 13 genes were detected by the iHS analyses, representing regions putatively under positive selection for the Ethiopian chicken populations. Strong selection sweep regions were detected on chromosomes 5, 7, and 9 (Figure 4.7A). A total of 4 GO terms were enriched. The top enriched terms were involved in signaling (Table 4.11, Figure 4.7B).

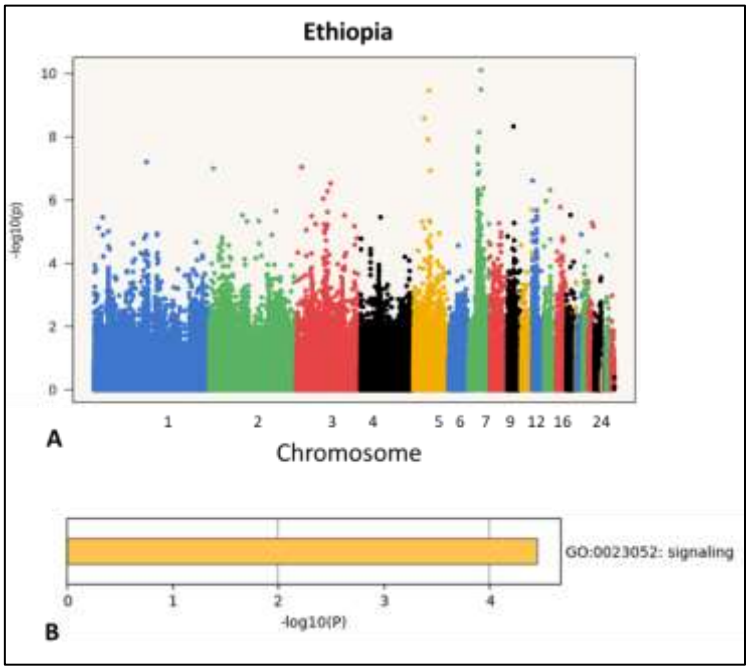

Figure 4.7: Distribution of iHS Scores, and GO Enrichment Analysis Comparing Amongst Chicken Population from Ethiopia.

Table 4.11: A Subset of GO Enrichment of iHS Analysis in Chicken Genomes from Ethiopia

Pathway	GO Term	GO ID
Signaling	Regulation of Ras protein signal transduction	GO:0046578
	GPCR downstream signaling	R-HSA-388396

4.1.3.5.1 Annotated Genes on Enriched Pathways in Chicken Genomes from Ethiopia

Most of the genes that were annotated in the various pathways in the chicken genomes from Ethiopia were involved in the signaling pathway (GO:0023052). Some of these genes include GPR18, GPR20, DENND3, and GPR183.

4.1.3.6 Genomic Signatures of Selection Common in Chicken Populations from China

A total of 43 genes were detected by the iHS analyses, representing regions putatively under positive selection for the chicken populations from China. Strong selection sweep regions were detected on chromosomes 1, 3, 5, and 16-19 (Figure 4.8A). A total of 9 GO terms were enriched. The top enriched terms were involved in localization, and response to stimulus (Table 4.12). Other enriched terms were involved in metabolic process, and positive regulation of biological process (Figure 4.8B).

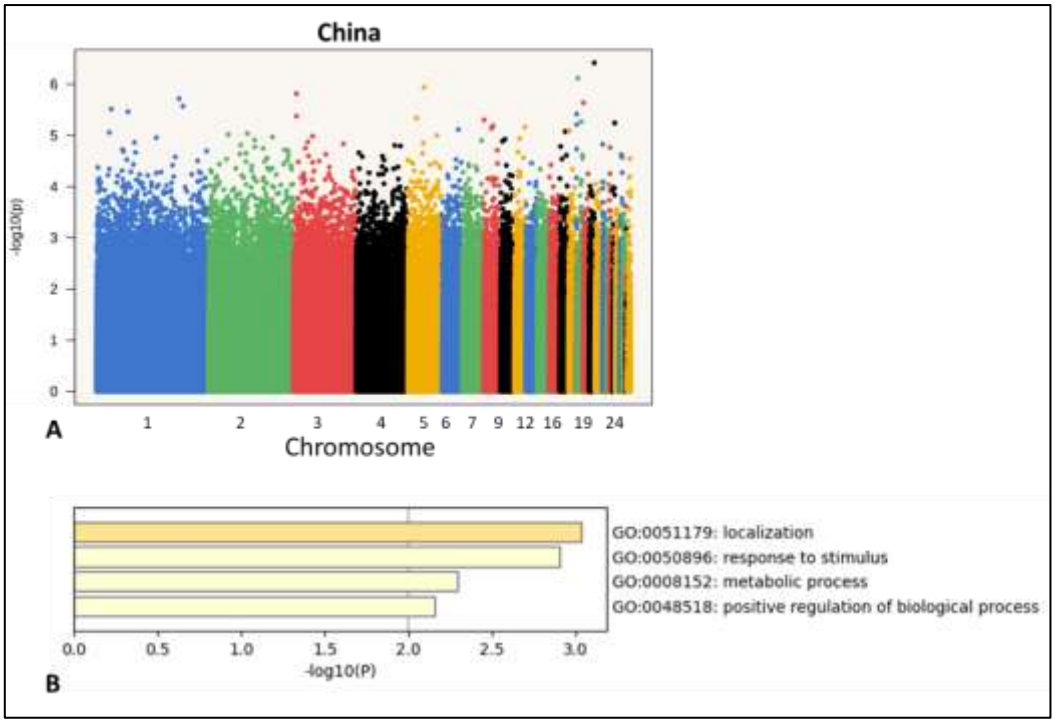

Figure 4.8: Distribution of iHS Scores, and GO Enrichment Analysis Comparing Amongst Chicken Population from China.

Table 4.12: A Subset of GO Enrichment of iHS Analysis in Chicken Genomes from China

Pathway	GO Term	GO ID
Localization	Protein-containing complex localization	GO:0031503
	Endosomal transport	GO:0016197
Response to stimulus	Response to oxidative stress	GO:0050896

4.1.3.6.1 Annotated Genes on Enriched Pathways in Chicken Genomes from China

Several genes were annotated for the two enriched terms in the chicken genomes from China. These genes include the PPP2CB, and WRN in the response to stimuli and HNRNPU, and KIF5C in the localization pathway (Table 4.13).

Table 4.13: Annotated Genes in Enriched Pathways in Chicken Genomes from China

Pathway	Genes	GO ID
Localization	HNRNPU,KIF5C,GRIP1,TBC1D5,GRIP1, HEATR5A	GO:0051179
Response to stimuli	PPP2CB,WRN,DYRK2,COX20,CNOT6L,MGMT, PPP2CB,WRN,RBPMS	R-HSA-3700989

4.2 Genomic Signatures of Selection for Heat Stress in Guineafowl Genomes

4.2.1 Annotation of Guineafowl SNPs

The mapping of the guineafowl genome samples to the NumMel1.0 resulted in an average of 95.9% coverage and a 7.2X depth. The number of variants obtained after filtration was 38,031,218. The guineafowl genome total length is 1,043,264,150bp. The variant rate was found to be 1 variant for every 24 bases Table (4.14). The intronic region had the highest number of SNPs at 58.38%, followed by the intergenic regions at 20.25%. Moreover, the exon regions contained approximately 1.95% of the total SNPs. As expected, there are some SNPs within downstream, upstream, 3' untranslated region, and 5' untranslated region which may regulate gene expression (Table 4.15). Chromosome 1 had the greatest number of variants (7,175,732), followed by chromosomes 2 and 3 at 5,265,867 and 4,086,399 variants respectively. Chromosome 15 had the least number of variants at 15, 426.

Table 4.14: Summary of the Guineafowl Variant Rates Details

Variant	Details
Genome	NumMel1.0.99
Number of variants after filter	38,031,218
Number of effects	83,843,446
Genome total length	1,043,264,150
Genome effective length	928,860,929
Variant rate	1 variant every 24 bases

Table 4.15: Summary of the Number of Effects by Region in the Guineafowl Genome

Region	Count	Percentage
Downstream	7,788,879	9.29%
Exon	1,634,813	1.95%
Intergenic	16,979,831	20.25%
Intron	48,950,859	58.38%
Upstream	7,993,327	9.53%
UTR_3_Prime	205,752	0.25%
UTR_5_Prime	108,732	0.13%

4.2.2 Population Structure Analysis in Guineafowls

4.2.2.1 Principal Component Analysis for the Guineafowl Genome

The plot of the first (PC1) and second (PC2) eigenvectors in guineafowls explained about 24.5% and 12.3% of the proportion of variations respectively. Principal components (PCs) completely separate the three guineafowl populations according to their sampled locations and species. The helmeted, crested, and vulturine guineafowl species were separated by the PC1. The helmeted guineafowls from Asia, Europe, and

some African populations clustered together. However, helmeted guineafowls from Africa were separated by the PC2. The vulturine guineafowls were quite distant from the crested and helmeted guineafowls (Figure 4.9).

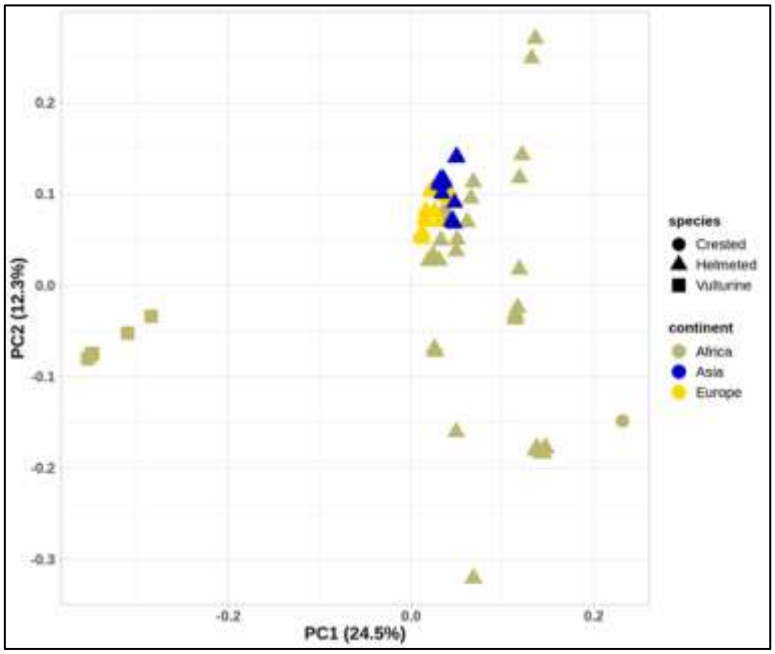

Figure 4.9: Guineafowl Population Structure as Revealed by PCA.

4.2.2.2 ADMIXTURE Analysis for the Guineafowl Genome

The ADMIXTURE test was also used to test for genetic clustering in the guineafowl populations. The hypothetical ancestral populations *(K)* varied from 2 to 6. A cluster with *K*=6 was regarded as the most appropriate model because the average likelihood was the highest. The results clearly show no admixture across all *K* values in 3 vulturine guineafowl species. At *K*=6, no admixture was observed in some guineafowls from Kenya (1 crested guineafowl, 4 vulturine guineafowls, and, 1 wild helmeted guineafowl), 1 domestic helmeted guineafowl from Nigeria and 1 domestic helmeted guineafowl from Sudan (Figure 4.10).

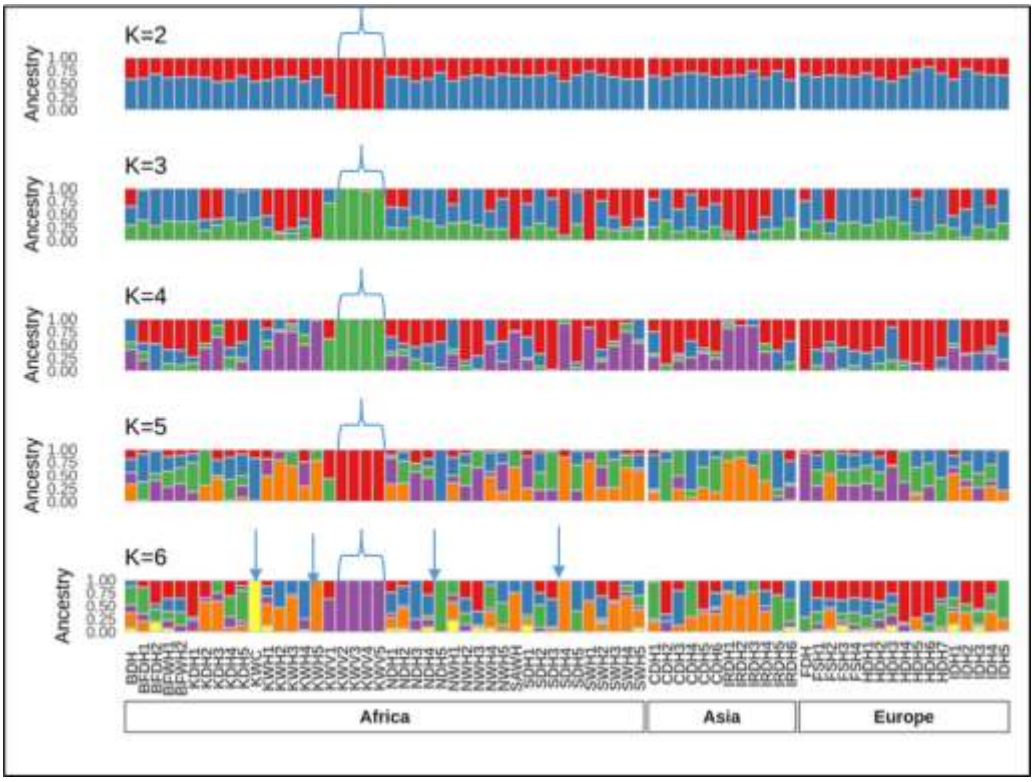

Figure 4.10: Guineafowl Population Structure as Revealed by Admixture Analysis.

