GENETIC BACKGROUND AND SIGNATURES OF SELECTION ASSESSMENT IN WILD AFRICAN HARLEQUIN AND DOMESTIC JAPANESE QUAILS USING A GENOMIC AND TRANSCRIPTOMIC APPROACH

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Genetic Background and Signatures of Selection Assessment in Wild African Harlequin and Domestic Japanese Quails Using a Genomic and Transcriptomic Approach

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DECLARATION

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

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DEDICATION

This work is dedicated to my treasured mother, Mrs. Margaret Ogada, and my lovely siblings, who have been a pillar of support, hope, and encouragement throughout my study.

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LIST OF ACRONYMS/ABBREVIATIONS

CLR	Composite Likelihood Ratios
DE	Differentially expressed
DEGs	Differentially expressed genes
DNA	Deoxyribonucleic acid
F_{ST}	Fixation Index
GBS	Genotyping by sequencing
GWAS	Genome-wide association studies
iHS	Integrated haplotype score
ka	Thousand years ago
LD	Linkage disequilibrium
Ma	Million years ago
mtDNA	Mitochondrial DNA
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
SNP	Single-nucleotide polymorphism

ABSTRACT

Quails are increasingly becoming a vital complementary protein source, contributing to food security. In Kenya, the indiscriminate and destructive harvesting of wild African harlequin quails using traditional methods has been ongoing for generations. Yet, their genetic diversity and evolutionary history are largely unknown, thus posing a threat to extinction and genetic diversity. In this study, the genetic variation and demographic history of captured wild African harlequin quails (n = 78) were determined using mitochondrial DNA (mtDNA) and genotyping by sequencing (GBS) data. Selection signatures were also examined using composite likelihood ratio (CLR) and integrated haplotype score (iHS) tests in comparison to domestic Japanese quails (n = 22). Domestic Japanese quail transcriptome data (n = 6) was also examined. The genetic variation in wild African harlequin quails was predominantly among individuals rather than populations. Demographic analyses indicated a signal of rapid expansion. The estimated time since population expansion was 150 to 350 thousand years ago (kya), corresponding to around the Pliocene–Pleistocene boundary. A gradual decline in their effective population size was also observed, which raised concerns about their conservation status. 352 and 424 candidate genes were detected in the wild African harlequin quail and domestic Japanese quail through the CLR test, respectively, whereas 150 and 457 candidate genes were identified through iHS analysis. Candidate genes under positive selection identified in the wild African harlequin quail were associated with important traits such as immune response (MAPK13, CREB1, ITGB3, and PPP1CA) and morphological traits (WNT5A, GRIA1, and ALK), whereas, in domestic Japanese quail, production-related genes such as VIPR2, DYNLL2, PRF1, COL11A1, and GNA12 were identified. RNA-Seq analysis revealed differentially expressed genes associated with production processes such as spermatogenesis and muscle structure development. This is the first study on the wild African harlequin quails to provide information useful in biodiversity conservation and proper utilization of its genetic resources.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Poultry farming is widely practiced globally since it provides high-quality and affordable animal protein. Additionally, poultry meat and egg production play a key role by offering economic empowerment to especially rural smallholder farmers. Some poultry species reared in Kenya include chickens, quails, ducks, guinea fowls, geese, turkeys, pigeons, and ostriches (MOLD, 2010). However, indigenous chickens are the most common. They are mainly reared under the free-range (backyard or scavenging) system as it is less capital-intensive and applies little to no biosecurity and biosafety measures (Nyaga, 2007). In Kenya and other developing countries, these poultry species are mainly reared in rural settings and are commonly referred to as village poultry (Khobondo et al., 2015). Village poultry meat, which is classified under white meats, is preferred by consumers due to its distinct flavor, leanness, and perception that they are healthier compared to commonly available red meats (Bett et al., 2012). This, among other factors, has led to an increase in their demand.

1.1.1 Complementary sources of poultry meat and eggs

The primary source of poultry protein for many rural households in Africa and other developing countries is chicken, though progressively, other poultry species have become a vital supplement, increasing their socioeconomic value (MOLD, 2010). These include quails, guinea fowls, ducks, turkeys, pigeons, geese, and ostrich. Quails, especially in developing countries, are complementary poultry to chicken, thereby contributing to food security. They also provide an extra source of income and have become popular among smallholder farmers. Quails are hardy, more resilient to common poultry diseases, and can survive unfavorable climatic conditions with little maintenance costs. In addition, their low feed requirements, rapid growth, short generation, and gestation periods, among

other attributes, make them favorable to smallholder farmers with limited resources (Jeke et al., 2018).

The growing demand for healthier sources of animal protein led to the increase in demand for quail meat and eggs in Kenya during the quail "bubble" of 2013 – 2015, which was followed by a sudden influx of exotic quail species and increased harvesting of wild quails in Kenya (Ogada et al., 2022). This resulted from speculations purporting that quail meat and eggs contained vital minerals and medicinal properties. Quails have been shown to contain less fat and more minerals that are vital to the body. According to findings by Chepkemoi et al. (2017), quail meat and eggs contain higher protein content and minerals (zinc, potassium, calcium, and iron) when compared to guinea fowl, indigenous and commercial chickens. The Food and Agriculture Organization (FAO) of the United Nations has emphasized identifying alternative food resources to address world issues relating to malnutrition due to the increased human population, especially in developing countries (Jeke et al., 2018). Quail meat and eggs, as a complementary source of animal protein to chicken, can help alleviate protein malnutrition in Kenya and other developing countries (Mohammed & Ejiofor, 2015).

1.1.2 History of quails in Kenya

Old-world quails occur widely across the globe and are mainly divided into wild and domestic categories (Chang et al., 2005). Wild quail species such as the common quail (*Coturnix coturnix),* African harlequin quail (*Coturnix delegorguei delegorguei*), African blue quail (*Coturnix adansonii*), and rain quail (*Coturnix coromandelica*) are common in East and Southern Africa where they migrate seasonally within and across country borders (Lewis & Pomeroy, 1989). In Kenya, the wild African harlequin quail is the most notable wild quail species, whereas the Japanese quail (*Coturnix japonica*) is the most common domesticated species (Wamuyu et al., 2017). The domestic Japanese quail are mainly kept for meat and egg production (Nishibori et al., 2001), while the wild African harlequin quail is hunted primarily by rural smallholder farmers in Western Kenya for consumption and sale. The wild African harlequin quail can be found in Siaya, Kisumu, Vihiga,

Homabay, Kakamega, Busia, and Bungoma Counties (Wamuyu et al., 2017). They are usually found in the wild and farmlands during the grain-planting season.

The domestication of Japanese quail during the late 19th century and early 20th century has contributed immensely towards improved poultry meat and egg production and has made it the most commonly consumed quail species globally (Nishibori et al., 2001; Lukanov & Pavlova, 2020). Currently, the domestic Japanese quail can be found in many countries where they are selectively bred into meat and egg types (Jeke et al., 2018). Several quail breeds have been developed from the domestic Japanese quail, and the most commonly found in commercial farms include the English white, golden range, Texas A&M, and Manchurian.

1.1.3 Harvesting and conservation status of the Wild African harlequin quail

Wild quail populations are continuously threatened primarily by illegal hunting, which has led to their declining numbers over time (Puigcerver et al., 2014). The ongoing unlawful hunting of wild African harlequin quails by rural smallholder farmers in Western Kenya is trending to unsustainable levels leading to declining numbers over time (Wamuyu et al., 2017). The seasonal migration of wild African harlequin quails into farmlands encourages their incessant capture for sale and consumption. Currently, control measures to check unlawful and uncontrolled hunting are nonexistent, and little to no effort has been made to monitor and study their conservation status.

Introducing domestic Japanese quails in Kenya and other developing countries has provided entrepreneurial opportunities and an alternative way of alleviating animal protein deficiency in their populations (Nasar et al., 2016). However, this introduced new challenges, such as the quail "bubble" of 2013-2015, which contributed to the uncontrolled introduction of several exotic Japanese quail breeds in Kenya and other East African countries (Ogada et al., 2022). Considering the domestic quail business bubble soon deflated, these exotic quail breeds might have been released, presenting the risk of breeding with the local wild quail populations. Planned or accidental interaction of domestic and wild quail populations would bring adverse genetic changes to wild quail

populations, such as loss of genetic variation, loss of survival and adaptive ability, and altered population structures, as has been observed in other parts of the world (Laikre et al., 2010). In addition, admixture between the domestic and wild quail populations in some parts of the world has been observed, where wild quails were found to be hybrids of the domestic Japanese quail (*C. Japonica*) and the wild common (*C. Coturnix*) quail (Barilani et al., 2005; Amaral et al., 2007; Chazara et al., 2010; Sanchez-Donoso et al., 2012). Therefore, characterization studies are essential to detect or monitor any changes in genetic variation and population structure that may arise among populations.

Human activities, such as urbanization and the creation of farmlands due to the increased human population, have led to the fragmentation and destruction of many wild avian species' habitats (Scanes, 2018). However, the impact of such activities and the effects of climate change on the wild African harlequin quail population size and structure is yet to be examined. Such information would also prove helpful in its conservation status management.

1.2 Problem statement

Rural smallholder farmers have hunted the wild African harlequin quail for generations. Even though it has served as a healthy source of protein nutrition, the wild African harlequin quail is threatened by habitat destruction, incessant harvesting, the uncontrolled introduction of exotic breeds into the country, and climate change, among others (Wamuyu et al., 2017). Little to no attention has been directed toward understanding its conservation status and possible utilization of its genetic resources. Several genetic studies have been conducted on wild common quails and domestic Japanese quails using mitochondrial DNA and microsatellite markers (Barilani et al., 2005; Amaral et al., 2007; Chazara et al., 2010; Sanchez-Donoso et al., 2012; Puigcerver et al., 2014). Genetic information on the wild African harlequin quail that can be used to understand its diversity, demography history, and mechanisms underlying production traits is unavailable.

Previous studies have profiled genomic regions with functional loci associated with crucial traits such as meat and egg production in poultry (Boschiero et al., 2018; J. Wu et al., 2018; Guo et al., 2020; Rostamzadeh Mahdabi et al., 2021). However, these loci are under natural and artificial selection, which has left footprints in the genome as a result of altered allele frequencies, thereby affecting the genetic and phenotypic variation (Gouveia et al., 2014; Boschiero et al., 2018; Khalkhali-Evrigh et al., 2022; Seo et al., 2022). Furthermore, the positive selection of genes involved in molecular pathways associated with key production traits influences gene expression and the quantity levels of key metabolites in cells (Kosiol et al., 2008). Artificial selection in domestic Japanese quail species in other parts of the world has resulted in improved meat and egg traits at the expense of others, such as disease tolerance (Jeke et al., 2018). So far, the effects of natural and artificial selection on important traits have yet to be examined in quail populations of Kenya.

Developing countries rely heavily on traditional phenotype-based breeding methods due to insufficient molecular information that can be produced through multi-omics studies (Muchadeyi et al., 2020). Additionally, using a single molecular marker or approach to studying the diversity, demography history, and genetic mechanisms of meat and egg production in poultry may need to provide more information to facilitate the utilization of molecular breeding techniques.

1.3 Justification

Protein-energy undernutrition remains a persistent problem in Kenya and other developing countries (WHO, 2007; Sokhela et al., 2023). In these regions, plant sources of dietary protein, such as cereals, are more common when compared to animal sources. Moreover, animal-based protein sources generally contain higher amounts of protein per unit energy, which is better quality with adequate proportions of dietary essential amino acids (Schönfeldt & Gibson Hall, 2012). Hence, animal protein sources are crucial in the global food chain.

The global demand for animal protein is rapidly increasing, mainly due to population growth and increased wages from urbanization. Increased demand for poultry meat and eggs, in particular, has also been greatly influenced by consumer preference, where poultry products are preferred for their nutritional value relative to widely available beef (Bett et al., 2012). Following the consumer shift and market demand for indigenous poultry, genetic improvement of indigenous poultry to meet this demand is highly encouraged. Alternative and complementary sources, such as quails, have also been advocated to combat undernutrition and ensure food security (Jeke et al., 2018).

Genomic selection through modern genetic and statistical tools such as genome-wide association studies (GWAS) has improved production by large margins (Rubin et al., 2010). However, such techniques haven't been applicable in Kenya due to insufficient molecular information, such as identifying quantitative trait loci and molecular markers associated with production traits. Molecular markers discovered through genomic studies help select genetically superior individuals used as breeding parents (Hickey et al., 2017). Genome-wide sequence data allows for in-depth molecular studies that help shed light on the genetic mechanisms that govern production and phenotypic traits. Signatures of selection tests are crucial for examining and understanding genomes as they can provide an accurate and deep understanding of the processes that affect population diversity and trait selection in the genome (Saravanan et al., 2020). Additionally, transcriptome analysis also allows for a proper understanding of the functional elements of the genome and gene expression patterns (Beauclercq et al., 2017). Using different genetic markers and methodologies together will further advance this understanding of complex traits involved in animal production.

The wild African harlequin quail is under threat, and studying its molecular information can help conserve and appropriately utilize its genetic resources. Population genetic studies can monitor changes in the effective population size of populations. These studies could reveal the effects of incessant harvesting, habitat destruction, the uncontrolled introduction of exotic breeds into the country, and climate change on the population size

of wild African harlequin quails in the study areas. Moreover, they can also assess the genetic integrity of the wild African harlequin quails following the uncontrolled introduction of several exotic Japanese quail breeds into the country. Comparative genetic analyses are equally crucial as they can be used to study the evolutionary relationships between wild and domesticated quail populations, possibly revealing similarities and differences observed when examining the effects of natural and artificial selection.

Generating and introducing such molecular information into the breeding programs will encourage modern molecular-based breeding technologies, complementing already existing and widely used traditional methods.

1.4 Objectives

1.4.1 General objective

To assess the genetic background and signatures of selection in wild African harlequin and domestic Japanese quails using genomic and transcriptomic approaches

1.4.2 Specific objectives

1. To characterize the genetic background of wild African harlequin quails using mtDNA marker.
2. To investigate the genetic diversity and genomic regions associated with growth, meat, and egg production of wild African harlequin quail using GBS data.
3. To analyze signatures of selection for growth, production, and reproduction in wild African harlequin and domestic Japanese quails.
4. To investigate the transcriptomic landscape of growth and reproduction traits of the domestic Japanese quail.

1.5 Null hypotheses

1. MtDNA analysis does not reveal the genetic background of wild African harlequin quails.
2. There is no genetic diversity and selection signatures present in the genomic regions associated with growth, meat, and egg production present in wild African harlequin quails based on genotyping by sequencing data.

3. There are no differences in genes under selection in wild African harlequin and domestic Japanese quails.
4. Studying the differential expression of genes in organs associated with the domestic Japanese quail's growth and reproduction traits is impossible.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter describes the reviews and concepts that guided the study to achieve its objectives. A conceptual framework was also developed to indicate the variables involved in the study. Previous research concerning the molecular characterization of poultry and the approaches used in domesticated poultry and avian species, in general, are also discussed here.

2.2 Theoretical review

This sub-section highlights the dependency of poultry production on genetic factors. It also shows the importance of molecular characterization in poultry and other avian species consumed for their protein. Theories on how molecular characterization can help us comprehend their full potential, allowing for proper utilization of their genetic resources and biodiversity conservation, are also discussed.

2.2.1 Poultry meat and egg production

Poultry meat and egg production are commonly defined by traits such as growth rate, body size, body weight, body composition, broodiness, ovulation rate, feed conversion, egg number, egg quality, health, and sexual maturity, among others (King'ori, 2011; Nyalala et al., 2021). These production traits have been shown to depend on genetic factors. Yet, poultry breeding programs, especially in developing countries of Africa and Asia, still rely on phenotype-based approaches where individuals with superior traits are selected as parents for future generations. However, these phenotype-based methods are continuously being improved over time through genetic and statistical procedures that have allowed for better evaluation of crucial traits and breeding values (Jorge et al., 2007). For instance, artificial selection in combination with quantitative genetics-based methods has led to improved poultry productivity and the establishment of specialized layer and broiler breeds in chicken (Rubin et al., 2010). Commercial breeding companies have improved

growth rate by 85 to 90% and nutrition by 10 to 15% in broilers over 45 years through genetic selection (Havenstein et al., 2003). Although specialized layer and broiler chicken breeds are highly encouraged for better performance, dual-purpose chicken breeds and poultry are still more common in Africa and the developing world.

Genome-wide association studies (GWAS), signatures of selection detection, and differential gene expression analyses are essential in the identification and examination of genomic regions and genetic variants associated with phenotypes and for a better understanding of population history and genetic mechanisms affecting phenotypic differentiation in humans, livestock, and wild animals (González-Prendes et al., 2017; Almeida et al., 2019; Jiang et al., 2019). In developed countries, modern genomic and bioinformatics approaches involving GWAS and selection signatures detection are currently being used to complement traditional selection methods to ensure a more accurate selection of superior individuals for breeding (Kadarmideen, 2014).

Modern breeding approaches and technologies led to the development of specialized broiler and layer breeds for meat and egg production, respectively (Cheng, 2010). Developing specialized broiler and layer chicken breeds requires time, expertise, and financial strength; therefore, only large breeding companies can produce them. For instance, the TT broiler and CC layer lines were selected over six and eight generations, resulting in improved meat and egg production traits, respectively (Pértille et al., 2017). However, these breeds require intensive systems and are expensive for rural smallholder farmers to keep. Village poultry, in contrast, are primarily dual-purpose breeds kept for meat and eggs (Dana et al., 2010). Due to the antagonistic properties of meat and egg production traits in poultry, dual-purpose indigenous chicken breeds do not produce high quantities of meat and egg compared to commercial specialized broiler and layer breeds (Silva et al., 2013). Commercial layer chicken breeds can produce around 300 eggs annually, whereas dual-purpose indigenous breeds produce about 100 eggs (Grobbelaar et al., 2010). Additionally, broilers under the intensive system can reach 2.0 kg live weight at five weeks of age, whereas indigenous-breed male birds often weigh around 1.0 kg in

more than five weeks (Sørensen, 2010). This has also been witnessed in other poultry species, such as quails and guinea fowls, where domestic breeds subjected to modern breeding technologies are highly productive compared to wild individuals.

2.2.2 Characterization of poultry using molecular markers

Meat and egg production traits are greatly influenced by the genetic architecture of poultry birds (Muchadeyi & Dzomba, 2017). Genomic technologies that currently exist have allowed for genetic studies that have provided information on the genetic make-up of individuals, species and comparisons between species. In Kenya, the indigenous chicken (*Gallus gallus domestiscus*) is the most widely studied poultry species. However, most of these studies have focused on its origin and genetic diversity (Mwacharo et al., 2007, 2011; Ngeno et al., 2015; Kennedy, 2016; Ogada, 2017; Okoth et al., 2017). On the contrary, quails are among the least studied poultry species and are classified under the emerging livestock (Wamuyu et al., 2017). Currently, there is little information about the origin, diversity, dispersal patterns, domestication events, and genetic divergence time of quails in Kenya. This also includes information about the genetic mechanisms behind meat and egg production traits which are yet to be thoroughly studied. However, it is important to note that chicken and quail genomes are highly similar in terms of contiguity, assembly statistics, gene content, and chromosomal organization (Kayang et al., 2006; Morris et al., 2020); hence the genetic mechanisms behind meat and egg production traits are most likely shared between the two species.

Biodiversity studies on poultry have affirmed the existence of different genetic variants among individuals, populations, breeds, and species. However, there is still insufficient information on the genetic variation, structure, and composition of poultry and game birds found in developing countries, even among widely-studied species such as chicken (Muchadeyi & Dzomba, 2017). The genetic diversity in poultry is crucial as it provides room for further genetic improvements that may be required to meet market and consumer preference demands (Padhi, 2016). Natural selection facilitated by factors such as nutrition, climate, and disease, has led to genetic variation and the development of poultry

phenotypes that vary in productivity level, disease resistance, heat tolerance, and feed intake capacity (Muchadeyi & Dzomba, 2017). However, these genotypes and phenotypes that provide adaptive capabilities to village poultry to survive under such harsh conditions haven't been well-examined and understood. Additionally, Muchadeyi & Dzomba (2017) also noted that the absence of intensive management of village poultry, like indigenous chickens, has led to undocumented breeding and artificial selection practices that target traits of economic importance, resulting in sometimes good but non-reproducible outcomes.

Genetic characterization studies previously done on indigenous poultry of Africa were mainly based on mtDNA and microsatellite markers and are yet to fully explore the genomic architecture and adaptation traits of local indigenous poultry (Mwacharo et al., 2011; Muchadeyi & Dzomba, 2017). Recently, genotyping arrays, reduced-representation sequencing methods (Genotyping-by-sequencing (GBS) and restriction site associated DNA (RAD) sequencing), and whole-genome resequencing have produced genome-wide single nucleotide polymorphism (SNP) data that has been used for characterization and advanced genomic studies on poultry (Guo et al., 2016; Wragg et al., 2012). There are about 3.3 million SNPs that have been identified in the chicken genome, which have proved helpful in the identification of quantitative trait loci (QTL) associated with key traits such as growth, body size, body composition, egg production, disease resistance, and heat tolerance, among others (Pym, 2013).

2.2.3 Genetic characterization of the harlequin quail

The harlequin quail (*Coturnix delegorguei*) is among the least-studied quail species, as many studies focus on the more familiar Japanese and common quail species. Currently, no reference whole genome sequence is available for the harlequin quail. Threatened species and non-model organisms like the harlequin quail often lack reference genomes, which are preferred for conservation genomic research (Galla et al., 2019).

In addition, inadequate funding has hampered efforts aimed at sequencing such genomes posing a significant challenge to the effective global biodiversity conservation (Waldron

et al., 2013). However, the recently sequenced whole genome of the Japanese quail allows for studying the Japanese quail and other closely-related quail species, such as the harlequin quail, which also belongs to the *Coturnix* genus. Studies have shown that in the absence of a conspecific (species-specific) reference genome to map genomic sequence reads to, the availability of a high-quality reference genome of related species, such as the Japanese quail, can provide highly correlated diversity estimates (Galla et al., 2019). Closely related species, such as chicken, have also been used to study quails. For example, chicken *VIPR1* and *PRL* gene primers were used to amplify quail *VIPR1* and *PRL* gene fragments successfully by Macharia (2018) to study allele variation between quail populations of Western Kenya. However, it is essential to note that these alternatives still won't be able to showcase the full resolution of SNPs present in the species, especially in line with missing genotypes.

2.3 Conceptual framework

A conceptual framework was generated to illustrate the potential factors considered in the molecular characterization of the wild African harlequin quail (Figure 2.1). This was done to examine the association between the genetic architecture of the wild African harlequin quail and other factors like migration, population size, and adaptation. The independent variables included wild African harlequin quail migration, population size, and adaptation. Unlawful harvesting of wild African harlequin quails and the introduction of exotic domestic quail breeds were considered mediator variables. This is because the demand for quail protein has led to an increase in the harvesting of wild African harlequin quails and an introduction of exotic quail species into the country. Climate change, which has been shown to affect the migration of wild birds (Carey, 2009; Clairbaux et al., 2019), and habitat fragmentation and destruction were considered as moderator variables.

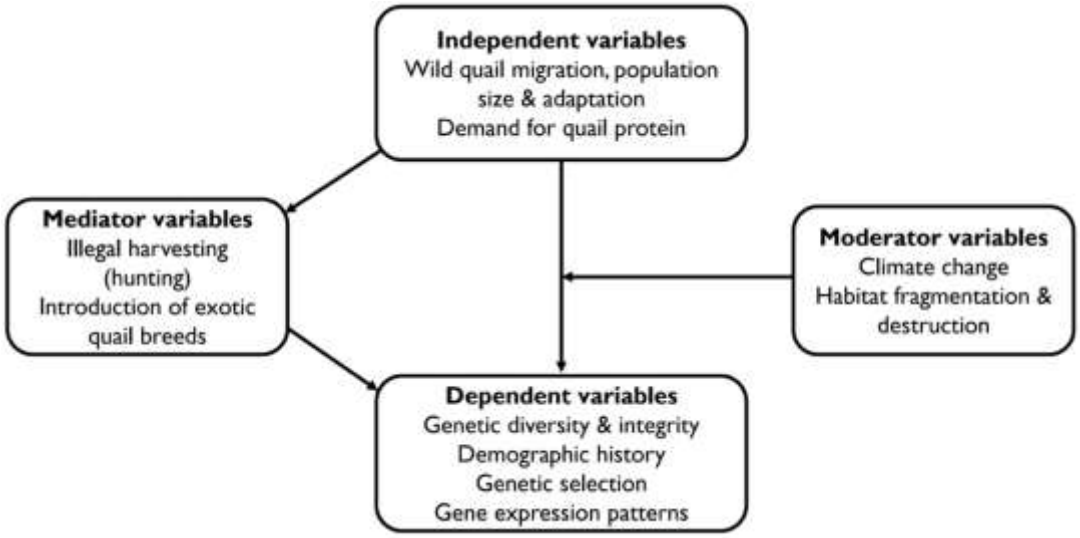

Figure 2.1: A conceptual framework of the molecular characterization of wild African harlequin.

The components of the research study in terms of molecular markers used and the types of analyses to be conducted with each marker in the molecular characterization of the quails were illustrated in a chart (Figure 2.2). However, transcriptomic analysis, which relies on RNA-Seq count data, was not included in the chart but discussed further in the empirical review.

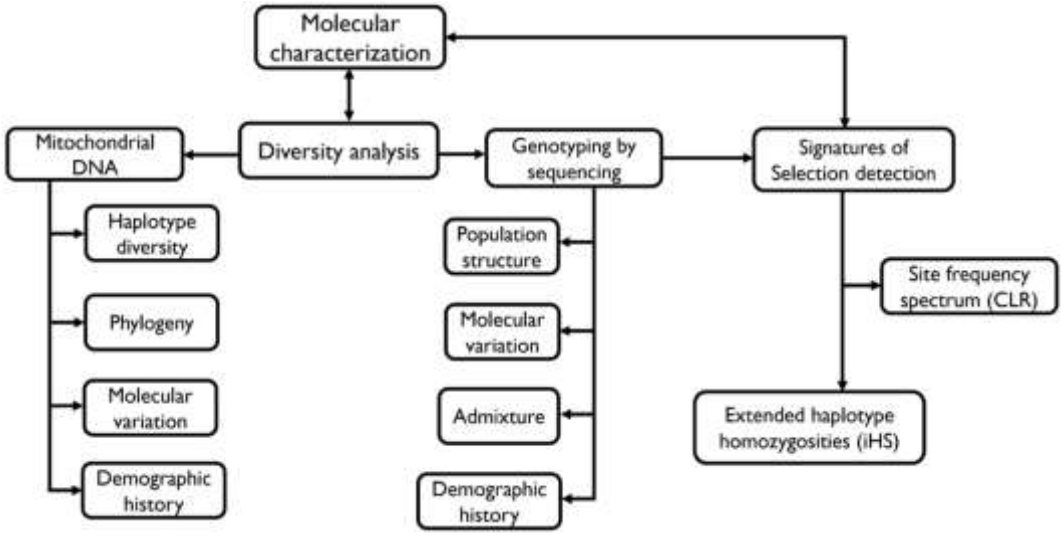

Figure 2.2: Chart showing the components of the research study

2.4 Empirical review

The molecular characterization of domesticated poultry and wild avian species has been conducted using the mitochondrial DNA, genotyping-by-sequencing, and RNA-Seq approaches. This empirical review discusses these molecular characterization approaches in-depth and highlights research that has used them for genetic diversity, demographic history, signatures of selection detection, and transcriptome analyses. This review section also discusses studies showing how genome and transcript molecular data can be used to identify crucial biological processes associated with production traits.

2.4.1 Molecular characterization approaches

Over the years, several methods and technologies have been used to study poultry genomics. Molecular markers such as Restriction fragment length polymorphisms (RFLPs), Amplified fragment length polymorphisms (AFLPs), mitochondrial DNA (mtDNA), Randomly amplified polymorphic DNAs (RAPDs), Microsatellites/simple sequence repeats (SSRs), Expressed sequence tag (EST) based marker system and Single nucleotide polymorphisms (SNPs) have been used to study different poultry and wild avian species depending on the objectives of the research (Al-samarai & Al-kazaz, 2015). For an extended period, microsatellites have been continually used mainly for genetic characterization studies as the Joint International Society for Animal Genetics (ISAG) and Food and Agriculture Organization of the United Nations (FAO) working group approved them (Andres & Kapkowska, 2011). However, with the development of sequencing technology, more studies are adopting new methods that have enabled the production of highly informative sequence data results. Next-generation sequencing technology has allowed for sequencing applications such as methylation sequencing, transcriptome sequencing, genotyping by sequencing, and whole-genome resequencing of individuals within a short period (Pértille et al., 2016). Recently, genome-wide SNP data has been highly used in studying production animals like chickens compared to other molecular markers due to their high genomic density and abundance (Keats & Sherman, 2013).

2.4.2 Mitochondrial DNA

The Mitochondrial DNA (mtDNA) is a molecule that spans 16,775 base pairs in size, on average (Figure 2.1), in several animal species (Desjardins & Morais, 1990). It is a valuable marker with a maternal mode of inheritance that is commonly used to examine genetic diversity, population structure, population dynamics, and demographic history of a study population (Amorim et al., 2019; Galtier et al., 2009; Harrison, 1989; Richards et al., 1998; Torroni et al., 1994). Animal mtDNA evolves faster than a nuclear genetic marker and allows for faster examination of the genetic variation of populations (Ladoukakis & Zouros, 2017). Mitochondrial DNA haplotypes are commonly used to group individuals among populations based on shared ancestry and origin (Mitchell et al., 2014).

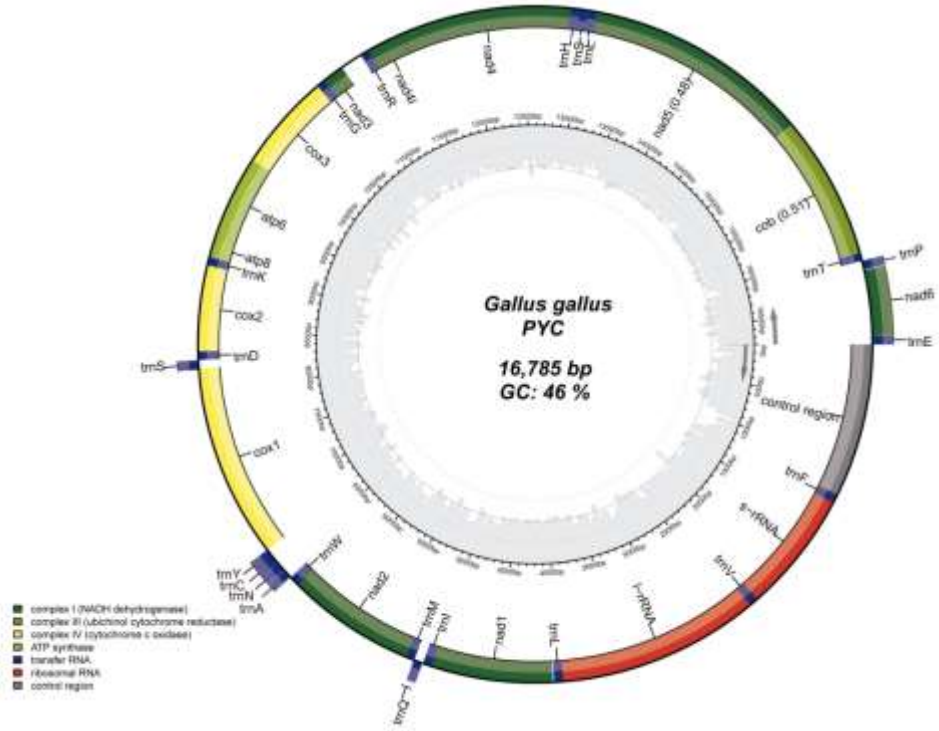

Figure 2.3: Avian mitochondrial DNA molecule. The displacement loop (D-loop) is located within the control region (grey).

The genealogical origin of the domestic chicken was determined using the mtDNA D-loop region, a highly variable region within the avian mitochondrial genome (Fumihito et al., 1994). This was after the red junglefowl was first hypothesized to be the direct ancestor of the domestic chicken (Cramer, 1896). The introduction and dispersion of domestic chicken in Kenya, Uganda, Ethiopia, and Sudan were also studied using the mtDNA D-loop (Mwacharo et al., 2011). The mtDNA D-loop features warrant its suitability for phylogeny studies using PCR-based methods, making it popular and more affordable to researchers who do not have resources for whole genome sequencing, especially in the developing world.

2.4.3 Genotyping by Sequencing (GBS)

Recently, SNPs are increasingly being used in population genetic studies. This type of genetic variant is obtained from direct DNA sequencing of whole genomes or specific regions. Advances in technology have contributed to a substantial reduction in sequencing costs (Ng & Kirkness, 2010). However, whole-genome sequencing costs are still high for many researchers in the developing world who also lack or need more computational resources for whole-genome sequence data analysis. This has encouraged the adoption of cost-intensive and economic approaches such as genotyping arrays and reduced-representation sequencing methods like genotyping-by-sequencing (GBS). The GBS method can retrieve sufficient genome-wide SNP information for population genetic studies at a more affordable cost. In addition, the reduced sequencing costs allow for the incorporation of more samples for sequencing.

The GBS approach involves the use of restriction enzymes to digest complex genomes, followed by PCR amplification of the fragments, reduced representation library construction, and sequencing (Elshire et al., 2011; He et al., 2014). Apart from being cost-effective, GBS is a simple, rapid, and highly reproducible approach that can identify SNPs, insertions, deletions, and microsatellites even in non-model organisms with no prior genome information (Elshire et al., 2011). In addition, GBS is a high-throughput genotyping technology that can efficiently identify a large number of SNPs accurately

hence applicable in livestock studies (Gurgul et al., 2019). Aside from GBS, there are other protocols based on restriction site-associated genomic DNA sequencing such as restriction-associated DNA sequencing (RAD-Seq), diversity arrays technology (DArT), complexity reduction of polymorphic sequences (CRoPS), which are equally effective (Van Orsouw et al., 2007; Baird et al., 2008; Sansaloni et al., 2011). However, RAD-Seq and GBS are the most commonly applied.

Genotyping by sequencing approach has been used to study a variety of livestock and poultry species such as chicken, geese, camels, cattle, sheep, and pigs, among others. For example, Pértille et al. (2017) used the genotyping by sequencing approach to perform genome-wide association studies (GWAS) in chickens to identify regions on the chicken genome associated with performance traits. Yang et al. (2021) identified a major QTL and four minor QTLs for growth traits in commercial chicken using GBS. Grzegorczyk et al. (2021) studied the genetic variation and diversity of Polish geese using GBS and validated key SNPs using Sanger sequencing. GWAS analysis to identify candidate genes associated with pigmentation in camels was studied using SNPs acquired from GBS data (Bitaraf Sani et al., 2022). In summary, the GBS approach has proved useful, especially in studying non-model species with no defined custom SNP-array chips.

2.4.4 Signatures of selection detection in poultry

The contribution of selection to molecular evolution in species is significant. Over the years, studying quantitative traits and the actual genes behind these traits have proved difficult since phenotypic variance is controlled by multiple loci (Consortium, 2004). However, signatures of selection detection using genome-wide SNP data has helped locate genomic regions with relevant genes associated with important traits such as meat and egg production (Liu et al., 2016). Genomic regions affected by selection pressures tend to develop signatures such as high allele frequencies, substantial linkage disequilibrium, increased homozygous genotypes, and long haplotypes (Nielsen, 2005; Qanbari & Simianer, 2014).