Key: Figure 4.10 shows the population structure analysis of the guineafowls as revealed by ADMIXTURE. The white lines separate the experimental samples. Each sample is represented by a vertical bar partitioned into stained segments according to the proportion of ancestry in each cluster. The samples represent selected countries from Africa, Asia, and Europe. The codes for the countries are presented in Appendix II.

4.2.3 Genomic Signatures of Selection Analysis in Guineafowls

4.2.3.1 Genomic Signatures of Selection Common in Guineafowl Populations from Africa and Asia

A total of 45 genes and 28 genes were detected by the F_{ST} and XP-EHH analyses respectively, representing regions putatively under positive selection for the African and Asian guineafowl populations. Strong selection sweep regions were detected on chromosomes 1,3,4,6,9 and 14 by the F_{ST} method (Figure 4.11A) and on chromosomes 6 and 11 by the XP-EHH method (Figure 4.11B). A total of 61 GO terms were enriched. The top enriched terms were related to response to stimulus, signaling, regulation of biological process, cellular process, localization, metabolic process, and developmental process (Figure 4.11C, Table 4.16).

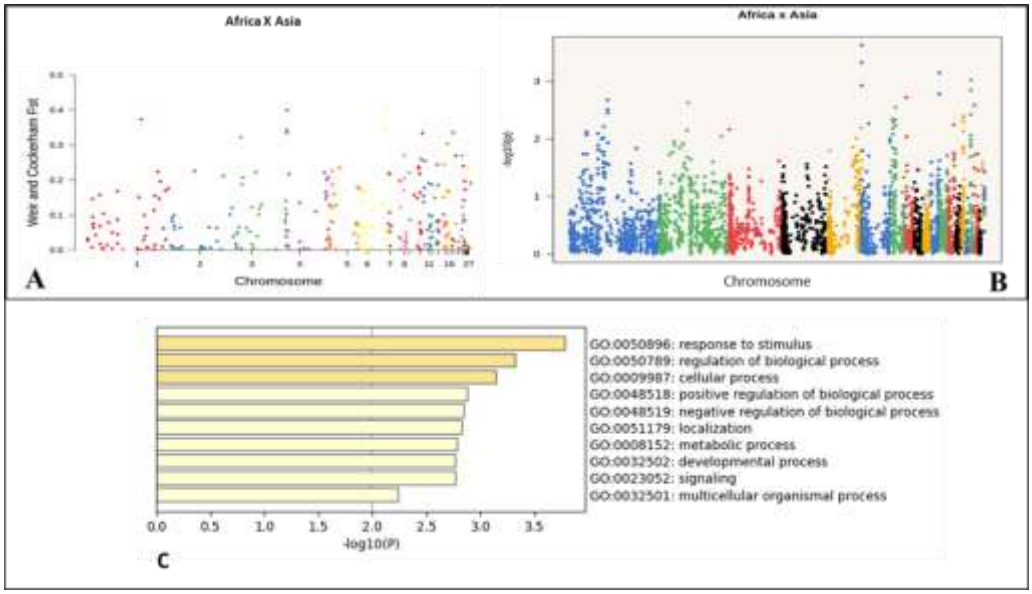

Figure 4.11: Distribution of F_{ST}, XP-EHH, and GO Enrichment Analysis Among Guineafowl Populations from Africa and Asia.

Table 4.16: A subset of GO Enrichment Pathways of F_{ST} and XP-EHH Analysis in Guineafowl Genomes from Africa and Asia

Pathway	Enriched term	GO ID
Response to stimulus	Response to mechanical stimulus	GO:0009612
	Response to radiation	GO:0009314
Regulation of biological process	Regulation of mitochondrion organization	GO:0010821
	Positive regulation of proteasomal ubiquitin-dependent protein catabolic process cellular processes	GO:0032436
Cellular process	DNA duplex unwinding	GO:0032508
Localization	Organelle localization	GO:0051640
	Localization within membrane	GO:0051668
Metabolic process	Regulation of kinase activity	GO:0043549
	Glycerolipid metabolism	hsa00561
	Fatty acid metabolism	HSA-8978868
Developmental process	Hematopoietic progenitor cell differentiation	GO:0002244
Signaling	MAPK cascade	WP422

4.2.3.1.1 Annotated Genes on Enriched Pathways in Guineafowl Genomes from Africa and Asia

Several genes were annotated for the enriched terms in the guineafowl genomes from Africa and Asia. These genes include the ANGPT2, ZNF236, and MAP3K2 in the response to stimuli, GCLC, BRAF, and HTT in the regulation of biological process, ERCC3, and XRCC5 in the cellular response (Table 4.17).

Table 4.17: Annotated Genes in Enriched Pathways in Guineafowl Genomes from Africa and Asia

Pathway	GO ID	Genes
Response to stimuli	GO:0050896	ANGPT2,BRAF,ERCC3,PTPRK,XRCC5, MAP3K2,GCLC,HTT, PTN,ZNF236
Regulation of biological processes	GO:0050789	GCLC,HTT,SSBP1,TMEM14A,XRCC5,MCPH1, ERCC3,DLGAP5,KIF13A,MCPH1,BRAF
Cellular response	GO:0009987	GCLC,TMEM14A,AGK,SSBP1,SIRT5,ERCC3, XRCC5,CDKN3,HTT,MCPH1,WEE2

4.2.3.2 Genomic Signatures of Selection Common in Guineafowl Populations from Africa and Europe

A total of 686 GO terms were enriched in the African and European guineafowl populations. Strong selection sweeps were observed on chromosomes 5,7 and 10 by the F_{ST} method (Figure 4.12A) and on chromosomes 1,2,4,6 and 7 by the XP-EHH method (Figure 4.12B). Genes located in the selected regions were used for the detection of enriched GO terms. Some of the top-level enriched terms were involved in localization, cellular process, DNA metabolic process, response to stimuli, developmental process, regulation of biological process, biological regulation (Figure 4.12C, Table 4.18).

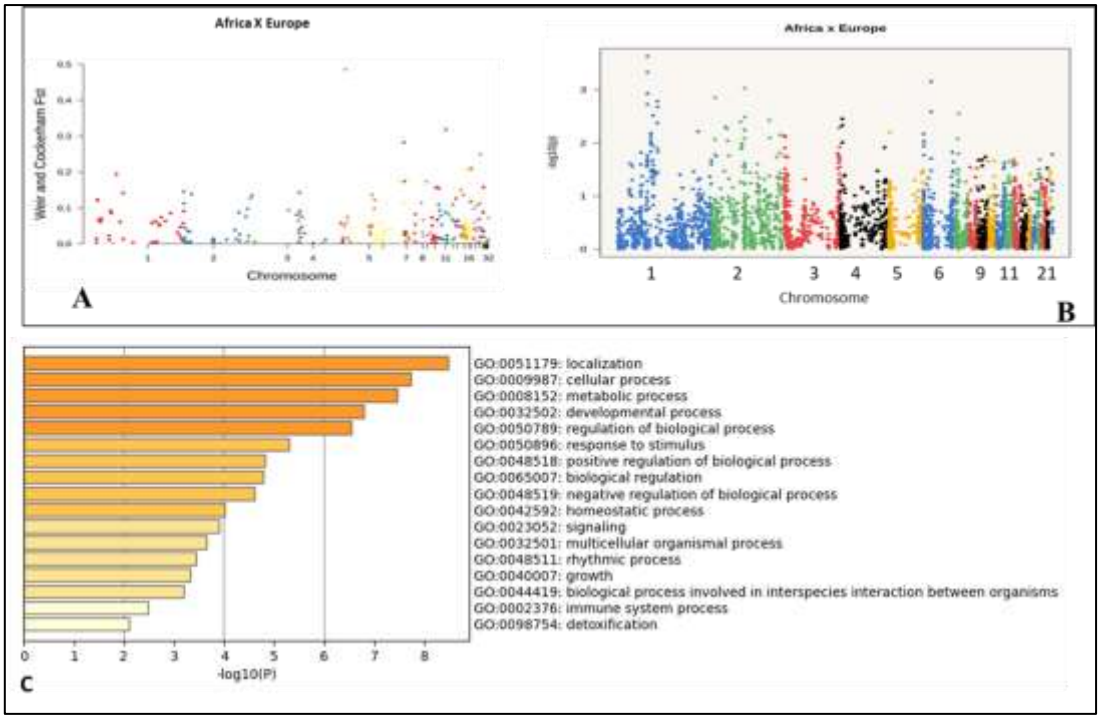

Figure 4.12: Distribution of $F_{ST,}$ XP-EHH Scores, and GO Enrichment Analysis Comparing Amongst Guineafowl Population from Africa and Europe.

Table 4.18: A Subset of GO Enrichment Pathways of F_{ST} and XP-EHH Analysis in Guineafowl Genomes from Africa and Europe

Pathway	Enriched term	GO ID
Localization	Vesicle-mediated transport	R-HSA-5653656
	Regulation of protein transport	GO:0051223
	Maintenance of location	GO:0051235
	Regulation of protein secretion	GO:0050708
	Regulation of protein secretion	GO:0050708
	Endocytosis	GO:0006897
Cellular process	Mitochondrion organization	GO:0007005
	Membrane organization	GO:0061024
Metabolic process	DNA metabolic process,	GO:0006259
	Lipid catabolic process	GO:0016042
	Ubiquitin-dependent protein catabolic process	GO:0006511
	Organophosphate catabolic process	GO:0046434
	Regulation of proteolysis	GO:0030162
Developmental process	Limbic system development	GO:0021761
Regulation of biological process	Regulation of mitochondrion organization	GO:0010821
	Biological regulation: regulation of membrane potential	GO:0042391
Response to stimuli	Response to carbohydrate	GO:0009743

4.2.3.2.1 Annotated Genes on Enriched Pathways in Guineafowl Genomes from Africa and Europe

Some of the genes that were annotated for the different pathways include PPARG, SREBF1, and LRRC8A in the localization, BAK1, and BCL2A1 in the cellular process, APOA4, and HMGCS2 in the metabolic process (Table 4.19).

Table 4.19: Annotated Genes in Enriched Pathways in Chicken Genomes from Africa and Europe

Pathway	GO ID	Genes
Localization	GO:0051179	GCK,GHSR,HNF4A,LLGL1,PPARG,SREBF1, ATP13A2,SERGEF,SIDT2,LRRC8A,RHBDF1
Cellular process	GO:0009987	BAK1,BCL2A1,ENDOG,EYA2,NPTX1,TOP3A
Metabolic process	GO:0008152	APOA1,APOA4,HMGCS2,ITPKB,PLA2G2A

4.2.3.3 Genomic Signatures of Selection Common in Guineafowls Populations from Europe and Asia

Strong putative selection signature regions were detected on chromosomes 1,2,3, 4, 8, 12, and 16 by the F_{ST} method (Figure 4.13A) and on chromosomes 6 and 7 by the XP-EHH method (Figure 4.13B). A total of 206 GO terms were enriched. Some of the top-level enriched terms were involved in localization, metabolic process, positive regulation of biological process, developmental process, cellular process, response to stimulus, negative regulation of biological process (Figure 4.13C, Table 4.20).

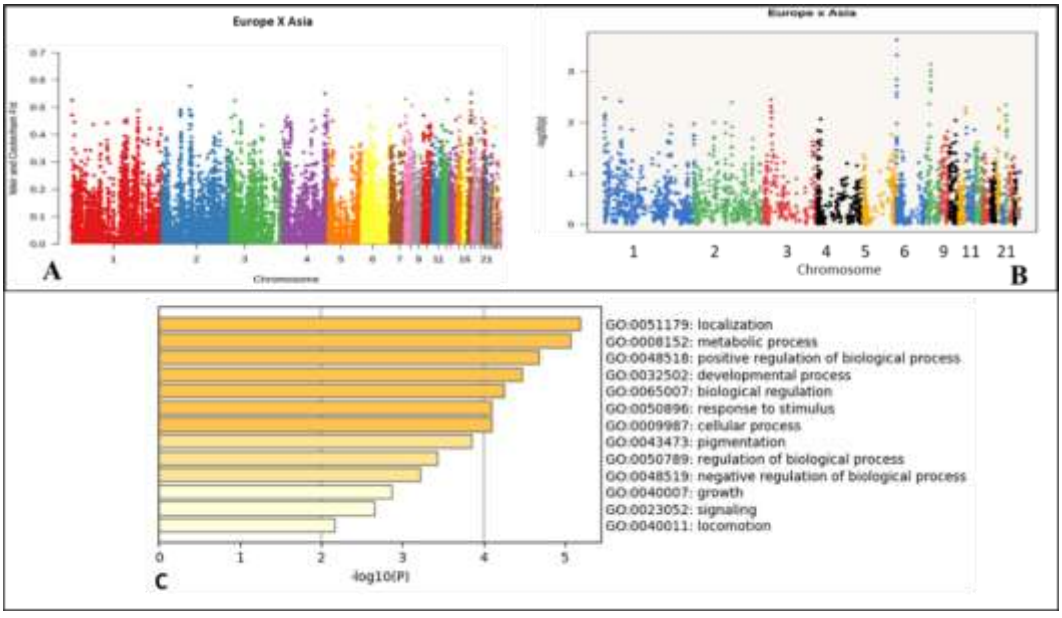

Figure 4.13: Distribution of F_{ST}, XPEHH Scores, and GO Enrichment Analysis Comparing Amongst Guineafowl Population from Europe and Asia.