QTLs provide a basic overall understanding of the genetic architecture of an individual (Abasht et al., 2006). There are definite genomic regions that contain QTLs associated with poultry meat and egg traits such as growth, breast meat yield, tarsal length and width, abdominal fat, meat color, feed intake, feed efficiency, age at first egg, egg number, albumin, and eggshell properties, that were noted by Abasht et al. (2006). Other important traits that affect and are associated with production include adaptation to hot climates, harsh local environments, and immune response, among others. Nonetheless, pedigree data at different development stages is highly required to properly understand the QTLs behind these traits. Lack of pedigree data from village poultry raised under the scavenging system and game birds hinders proper breeding practices. Thus, signatures of selection methods, such as those that are based on linkage disequilibrium determination from SNP data, have enabled the calculation of genetic parameters without data from various poultry development stages (Khanyile et al., 2015).

Meat and egg production QTLs have been identified for different traits of interest. For instance, QTLs associated with breast meat yield in chicken were found on chromosomes 1, 3, 4, 6, 10, 20, and Z whereas those for thigh weight were found in chromosomes 1, 5, 13, 20, 26, and Z (Abasht et al., 2006). The *TBC1D1* locus, which is involved in muscle tissue development through insulin signaling, was detected in a selective sweep involving a study on broiler and layer comparative genetics which helped explain the differences in growth between the two breeds (Ambo et al., 2009). To better understand these important QTLs, transcriptomic data is usually integrated by detecting differentially expressed genes through RNA sequencing.

2.4.4.1 Types of selection

Selection determines the genetic variation in a population by selecting favorable phenotypes and removing unfavorable ones while ignoring the neutral ones. The varying effects of selection on a phenotypic trait have been used to characterize and describe the different types of selection that have been observed in a population. There are several types of selection as shown in Figure 2.4.

i. **Directional selection** – This refers to the directional shift in favor of a particular phenotype at one end of the spectrum of existing genetic variation. This results in the continuous shifting in one direction of the population's genetic variance toward the more favorable trait.

   (a) ***Positive directional selection*** – This refers to the increase in the frequency of new mutations or rare variants that improve an individual's fitness. It is also known as adaptive selection and is commonly studied by population geneticists.

   (b) ***Negative directional selection*** – It's the most prevalent form of natural selection, also known as purifying selection. It involves the removal of deleterious alleles that decrease fitness in individuals.

ii. **Balancing selection** – This refers to the maintenance of multiple alleles in a population at the selected site. This also maintains the genetic variation present in a population as no single phenotype is favored.

iii. **Stabilizing selection** – This occurs when selection favors a particular phenotype resulting in it being stabilized in a population. This results in a decrease in the population's genetic variance in favor of that particular trait. Phenotypes near the middle of the range of phenotypic variation are favored.

iv. **Disruptive selection** – This refers to selection that occurs when extreme values of a trait (on either end of the spectrum) are favored over intermediate ones. This type of selection often results in increased genetic variance as the population becomes more diverse hence leading to speciation.

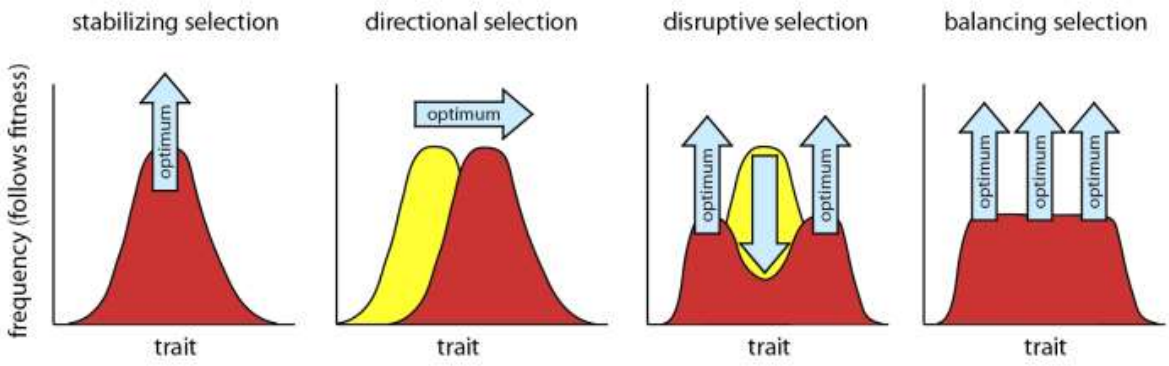

Figure 2.4: Types of selection and its effect on phenotypic traits.

2.4.4.2 Methods for signatures of selection detection

Signatures of selection tests are crucial for understanding genomes as they can provide an accurate and deep understanding of the processes that affect population diversity and trait selection (Oleksyk et al., 2010; Ma et al., 2015). There are several methods for detecting loci under selection (selective sweep), commonly referred to as outliers, with population genetic data. The key methods comprise methods that are based on:

(i) population differentiation patterns
(ii) linkage disequilibrium (LD) and haplotype structure
(iii) site frequency spectrum (SFS).

Some of the most commonly used methods or tests include:

i. **Composite likelihood ratios (CLR)** – This method adopts the use of site frequency spectrum (SFS) patterns of SNP to detect selective sweeps (Williamson et al., 2007). This is achieved through the evaluation of the skewness of the frequency spectrum across SNPs under selection versus neutrality within a population while taking into consideration recombination rate information and demographic events (Nielsen et al., 2005).
ii. **Integrated haplotype-homozygosity score (iHS)** – This is a haplotype-based method that examines the homozygosity of extended haplotypes that are generated by a selective sweep (Voight et al., 2006). In this method, differential levels of

linkage disequilibrium surrounding positively selected allele are compared to ancestral allele at the same position within a population (Randhawa et al., 2016).

iii. **Cross-population extended haplotype homozygosity (XP-EHH)** – A haplotype-based method that compares the amount of extended haplotype homozygosity among populations, observed versus reference population (Ma et al., 2015). Extended haplotype homozygosity (EHH) detects selection signatures by comparing positively selected regions carrying frequent haplotypes with unusually high long-range LD patterns within a population. However, the XP-EHH test compares the integrated EHH profiles between two populations at the same SNP (Eydivandi et al., 2021).

iv. **Fixation index (F_{ST})** – Wright's fixation index (F_{ST}) is a widely used method for evaluating variation among populations that has the ability to detect loci undergoing selection (Weir & Cockerham, 1984). The F_{ST} is a highly used single marker-based estimation approach compared to other haplotype-based (patterns at multiple SNPs) such as iHS and XP-EHH.

v. **hapFLK** – The extended Lewontin–Krakauer F_{ST} outlier test is a haplotype-based population differentiation method for the detection of positive selection from multiple population data. Haplotype frequencies are computed based on a multipoint linkage disequilibrium model which is then used to measure differentiation between populations (Bonhomme et al., 2010). Unlike F_{ST}, FLK method accounts for sample size, admixture and hierarchal structures between populations (Fariello et al., 2013).

vi. **Z-transformed pooled heterozygosity values (ZHp)** – This method explores the distribution of genetic diversity across the genome using the pooled heterozygosity indexes (Rubin et al., 2010; Aramburu et al., 2020). This statistic estimates local heterozygosity depression in chromosomal regions compared to the whole genome thereby noting loci that are under selection.

vii. **Integrated extended haplotype homozygosity across population (Rsb)** – This involves signatures of selection using integrated extended haplotype

homozygosity at the SNP site (iES). The iES approach detects recent positive selection by estimating extended haplotype homozygosity decay levels at each SNP site in genotypic data within a population (Tang et al., 2007). Rsb method detects recent selection on completely or nearly fixed selective sweeps by comparing the single locus iES (Randhawa et al., 2016).

viii. **Runs of homozygosity (ROH)** – Runs of homozygosity are continuous homozygous segments of the DNA sequence (identical haplotypes) that are inherited from each parent (Peripolli et al., 2017). Studying ROH patterns and how they are shaped by selection (generation of ROH islands) can be used to detect recent positive selection in genomes.

2.4.4.3 Effects of selection on wild and domesticated quail species

For generations, rural smallholder farmers in Kenya have hunted quails for consumption as a complementary source of poultry protein (Urban et al., 1986). Other than continuous and uncontrolled harvesting, breeding attempts by rural farmers, climate change, habitat destruction by humans, migration, population bottlenecks, and inbreeding are among the main challenges facing the wild African harlequin quail in Kenya (Wamuyu et al., 2017). These factors interfere with the normal evolutionary processes experienced by wild African harlequin quails and might influence natural, artificial, or sexual selection (Allendorf & Hard, 2009).

In contrast, commercial quail breeds such as the domestic Japanese quail have become the most commonly consumed quail species globally since their domestication in the late 19th century and early 20th century (Nishibori et al., 2001). The domestic Japanese quails have undergone intense selection pressure since their domestication through modern breeding methods that are aimed at increased egg and meat production (Mills et al., 1997; Lukanov & Pavlova, 2020). The domestication and artificial selection of the Japanese quail has brought about genetic variation changes, altering its phenotype and resulting to an increase in its size and number of eggs laid (Lukanov & Pavlova, 2020).

The main differences between wild and domestic quail species lie in the morphological, behavioural and productivity characteristics (Chang et al., 2009). The wild African harlequin quail meat was found to contain the highest protein content (25.50%), the least saturated fatty acids (24.86%), and the highest polyunsaturated fatty acids (74.85%) compared to the domestic Japanese quail, helmeted guinea fowl, indigenous and commercial chickens (Chepkemoi et al., 2017). Wild African harlequin quail meat and eggs were also found to contain higher amounts of minerals such as zinc, potassium, calcium, and iron in the same study. Therefore, understanding the genetic architecture of the wild African harlequin quail could help elucidate if there is a relationship between the effects of selection and some of the observed traits.

2.4.5 Transcriptomic assessment of poultry production traits

The utilization of transcriptome data from specific tissues at different stages of development has ushered in a new age of functional analyses that have provided a better understanding of molecular pathways and gene expression (Beauclercq et al., 2017). Transcriptome data provides insight into differential gene expression and gene pathways behind key traits. This allows for exploration into molecular mechanisms underlying a phenotype.

Transcriptomic studies have been done on several poultry species such as chicken, duck, quail, turkey and pigeon (Bao et al., 2020; Brady et al., 2021; Cai et al., 2019; X. Huang, Zhou, et al., 2022; Yin et al., 2022) and several genes associated with key traits such adaptation, production, immunity, response to environment, among others, have been identified. These studies utilized different tissues depending on the traits of interest. For instance, Dhanasekaran et al (2014) highlighted some of the genes associated with egg production that showed increased expression in ovarian follicles, uterus and shell glands. These genes included Osteopontin, acetyl-CoA dehydrogenase long chain, ovocalyxin 32, ovocalyxin 21, and thioredoxin, among others. Whole transcriptome analyses were carried out by Désert et al (2008) during feeding and fasting periods, remarkably, the findings were able to show how biological processes such as energy metabolism and cholesterol

synthesis were affected. This information could help breeders understand the effects of feed and nutrient intake on the genetic architecture of domestic quails compared to wild species. Breast and thigh muscles are determined by body composition, size, and weight parameters, which are often used to evaluate meat production. Total RNA extraction from breast muscle and leg muscle tissues revealed high expression of FOXO3, a member of the Forkhead box class O (van der Vos & Coffer, 2011). FOXO3 was found to contribute towards cell-cycle control, cell growth, and differentiation in the striated muscle (Sanchez et al., 2014). Transcription factors CEBPB, FBXO32, and MYOD1 were also found to interact with FOXO3, which are believed to be linked to the growth of chicken and other poultry (Chen et al., 2015). Gene expression patterns of chicken, mainly commercial breeds, have been widely studied. However, little information is available on the gene expression patterns of other poultry species such as quails and game birds.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter outlines the utilized materials and methods that were followed in the study. The information provided here includes the study design, study area, study clearance, and permit information, sample size determination, sample collection procedure, and data analysis approach.

3.2 General methodology

3.2.1 Study areas

The study was carried out in Siaya County, which lies in the Lake Victoria basin. Three populations were selected: Siaya North, Siaya Central, and Siaya South (Figure 3.1). The Lake Victoria basin lies at an altitude of 1,134 meters above sea level. It has an annual rainfall of 500-1000mm with temperatures ranges 17.1°C–29.4°C. Lake Victoria basin experiences a climate that is characteristic of zone III of the agro-climatic zones of Kenya (Ogada et al., 2016). Kajiado County lies at an altitude of 1,732 meters above sea level and has an annual rainfall of 300-800 mm. It is characterized by a semi-arid and arid climate that is characteristic of zone IV of the agro-climatic zones of Kenya (Bobadoye et al., 2014). The northern part of Kajiado County is a peri-urban area adjacent to Nairobi, the capital city of Kenya.

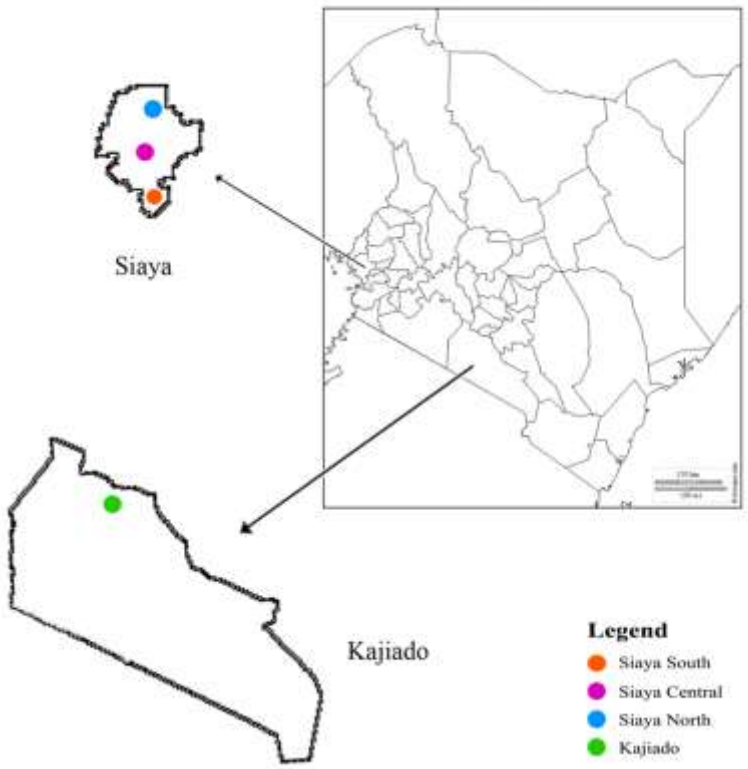

Figure 3.1: Map of Kenya showing the sampled areas.

3.2.2 Study clearance and permit of compliance

This study received ethical clearance from the Kenya Wildlife Service under permit number KWS/BRM/5001 to sample wild African harlequin quails and "permission to sample" from the Director of Veterinary Services, Ministry of Agriculture, Livestock and Fisheries in Kenya under permit number RES/POL/VOL.XXVII/162 to sample domestic Japanese quails. Additionally, the Institutional Animal Care and Use Committee (IACUC) of Jomo Kenyatta University of Agriculture and Technology approved the poultry sample collection procedures used. Permission from the local County governments and farmers was also sought prior to the sample collection.

3.2.3 Study design

A purposive and cross-sectional approach was used for sample collection. Siaya County was selected due to the significant seasonal presence of wild African harlequin quails in

the region, compared to others, and their human-wildlife interaction with rural smallholder farmers. Harvesting of the wild African harlequin quails in Siaya County by rural smallholder farmers is more common when compared to other Counties in the region and has been ongoing for generations. Interviews were conducted to determine if there were any observed changes in the annual presence and seasonal migration patterns of wild African harlequin quails in the region. In contrast, Kajiado County is a peri-urban area and was selected due to the presence of several commercial poultry farms. Commercial poultry farmers in the region tend to rear various poultry species, including purebred domestic Japanese quails. Domestic quail species are more common in the peri-urban areas of Kajiado and Kiambu when compared to other Counties.

Samples for population genomics studies were collected as per the recommendations of Hale et al. (2012) which suggests 24-32 sampled individuals per population for adequate representation of all the allele frequencies present in that population. Households were selected based on willingness to participate in the study and the availability of captured wild quails. Sampling was conducted in households that were 0.5 km apart to reduce the chances of sampling related individuals that were migrating together. For transcriptome analysis, six domestic Japanese quail samples (3 biological replicates each) belonging to two experimental groups (heavy-weight line and low-weight lines) were selected based on a non-experimental research design. Therefore, no control biological replicates were required for differential gene expression comparisons between the two experimental groups.

3.2.4 Sample collection

3.2.4.1 Sample collection for DNA sequence analysis

Sample collection for DNA extraction was conducted to achieve objectives one, two, and three. Wild African harlequin quails were captured from the wild by farmers using traditional methods described by Wamuyu et al. (2017). A man-made thicket with traps was prepared using local materials. Long poles containing bait quails in woven baskets were erected at the edge of the man-made thicket (Figure 3.2). Female wild African

harlequin quails were mainly used as bait quails to attract other quails through their calls. The captured wild African harlequin quails in the man-made thicket were placed in woven baskets.

Figure 3.2: Wild African harlequin quail capture using traditional methods.

Whole blood samples were drawn from the wing vein of 78 wild African harlequin (Siaya) and 22 domestic Japanese (Kajiado) quail individuals (Table 2.1). This comprised an equal number of male and female individuals for every visited farm. The blood was collected in 2ml cryovials containing 70% alcohol and preserved in liquid nitrogen (-196ºC) during the sample collection period. After sampling, the collected blood samples were then stored in a -80°C freezer at the Jomo Kenyatta University of Agriculture and Technology (JKUAT).

Table 2.1: Number of quails sampled per study population

County	Population	Number of individuals
Siaya	Siaya North	24
Siaya	Siaya Central	28
Siaya	Siaya South	26
Kajiado	Kajiado	22
Total		**100**

3.2.4.2 Sample collection for transcriptome analysis

To undertake the fourth objective of this study, testis tissue samples were harvested from six domestic Japanese quails (three heavy-weight line (> 250g) and three low-weight line (< 200g) individuals each). Domestic Japanese quail meat and egg lines have been bred for decades, under controlled conditions, towards improved meat and egg production. Tissue samples were collected in 2ml cryovials containing 1ml RNA*later*™ Stabilization Solution (ThermoFisher Scientific) and immediately preserved in liquid nitrogen (-196ºC). The collected tissue samples were later stored in a -80°C freezer at the Jomo Kenyatta University of Agriculture and Technology (JKUAT).