Table 4.20: A Subset of GO Enrichment Pathways of F_{ST} and XP-EHH Analysis in Guineafowl Genomes from Europe and Asia

Pathway	Enriched term	GO ID
Localization	Establishment of organelle localization	GO:0051656
	Positive regulation of secretion	GO:0051047
Metabolic process	Modification-dependent macromolecule catabolic process	GO:0043632
	Regulation of protein catabolic process	GO:0042176
	Regulation of protein serine/threonine kinase activity	GO:0071900
Positive regulation of biological process	Positive regulation of protein-containing complex assembly	GO:0031334
	Positive regulation of cell-substrate junction organization	GO:0150117
Developmental process	Ventricular septum morphogenesis	GO:0060412
	Regulation of fat cell differentiation	GO:0045598
	Cell morphogenesis	GO:0000902
	Brain development	GO:0007420
	Lung epithelium development	GO:0060428
Response to stimulus	Cellular response to prostaglandin stimulus	GO:0071379
Cellular process	Necroptotic process	GO:0070266
Negative regulation of biological process	Negative regulation of mitotic nuclear division	GO:0045839

4.2.3.3.1 Annotated Genes on Enriched Pathways in Guineafowl Genomes from Europe and Asia

Some of the genes that were annotated in the enriched pathways include ARF6, BBS7, ALOX5, DNM1L, and MAP2K1 for the localization pathway, BMP4, and NUP153 for the metabolic process, EXOSC9, and DNM1L for the positive regulation of biological process (Table 4.21).

Table 4.21: Annotated Genes in Enriched Pathways in Guineafowl Genomes from Europe and Asia

Pathway	Genes	GO ID
Localization	GO:0051179	ARF6,CYBA,INHBB,DNM1L,ALOX5,BMP4, EPB41L5,BLTP1,MAP2K1,APRT,BBS7,CDT1
Metabolic process	GO:0008152	BMP4,MAP2K1,RALB,EXOSC9,FAM8A1,SPSB4, TRIM9,TRNT1,NUP153,MAD2L1
Positive regulation of biological process	GO:0048518	MAP2K1,RALB,FERMT2,CBFA2T3,EXOSC9, RBM24,ARF6,CLSTN2,DNM1L,ALOX5

4.2.3.4 Genomic Signatures of Selection Common in the Guineafowl Population from Africa

The genome-wide distribution of iHS values across the genome of the guineafowl populations from Africa is shown below (Figure 4.14A). Strong selective sweeps were observed on chromosomes 1 and 2 (Figure 4.14A). Genes located in the selected regions were used for the detection of enriched GO terms. Some of the top-level enriched terms were involved in metabolic process, positive regulation of biological process, regulation of biological process, cellular process, developmental process, positive regulation of biological process, negative regulation of biological process, localization, response to stimulus, biological regulation, signaling (Figure 4.14 B, Table 4.22).

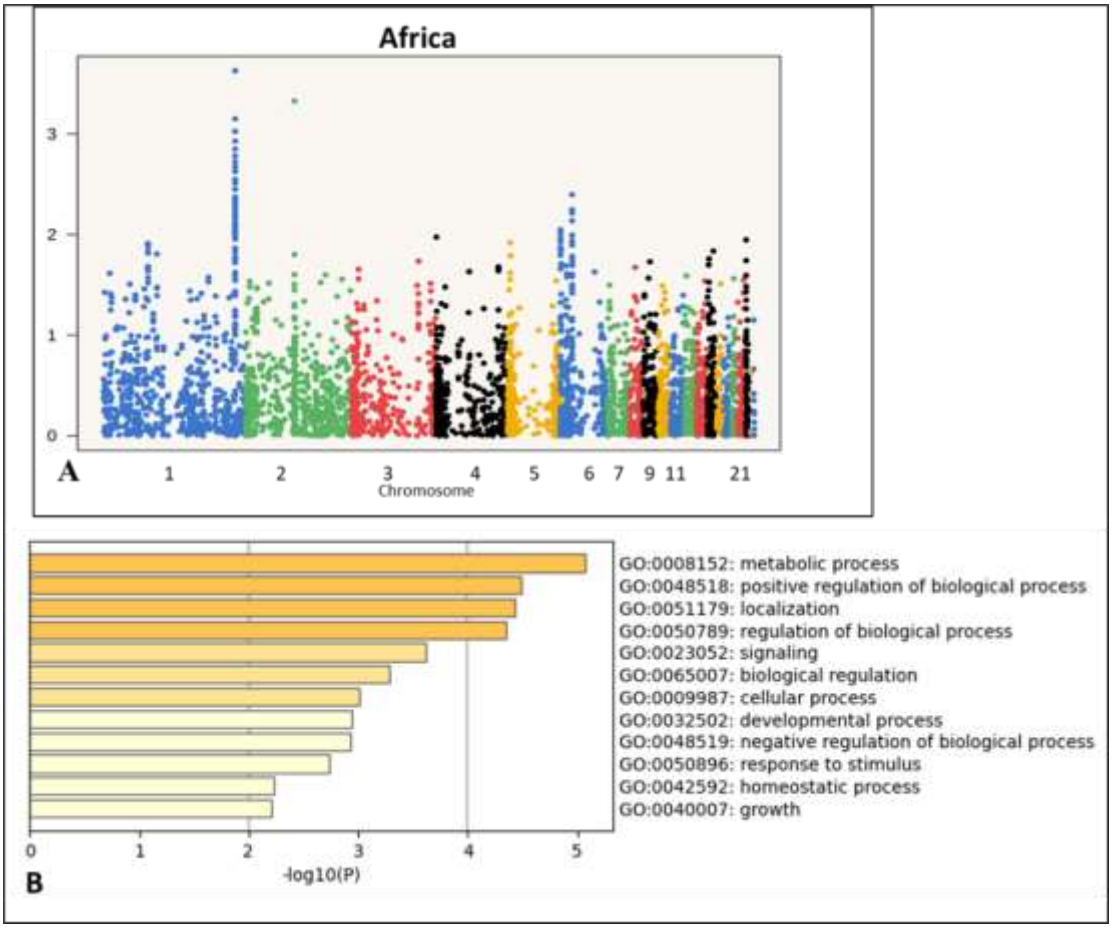

Figure 4.14: Distribution of iHS Scores, and GO Enrichment Analysis Comparing Amongst Guineafowl Populations from Africa.

Table 4.22: A Subset of GO Enrichment Pathways of iHS Analysis in Guineafowl Genomes from Africa

Pathway	Enriched term	GO ID
Metabolic process	Organophosphate catabolic process	GO:0046434
Positive regulation of biological process	Positive regulation of organelle organization	GO:0010638
	Positive regulation of chromosome organization	GO:2001252
	Positive regulation of cellular catabolic process	GO:0031331
Localization	Intracellular protein transport	GO:0006886
	Regulation of vesicle-mediated transport	GO:0060627
Regulation of biological process	Regulation of mitochondrion organization	GO:0010821
Signaling	Cell surface receptor signaling pathway involved in cell-cell signaling	GO:1905114
Biological regulation	Positive regulation of binding	GO:0051099
Cellular process	Protein complex oligomerization	GO:0051259
Developmental process	Regulation of synapse assembly	GO:0051963
	Regulation of neuron differentiation	GO:0045664
	Epidermal cell differentiation	GO:0009913
Negative regulation of biological process	Negative regulation of response to external stimulus	GO:0032102
	Negative regulation of cellular component organization	GO:0051129
Response to stimulus	Regulation of cellular response to stress	GO:0080135

4.2.3.4.1 Annotated Candidate Genes on Enriched Pathways in Guineafowl Genomes from Africa

Some of the genes that were annotated for the guineafowl populations from Africa include the FASLG, and BAK1 for the metabolic process, NFSF10, and XDH for the Positive regulation of biological process, PLCG1, and STK4 for localization (Table 4.23).

Table 4.23: Annotated Genes in Enriched Pathways in Guineafowl Genomes from Africa

Pathway	Genes	GO ID
Metabolic process	GO:0008152	FASLG,BAK1,XDH,TNFSF10,SERPINF2, ACAT1,PLCG1,PLD1,STK4,SSBP1,ATP5MF
Positive regulation of biological process	GO:0048518	ASLG,PLCG1,STK4,SERPINF2,SSBP1, FLRT3,CDT1,RNF144B,ECT2,ERCC3
Localization	GO:0051179	FASLG,MCUR1,BAK1,PLCG1,ATP5MF, SERPINF2,XDH,TNFSF10,STK4,ECT2

4.2.3.5 Genomic Signatures of Selection Common in Guineafowl Populations from Asia

The strongest putative regions of selection were on chromosomes 2, 3, and 6 (Figure 4.15A). The selected genes were subjected to gene ontology and some of the enriched terms were involved in the positive regulation of biological processes, localization, response to stimulus, cellular process, metabolic process, growth, response to stimulus, biological regulation (Figure 4.15B, Table 4.24).

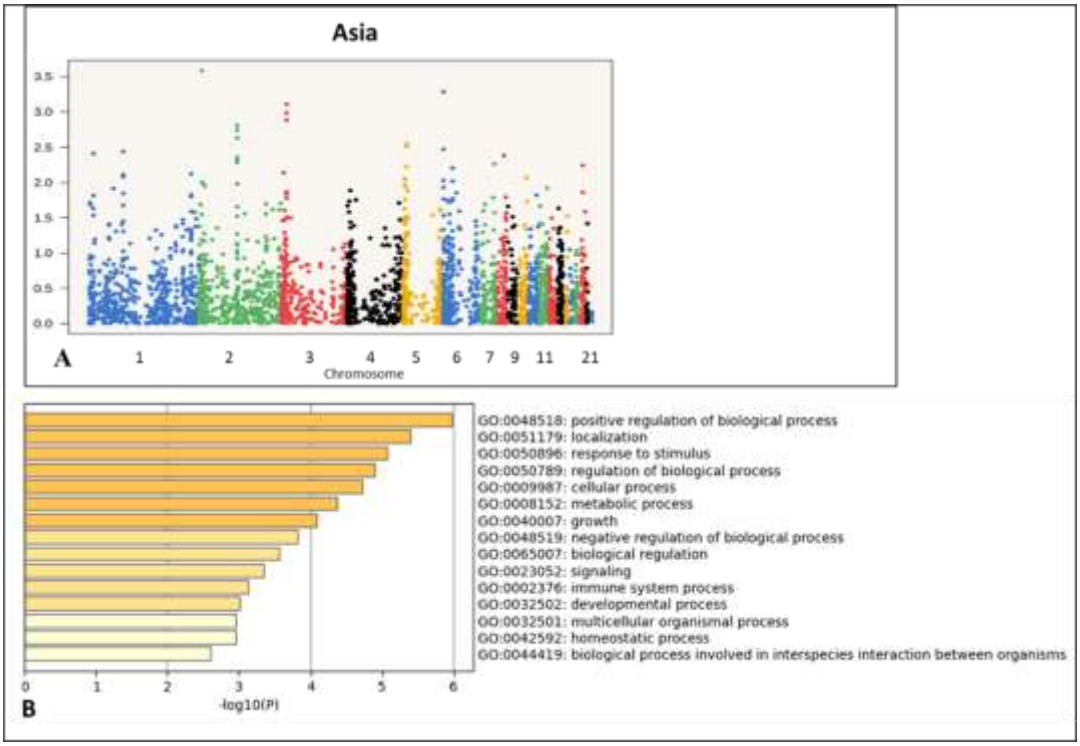

Figure 4.15: Distribution of iHS Scores, and GO Enrichment Analysis Comparing Amongst Guineafowl Populations from Asia.

Table 4.24: A Subset of GO Enrichment of iHS Analysis, and Annotated Genes in Guineafowl Genomes from Asia

Pathway	Enriched term	GO ID
Positive regulation of biological processes	Positive regulation of mitochondrion organization	GO:0010822
Localization	Maintenance of location	GO:0051235
	Regulation of protein transport	GO:0051223
	FOXO-mediated transcription of cell death genes	R-HSA-9614657
	Apoptosis	hsa04210
Response to stimulus	Response to carbohydrate	GO:0009743
	Cellular response to oxygen levels	GO:0071453
	Fat digestion and absorption	hsa04975
Cellular process	Mitochondrion organization	GO:0007005
Metabolic process	Cellular macromolecule catabolic process	GO:0044265
	Histone ubiquitination	GO:0016574
	Regulation of small molecule metabolic process	GO:0062012
	GP1b-IX-V activation signaling	R-HSA-430116
Growth	Negative regulation of growth	GO:0045926
Biological regulation	Regulation of membrane potential	GO:0042391
	Metabolism of lipids	R-HAS-556833

4.2.3.5.1 Annotated Genes on Enriched Pathways in Guineafowl Genomes from Asia

Among the genes that were annotated were BRCA1, SREBF1, and PINK1 for the positive regulation of biological process, LRRC8A, SERINC3, and SLC27A4 for localization, BAK1, and BRCA1 for the response to stimulus (Table 4.25).

Table 4.25: Annotated Genes in Enriched Pathways in Guineafowl Genomes from Asia

Pathway	Genes	GO ID
Positive regulation of biological process	GO:0048518	BRCA1,PINK1,BAK1,CASP9,SRC,FASLG,ECT2, ENDOG,STK4,SLC27A4,SPTAN1,SREBF1, GHSR,SCARB1,MAP2K3,COL16A1
Localization	GO:0051179	SERINC3,SLC27A4,SLC66A1,YWHAB,TRPM1, PINK,GHSR,SDC4,SREBF1,TREM2,LRRC8A
Response to stimulus	GO:0050896	SREBF1,BAK1,BRCA1,TRPM1,TREM2,ENDOG, AP2K3,ATP6V0,PINK1,GHSR,RAF1,SLC27A4

4.2.3.6 Genomic Signatures of Selection Common in Guineafowl Populations from Europe

The genome-wide distribution of iHS values across the genome of the guineafowl populations from Europe are shown in Figure 4.16A. Strong signals of selection were seen on chromosomes 1,2, and 6 (Figure 4.16A). A total of 39 GO terms were enriched. The significantly enriched terms were involved in signaling, developmental process, metabolic process, positive regulation of biological process, localization, and homeostatic process (Figure 4.16B, Table 4.26).