3.3 DNA extraction and sequencing

Genomic DNA extraction was required for mitochondrial DNA sequencing for objective one and genotyping by sequencing for objectives two and three. The extracted DNA was divided into two portions; one was used for mtDNA amplification reactions for objective one, and the second was sent to the sequencing company for genotyping by sequencing analysis for objectives two and three.

3.3.1 Genomic DNA extraction and quantification

Genomic DNA was extracted from 100 whole blood samples using the phenol-chloroform method (Sambrook & Russell, 2006). 400µl STE (30mM Tris-HCl, 200mM EDTA, 50mM NaCl pH 8.0), 50 µl SDS 10% and 20 µl proteinase K were added. Incubation was then done at 60°C for 6 hours. 470 µl phenol was added followed by 8 hours of rotation.

Centrifugation was done at 9000 rpm for 10 min and the supernatant was transferred to another microtube. 235µl phenol and 235µl chloroform were then added and the solution rotated for 8 hours. Centrifugation was done at 9000rpm for 10 min and the supernatant was transferred to another microtube. 470 µl chloroform was added and rotation was done as above. 1000 µl isopropyl alcohol was added to the supernatant and then cooled at -20°C for 8 hours. Centrifugation was done at 10000 rpm for 10 min and the alcohol was poured out. 1000 µl 70% ethanol was added then centrifugation was done at 10000rpm for 10 min and the alcohol poured out. The cleaning step was repeated. Finally, 50 µl TE was added to the DNA and then stored at -20°C. The extracted DNA was run on a 0.6% gel to check the quality of the genomic DNA. The concentration of the DNA was also checked on Nanodrop 2000.

3.3.2 Mitochondrial DNA amplification and sequencing

To characterize the genetic background of wild African harlequin quails using the mtDNA marker, polymerase chain reaction (PCR) was done on 56 wild African harlequin and 17 Japanese quail samples using 24.85 µl reaction volumes which contained 17.5 µl PCR water, 2.8 µl buffer, 2 µl dNTP mixture, 0.6 µl forward primer, 0.6 µl reverse primer, 0.15 µl r Taq polymerase, and 1.2µl DNA template. The mtDNA D-loop region was amplified via primers AV1F2 (5‟-AGGACTACGGCTTGAAAAGC-3‟) and CR1b (5‟-CCATACACGCAAACCGTCTC-3‟) that were used to amplify 760bp chicken mtDNA D-loop fragment (Mwacharo et al., 2011), hence chicken samples were used as positive control. Amplification was carried out in an Eppendorf mastercycler Pro S thermocycler. The temperature profile was as follows; initial denaturation at 94°C for 5 min followed by denaturation temperature of 94°C for 30s, annealing temperature of 60°C for 1 min, 72°C for 1 min, and a final elongation temperature of 72°C for 7 min. Gel electrophoresis was done to check whether amplification was successful. The mtDNA amplicons were loaded onto the 2% agarose gel using 1X Tris-Boric Ethylenediaminetetraacetic acid (TBE) buffer (89Mm Tris, 89mM boric acid, 2Mm Na2 EDTA) in a voltage of 70V for 1 hour. The gels were stained with gel red and visualized under UV light (BTS-20 model, UVLtec Ltd., UK).

The PCR product was purified using the ExoSAP protocol as per the manufacturer's instructions (Affymetrix). The reaction mixture constituted 0.3 µl of shrimp alkaline phosphatase, 0.15 µl exonuclease 1, and 0.55µl distilled water. The thermocycler temperature profile for the purification process was 37°C for 40 min then 80°C for 20 min. Cycle sequencing was done using the BigDyeTM Terminator Cycle Sequence Kit 3.1 Ready Reaction Cycle Sequencing Kit (ABI Applied Biosystems). A reaction volume of 7µl (3µ kit, 3µ primer, and 1µ DNA template) was used. The thermocycler temperature profile was 96°C for 10s, 50°C for 5 s, and 60°C for 4 min. Capillary electrophoresis was done using the Applied Biosystems 3730xl DNA analyzer at Macrogen (South Korea).

3.3.3 GBS library preparation and sequencing

Wild African harlequin quails (n = 78) and domestic Japanese quails (n = 22) samples were subjected to genotyping-by-sequencing (GBS). A GBS pre-design experiment approach was used to evaluate the enzymes and sizes of restriction fragments using training data. The following criteria were applied:

(i) the number of tags must be suitable for the specific needs of the research project;

(ii) the enzymatic tags must be evenly distributed through the sequences to be examined;

(iii) repeated tags should be avoided.

These considerations improved the effectiveness of GBS for the dataset at hand. A strict length range was selected to maintain the sequence depth uniformity of different fragments (~50 bp).

We then constructed the GBS library in accordance using the pre-designed scheme. Genomic DNA was incubated at 37℃ with *Mse*I (New England Biolabs, NEB), T4 DNA ligase (NEB), ATP (NEB), and *Mse*I Y adapter N-containing barcode. The restriction-ligation reactions were heat-inactivated at 65℃ and then digested with the *Hae*III (GGCC) restriction enzyme at 37°C. The restriction ligation samples were purified with Agencourt AMPure XP (Beckman, United States). Polymerase chain reaction (PCR) was conducted using the purified samples, the Phusion Master Mix (NEB, United States)

universal and index primers, and i5 and i7 sequences. The PCR products were purified using Agencourt AMPure XP, pooled, and electrophoresed on a 2% agarose gel. A Gel Extraction Kit (Qiagen, Germany) was used to isolate 375-400 base-pair fragments (with indexes and adaptors). These fragments were purified using Agencourt AMPure XP, and the resulting products were diluted for sequencing. Then, paired-end sequencing of the selected tags was performed using the Illumina NovaSeq high-throughput sequencing platform at the Novogene Bioinformatics Technology Company, in China.

3.4 Total RNA extraction and sequencing

Total RNA required for objective four analysis was isolated from six domestic Japanese quail testes tissue samples using the Trizol-based method. The frozen tissue samples were first ground into powder using a mortar and pestle, after the addition of liquid nitrogen. 1 ml Trizol®LS reagent (Invitrogen, California, USA) was added per 50 – 100 mg of the tissue sample and shaken by hand for 20 seconds to mix. The homogenate was incubated for 5 min at room temperature. 200 µl Chloroform was added, shaken vigorously for 15 seconds, and incubated for 2-3 minutes. Centrifugation was performed at 12,000 RCF (Relative Centrifugal Force) for 15 minutes at 4ºC. The uppermost layer was then pipetted out into a fresh RNase-free tube. 500 µl isopropanol was added, mixed, and incubated for 10min at room temperature. Centrifugation was then done at 12,000 RCF for 10 minutes at 4ºC. The supernatant was discarded and the pellet was washed using 1ml of 75% ethanol. After centrifugation for 5 minutes at 7500 RCF, the pellet was air-dried and resuspended in RNase-free water. Total RNA was then quantified and its integrity was checked using Invitrogen Qubit 4 fluorometer. RNA samples that were of the highest quality were then shipped to BGI Genomics (China) for cDNA synthesis and sequencing using the Illumina platform.

3.5 Data analysis

Genetic diversity and demographic history analyses were conducted on the wild African harlequin and domestic Japanese quails using mtDNA and GBS data for the first and second objectives, respectively. This was done to compare whether short DNA fragment

data can be as informative as genome-wide SNP data. On the other hand, signatures of selection analyses were conducted on the wild African harlequin and domestic Japanese quails for objectives two and three respectively. Finally, differential gene expression analysis was conducted on the RNA sequencing data to achieve the fourth objective.

3.5.1 Mitochondrial DNA and GBS sequence data processing

Forward and reverse mtDNA sequences of each sample were edited using SeqMan Pro version 7.1.0 (DNASTAR, Inc.), and used to generate a consensus sequence. Sequence alignment was done using Clustal X version 2.1 (Thompson et al., 1997) and MUSCLE version 3.6 (Edgar, 2004).

GBS sequence processing was conducted as shown in Figure 3.3. Raw sequence reads were processed through a series of quality control procedures which involved removing reads with ≥10% unidentified nucleotides (N), with >50% bases with a Phred quality < 5, with >10 nucleotides alignment to the adapter, with >10% mismatches, and that contain the *Hae*III enzyme sequence. Burrows-Wheeler Aligner (BWA) version 0.7.17 was used to align the retained reads against the *Coturnix Japonica* 2.0 genome (Assembly accession number GCF_001577835.1) with the parameters 'mem -t 4 -k 32 -M' (Li & Durbin, 2009). Variant calling was performed using SAMtools version 1.11 *mpileup* command (Li et al., 2009) in conjunction with BCFtools version 1.11 *call* command (Li, 2011). Variant filtering was performed by restricting the dataset to biallelic SNPs found in at least 80% of samples, with a minimum depth of 2 reads, minimum Phred score of 30, and minimum minor allele frequency (MAF) of 0.05 using VCFtools version 0.1.13 (Danecek et al., 2011). For signatures of selection analysis, additional filtering was also done using PLINK version 1.9 (Purcell et al., 2007) to remove individuals with missing genotype data (--mind 0.1); variants with missing genotype data (--geno 0.05); minor allele frequency threshold (--maf 0.05) and Hardy–Weinberg exact test threshold (--hwe 1e6).

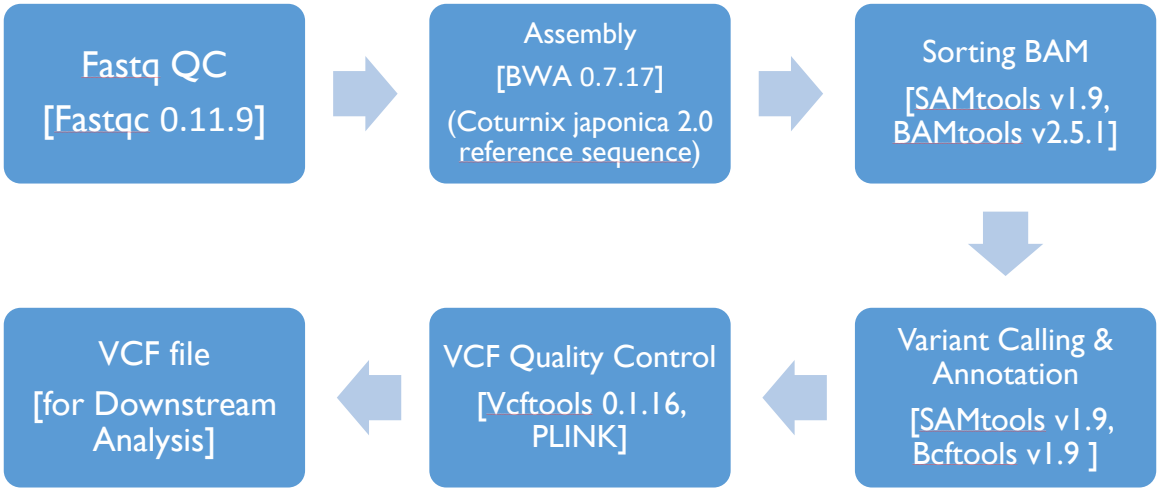

Figure 3.3: Flow chart showing GBS sequence analysis.

3.5.2 Genetic diversity and population structure of wild African harlequin quails

Mitochondrial DNA haplotype diversity was assessed using DnaSP version 5.10.1 (Librado & Rozas, 2009). The phylogenetic relationship between the study mtDNA haplotypes and GenBank reference mtDNA haplotypes (*C. japonica* and *C. coturnix*) was estimated using a median-joining network and a maximum likelihood tree generated with network version 5.0 (Bandelt et al., 1999) and MEGA version 10.2 (Kumar et al., 2018), respectively. Analysis of molecular variation among populations (F_{ST}) and diversity indices of the mtDNA haplotypes were calculated using Arlequin version 3.5.2.2 (Excoffier et al., 2010).

Molecular variation among populations test (F_{ST}) and principal component analysis (PCA) of the genotyping-by-sequencing SNP dataset were used to assess the distribution of genetic variation among wild African harlequin quail individuals and populations. Both tests were performed using SNPRelate R package version 1.22.0 (Zheng et al., 2012) with additional filtering based on linkage disequilibrium ($r^2 = 0.2$). To investigate the genetic ancestry of the wild African harlequin quail individuals and admixture between the wild and domestic quail species, a maximum-likelihood-based clustering algorithm in

ADMIXTURE version 1.3.0 was applied (Alexander & Lange, 2011) for *K* values 1 to 10. *K*-value with the lowest cross-validation (CV) error value was considered most optimal. To investigate the evolutionary relationship between the wild African harlequin and domestic Japanese quails based on the genetic distance between the species, a neighbor-joining tree was developed using vcfR R package version 1.14.0.

3.5.3 Demographic history of wild African harlequin quails

MtDNA neutrality tests (Tajima's D and Fu's Fs) and mismatch analysis (sum of squares deviation (SSD), raggedness index (rag.), theta0 (θ_0), theta1 (θ_1) and Tau (τ)) were calculated using Arlequin version 3.5.2.2 (Excoffier et al., 2010) to check for signals of demographic expansion through investigating mismatch distributions of pairwise differences among mtDNA haplotypes. Time since population expansion (*t*) was calculated using the equation $\tau = 2\ \mu Lt$, where τ is the tau value, μ is the substitution rate, and *L* is the length of the sequence (Aoki et al., 2018). Like most other avian species, the mitochondrial genome mutation rate of wild African harlequin quail or other quail species, especially for the mtDNA control region, is unknown. Therefore, the commonly used mitochondrial genome mutation rate of 2% per million years (Myr) was applied in this study (Nabholz et al., 2016). The divergence times between African harlequin quails and related quail species such as the Japanese and common quails were estimated using the TimeTree database (Kumar et al., 2017).

To examine the recent demographic history of the wild African harlequin quail using the SNP dataset, all populations were combined, and easySFS (Gutenkunst et al. 2009) was used to generate folded site frequency spectrum (SFS) information. The historical population size changes were then estimated using Stairway plot version 2.1 (Liu & Fu, 2015). The stairway plot has proved applicable to low-depth sequencing and reduced-representation sequence data (Liu & Fu, 2020). The mutation rate of the wild African harlequin quail is unknown, but since the mean mutation rate of most avian genomes ranges between $1.23 - 2.21 \times 10^{-9}$ site^{-1} year^{-1}, an intermediate rate observed in chicken (1.91e-9) and a generation time of 1 year was assumed (Nam et al., 2010).

3.5.4 Signatures of selection detection in wild and domestic quails

Signatures of selection techniques were used to examine genomic regions associated with growth, meat, and egg production. Here, two within-population complementary methods (CLR and iHS) that were found to have the most power (> 70%) to detect completed selection signatures after fixation of the advantageous allele were applied (Qanbari et al., 2014; Ma et al., 2015). CLR values which are based on the variation in the site-frequency spectrum along the chromosome were computed at 1000 grids (-grid 1000) using the SweeD program version 4.0.0 (Pavlidis et al., 2013) at each SNP. The iHS analysis requires phased haplotypes thus SHAPEIT version 2.r900 (Delaneau et al., 2013) was used to perform haplotype phasing with default settings. The ratio of extended haplotype homozygosities (EHH) associated with each allele were calculated into standardized iHS values using the function "*ihh2ihs"* in *rehh* version 3.2.1 R package (Gautier et al., 2017). The iHS analysis was conducted using unpolarized alleles which is ideal for non-model organisms that lack representative studies to allow for the correct designation of 'ancestral' or 'derived' alleles (Santos et al., 2021). Candidate genes under selection were determined using an outlier approach where SNPs that lie above the cutoff value were highly considered (Wang et al., 2018). The 99th percentile of the observed genome-wide distribution of all iHS values and CLR values was used as the threshold to identify outliers. SNPs that met the threshold and were located within a 250 kb window were highly considered and used to determine candidate genes under positive selection. The annotation of the candidate regions was based on the Japanese quail genome assembly (Accession number GCF_001577835.1) from NCBI. Gene Ontology (GO) terms and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways identification was performed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) version 6.8 browser tool (Huang et al., 2009) based on the Japanese quail annotation file as a reference genome.

Comparative analysis between wild African harlequin and domestic Japanese quails was based on the two signatures of the selection tests i.e., CLR and iHS. Genomic regions that

were found to be under positive selection were compared to determine how wild and domesticated quail species are affected by natural and artificial selection.

3.5.5 Transcriptomic landscape of growth and reproduction traits in quail

Transcriptome analysis was executed in a flowchart using a non-experimental research design, as shown in figure 3.4. The paired-end raw sequence data from RNA sequencing (RNA-Seq) of the six domestic Japanese quails belonging to two experimental groups (heavy-weight line and low-weight line) was thoroughly examined using FastQC version 0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to ensure only good-quality reads with at least 5 million reads per sample were selected. Mapping of reads against the reference genome of Japanese quail (*Coturnix_japonica*_2.0) was done using TopHat2 version 2.1.1 (Kim et al., 2013) with default parameters. To quantify how many reads map to each gene and to develop a counts file for differential gene expression analysis in R programming software, FeatureCounts version 2.0.0 (Liao et al., 2014) was used with the following parameters: -T 5 -p -B -C -t exon -g gene_id. Differential expression analysis was conducted using DESeq2 R package version 1.32.1 (Love et al., 2014). Genes were considered as candidates of Differentially expressed genes (DEGs) by the following thresholds: $|\log2FoldChange| > 1$, and $padj < 0.05$. Differentially expressed genes were subjected to GO analysis and KEGG pathway enrichment analysis with the DAVID Functional Annotation Tool (D. W. Huang et al., 2009). GO and KEGG items with $pvalue < 0.05$ and $qvalue < 0.05$ were treated as enrichment.