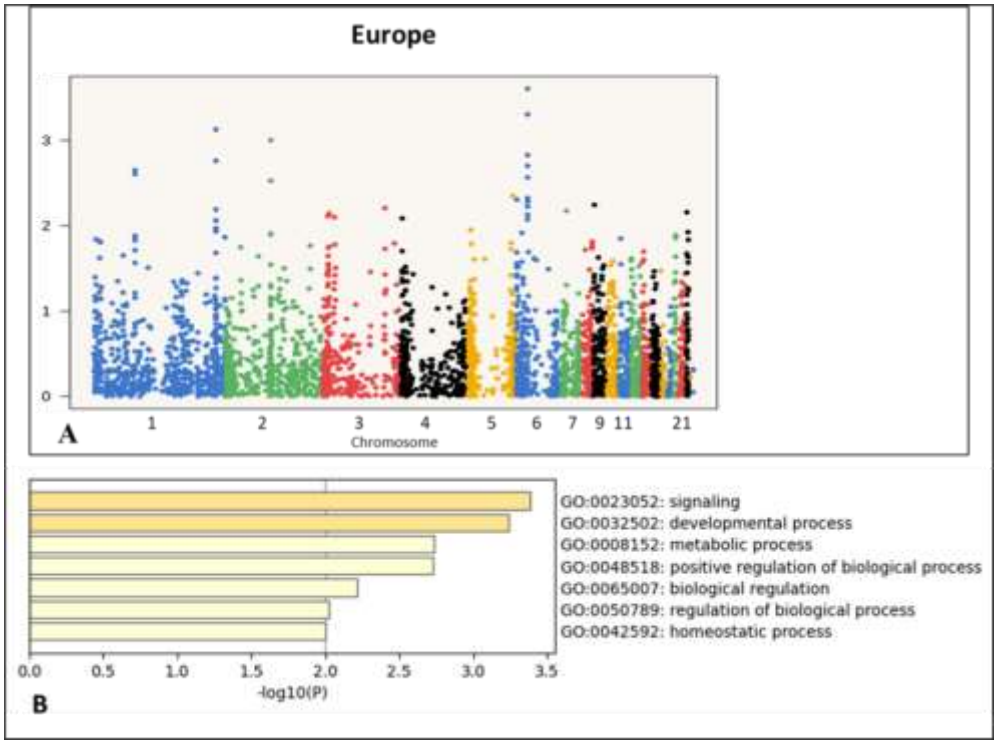

Figure 4.16: Distribution of iHS Scores, and the GO Enrichment Analysis Comparing Amongst Guineafowl Populations from Europe.

Table 4.26: A Subset of GO Enrichment of iHS Analysis, and Annotated Genes in Guineafowl Genomes from Europe

Pathway	Enriched term	GO ID
Signaling	Wnt signaling pathway	GO:0016055
	Small GTPase mediated signal transduction	GO:0007264
Developmental process	Chordate embryonic development	GO:0043009
	Cell morphogenesis	GO:0000902
Metabolic process	Alpha-amino acid metabolic process	GO:1901605
	Regulation of kinase activity	GO:0043549
Positive regulation of biological process	Positive regulation of plasma membrane bounded cell projection assembly	GO:0120034
Homeostatic process	Cellular calcium ion homeostasis	GO:0006874

4.2.3.6.1 Annotated Genes on Enriched Pathways in Guineafowl Genomes from Europe

Several genes were annotated in the enriched pathways in guineafowl genomes from Europe. Some of these genes include the CDH13, and DOCK10 for signaling, HSD17B2, and OPA1 for the developmental process, XIAP, and CST7, for the metabolic process (Table 4.27).

Table 4.27: Annotated Genes in Enriched Pathways in Guineafowl Genomes from Europe

Pathway	Genes	GO ID
Signaling	GO:0023052	CDH13,DOCK10,PLCE1,XIAP,MITF,TCF7,TNKS2
Developmental process	GO:0032502	HSD17B2,OPA1,NR2F2,CDH13,DOCK10,NYAP2
Metabolic process	GO:0008152	XIAP,CST7,GRAMD4,NR2F2,LMO4,ABI1,TENM1, PLCE1,MPHOSPH6,POLR1B,TPRKB,TRMU

4.3 Candidate Genes Selected for Heat Stress in Chicken and Guineafowls

4.3.1 Specific Candidate Genes Associated with Heat Stress Tolerance in Chicken and Guineafowl Genomes

Several candidate genes that are associated with heat stress tolerance were selected in the chicken and guineafowl genomes from Africa, Asia, and Europe. Some of these genes include the VDAC2, TRPA1, HYOU1, ARPC3 (Table 4.28).

Table 4.28: Candidate Genes Associated with Heat Stress Tolerance in Chicken and Guineafowls

Gene	Poultry species	Sampled Region
Voltage Dependent Anion Chanel 2 (VDAC2)	Chicken	Africa
Transient Receptor Potential Ankyrin 1(TRPA1)	Chicken	Africa
Hypoxia up-regulated protein 1 (HYOU1-member of HSP70)	Chicken	Africa, Asia
Actin-relate protein 2/3 complex (ARPC3)	Chicken	Africa, Asia
Actin Related Protein 10 (ACTR10)	Chicken	Africa, Asia
Peroxisome proliferator-activated receptor gamma (PPARG)	Chicken & Guineafowl	Chicken: Africa, Asia Guineafowl: Africa, Europe
Sterol regulatory element-binding transcription factor 1 (SREBF1)	Chicken & Guineafowl	Chicken: Africa, Asia Guineafowl: Africa, Europe, Asia
Transient receptor potential cation channel, subfamily M, member 2 (TRPM2)	Chicken	Africa, Asia
Ubiquitin-conjugating enzyme E2 G2 (UBE2G2)	Chicken	Africa, Asia
14-3-3 protein epsilon (YWHAE)	Chicken	Africa, Asia

Gene	Poultry species	Sampled Region
Period Circadian Regulator 2 (PER2)	Chicken	Africa, Asia
Adiponectin (ADIPOQ)	Chicken	Africa, Asia
Serine/threonine-protein kinase 25 (STK25)	Chicken	Africa, Asia
Keratin 5 (KRT5)	Chicken	Africa, Asia
Caspase 14 (CASP14)	Chicken	Africa, Asia
V-type proton ATPase 16 kDa proteolipid subunit (ATP6V0)	Chicken & Guineafowls	Chicken: Africa, Asia Guineafowl: Asia
sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (SERCA2)/ATP2A2	Chicken	Africa, Asia
Plasma membrane calcium-transporting ATPase 4 (ATP2B4)	Chicken	Africa, Asia
Leucine-rich repeat-containing protein 8A (LRRC8A)	Chicken & Guineafowls	Chicken: Africa, Asia Guineafowl: Africa, Europe
Atrial natriuretic peptide (ANP)/atrial natriuretic factor (ANF) (NPPA)	Chicken	Africa, Asia
Thromboxane A synthase 1 (TBXAS1)	Chicken	Africa, Asia

4.4 Transcriptomic Landscape in Chicken Kidney Tissue Samples

4.4.1 Population Structure Analysis in Chicken Kidney Tissue Samples

To characterize the transcriptomic profile of chicken kidney tissue samples from Lamu and Mombasa, a PCA of all the samples was performed. Components one (PC1) (26%) and two (PC2) (13%) of the chicken kidney tissue PCA analysis jointly account for 39% of the total variance as shown in Figure 4.17. The samples were clustered into 4 groups. Cluster 1, 3 and 4 had samples from Mombasa and Lamu while cluster 2 was composed of samples from Mombasa.

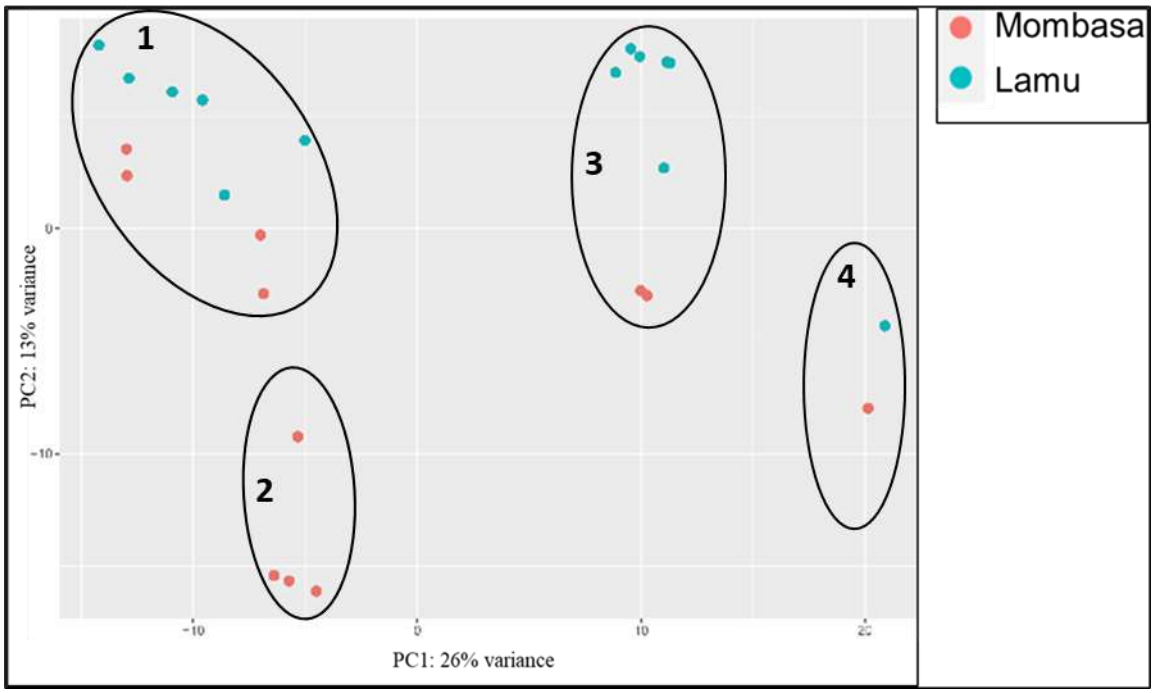

Figure 4.17: PCA Analysis for the Chicken Kidney Tissue Samples.

4.4.2 Clustering Analysis of Differentially Expressed Genes in Chicken Kidney Tissue Samples

To compare the transcriptomic profile of kidney tissue samples of the chicken from Lamu and Mombasa, an analysis of the DEGs was performed. In total, 278 DEGs were identified between chicken from Lamu and chicken from Mombasa, out of which 129 were up-regulated, and 149 were down-regulated (Figure 4.18). Some of the upregulated genes include CD36, ANGPTL4, PLIN1, GLRA3, GABRA4, ADORA1,

GRIN2C. The downregulated genes include the GABRA2, C3, GHRHR, GHSR, GRIK4, GRIA3, ACSL6, SOCS3, VTN, and SPP1.

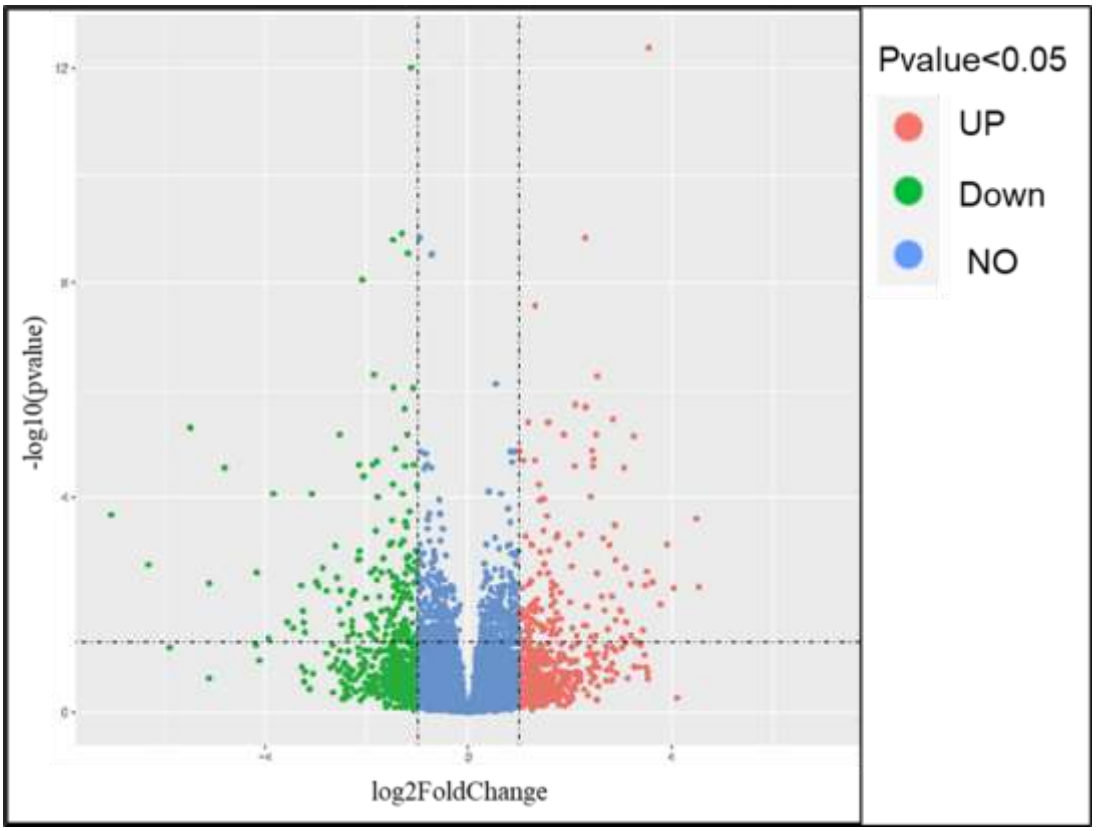

Figure 4.18: Visualization of all DEGs in Chicken Kidney Tissue Samples by Volcano Plot.

Key: The X-axis represents the log2 fold change, the Y-axis represents the statistical significance. Significantly up-regulated and down-regulated genes are indicated in orange and green, respectively. Genes that were not differentially expressed are depicted in blue.

4.4.3 Gene Ontology Enrichment Analysis of the Chicken Kidney Tissue Samples

The functional enrichment analysis was performed using the DEGs. A total of 54 enriched GO terms are involved in the biological processes of metabolic, and developmental processes (Figure 4.19).

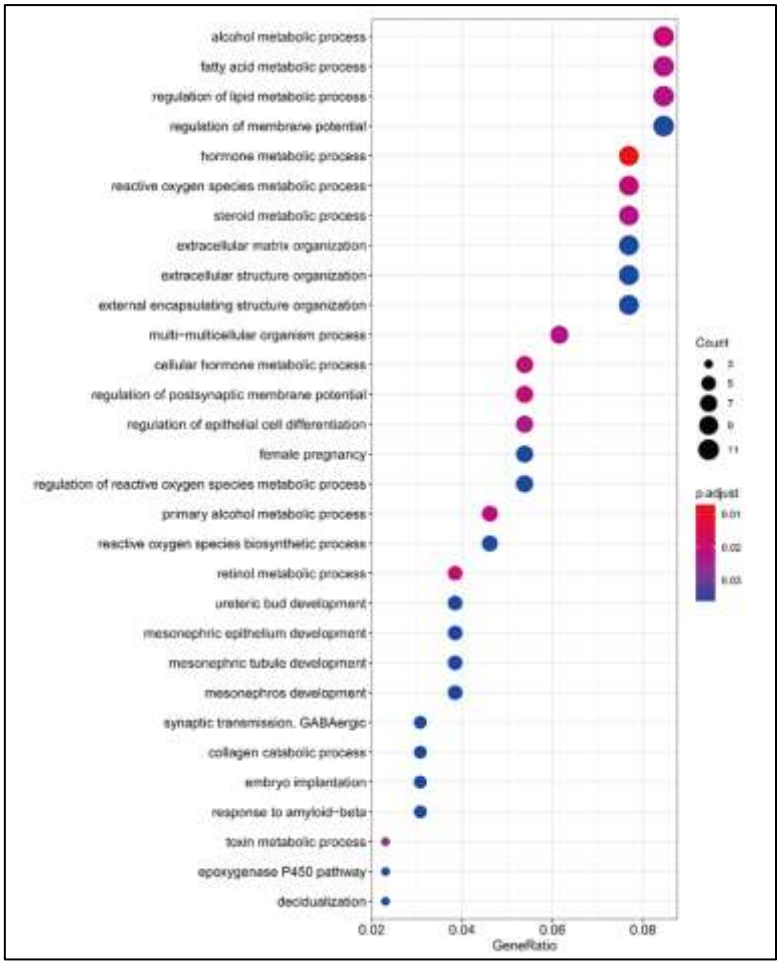

Figure 4.19: The GO Enrichment Analysis of DEGs of Chicken Kidney Tissue Samples.

Key: The top 30 enriched GO terms that are involved in biological processes are shown in a dot plot. The GO items that had a *p*-value of <0.05 and a qvalue of <0.05 were considered enriched. The spot size represents the enriched gene number, and the spot color represents the *p*-value.

4.4.4 Pathway Enrichment Analysis in Chicken Kidney Tissue Samples

The KEGG pathway analysis found that the differentially expressed genes were significantly enriched in the Neuroactive ligand-receptor interaction, PPAR signaling pathway, Adipocytokine signaling pathway, and ECM-receptor interaction (Table 4.29).

Table 4.29: The Enriched KEGG Terms of DEGs of Chicken Kidney Tissue Samples

ID	Description	*p*-value	FDR	Genes
gga04080	Neuroactive ligand-receptor interaction	0.000049123	0.002112286	GABRA2, C3, GHRHR, GHSR, GLRA3, GABRA4, ADORA1, GRIK4, GRIN2C, GRIA3
gga03320	PPAR signaling pathway	0.007788768	0.167458502	ACSL6, ANGPTL4, CD36, PLIN1
gga04920	Adipocytokine signaling pathway	0.065228681	0.934944429	SOCS3, ACSL6, CD36
gga04512	ECM-receptor interaction	0.099989711	1	VTN, SPP1, CD36

4.5 Transcriptomic Landscape in Guineafowl Kidney Tissue Samples

4.5.1 Population Structure Analysis of Guineafowl Kkidney Tissue Samples

To characterize the transcriptomic profile of guineafowl kidney tissue samples from Lamu and Mombasa Counties using the kidney tissue, a PCA of all the samples was performed. Components one (PC1) (22%) and two (PC2) (13%) of the guineafowl kidney tissue samples PCA analysis jointly account for 35% of the total variance as shown in Figure 4.20. The samples were clustered into 4 groups. Cluster 1 had samples from Lamu while cluster, 2 and 4 had samples from Mombasa. Cluster 3 had samples from Mombasa and Lamu.

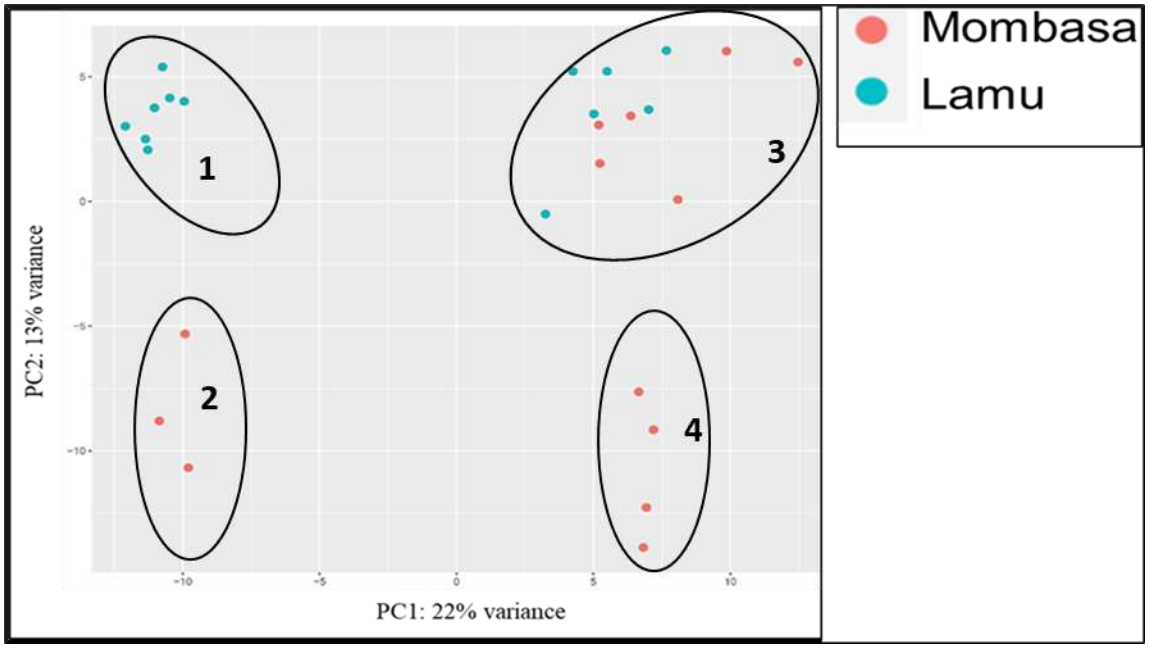

Figure 4.20: PCA Analysis for the Guineafowl Kidney Tissue Samples.

4.5.2 Clustering Analysis of Differentially Expressed Genes in Guineafowl Kidney Tissue Samples

To compare the transcriptomic profile of guineafowl kidney tissue samples from Lamu and Mombasa, an analysis of the DEGs was performed. In total, 349 DEGs were identified, out of which 190 were up-regulated, and 159 were down regulated (Figure 4.21). Some of the upregulated genes include SDR16C5, RETSAT, ALDH1A1, BCO1, ACSM3, AKR1D1, MDH2, CYP24A1, CKMT1B, AGMAT, LDHA, G6PC, ALDOB, FBP1, NPY6R, GRP, CHRM5, GRIN2C, GIP, NMU, IL4I1, HMGCS2, CDKN2A, DHFR, ALPI. The downregulated genes included PAH, MAPK12, GRM1, RAG1, CARNS1, CNDP1, FTCD, HTR2A, MYLK2, AOX1, AOX2, SSTR3, ENO2, and CKMT2.

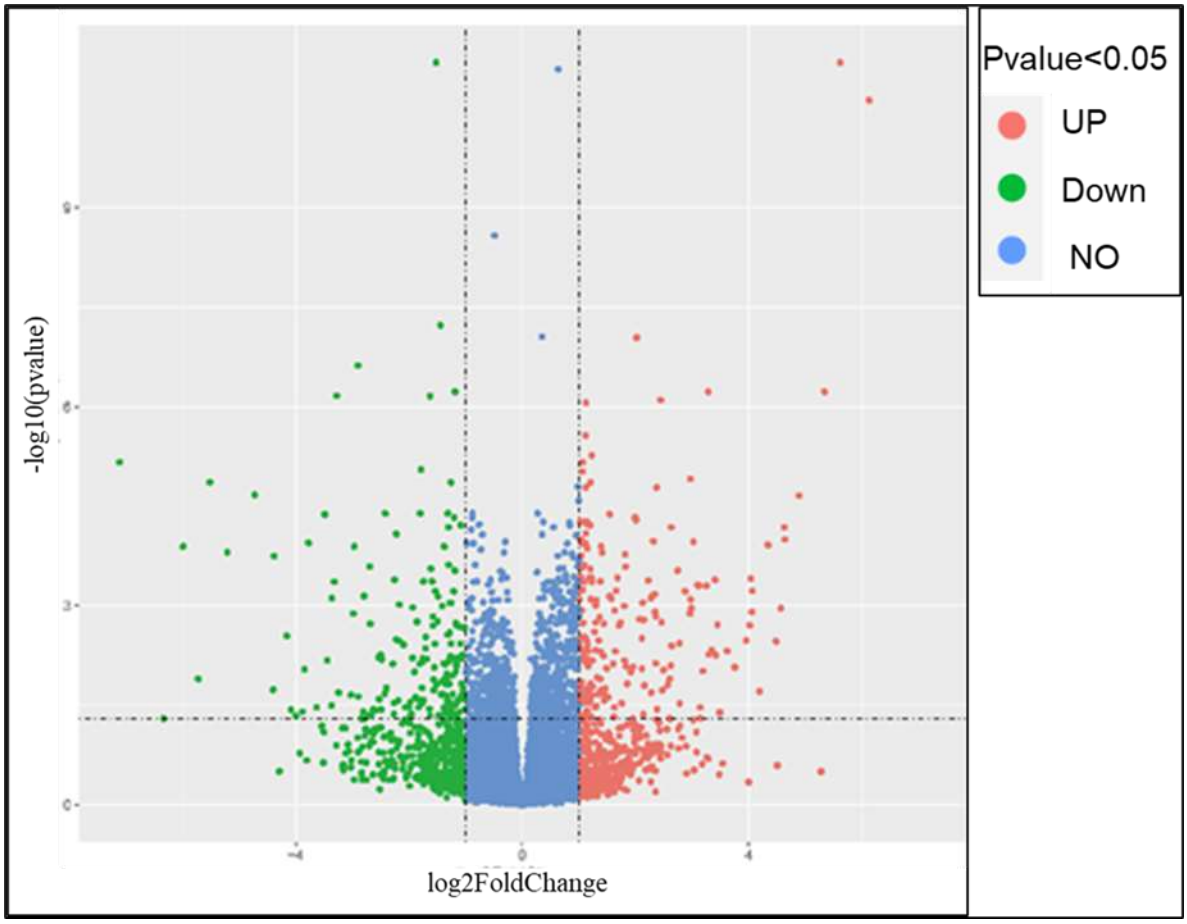

Figure 4.21: Visualization of all DEGs in Guineafowl Kidney Tissue Samples by Volcano Plot.

Key: The X-axis represents the log2 fold change, the Y-axis represents the statistical significance. Significantly up-regulated and down-regulated genes are indicated in orange and green, respectively. Genes that were not differentially expressed are depicted in blue.

4.5.3 Gene Ontology Enrichment Analysis in Guineafowl Kidney Tissue Samples

The functional enrichment analysis was performed using the DEGs. A total of 103 GO terms were enriched and most of them were significant in the biological processes for the metabolic and catabolic processes (Figure 4.22).