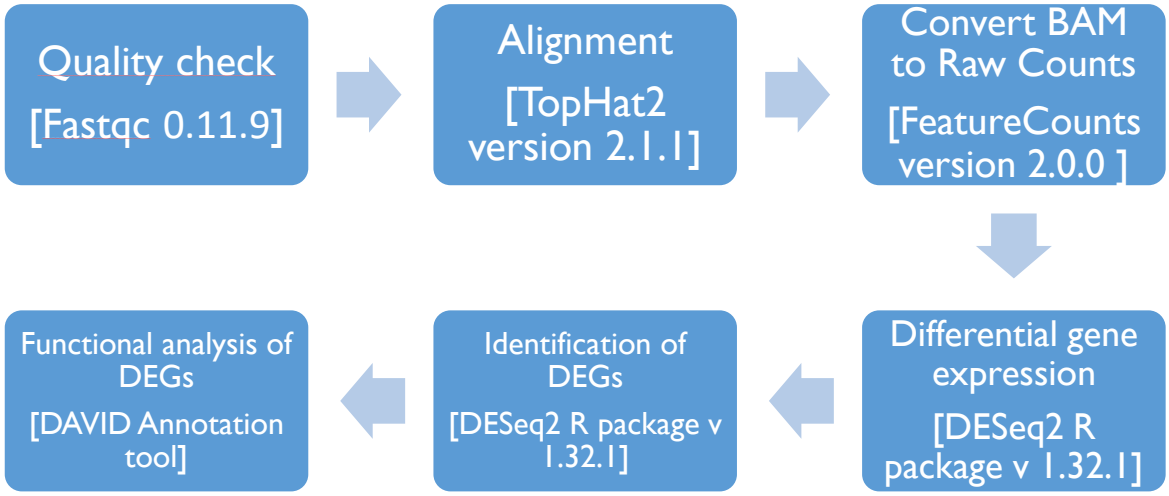

Figure 3.4: Flow chart showing RNA-Seq analysis flow chart.

CHAPTER FOUR

RESULTS

This chapter contains the results of the data analyses conducted in this study. The results are presented and described under each objective.

4.1 Genetic background assessment of wild African harlequin quail using mtDNA

The genetic origin, diversity, variation, and demographic history of the wild African harlequin were assessed using mtDNA haplotype data under the first objective. The results of the mtDNA data analyses are presented here.

4.1.1 Gel electrophoresis and sequence editing of Mitochondrial DNA

A total of 56 wild African harlequin and 17 domestic Japanese quail samples were successfully amplified using PCR. The agarose gel electrophoresis was used to confirm the correct amplified expected fragment size of 760bp before sequencing (Figure 4.1). The chicken sample was used as the positive control (+VE), whereas distilled water was the negative control (-VE).

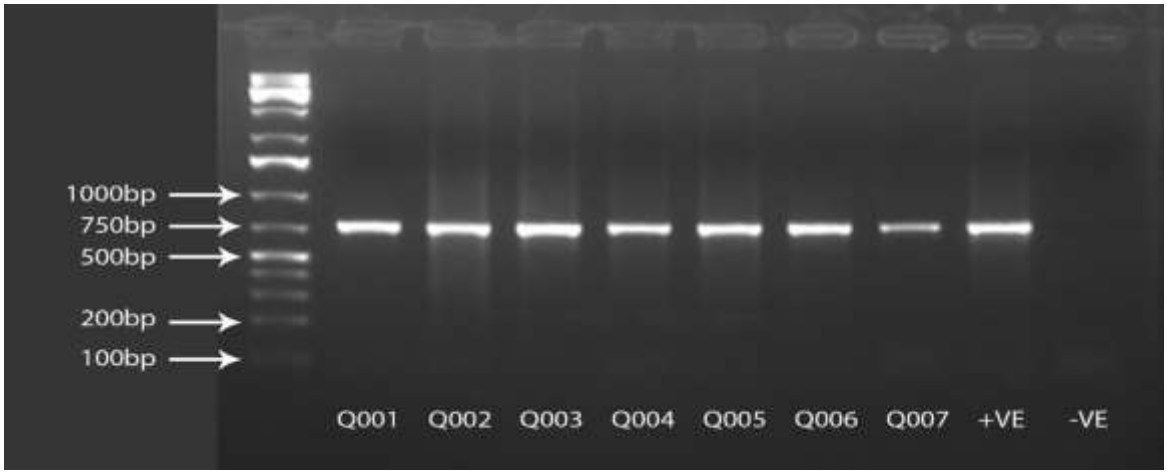

Figure 4.1: Agarose gel image showing the amplified 760bp mtDNA D-loop fragment in wild African harlequin quail.

After sequence editing of the forward and reverse sequences of each sample to generate a single consensus sequence, a multiple sequence alignment of all the edited mtDNA D-loop sequences was done. This was followed by the trimming of highly conserved regions of the sequences, which showed no genetic variation between the sequences (Figure 4.2). A highly variable 347bp fragment was retained for further analysis.

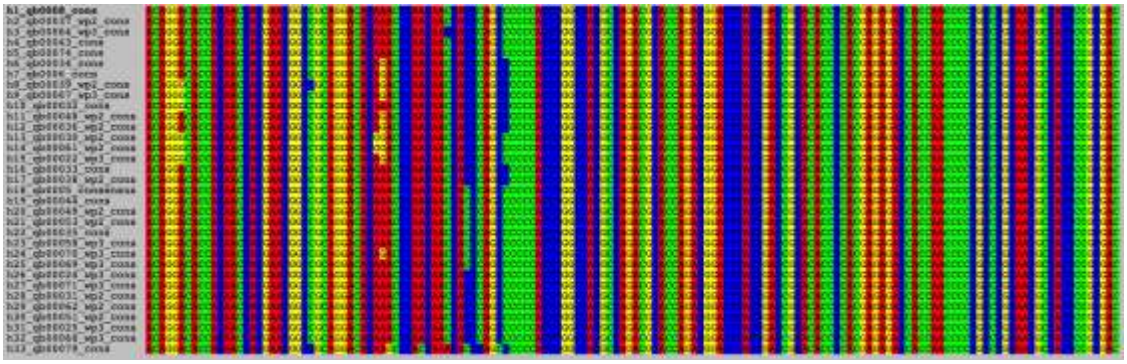

Figure 4.2: A multiple sequence alignment image showing both the conserved (right) and variable regions (left) among the mtDNA D-Loop haplotype (h1-h33) sequences.

4.1.2 Mitochondrial DNA haplotype diversity and phylogenetic relationships

The mtDNA D-loop sequences of the wild African harlequin quails revealed 32 haplotypes in total after haplotype analysis in DnaSP software. This translated to a haplotype diversity of 0.949±0.016 showing diversity among wild African harlequin quail individuals. In contrast, all the mtDNA D-loop sequences of the domestic Japanese quails revealed only a single haplotype (haplo 33 farm reared). Four of the wild African harlequin quail haplotypes were observed in more than two individuals, three haplotypes were observed in two individuals only, and the remaining haplotypes were singletons (present in one individual only). Haplotype 1 was the most common and present in 9 individuals (Table 3.1).

Table 4.1: Haplotype distribution in wild African and domestic Japanese quails

Haplotype	Number of individuals
Haplotype 1	9
Haplotype 2	1
Haplotype 3	1
Haplotype 4	1
Haplotype 5	1
Haplotype 6	3
Haplotype 7	1
Haplotype 8	1
Haplotype 9	1
Haplotype 10	1
Haplotype 11	2
Haplotype 12	1
Haplotype 13	1
Haplotype 14	1
Haplotype 15	1
Haplotype 16	1
Haplotype 17	1
Haplotype 18	2
Haplotype 19	6
Haplotype 20	1
Haplotype 21	2
Haplotype 22	1
Haplotype 23	7
Haplotype 24	1
Haplotype 25	1
Haplotype 26	1

Haplotype	Number of individuals
Haplotype 27	1
Haplotype 28	1
Haplotype 29	1
Haplotype 30	1
Haplotype 31	1
Haplotype 32	1
Haplotype 33 farm reared*	17

*Only 1 haplotype observed in all domestic Japanese quail individuals (Haplotype 33 farm reared)

Two clades were observed in the maximum likelihood phylogenetic tree, which separated the wild African harlequin quails from the other reference quail species with strong support (Figure 4.3). None of the other reference quail species haplotypes (Table 4.2) huddled together with the wild African harlequin haplotypes; therefore, no haplotype sharing was observed. The single domestic Japanese quail haplotype from our study (haplo 33 farm reared) clustered together with other reference Japanese quail haplotypes.

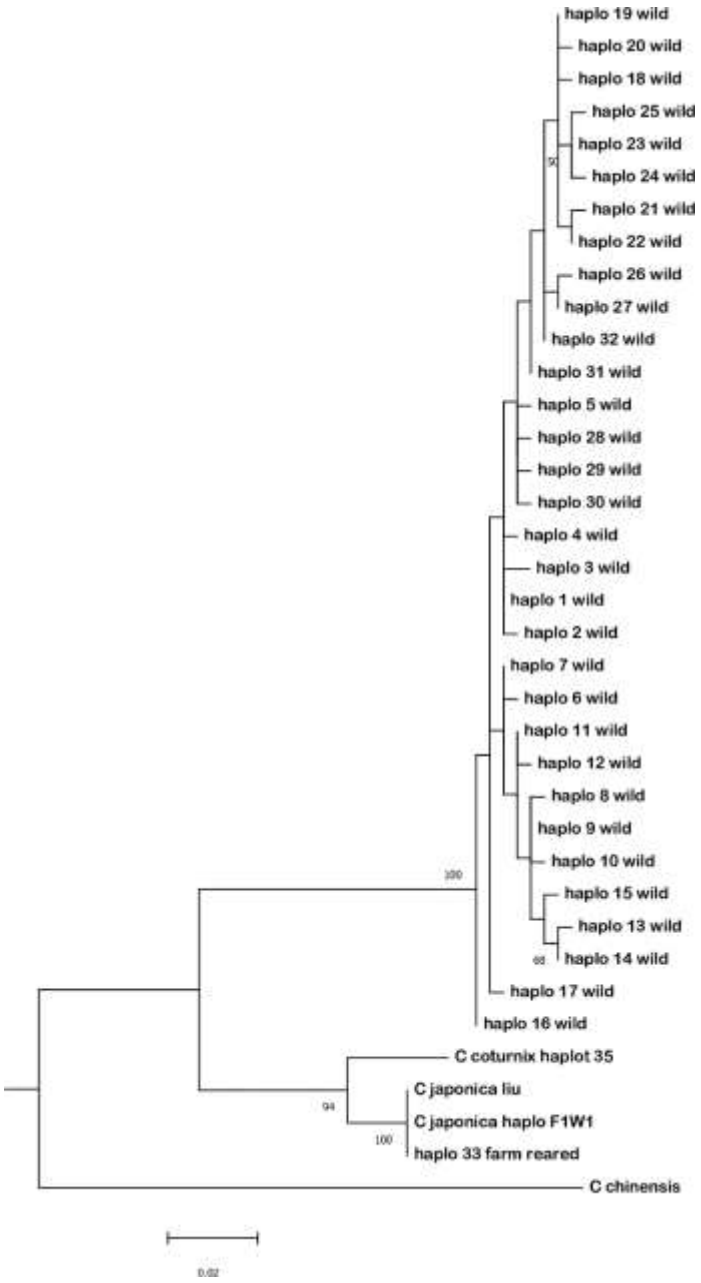

Figure 4.3: A maximum-likelihood tree showing the phylogenetic relationships between wild African harlequin quails (haplo 1 wild – haplo 32 wild) and other *Coturnix* species.

Table 4.2: Phylogenetic tree reference quail mtDNA haplotypes

Reference Haplotype	Accession Number
Coturnix chinensis mitochondrial DNA (C. chinensis)	AB073301.1
Coturnix coturnix haplotype 35 D-loop (C. coturnix haplot 35)	KJ623801.1
Coturnix japonica mitochondrion (*C. japonica* liu)	KX712089.1
Coturnix japonica haplotype F1W1 (*C. japonica* haplo F1W1)	KF410830.1

4.1.3 Median-joining network analysis

The median-joining network of our study haplotypes together with reference quail mtDNA haplotypes (*C. japonica* and *C. coturnix*) from GenBank formed three haplogroups (Figure 4.4). All the wild African harlequin quail mtDNA haplotypes (yellow) clustered under haplogroup 1, whereas haplogroup 2 contained the single domestic Japanese quail mtDNA haplotype from this study (blue), reference Japanese quail mtDNA haplotype that was similar to the Japanese quail mtDNA haplotype of this study (orange) and other reference Japanese quail mtDNA haplotypes (purple) from GenBank (Table 4.3). Haplogroup 3 contrarywise contained reference common quail mtDNA haplotypes (green). The king quail (*C. chinensis*) mtDNA haplotype was the chosen outgroup. Currently, there are no other harlequin quail mtDNA haplotypes deposited on GenBank that would have allowed for investigation of the ancestral origin.

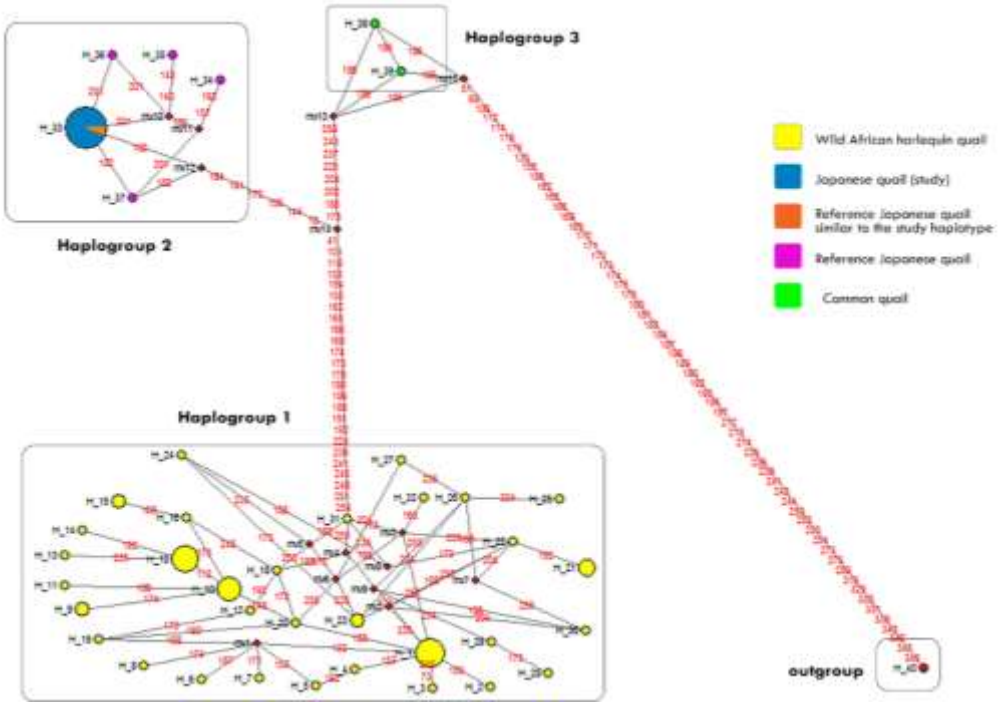

Figure 4.4: Median-joining phylogenetic network constructed for quail mtDNA haplotypes. Circled areas show the haplotype frequencies.

Table 4.3: Median network reference quail mtDNA haplotypes

Reference Haplotype	Accession Number
Coturnix chinensis mitochondrial DNA	AB073301.1
Coturnix japonica haplotype F2 control region	KF410831.1
Coturnix japonica haplotype F3 control region	KF410832.1
Coturnix japonica haplotype F4 control region	KF410833.1
Coturnix coturnix haplotype W2 control region	KF410838.1
Coturnix japonica haplotype W6 control region	KF410842.1
Coturnix coturnix haplotype W9 control region	KF410845.1
Coturnix japonica haplotype F1W1	KF410830.1

4.1.4 Diversity analysis

The percentage genetic variation among the three wild African harlequin quail populations from Siaya (Siaya North, Siaya Central, and Siaya South) was small (1.86%). This was backed by the non-significant low F_{ST} value (F_{ST} = 0.019, P > 0.05) calculated using AMOVA. The nucleotide diversity in the three wild African harlequin quail populations was 0.00775 ± 0.00482, 0.0109 ± 0.00641, and 0.00947 ± 0.00572, respectively, which was relatively low.

4.1.5 Demographic history of the wild African harlequin quails

The population expansion size values (θ_0 and θ_1) were 0.00234 and 1169.888, respectively, indicating a high population expansion. The tau (τ) value for the estimated age of expansion was 3.58 ± 0.504 (4.88–2.12) at a 95% confidence interval (CI). This translated to an estimated time since population expansion of 257,000 ka (Thousand years ago), 95% CI 150–350 ka, corresponding to around the Pliocene–Pleistocene boundary (Figure 4.5). The estimated mean Tajima's D (−0.424 ± 0.366, p > 0.05) and Fu's Fs (−7.67 ± 2.56, p < 0.05) values based on the mtDNA dataset were both negative for the wild African harlequin quail populations of Siaya County, also indicating a signal of demographic expansion and/or positive selection. This observation was further supported by the unimodal mismatch distribution (Figure 4.6) and the small but non-significant SSD and Raggedness index that was 0.00573 ± 0.00170 (p > 0.05) and 0.0343 ± 0.00571 (p > 0.05), respectively.

The molecular divergence time between the African harlequin, Japanese, and common quails was estimated by the TimeTree database to range between 5.13 and 17.60 Ma (million years ago). However, the estimated median time was 11.03 Ma during the Miocene epoch of the Neogene period.