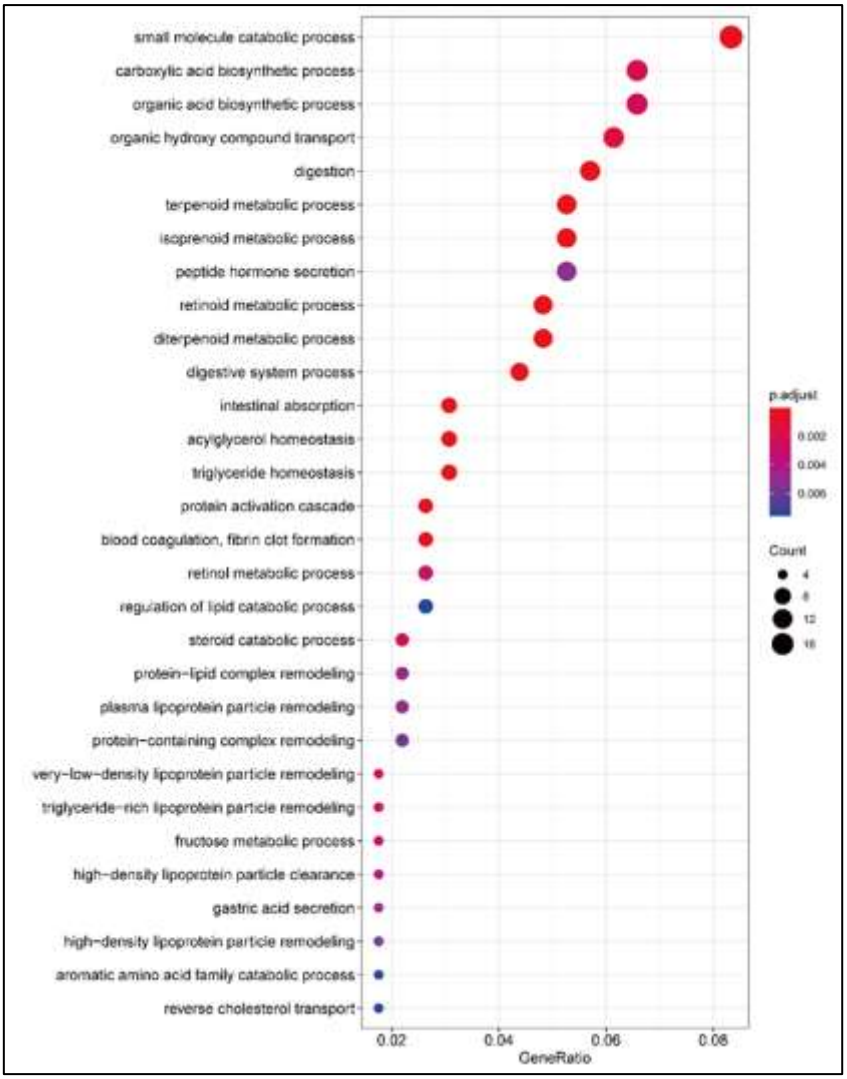

Figure 4.22: The GO Enrichment Analysis of DEGs of Guineafowl Kidney Tissue Samples.

Key: The top 30 enriched GO terms that are involved in biological processes are shown in a dot plot. The GO items that had a *p*-value of <0.05 and a qvalue of <0.05 were considered enriched. The spot size represents the enriched gene number, and the spot color represents the *p*-value.

4.5.4 Pathway Enrichment Analysis in Guineafowl Kidney Tissue Samples

The KEGG pathway analysis found that the differentially expressed genes were significantly enriched in the retinol metabolism, metabolic pathways, arginine and proline metabolism, glycolysis, and neuroactive ligand-receptor interaction, among other pathways (Table 4.30)

Table 4.30: The Enriched KEGG Terms of DEGs of Kidney Tissue Samples of Guineafowls

ID	Description	*p*-value	FDR	Genes
gga00883	Retinol metabolism	0.000688395	0.064709166	SDR16C5, RETSAT, AOX2, ALDH1A1, AOX1, CO1
gga01100	Metabolic pathways	0.003271019	0.153737888	ACSM3, AKR1D1, ENO2, AOX2, MDH2, CYP24A1
gga00330	Arginine and proline metabolism	0.006607419	0.207032486	CKMT2, CKMT1B, CNDP1, AGMAT
gga00010	Glycolysis / Gluconeogenesis	0.013046538	0.306593651	LDHA, G6PC, ALDOB, ENO2, FBP1
gga04080	Neuroactive ligand-receptor interaction	0.022145162	0.416329039	NPY6R, GRP, CHRM5, GRIN2C, SSTR3, GRM1, GIP, NMU
gga00380	Tryptophan metabolism	0.028472789	0.446073690	IL4I1, AOX2, AOX1
gga00280	Valine, leucine and isoleucine degradation	0.044369281	0.518895824	IL4I1, AOX2, AOX1, HMGCS2
gga04020	Calcium signaling pathway	0.046182573	0.518895824	MYLK2,HTR2A,GRIN2C,GRM1
gga00340	Histidine metabolism	0.049681515	0.518895824	CARNS1, CNDP1, FTCD
gga04068	FoxO signaling pathway	0.068769421	0.646432557	G6PC, CDKN2A, MAPK12, GRM1, RAG1
gga00790	Folate biosynthesis	0.084677277	0.652624739	DHFR, PAH, ALPI
gga00400	Phenylalanine, tyrosine and tryptophan biosynthesis	0.087159431	0.652624739	IL4I1, PAH

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Population Stratification in Chicken and Guineafowl Populations

The PCA and ADMIXTURE analysis revealed that chicken populations from Kenya and China are closely related, with a partial overlapping of some chicken from Kenya with chicken from China, suggesting a relatively high gene flow between them. On the other hand, chickens from Ethiopia formed two distinct clusters, which could be an indication of reduced gene flow between chicken in Ethiopia allowing the genetic differences to persist and accumulate (Kim et al., 2016).

In guineafowls, both PCA and ADMIXTURE analysis confirmed the genetic similarity between helmeted guineafowl populations from Africa, Asia, and, Europe. The ADMIXTURE analysis shows gene flow between the helmeted guineafowls (Weimann et al., 2016). This may also be a result of farmers engaging in uncontrolled breeding practices, to obtain specific characteristics. On the other hand, the vulturine guineafowls exhibited no signs of admixture, suggesting a genetic purity. This can be attributed to the vulturine guineafowls' distinct evolutionary past and their adaptation to natural habitats. As a result, they display minimal admixture due to the preservation of a stable genetic makeup well-suited to their ecological niche (Shen et al., 2021).

5.2.2 Genomic Signatures of Selection for Heat Stress in Chicken Genomes

The study of genomic signatures of selection in chicken provides insights into the evolutionary forces that have shaped the genetic makeup of the domesticated chicken. Through advanced genomic techniques, this study identified regions of the chicken genome that have been subject to natural or artificial selection, shedding light on the genetic adaptations that have occurred during the process of domestication, breed formation, and environmental adaptation. The study identified several genes and pathways that play a role in adaptation in the different environments in chicken.

Heat stress in chickens can elevate the production of reactive oxygen species (ROS) within their cells, leading to oxidative stress (Ramiah et al., 2022; Rehman et al., 2018). ROS, which are highly reactive molecules, can damage DNA by modifying its bases and causing breaks in the DNA strands, including single-strand and double-strand breaks (Belhadj et al., 2016). This DNA damage can disrupt the genetic material's integrity, triggering cellular responses aimed at repair. However, under conditions of chronic heat stress and high ROS levels, the rate of DNA damage may outpace the cell's repair capacity (Ramiah et al., 2022). This accumulation of DNA damage can lead to genetic instability, mutations, reduced cell viability, and impaired cellular functions, ultimately posing a threat to the overall health and well-being of heat-stressed chickens.

The cellular nucleic acid-binding protein (CNBP) also known as zinc-finger protein 9 (ZNF9), was involved in the metabolic process of transcription by RNA polymerase II pathway in the chicken from Africa and Asia. The CNBP is a highly conserved zinc-finger protein that has been associated with diverse cellular functions, including transcription and translation (Chen et al., 2018). Liang et al. (2008) highlighted the role of CNBP in the regulation of immunity and inflammatory responses. The gene may have an important function in supporting the immune system of chicken from Africa and Asia. This could be due to the challenging conditions they face, such as having to scavenge for their food thus making them prone to diseases.

The skin plays a crucial role in regulating heat stress in chicken by facilitating heat dissipation through vasodilation, sweat production, and protection against UV radiation (Kennedy et al., 2022; Mascarenhas et al., 2023). Skin pigmentation can also impact the absorption of vitamin D, which is necessary for bone health (Akinyemi & Adewole, 2021). These mechanisms are important adaptations that help chicken cope with high temperatures and prevent overheating, maintaining their thermal balance and overall well-being. Skin development was one of the enriched terms in chicken from Africa and Asia. One of the selected genes in the developmental process of the skin was the COL1A1 gene, which codes for type I collagen, and is the main structural component of the extracellular matrix of the skin (You et al., 2023). This gene is essential for the development and maintenance of healthy skin as it provides tensile strength and elasticity to the tissue.

The Ras protein is responsible for activating a variety of signaling molecules by transporting them to the plasma membrane (Simanshu et al., 2017). This protein is vital for regulating cell growth, differentiation, apoptosis, cell migration, and neuronal activity (Olson & Marais, 2000; Simanshu et al., 2017). Given its crucial role in multiple signaling pathways involved in several cellular functions, the Ras protein identified in this study seems to play a critical role in the adaptation of chicken from Africa to their local environment.

5.2.3 Genomic Signatures of Selection for Heat Stress in Guineafowl Genomes

The top most significant genes functional annotation results revealed that most of the genes in regions selected by the F_{ST}, XP-EHH, and iHS methods were clustered into the response to stimulus, signaling, regulation of biological process, cellular process, localization, metabolic process, and developmental process. The enriched functions play a role in the adaptation of the guineafowls to the harsh environments in Africa, Asia, and Europe. For instance, the mitogen-activated protein kinase family like the MAP2K3, MAP3K2, MAP4K5, and MAP2K1 were positively selected and they played a role in several pathways like localization, metabolic process, and positive regulation of biological processes in the guineafowl populations from the various regions. The MAPK is a series of protein kinases that transmit signals from extracellular stimuli to the nucleus (Guo et al., 2020). They, therefore, play a crucial role in regulating a variety of physiological processes in guineafowls, including cell proliferation, cell differentiation, reproduction, immunity, secretion, and stress response (Wang et al., 2020; Guo et al., 2020; Sheng et al., 2020).

One of the selected genes, the BRAF gene plays a significant role in the response to stimuli and in the regulation of biological process pathways, and it has been shown to mediate the SLIT2-SRGAP1-CDC42-induced granulosa cell proliferation and differentiation in chicken (Shen et al., 2022). The BRAF and MAPK1 genes which were positively selected in the guineafowl populations from Africa and Asia, are known to be involved in neural crest cell development and migration (Gaudet et al., 2011; Kumar et al., 2014) which appears to support the neural crest hypothesis of domestication syndrome (Todd et al., 2006). It is possible that the selection of this gene, may be responsible for the guineafowls' behavioral and morphological traits during

domestication inadvertently affecting the development of neural crest-derived structures that led to the characteristic features seen in domesticated guineafowls.

The lipid catabolic process (GO:0016042) in the guineafowl populations from Africa and Europe, glycerolipid metabolism (hsa00561), and fatty acid metabolism (R-HSA-8978868) in the guineafowl populations from Africa and Asia were among the top enriched GO terms that play a role in lipid metabolism. The embryo's development is fueled by the metabolism of lipids that originate from feed or liver synthesis. These lipids are transported in small molecules through the blood to the ovaries and are then deposited in the yolk, which provides the necessary energy (Wang et al., 2020). Lipid metabolism may be necessary for the growth and reproduction performance of guineafowls in stressed environments.

Response to stimuli, which included regulation of cellular response to stress (GO:0080135) in the guineafowl populations from Africa and response to radiation (GO:0009314) in the guineafowl populations from Africa and Asia were among the enriched terms. Response to radiation which includes ultraviolet and ionizing radiation most likely enhances the adaptation of guineafowls to high-intensity solar radiation in Africa and Asia.

The CRYGN gene, which was selected in the guineafowl populations from Africa and Europe has been shown to play a role in eye development since it is localized to the refractive structure of the eye lenses of the vertebrates (Graw, 2009). Prolonged exposure to ultraviolet radiation can result in various eye-related conditions. The findings indicate that there may be a genetic explanation for why guineafowls can tolerate extended periods of exposure to ultraviolet light without experiencing harm to their visual system (Graw, 2009).

The results also showed that some genes like the SLC27A4, SLC66A1, SLC16A2, SLC20A1, and SLC16A14, which belong to the SLC family were positively selected. These genes played a role in the response to stimuli, localization, positive regulation of biological process, and metabolic pathways in the guineafowl populations from all the guineafowl populations. The SLC family facilitates the transfer of various molecules like sugars, nucleotides, and amino acids to maintain a constant internal environment (Shi et al., 2022; Mueckler & Thorens, 2013). Several other genes within the SLC family have been proposed as having high potential as heat stress biomarkers for

different organisms (Shi et al., 2022; Bao et al., 2017; Yadav et al., 2018). Therefore, this group of genes within the SLC family could be playing an important role in the guineafowl adaptation in varying environments.

The ATP5MF, ATP6V0A2, ATP13A4, and ATP13A5 genes were involved in the metabolic, localization, response to stimuli, and cellular calcium ion homeostasis pathways in all the guineafowl populations. These genes belong to the large family of genes involved in ATP-dependent ion transport involved in calcium homeostasis (Sim & Park, 2023; Szigeti & Kellermayer, 2006). Maintenance of calcium ion homeostasis has been reported to be related to temperate environment adaptation in previous studies (Nan et al., 2023; Zhang et al., 2016; Wang et al., 2015). These genes, therefore, play an important role in the adaptation of the guineafowls to the temperate environment in Europe.