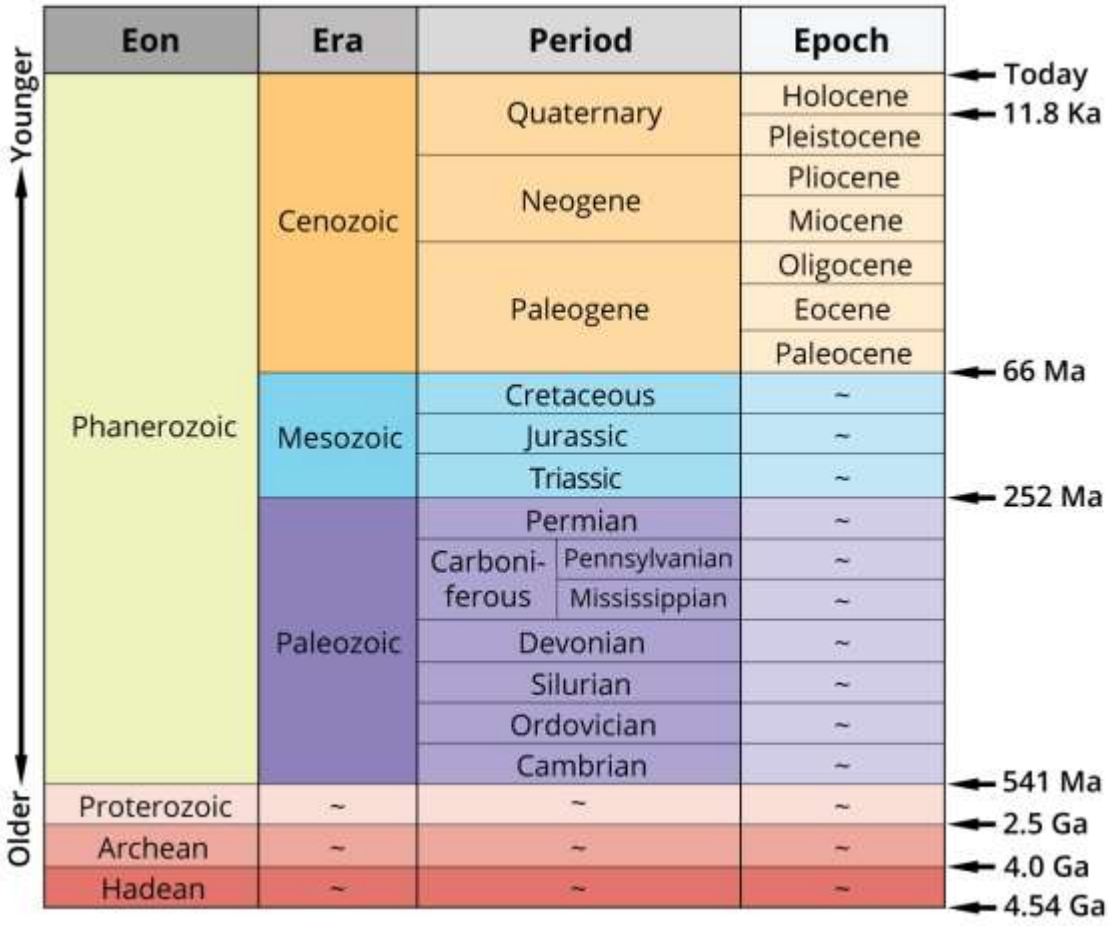

Figure 4.5: Geological timescale chart.

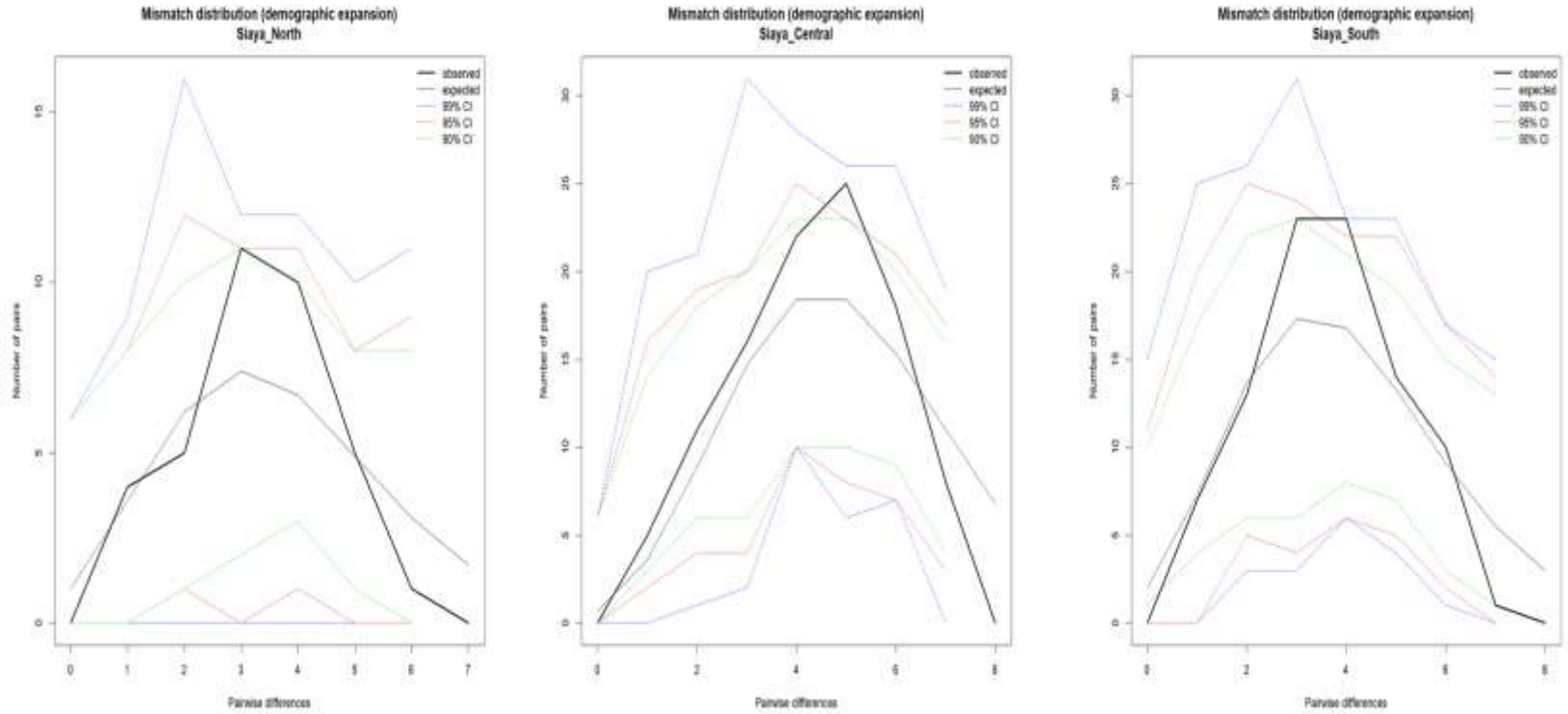

Figure 4.6: Unimodal mismatch distribution observed in the three wild African harlequin quail populations (Siaya North, Siaya Central, and Siaya South).

4.2 Genetic diversity and selection signatures in wild African harlequin quail

The information under this second objective contains the results of the wild African harlequin quail's genetic diversity and demographic history using SNP data from GBS. Additionally, the results of the selection signatures detected in the wild African harlequin quail are also presented here.

4.2.1 Genetic diversity and demographic history of the wild African harlequin quail

The genetic diversity and demographic history of the wild African harlequin quail using genome-wide SNP information was examined in this first section of the second objective.

4.2.1.1 Sequence data processing, SNP identification and analysis

The quality of the GBS data was first examined for read quality, sequence depth and missing data profiles. This was done to evaluate the quality of the data in relation to the quality of the reads, depth and amount of missing data per sample in order to provide guidelines in relation to variant filtering parameters. Genotyping by sequencing data is known to have a high proportion of missing SNP data (Munyengwa et al., 2021). Thus, preliminary observations are crucial to ensure that only high-quality data is retained for further analysis. The minimum depth of the data was 1 (Figure 4.7), whereas the proportion of missing data was low (Figure 4.8). The quality of the reads was good after observation thus enabling downstream analysis.

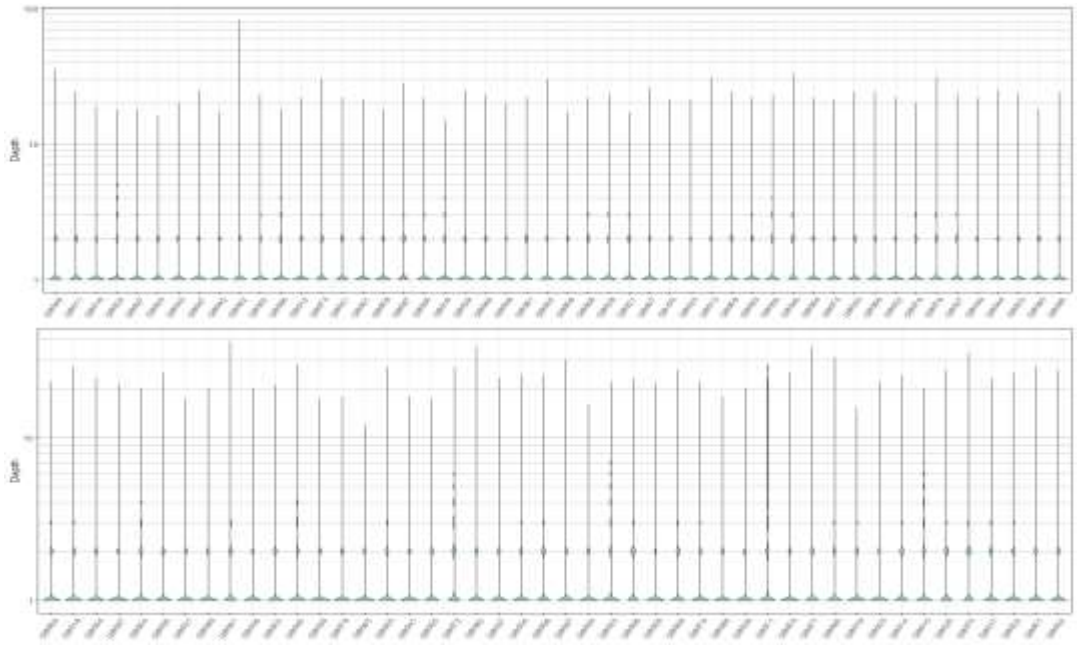

Figure 4.7: Sequence depth plot of the quail genotyping by sequencing data per individual.

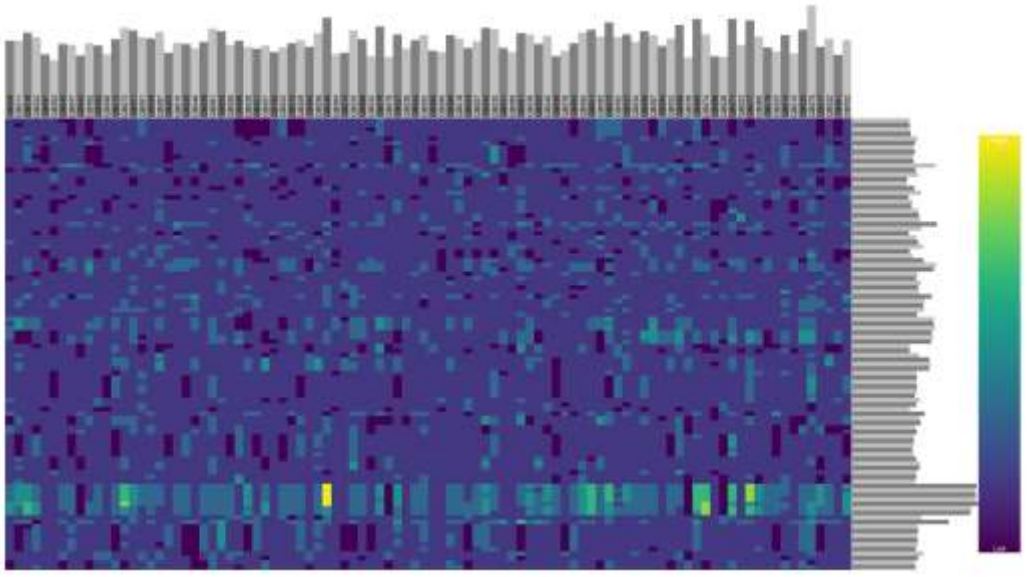

Figure 4.8: Heatmap showing the proportion of missing data present in the individual quail samples.

More than 95% of the wild African harlequin quail sequenced reads in all individuals were mapped to the Japanese quail reference genome (*Coturnix Japonica* 2.0), thereby ascertaining confidence in the SNP identification process. A total of 8,237,001 variants were called in 96 individuals, and after variant filtering, 119,339 SNPs were retained for further analysis.

4.2.1.2 Phylogenetic analysis

An NJ (neighbor-joining) algorithm was used in constructing the phylogenetic tree since it is less computationally intensive and relatively fast. The phylogenetic tree was able to group quail individuals based on genetic distance. Genetic distance represents the number of mutation events between the species since their divergence.

No domestic Japanese quail individuals clustered together with the wild African harlequin quails showing their divergence and distinction from each other (Figure 4.9). Domestic Japanese quails generally had longer branch lengths signifying greater genetic distances hence greater mutation events than wild African harlequin quails. The genetic distance clustering of the wild African harlequin quail individuals separated the individuals into two main groups containing individuals from all populations.

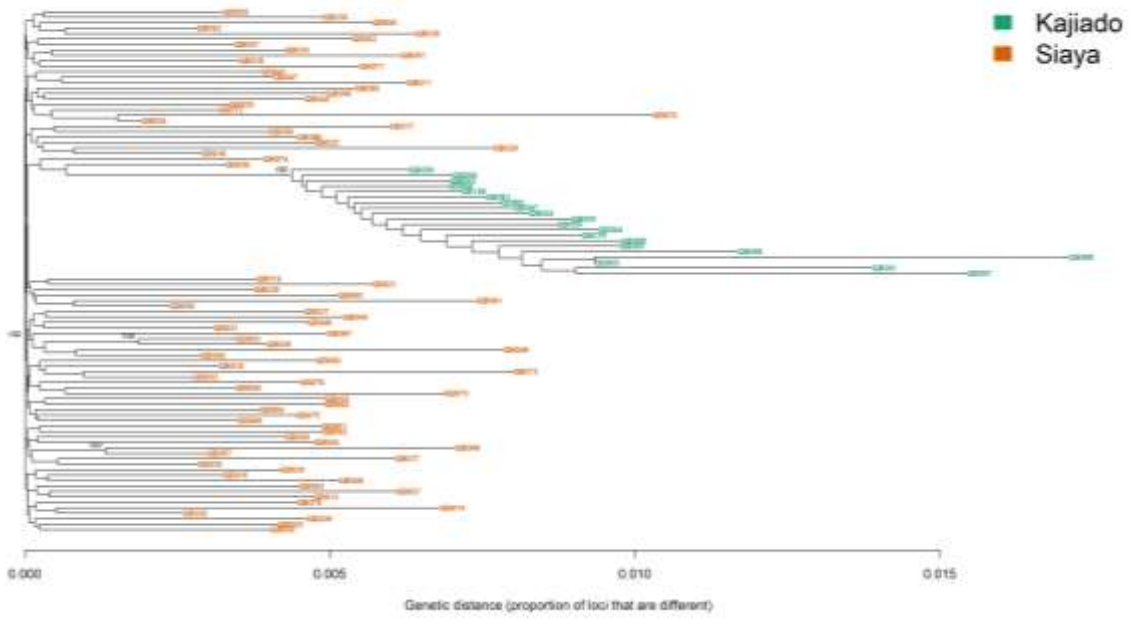

Figure 4.9: A neighbor-joining tree showing the evolutionary relationship between wild African harlequin quail populations (Siaya) and domestic Japanese quail (Kajiado).

4.2.1.3 Principal component analysis

Principal component analysis showed no signs of population structuring in wild African harlequin quails of Siaya County as most individuals were grouped in a single cluster (Figure 4.10). The first and second eigenvectors (EV) accounted for 6.6% and 2.1% of the total observed variation, respectively. This observation was also supported by a low average pairwise F_{ST} value of 0.000075, signifying slight genetic variation among the three sampled wild African harlequin quail populations. Overall, the wild African harlequin and domestic Japanese quails were separated into two distinct clusters.

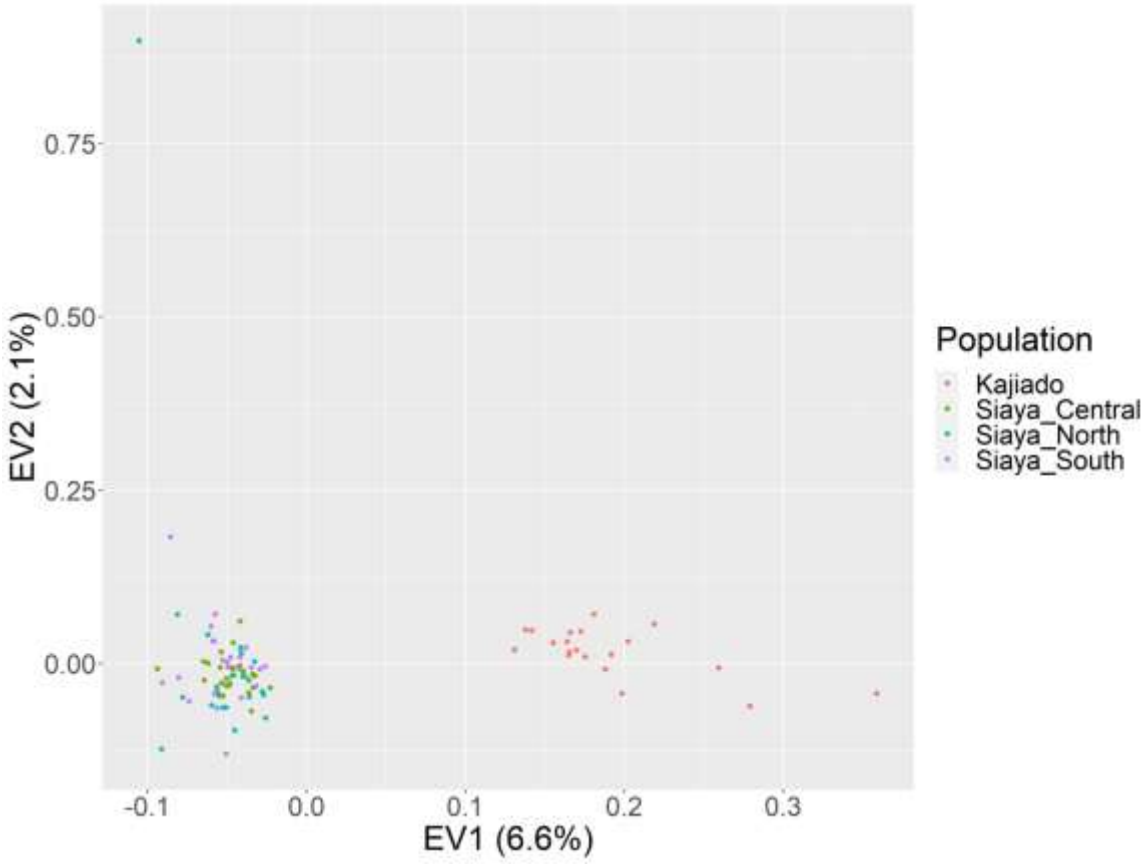

Figure 4.10: Principal component analysis of wild African harlequin quail populations (Siaya_North, Siaya_Central, and Siaya_South) and domestic Japanese quail (Kajiado).

4.2.1.4 Admixture

Admixture results were similar to PCA as the wild African harlequin quails were separated from domestic Japanese quails at K = 2, which had the least cross-validation error (Figure 4.11). The cross-validation procedure is crucial for identifying the K value for which the Admixture model can predict with high accuracy. One wild African harlequin quail sample exhibited low levels of shared genetic ancestry with Japanese quail at K = 2, an observation that was not supported by other K values (Figure 4.12). These results showed no hybridization between the sampled wild African harlequin quail individuals and purebred domestic Japanese quails.

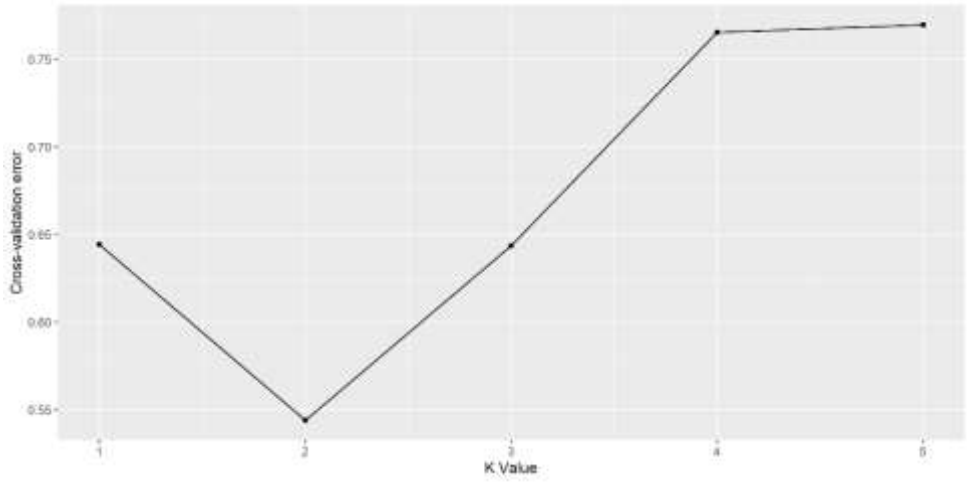

Figure 4.11: Plot showing the distribution of cross-validation error values.

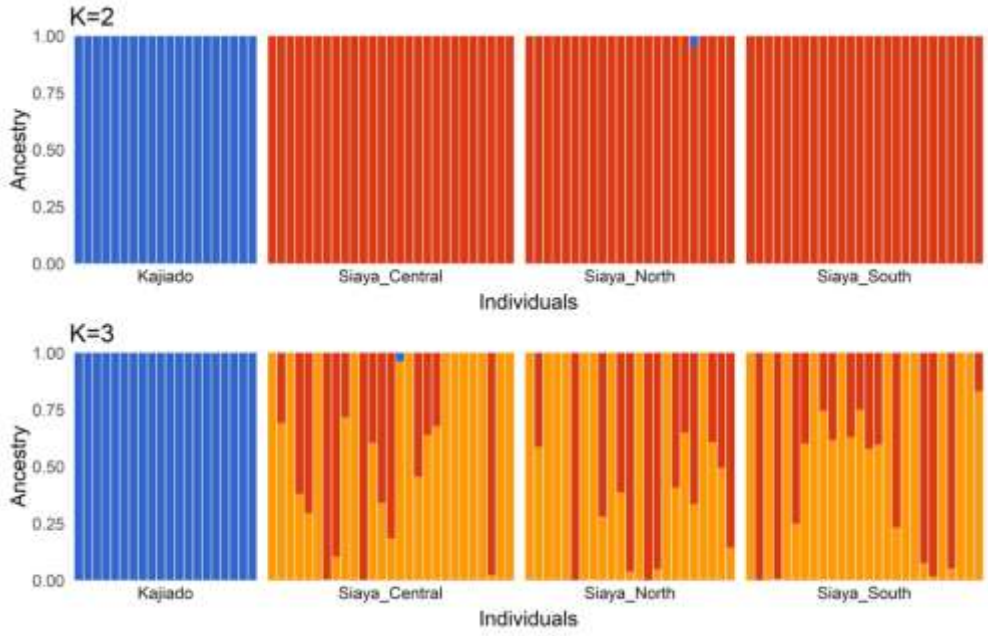

Figure 4.12: Admixture plot showing the relationship between wild African harlequin (Siaya) and domestic Japanese quails (Kajiado) at ancestry number (K) = 2–3.

4.2.1.5 Demographic history of the wild African harlequin quail

The demographic history and effective population size changes of the wild African harlequin quail of Siaya County spanning 560,000 years were displayed on a stairway plot (Figure 4.13). The folded site frequency spectrum information based on genome-wide SNP data revealed a population bottleneck, a drastic reduction in population size event, that occurred approximately 180 ka (Thousand years ago), followed by population expansion and a gradual decline in the effective population size to the present. The observed reduction in the effective population size, which is responsible for producing the next generation, validated observations by rural smallholder farmers that had observed the dwindling numbers of wild African harlequin quails in the region.

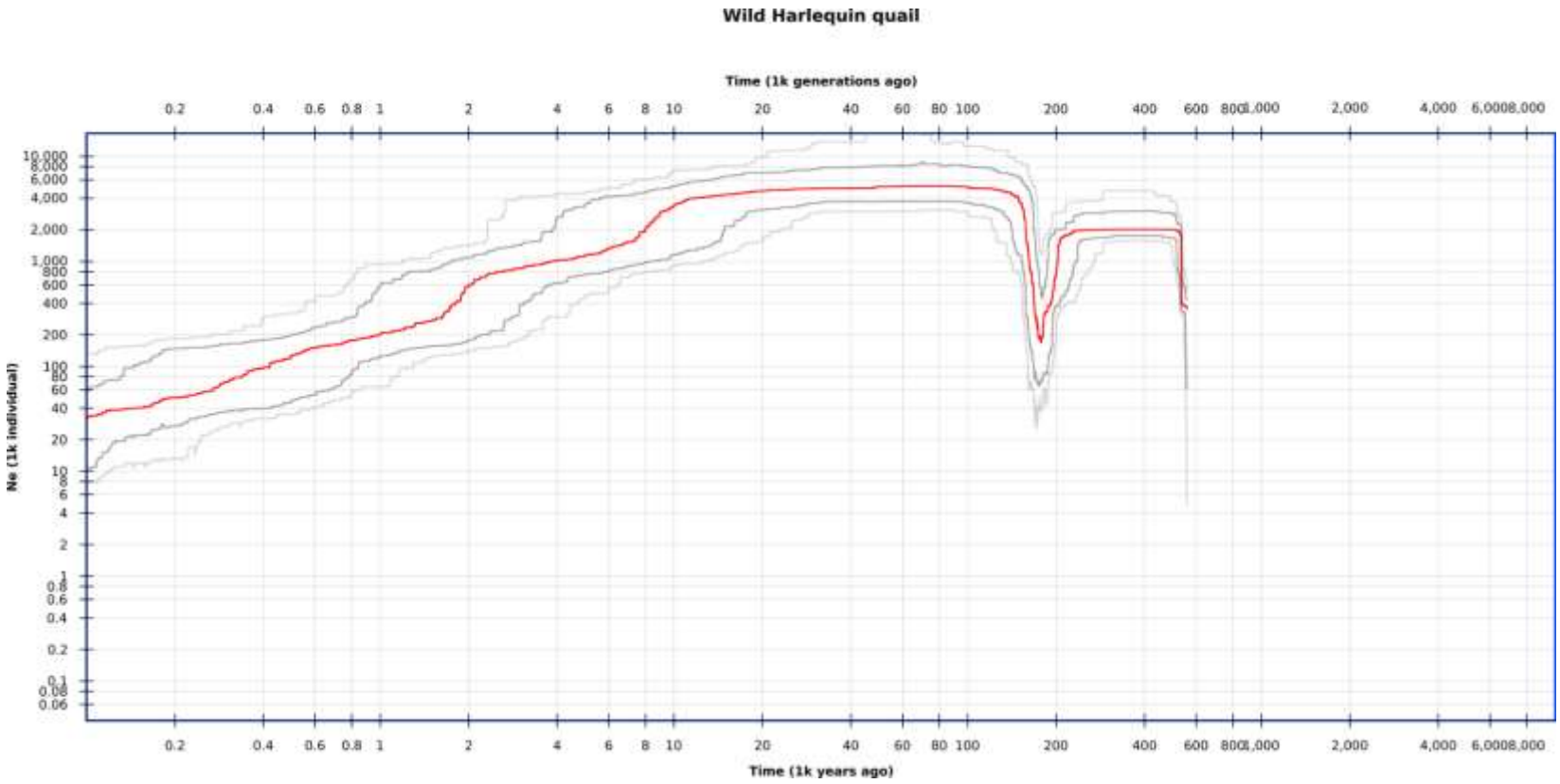

Figure 4.13: Stairway plot showing historical changes in effective population size of Siaya wild African harlequin quails.

4.2.2 Signatures of selection assessment in wild African harlequin quail

The presence of selection signatures in the wild African harlequin quail genome was investigated to examine genomic regions associated with growth, meat, and egg production. SNP information was examined and analyzed using signatures of selection detection tests in this second section of the second objective.

4.2.2.1 SNP identification and analysis

Additional variant filtering parameters were applied for signatures of selection detection tests. This was to ensure that only high-quality SNPs were retained for analysis. A total of 8,024 SNPs were retained after strict variant filtering and were used for downstream analysis.

4.2.2.2 Composite likelihood ratio (CLR) analysis

Genome-wide selection signatures were detected using the composite likelihood ratio statistical approach in the wild African harlequin quail. Potential candidate regions under selection were identified with outliers that fell into the 99th percentile of CLR scores distribution (CLR > 1.39). Chromosomes 1 and 2 had the highest number of significant CLR values (Figure 4.14) and a total of 352 potential candidate genes were identified across 28 autosomes.

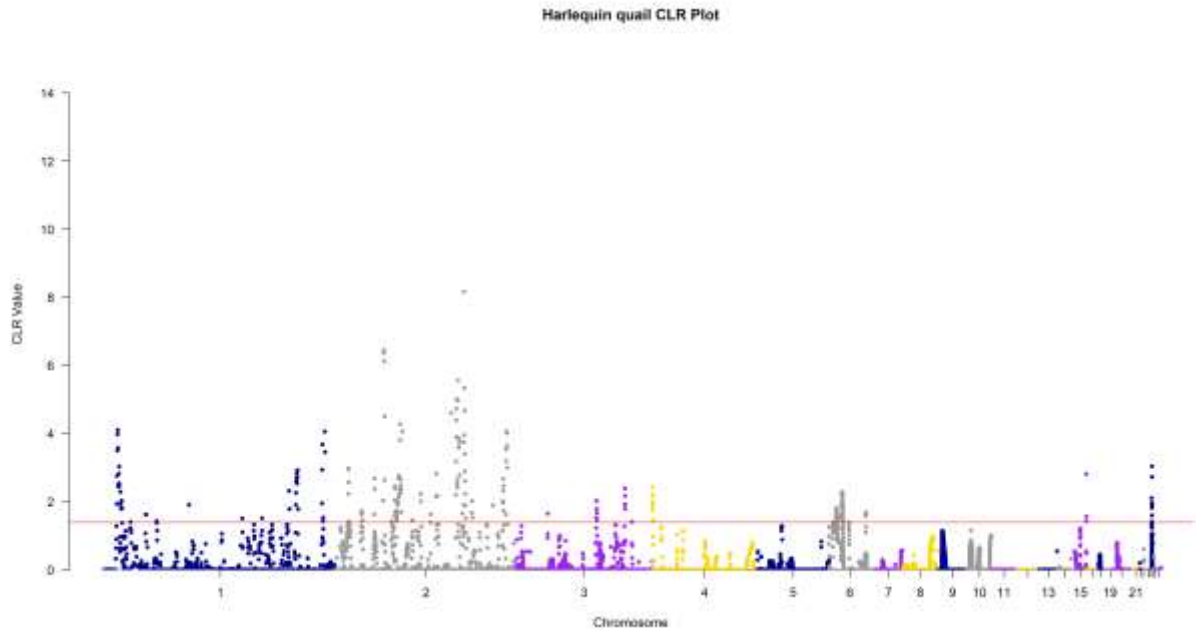

Figure 4.14: Manhattan plot showing CLR scores distribution across all wild African harlequin quail autosomes.

In wild African harlequin quails, functional analysis of the candidate genes showed an association with key biological processes, metabolic pathways and molecular functions. Wild African harlequin quail candidate genes were implicated in melanogenesis (WNT3, WNT4, FZD7, ADCY8, CAMK2B, WNT5A, WNT8B), behavior (ADCY8, ALK, DACH1, GLRB, GRIA1, NR3C1, KCNJ10, OPRM1, PEX13, PMCH, PTEN, PTPRZ1, TBX1, NRXN3, HOMER2, DKK1, NCSTN, RASD2, CSMD1, USP46, ADM2, KIRREL3, DMBX1), circadian entrainment (ADCYAP1, CAMK2B, GRIA1, PROX1, PRKG1, SPSB4), carbohydrates and glucose metabolism (PTPN11, SSTR5, IRS2), sex differentiation (CEBPB, DACH1, FSHB, FSHR, PTPN11, VEGFA, WNT5A, SOX8, WNT4, CSMD1), growth (FGF7, PRKG1, PTPN11, SOX2, FZD7), lipid metabolism (ALK, FSHB, PPP2R5A, IRS2, VAV3, WNT4, ADCYAP1, SLC2A1, VEGFA, PTPN11, GPC1, BAMBI), ear morphogenesis (COL11A1, FGFR2, PROX1, TBX1, WNT5A, LRIG1), and muscle tissue development (ACTA1, ADRA1A, ATF3, COL11A1, FGFR2, PROX1, VEGFA, WNT5A, EOMES, HAND1, SEMA3C, SOX8, CDON), among others.

Enrichment analysis of the candidate genes revealed cluster groups with enriched terms such as Wnt signaling pathway (GO:0016055), regulation of actin cytoskeleton organization (GO:0032956), gliogenesis and glial cell differentiation (GO:0042063, GO:0010001), cAMP signaling pathway (hsa04024), Hippo signaling pathway (hsa04390), Calcium signaling pathway (GO:0019722), regulation of MAPK cascade (GO:0043408), Tight junction (GO:0070160), and Endocytosis (GO:0006897).

4.2.2.3 Integrated haplotype score (iHS) analysis

The iHS values were used to identify genomic regions that showed signatures of selection patterns. Outliers that fell into the 99th percentile in wild African harlequin quails (iHS > 3.55) were considered to be potential candidate regions under selection. Chromosome 2 had the highest number of outlier SNPs (Figure 4.15) and a total of 150 potential candidate genes were detected across 28 autosomes in wild African harlequin quails.

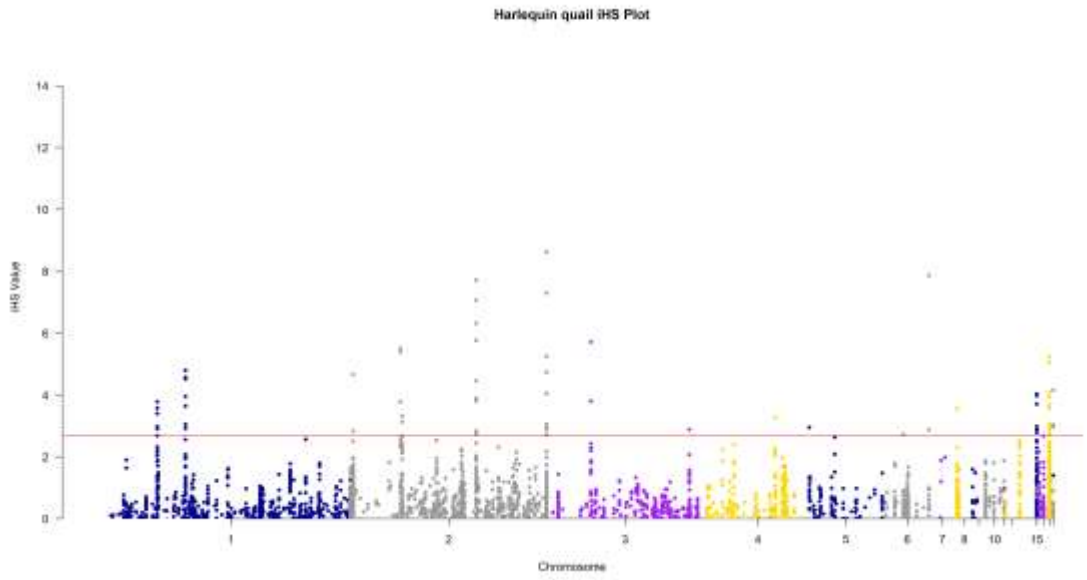

Figure 4.15: Manhattan plot showing iHS scores distribution across all wild African harlequin quail autosomes.