The ANGPT2 gene was positively selected in guineafowl populations from Africa and Asia. The gene was involved in the response to stimuli pathway. This gene is involved in various biological functions including the formation and stability of blood vessels, inflammation, wound healing, and glucose metabolism (Smeland et al., 2023; Wang et al., 2023). Studies indicate that exposure to high temperatures can cause an increase in ANGPT2 expression in poultry, which may affect blood flow and heat dissipation mechanisms, suggesting its involvement in the physiological response to thermal stress in poultry. In a study conducted by Wang et al. (2016), it was found that heat stress increased ANGPT2 expression in the liver and heart of broiler chicken, implying its role in regulating blood flow during thermal stress. Therefore, ANGPT2 may be crucial in the adaptation of guineafowl to tropical conditions in Africa and Asia.

The regulation of synapse assembly and the regulation of neuron differentiation were the two enriched terms for the developmental process in the guineafowl populations from Africa. Studies have shown that many genes are associated with changes in behavior related to domestication, such as decreased fear, increased exploration, and altered learning and memory capacity (Forrest et al., 2018; Sweatt, 2004). These changes are thought to be regulated by neurotransmissions and signal transductions that affect synaptic plasticity and neural circuits involved in emotional, social, and cognitive functions (Forrest et al., 2018; Schafe et al., 2001; Sheng & Kim, 2002; Sweatt, 2004).

These pathways could be playing an important role in the domestication of wild guineafowls in Africa.

5.2.4 Genomic Signatures of Selection for Heat Stress in Chicken and Guineafowl Genomes

Just like in the chicken genomes, the guineafowl genome scans revealed strong putative sweep regions that spanned several candidate genes with diverse functions that play a role in the adaptation to varying environments. Some of the candidate genes that were common in the chicken and guineafowl genomes included the SREBF1, which was one of the genes involved in localization and response to stimuli pathways in the chicken and guineafowl genomes. The LRRC8A candidate gene was also one of the genes involved in the localization pathway in the chicken and guineafowl genomes. The SLC group of genes were also involved in several pathways in chicken and guineafowl genomes.

The presence of several genes in each pathway implies that the ability of chicken and guineafowls to adapt to different environments is likely influenced by a network of genes working together, rather than being solely controlled by the actions of single candidate genes (Kemper et al., 2014; Lv et al., 2014). It is, therefore, not unexpected that the selected regions spanned several candidate genes that have a direct or indirect impact on several traits that are essential for surviving in varying heat-stressed environments. Enriched terms that were common to both chicken and guineafowls include localization, cellular process, developmental process, signaling, metabolic process, and homeostasis.

These selection signatures were located in regions enriched with genes of adaptive significance, and they could also have arisen from the differences in a population's history, like genetic drift, changes in population size, or inbreeding (Akey, 2009).
The guineafowl genome has not been fully annotated. Our results demonstrate that improvements in the assembly and annotation of the guineafowl genome are necessary to make it possible to compare common conserved regions under selection and investigate their evolutionary history and significance.

5.2.5 Specific Heat Stress Genes Expressed in Chicken and Guineafowl Genomes

Candidate genes are genes that are accountable for a constant amount of genetic variation of a trait (Moioli et al., 2007). From the results, it was evident that various candidate genes were involved in various pathways that play a vital role in economically important traits such as heat stress tolerance.

The VDAC2 (Voltage Dependent Anion Chanel 2), which was selected for the chicken populations from Africa, is a mitochondrial protein that plays a role in regulating mitochondrial membrane potential and the release of apoptotic factors. It has been suggested to play a role in regulating mitochondrial respiration and preventing the production of reactive oxygen species (ROS) during exposure to high temperatures (Shoshan-Barmatz et al., 2010). This gene has also been shown to interact with other proteins involved in heat stress response such as HSP90 and FKBP38 to regulate mitochondrial protein import and cell survival cellular responses to heat stress (Aolymat et al., 2023).

The transient receptor potential ankyrin 1 (TRPA1), which was positively selected in the chicken populations from Africa is expressed in the skins of chicken. This gene plays a role in the detection of exposure to heat and transducing the signal that initiates heat stress response (Bohler et al., 2021). It has been shown that during exposure to heat stress, the TRPA1 channels which are located on the sensory nerves are activated leading to the modulation of cutaneous vasodilation (McGarr et al., 2023). The HYOU1 gene may have a protective role against heat-induced cell damage. HYOU1 which is one of the HSP70 genes, was shown to be highly upregulated in the sea bass subjected to high temperatures (Sun et al., 2021). This gene was selected for chicken from Africa and Asia. ARPC3, which belongs to the actin-related protein 2/3 complex subunit family was found to be upregulated in broilers subjected to heat stress (Liu et al., 2022) and ACTR10 was involved in the cellular response to stress pathway in bovine oocytes which were under seasonal heat stress (Özmen & Karaman, 2021). The ARPC3 and ACTR10 genes which were selected in the chicken and guineafowl genomes could therefore have a significant role in heat stress adaptation.

The functional peroxisome proliferator-activated receptor gamma (PPARG) gene is involved in the heat shock response and has been implicated in the regulation of lipid metabolism and homeostatic lipid maintenance during heat stress (Wang et al., 2020; Wang et al., 2022). An overload of free fatty acid-induced lipotoxicity could be detrimental to an animal's ability to adapt to environmental stress by inducing oxidative stress (Wang et al., 2022). The PPARG gene was positively selected in all chicken populations, and also in the guineafowl population from Africa and Europe. SREBF1 (Sterol regulatory element-binding transcription factor 1) is a potent activator of the PPARG/RXRa activity during adipocyte differentiation via the co-activation of PPARGC1B (Zhao et al., 2022). Higher expression of this gene was found in the female fetal longissimus dorsi of the glitz that was subjected to heat stress (Zhao et al., 2022). This gene has also been expressed in the adipose tissue of neonatal piglets born to sows experiencing heat stress during late gestation and lactation (Heng et al., 2019). This gene was activated in all chicken populations and guineafowl populations from Africa and Europe.

TRPM2 which is a calcium-permeable channel that belongs to the TRP family, was positively selected in chicken populations from Africa and Asia. This gene is expressed in the sensory neurons and it is a candidate for the regulation of body temperature, specifically in the detection of heat in the body (Togashi et al., 2006). One of the mechanisms that poultry employ to reduce the effects of heat stress is to move to cooler environments like a shade (Kennedy et al., 2022). In heat-stressed environments above 38°C, TRPM2 is activated and creates an aversive signal that drives an animal to cooler temperatures (Tan & McNaughton, 2018). Genetic deletion of this gene in mice was shown to lead to the inability of the mice to detect heat (Tan & McNaughton, 2018). TRPM2 is also activated in the presence of ROS like H_2O_2 (Kashio et al., 2012). Heat stress stimulates the excessive accumulation of ROS. High concentrations of ROS limit energy production and utilization in heat-stressed animals, negatively affecting animal production performance (Slimen et al., 2016).

UBE2G2 gene, in association with heat shock proteins, interacts with cell surface receptors in response to environmental stress such as heat stress. The UBE2G2 is therefore important in signal transmission during heat-stress environments (Atkin et al., 2021). This gene was selected in all the chicken populations. YWHAE is a member of

the 14-3-3 family of proteins and has been implicated in the heat shock response. YWHAE facilitates the movement of phosphorylated heat shock factor 1 from the nucleus to the cytoplasm, increasing heat adaptation and regulating the activity of heat shock transcription factors (Liu et al., 2020; Özmen & Karaman, 2021). This gene was positively selected in all the chicken populations.

PER2 gene is a circadian clock gene that is involved in the heat shock response in mice. Circadian rhythms, which are regulated by the heat shock pathway, are involved in the adaptation to daily environmental changes such as the temperature cycle (Tamaru et al., 2011). This gene was selected in all chicken populations. ADIPOQ (adiponectin) gene is an adipokine that plays a role in energy homeostasis and appetite regulation (Laursen et al., 2017). It has also been shown to inhibit oxidative stress in the central nervous system of rats (Wang et al., 2018). This gene has been reported to be a novel candidate for heat stress response and it promotes thermotolerance by protecting animals under heat stress conditions against oxidative stress (Dou et al., 2022). This gene was selected in all chicken populations.

STK25 (The serine/threonine kinase 25) gene is associated with creatine kinase activity and has been reported to be involved in the response to oxidative stress in Taiwan indigenous chicken subjected to heat stress (Zhuang et al., 2020). This gene was positively selected in all chicken populations and could have a role in adaptation in poultry found in heat stressed environments.

KRT5 gene encodes for a protein that is a component of intermediate filaments in skin cells, which are important for skin structure and thermoregulation. Several phenotypes have been documented to better withstand heat stress. Among these phenotypes is the frizzled phenotype (Moraa et al., 2015). The KRT6A, KRT7, and KRT75 genes belong to the α-keratin family, and they are considered candidates for heat tolerance since they are associated with the frizzle feather in chicken (Chen et al., 2022). A study reported that a 69-bp deletion in KRT6A is responsible for the frizzle character in chicken (Chen et al., 2022; Nawaz et al., 2021). This mutation in the chicken genome is reflected as an adaptive mechanism for heat stress tolerance. This gene was selected for some chicken populations from Africa and Asia.

The CASP14 gene is a member of the cysteine aspartate-specific protease family. This gene is essential for regulating and maintaining the skin barrier homeostasis aiding in thermoregulation (Ding et al., 2022). This gene was selected in some chicken populations from Africa and Asia. ATP6V0, ATP2A2, and ATP2B4 genes encode calcium-ATPases, which play a crucial role in regulating intracellular calcium levels (Guo et al., 2018; Sávio et al., 2023). During heat stress, calcium signaling pathways can be disrupted, and these calcium-ATPases may help to restore calcium homeostasis and prevent cell damage (Guo et al., 2018). The ATP2A2 and ATP2B4 were selected for all the chicken populations, while the ATP6V0 was selected for guineafowl populations from Asia.

LRRC8A gene is a member of the Leucine-rich repeat-containing protein 8, and it encodes a member of the leucine-rich repeat-containing protein family (Kern et al., 2023). LRRC8A has been implicated in the regulation of cell volume and is involved in the response to osmotic stress (Lahey et al., 2020). During heat stress, cells may experience changes in osmotic pressure, and LRRC8A may play a role in regulating cell volume and preventing cell damage. This gene was selected for all chicken populations and also in guineafowls from Africa and Europe. NPPA gene is a member of the cGMP-PKG signaling and thermogenesis pathways. This gene regulates blood pressure in response to heat stress and is an important factor in the regulation of heart development and stress response (Liu et al., 2020; Raza et al., 2021). This gene was selected in all the chicken populations. TBXAS1 gene was found to be positively selected in chicken adapted to desert conditions in Saudi Arabia (Tian et al., 2020). This gene was selected for all the chicken populations and could have a role in the adaptation of poultry in stressed environments.

The TBXAS1 gene provides instructions for creating an enzyme called thromboxane A synthase 1, which is part of the arachidonic acid cascade. This pathway processes arachidonic acid to generate various molecules with different functions in the body. Thromboxane A synthase 1 specifically converts prostaglandin H2 into thromboxane A2 (Sun et al., 2022). Thromboxane A2 has vasoconstrictive properties that can help regulate blood flow and maintain blood pressure during heat stress, which is crucial for thermoregulation. Thromboxane A2 may also be involved in the regulation of platelet

function, which can impact blood clotting and inflammation during heat stress (Tian et al., 2020).

5.2.6 Transcriptomic Landscape in Chicken

During heat stress, high temperatures can cause birds to lose significant amounts of water and electrolytes through evaporation, respiration, and excretion (Johnson et al., 2019). The kidneys play an important role in maintaining fluid and electrolyte balance in response to these challenges. To infer whether selection extends beyond allelic variation and also affects gene expression, individual gene expression patterns in the kidney between 13 chicken from Lamu County and 13 chicken from Mombasa County were compared. Some of the DEGs included the TTR, GHRHR, STC2, CYP1B1, PCSK1, SLC25A33, MT3, MMP3, ADH6, CYP1A2, GLRA3, MMPP9, ACTA1, ACSL6, FOXJ1, and NR0B. These genes were involved in the various KEGG pathways.

The Growth hormone-releasing hormone receptor (GHRHR), was found to be downregulated in chicken from Mombasa and Lamu. This gene has also been shown to be downregulated in cattle (Cheruiyot et al., 2021) and ducks (Kim et al., 2017) subjected to heat stress. This gene is relevant in the metabolic homeostasis in ducks (Kim et al., 2017), cattle (Cheruiyot et al., 2021) and, in the regulation of respiratory rates in pigs (Kim et al., 2018) during heat stress. The GHRHR gene is involved in the neuroactive ligand-receptor interaction pathway. This pathway is involved in the binding of various neurotransmitters and neuromodulators to their respective receptors (Li et al., 2022). A study by Cheruiyot et al. (2021), found this pathway to be enriched in heat stressed dairy cattle. Previous studies have also shown this pathway to be involved in maintaining energy homeostasis during heat stress in ducks (Kim et al., 2017).

The PPAR signaling pathway, which was among the top enriched KEGG terms, plays a role in regulating energy metabolism and is involved in the response to heat stress in some studies (Kim et al., 2017; Wang et al., 2015). A study by Srikanth et al. (2019), subjected some chicken to chronic heat stress and they found this pathway to be enriched in chicken subjected to chronic heat stress. They, therefore, concluded that the enrichment of PPAR signaling in chicken subjected to chronic heat stress implied that heat stress-induced reactive oxygen species accumulation and oxidative stress. Also,

the PPAR signaling pathway might be due to the requirement of considerable energy for pumping blood to dissipate the accumulated heat and alleviate oxidative stress.

The adipocytokine signaling pathway was enriched in chicken from Lamu. This pathway has been shown to play an important role in the liver of *G. maculatum* in response to heat stress (He et al., 2023; Li et al., 2021). This pathway was also involved in the adaptive response to heat stress in pigs (Ma et al., 2019).

The ECM-receptor interaction pathway was enriched in all chicken from Lamu and Mombasa. This pathway has been shown to affect ROS synthesis through integrins (Hu et al., 2023). This pathway has been shown to enable *M. nipponense* to adapt to acute heat stress mainly through metabolic function and reduced ROS (Wu et al., 2023)

5.2.7 Transcriptomic Landscape in Guineafowls

Some of the genes that were involved in the various KEGG pathways in the guineafowls include the SDR16C5, RETSAT, AOX2, AOX1, GSTT1L, NDUFB3, CA2, PLA2G4EL2, and NMU. The NMU gene was upregulated in the guineafowls from Lamu. This gene encodes neuromeric U that in mammals induces locomotor activity, grooming, face washing behavior, and wing flapping behavior in chicken (Ozaki et al., 2002). In chicken, this gene has also been shown to suppress food intake by inducing an anorexigenic pathway downstream mediated by corticotropin-releasing factor (CRF) (Honda et al., 2015), which is a candidate signal molecule for regulating appetite and energy in poultry (Sun et al., 2015). The NMU gene was involved in the neuroactive ligand-receptor interaction pathway, which plays a role in energy homeostasis and respiration in heat-stressed animals (Kim et al., 2017; Wang et al., 2015; Kim et al., 2018).

Many studies have reported that under heat stress, the glycolysis/gluconeogenesis pathway and the calcium signaling pathways are activated in various livestock (Kumar et al., 2019; Li et al., 2019). The calcium signaling pathway plays a critical role in the heat stress response of living organisms. Heat stress triggers an increase in cytosolic calcium concentration, which activates a signaling cascade that leads to the expression of genes involved in heat shock response and the synthesis of heat shock proteins (HSPs) (Kim et al., 2021). HSPs are a family of proteins that are synthesized in response to various forms of stress, including heat stress. They help maintain protein stability

and prevent protein aggregation under stress conditions. HSPs are regulated by the heat shock factor (HSF) family of transcription factors, which are activated by the increased cytosolic calcium concentration (Hu et al., 2023).

In addition to the regulation of HSPs, calcium signaling also plays a role in the activation of other signaling pathways involved in the heat stress response, such as the mitogen-activated protein kinase (MAPK) pathway. This pathway is activated by a variety of stress stimuli, including heat stress, and regulates the expression of genes involved in stress response (Kim et al., 2021). The FoxO signaling pathway is linked to the process of cell-lipid differentiation, particularly the regulation of preadipocyte differentiation. Once preadipocytes differentiate, the production and storage of triglycerides in adipose cells increase, resulting in larger fat cells (Song et al., 2018). Studies have shown that FoxO can increase glucose synthesis and lipolysis, while also promoting preadipocyte differentiation in heat-stressed cattle (Chen et al., 2022).

Another pathway in the guineafowl genomes that was enriched is the tryptophan metabolism. Tryptophan is an aromatic amino acid that belongs to the α-amino acid family, and has been reported to play regulatory roles under heat stress (Parthasarathy et al., 2018). Arginine, proline, and histidine metabolism have been associated with heat dissipation through skin vasodilation (Jo et al., 2021). They have provided biochemical insights into metabolic changes due to heat-stressed environments (Zhou et al., 2020).

5.2.8 Transcriptomic Landscape in Chicken and Guineafowls

The GO pathways in both the chicken and guineafowls were involved in the metabolic, catabolic, and developmental processes. These processes maintain cellular homeostasis and protect against the damaging effects of heat stress. The metabolic and catabolic processes play an important role in heat stress regulation by providing energy and facilitating the breakdown and elimination of heat-producing molecules (Srikanth et al., 2019). During heat stress, metabolic processes, such as glycolysis, and oxidative phosphorylation, produce ATP that can be used to maintain cellular functions and support heat shock response. The breakdown of glucose via glycolysis produces NADH, which is used to generate ATP via oxidative phosphorylation (Vandana et al., 2021). This ATP can be used to support cellular repair and maintenance, as well as the

production of heat shock proteins (HSPs) that help protect against the damaging effects of heat stress.

On the other hand, the catabolic processes, such as proteolysis and lipolysis, also play important roles in heat stress regulation by breaking down proteins and lipids to generate energy and eliminate heat-producing molecules (Kennedy et al., 2022). For example, the breakdown of proteins via proteolysis releases amino acids that can be used for energy production or gluconeogenesis, which can help maintain glucose homeostasis and support cellular functions during heat stress (Chen et al., 2022; Parthasarathy et al., 2018). The breakdown of lipids via lipolysis also generates energy and can help reduce the accumulation of heat-producing molecules, such as triglycerides and free fatty acids.

Developmental processes can also play important roles in heat stress regulation by influencing an organism's ability to adapt to high temperatures and maintain homeostasis in response to heat stress (Duncan et al., 2014). During development, organisms undergo a variety of physiological and morphological changes that can influence their responses to heat stress. For example, changes in body size and shape, such as the development of thinner and more elongated bodies or the development of larger surface area-to-volume ratios, can help facilitate heat dissipation and reduce the risk of overheating (Tu et al., 2016). Similarly, changes in metabolic rates, such as the development of higher oxidative capacities or increased heat shock protein expression, can help support energy production and protect against the damaging effects of heat stress.

Additionally, developmental processes can also influence an organism's ability to acclimate to heat stress (Kennedy et al., 2022; Perini et al., 2020). Acclimation is a process by which organisms adjust their physiological and biochemical responses to high temperatures over time, allowing them to better cope with heat stress. For example, exposure to moderate heat stress during development can induce changes in gene expression and protein production that help enhance an organism's heat tolerance and improve its ability to withstand future heat stress.

5.2 Conclusions

An analysis of the population structure in chicken populations revealed a substantial genetic distinction between chicken from Ethiopia and those from Kenya and China. However, there was relatively little genetic variability between chickens from Kenya and China.

In the guineafowl samples, we observed limited genetic variability among helmeted guineafowls from Africa, Asia, and Europe. Conversely, there was a notable genetic divergence between vulturine guineafowls and helmeted guineafowls. Furthermore, vulturine guineafowls showed no signs of admixture, indicating their genetic purity.

Importantly, the study identified several candidate genes with significant roles in heat stress tolerance. These genes include PPARG, associated with the heat shock response; TRPM2, involved in body temperature regulation; UBE2G2, contributing to signal transmission during heat stress; YWHAE, aiding in the movement of phosphorylated HSF1 from the nucleus to the cytoplasm; PER2, regulating circadian rhythms and aiding adaptation to daily environmental changes; AIPOQ, involved in energy homeostasis and appetite regulation; KRT5, which is responsible for the frizzle phenotype, encoding a component of skin cell intermediate filaments crucial for skin structure and thermoregulation; ATP6v0, ATP2A2 and ATP2B4, which regulate intracellular calcium levels; LRRC8, responsible for cell volume regulation under osmotic stress; and NPPA, which regulates blood pressure, heart development, and blood flow.

These genes were found to be associated with enriched pathways in chicken and guineafowl genomes, including skin development, brain development, response to oxidative stress, response to radiation, developmental processes, as well as various cellular, metabolic and signaling pathways. These findings collectively provide valuable insights into the genetic adaptations of these poultry species to diverse environmental conditions.

In transcriptomics analysis of the kidney tissues of chicken, 278 DEGs were identified. These were primarily associated with biological processes like metabolism and development, as well as with pathways such as the PPAR signaling pathway and neuroactive ligand-receptor interaction.

In kidney tissue samples of guineafowls, 349 DEGs were identified. These genes were linked to metabolic pathways, neuroactive ligand-receptor interaction, the FoxO signaling pathway, and the calcium signaling pathway.

The identification and selection of genes associated with heat tolerance enhances our comprehension of the molecular mechanisms implicated in heat stress among poultry. Consequently, this provides a promising and sustainable approach for breeding heat-resistant poultry. This approach effectively addresses the challenges posed by heat stress, particularly in the context of climate change, and ultimately contributes to ensuring food security.

5.3 Recommendations

The findings of this study recommend that:

- More extensive genetic characterization studies to explore the genetic diversity within and among chicken and guineafowl populations from different regions to provide a comprehensive understanding of the genetic basis of heat stress tolerance
- Perform validation studies to confirm the candidate genes identified in the comparative genomics and transcriptomics analyses to help establish the reliability of genes as markers for heat stress tolerance
- Develop and implement cross-breeding programs that incorporate heat-tolerant genes like PPARG, and UBE2G2, from different chicken and guineafowl populations to enhance the overall heat stress tolerance in poultry
- Implement selective breeding programs based on the identified heat tolerant genes, like the KRT5 responsible for the frizzle phenotype, to develop poultry breeds that are better adapted to heat stress conditions to contribute to improved poultry production in regions susceptible to high temperatures
- Advocate for policies and regulations that support the inclusion of heat-resistant genetic traits in poultry breeding programs to enhance food security in regions prone to climate change-induced heat stress.

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APPENDICES

Appendix I: Summary of Chicken Samples Used for Comparative Genomics

Accession number	Country	Continent	Breed
SRR7062655	Ethiopia-Horro	Africa	Domestic
SRR7062656	Ethiopia-Horro	Africa	Domestic
SRR7062657	Ethiopia-Horro	Africa	Domestic
SRR7062658	Ethiopia-Horro	Africa	Domestic
SRR7062659	Ethiopia-Horro	Africa	Domestic
SRR7062660	Ethiopia-Jarso	Africa	Domestic
SRR7062661	Ethiopia-Jarso	Africa	Domestic
SRR7062662	Ethiopia-Jarso	Africa	Domestic
SRR7062663	Ethiopia-Jarso	Africa	Domestic
SRR1217526	China-Yunnan	Asia	Wild
SRR1217527	China-Yunnan	Asia	Wild
SRR1217530	China-Yunnan	Asia	Wild
SRR1217531	China-Yunnan	Asia	Wild
SRR1217533	China-Hainan	Asia	Wild

Appendix II: Summary of Guineafowl Samples Used for Comparative Genomics

Accession number	Country	Continent	Breed	Code
SRR12042133	Sudan	Africa	Domestic	SDH1
SRR12042236	Sudan	Africa	Domestic	SDH2
SRR12042135	Sudan	Africa	Domestic	SDH3
SRR12042136	Sudan	Africa	Domestic	SDH4
SRR12042137	Sudan	Africa	Domestic	SDH5
SRR12042155	Sudan	Africa	Wild	SWH1
SRR12042162	Sudan	Africa	Wild	SWH2
SRR12042161	Sudan	Africa	Wild	SWH3
SRR12042158	Sudan	Africa	Wild	SWH4
SRR12042159	Sudan	Africa	Wild	SWH5
SRR12042139	Nigeria	Africa	Wild	NWH1
SRR12042140	Nigeria	Africa	Wild	NWH2
SRR12042141	Nigeria	Africa	Wild	NWH3
SRR12042142	Nigeria	Africa	Wild	NWH4
SRR12042144	Nigeria	Africa	Wild	NWH5
SRR12042220	Nigeria	Africa	Domestic	NDH1
SRR12042221	Nigeria	Africa	Domestic	NDH2
SRR12042222	Nigeria	Africa	Domestic	NDH3
SRR12042223	Nigeria	Africa	Domestic	NDH4
SRR12042224	Nigeria	Africa	Domestic	NDH5
SRR8101535	Burkina Faso Dori	Africa	Domestic	BFDH1
SRR8101541	Burkina Faso Saradan	Africa	Domestic	BFDH2
SRR8101544	Burkina Faso Sarakongo	Africa	Domestic	BFDH3
SRR8101542	Burkina Faso Koflande	Africa	Wild	BFWH1
SRR8101543	Burkina Faso Yabe	Africa	Wild	BFWH2
SRR8101536	Benin	Africa	Domestic	BDH
SRR8101538	South Africa	Africa	Wild	SAWH
SRR12042264	China	Asia	Domestic	CDH1
SRR12042265	China	Asia	Domestic	CDH2
SRR12042266	China	Asia	Domestic	CDH3

Accession number	Country	Continent	Breed	Code
SRR12042267	China	Asia	Domestic	CDH4
SRR12042268	China	Asia	Domestic	CDH5
SRR12042269	China	Asia	Domestic	CDH6
SRR12042200	Iran	Asia	Domestic	IRDH1
SRR12042205	Iran	Asia	Domestic	IRDH2
SRR12042241	Iran	Asia	Domestic	IRDH3
SRR12042249	Iran	Asia	Domestic	IRDH4
SRR12042248	Iran	Asia	Domestic	IRDH5
SRR12042244	Iran	Asia	Domestic	IRDH6
SRR12042252	Hungary	Europe	Domestic	HDH1
SRR12042253	Hungary	Europe	Domestic	HDH2
SRR12042254	Hungary	Europe	Domestic	HDH3
SRR12042255	Hungary	Europe	Domestic	HDH4
SRR12042256	Hungary	Europe	Domestic	HDH5
SRR8101532	Hungary Godollo	Europe	Domestic	HDH6
SRR8101539	Hungary Hortobagy	Europe	Domestic	HDH7
SRR8101531	France Gromoud	Europe	Domestic	FDH
SRR8101533	France Galor	Europe	Domestic	FSH1
SRR8101534	France Galor	Europe	Domestic	FSH2
SRR8101540	France Grimaud	Europe	Domestic	FSH3
SRR8101537	France Beghin	Europe	Domestic	FSH4
SRR12042171	Italy	Europe	Domestic	IDH1
SRR12042172	Italy	Europe	Domestic	IDH2
SRR12042207	Italy	Europe	Domestic	IDH3
SRR12042208	Italy	Europe	Domestic	IDH4
SRR12042210	Italy	Europe	Domestic	IDH5