Enrichment analysis of the wild African harlequin quail candidate genes identified clusters linked to platelet activation (MAPK14, ITGB3, PPP1CA, MAPK13), Th1 and Th2 cell

differentiation (MAPK14, LOC107317826, MAPK13), regulation of secretion (CREB1, ACE, EGF, OSBP, KLF7, TMEM132A, MICAL1, SLC8B1, CMKLR2), growth hormone synthesis, secretion and action (CREB1, MAPK14, MAPK13, MAP3K3), cellular response to environmental stimulus (MAPK14, ITGB3, MAPK13, RAD9A, RRH, TLK2, TMEM109), glucose and carbohydrate homeostasis (ACE, CMKLR2, KLF7, SLC8B1) and Th17 cell differentiation (MAPK14, LOC107317826, MAPK13). Key enrichment terms and pathways include FoxO signaling (GO:0009267), MAPK signaling pathways (GO:0071883), positive regulation of gliogenesis and glial cell differentiation (GO:0014015; GO:0010001), Insulin signaling (GO:0008286), Follicle stimulating hormone (FSH) signaling pathway (GO:0042699), cellular senescence (GO:0090398), regulation of actin cytoskeleton (GO:0032956), and focal adhesion (GO:0005925).

4.3 Comparative signatures of selection analysis in wild and domestic quails.

Signatures of selection detection in the domestic Japanese quails were analyzed under the third objective. The results presented here were then compared to the signatures of selection results of the wild African harlequin quail from the second objective to check for similarities and differences between wild and domestic quail species.

4.3.1 SNP identification and analysis of the domestic Japanese quail data

Additional variant filtering parameters were applied to the domestic Japanese quail data for signatures of selection detection tests. This was to ensure that only high-quality SNPs were retained for analysis. A total of 491,441 SNPs were retained after strict variant filtering and were used for downstream analysis.

4.3.2 Composite likelihood ratio (CLR) analysis in domestic Japanese quail

Genome-wide selection signatures were detected using the composite likelihood ratio statistical approach. Potential candidate regions under selection were identified with outliers that fell into the 99th percentile of CLR scores distribution (CLR > 8.33). Outliers were observed in a majority of the domestic Japanese quail chromosomes except

chromosomes 10 and 12 (Figure 4.16). A total of 424 potential candidate genes were identified across 28 autosomes.

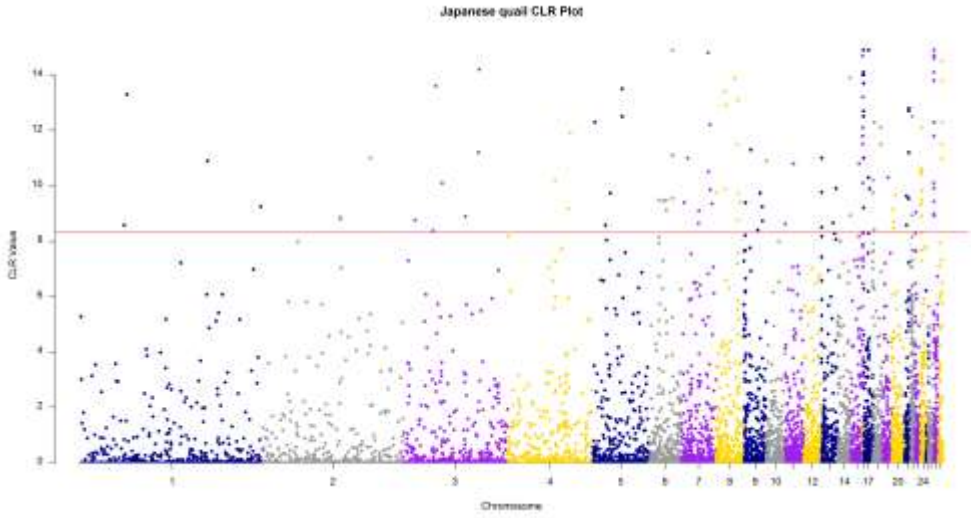

Figure 4.16: Manhattan plot showing CLR scores distribution across all domestic Japanese quail autosomes.

Some of the top enrichment terms observed in domestic Japanese quail include the regulation of actin cytoskeleton (GO:0032956), cAMP signaling pathway (GO:0019933) (VIPR2, MAPK1, RAC1, SLC9A1), focal adhesion (GO:0005925), MAPK signaling (GO:0071883), Fc gamma R-mediated phagocytosis (GO:0038096), B cell receptor signaling pathway (GO:0050853), autoimmune thyroid disease (cjo05320), tight junction (hsa04530). The top biological processes that were enriched were involved in muscle cell differentiation, tissue and structure development (CAV2, COL6A3, SLC9A1, SMARCD3, MSX2, PRF1), positive regulation of growth (FN1, PRKCQ, RPS6KA1, SLC9A1, RHOA, MAPK14, FGFR2,FN1, RPS6KA1, CXCL12, SLC9A1, TGFB2, VEGFA, PPM1F, TFCP2L1, SYT17, MAPKAP1) positive regulation of leukocyte activation and proliferation (RHOA, CBFB, CSK, PRKCQ, PTAFR, SMARCD3, ARID1A, SOCS1, ZBTB7B, KMT5B, PBRM1, PELI1, ZMIZ1, SLC39A10, TNIP2,

TNFRSF13C, BLOC1S3), insulin signaling (PRKCQ, MAPK1, RAC1, RPS6KA1, ICA1), embryonic morphogenesis (ACVR2B, COL11A1, FGFR2, FOXI1, FN1, GPI, HOXB1, HOXB2, LRP5, MBNL1, MSX2), tissue morphogenesis (AR, RHOA, COL11A1, FGFR2, GPI, MSN, MSX2, RET, SEMA3E, SIX2, TRAF3IP1, EXOC4, TMEM79, IRX2), and bacterial infection invasion of epithelial cells (EXOC4, MAPK1, RAC1, ACTR3B), (CAV2, FN1, MAPK1, RAC1, ACTR3B, RHOA), among others.

4.3.3 Integrated haplotype score (iHS) analysis in domestic Japanese quail

The iHS values were used to identify genomic regions that showed signatures of selection patterns. Outliers that fell into the 99th percentile in domestic Japanese quails (iHS > 3.2) were considered to be potential candidate regions under selection. In domestic Japanese quails, outlier SNPs numbers were high in all chromosomes (Figure 4.17). A total of 457 potential candidate genes were detected across 28 autosomes in Japanese quails.

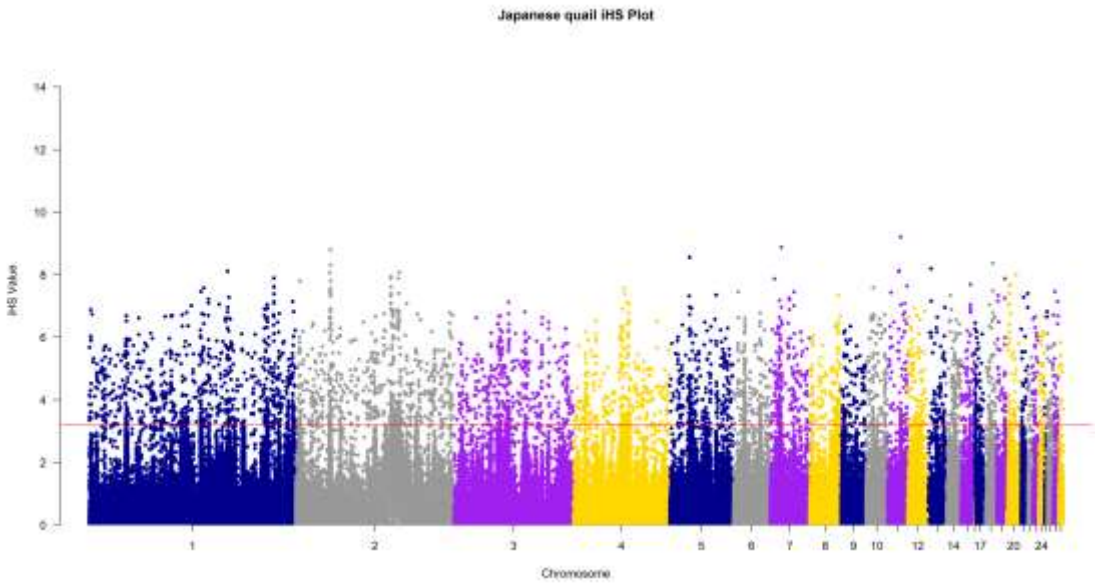

Figure 4.17: Manhattan plot showing iHS scores distribution across all domestic Japanese quail autosomes.

Functional enrichment analysis of the domestic Japanese quail candidate genes revealed an association with the regulation of lipid metabolic process (GO:0019216), antigen

processing and presentation (GO:0019882), autoimmune thyroid disease (cjo05320), Th1 and Th2 cell differentiation (GO: 0045063, GO:0045064), phagosome (GO:0045335) and intestinal immune network for IgA production (GO:0002387). Some of the genes were linked to Salmonella infection (DYNLL2, LOC107321367, LOC107321370), negative regulation of immune system process (AMBP, APOA1, RUNX3, CBFB, CNN2, PLCG1, PRKAR1A, PTPRJ, SDC4, SRC, FSTL3, SYT11, PHPT1, IL4I1, NRARP), regulation of insulin secretion (ENSA, IRS1, PRKAR1A, REST, SIRT4, SIDT2, MIDN), adipocytokine signaling pathway (STK11, IRS1, TRADD, TRAF2), FoxO signaling pathway (STK4, STK11, IRS1), cell adhesion (SDC4, CADM3), cholesterol transport and its regulation (APOA1, APOA4, APOA5, LI PC, STAR, ABCA2, ABCA5, ABCA7, LDLRAP1), Thyroid stimulating hormone (TSH) signaling pathway (GNA12, GNAO1, MAP2K6, RAP1GAP, SRC, GNA13), Sudden infant death syndrome (SIDS) susceptibility pathways (RUNX3, CHRNA4, ECE1, GRIN1, PRKAR1A, REST, RET, TPH1, YWHAB), kidney and renal system development (COL4A3, COL4A4, LHX1, SMAD5, RET, SDC4, HNF1B, WNT5A, ALDH1A2, APH1A), regulation of mRNA processing and metabolic process (CIRBP, HNRNPA2B1, PTBP1, SRSF4, TRAF2, EIF4ENIF1, REST, SRRM1, NUDT21, DAZAP1, SLTM), and protein processing in endoplasmic reticulum (SSR2, RNF185, RPN2, MBTPS, MAN1C1, DDOST, AMFR, TRAF2).

4.3.4 Comparative signatures of selection analysis

Candidate genes involved in crucial biological, molecular, and cellular processes such as immune response, growth, reproduction, and morphological and behavioral traits were positively selected in the wild African harlequin and domestic Japanese quails. However, the most common biological processes and molecular pathways were associated with key signaling pathways such as the MAPK, cAMP, and Wnt signaling pathways. Immune response candidate genes, which are crucial for survival and are hence favored by selection, were observed in both species. The Th1 and Th2 cell differentiation KEGG pathway, which is responsible for cell-mediated and humoral-type immune responses, was enriched in wild African harlequin and domestic Japanese quails. However, some

differences were observed, including platelet activation and intestinal immune network for IgA production, which were enriched only in the wild African harlequin and domestic Japanese quails, respectively, among others.

Positively selected candidate genes associated with morphological traits and biological processes (WNT5A, GRIA1, CREB1, ADCY8, and ALK) such as melanogenesis, eye, and inner ear development were more common in the wild African harlequin quail, whereas production-related candidate genes associated with processes like lipid and cholesterol metabolism (VIPR2, DYNLL2, COL6A3, MSX2, PR F1and GNA12) were more common in the domestic Japanese quail. Behavior-related candidate genes were also more common in the wild African harlequin quail than in the domestic Japanese quail. Growth and reproduction candidate genes were enriched in both species as cell proliferation and muscle development-related biological processes were observed. Spermatogenesis was enriched in the domestic Japanese quail, whereas the follicle-stimulating hormone signaling pathway was enriched in the wild African harlequin quail.

4.4 Transcriptomic landscape of domestic Japanese quail

The transcriptomic landscape of the domestic Japanese quail was examined under the fourth objective. This was done to investigate the expressed genes, biological processes, and molecular pathways involved in the growth and reproduction process of domestic Japanese quails. The raw counts gene expression data revealed 3501 genes expressed in the testes of both heavy-weight line and low-weight line domestic Japanese quails. The counts of the mapped reads for each gene were normalized using the median of ratios method to make accurate comparisons of gene expression between individuals. Normalization involved scaling raw count values to allow expression levels to be more comparable between samples.

The gene expression data of our two experimental groups using the non-experimental research design was a good fit for the DESeq2 model, as shown by the mean dispersion plot, since the dispersions decrease with increasing mean and cluster around the maximum likelihood (ML) line (Fig 4.18). This curve allows for more accurate identification of

differentially expressed genes with small sample sizes. 708 genes were upregulated (log2FoldChange > 1), 668 genes were downregulated (log2FoldChange < -1), and non-significant genes lie in-between (Fig 4.19).

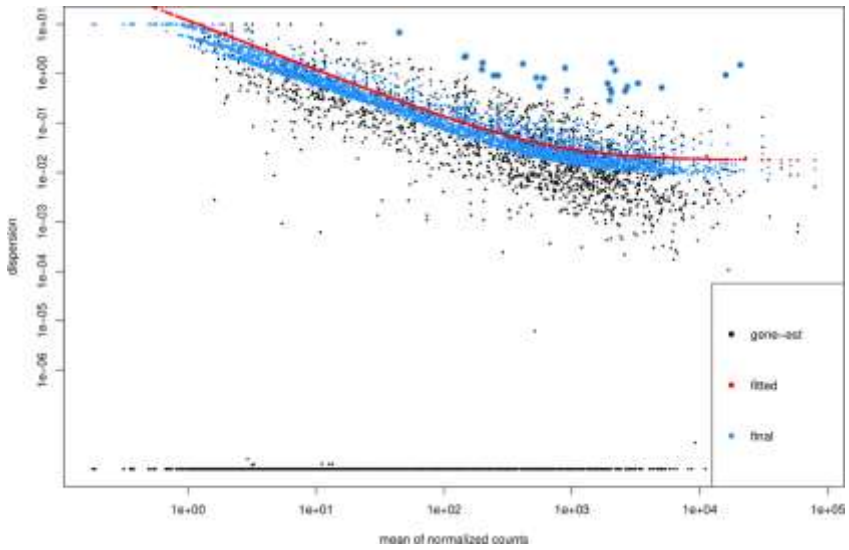

Figure 4.18: Mean dispersion plot showing domestic Japanese quail gene expression data

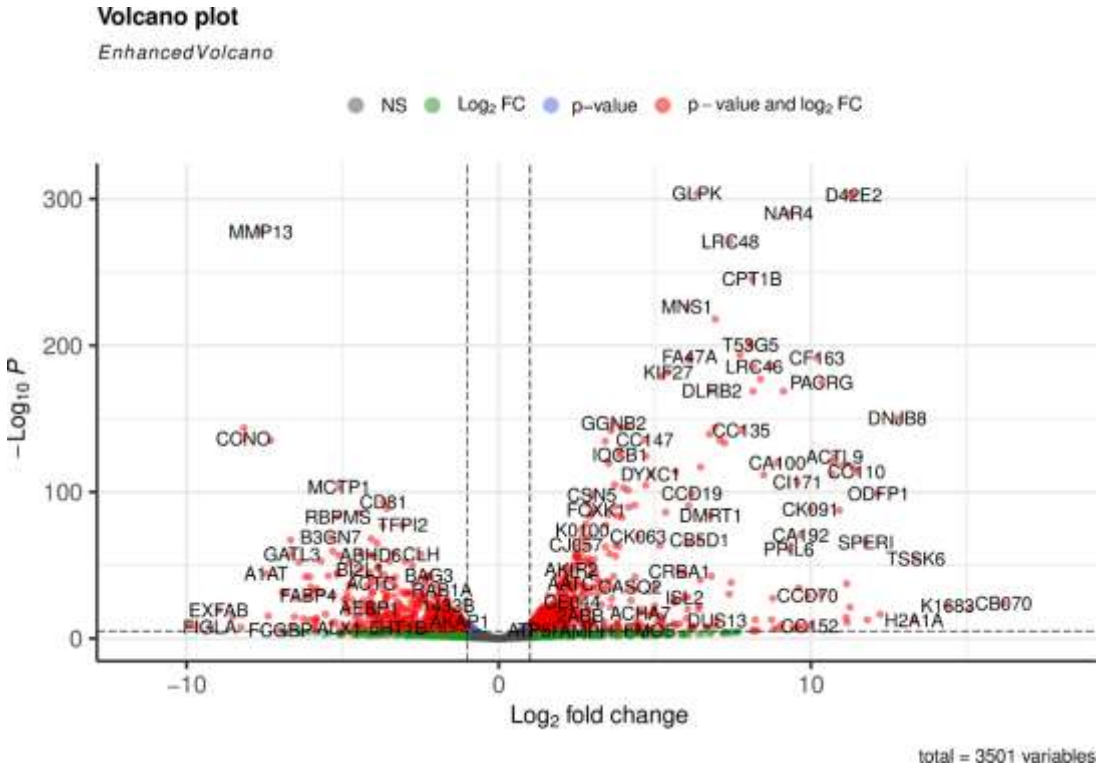

Figure 4.19: Volcano plot showing differentially expressed genes between heavy-weight and low-weight domestic Japanese quail.

Differential analysis showed which genes significantly differ in expression between heavy-weight and low-weight domestic Japanese quails. The expression pattern of the top 20 significant differentially expressed (DE) genes in heavy-weight and low-weight quails was plotted (Figure 4.20). The normalized gene expression counts of 19 out of the top 20 significant DE genes were higher in low-weight domestic Japanese quails when compared to the heavy-weight domestic Japanese quails.

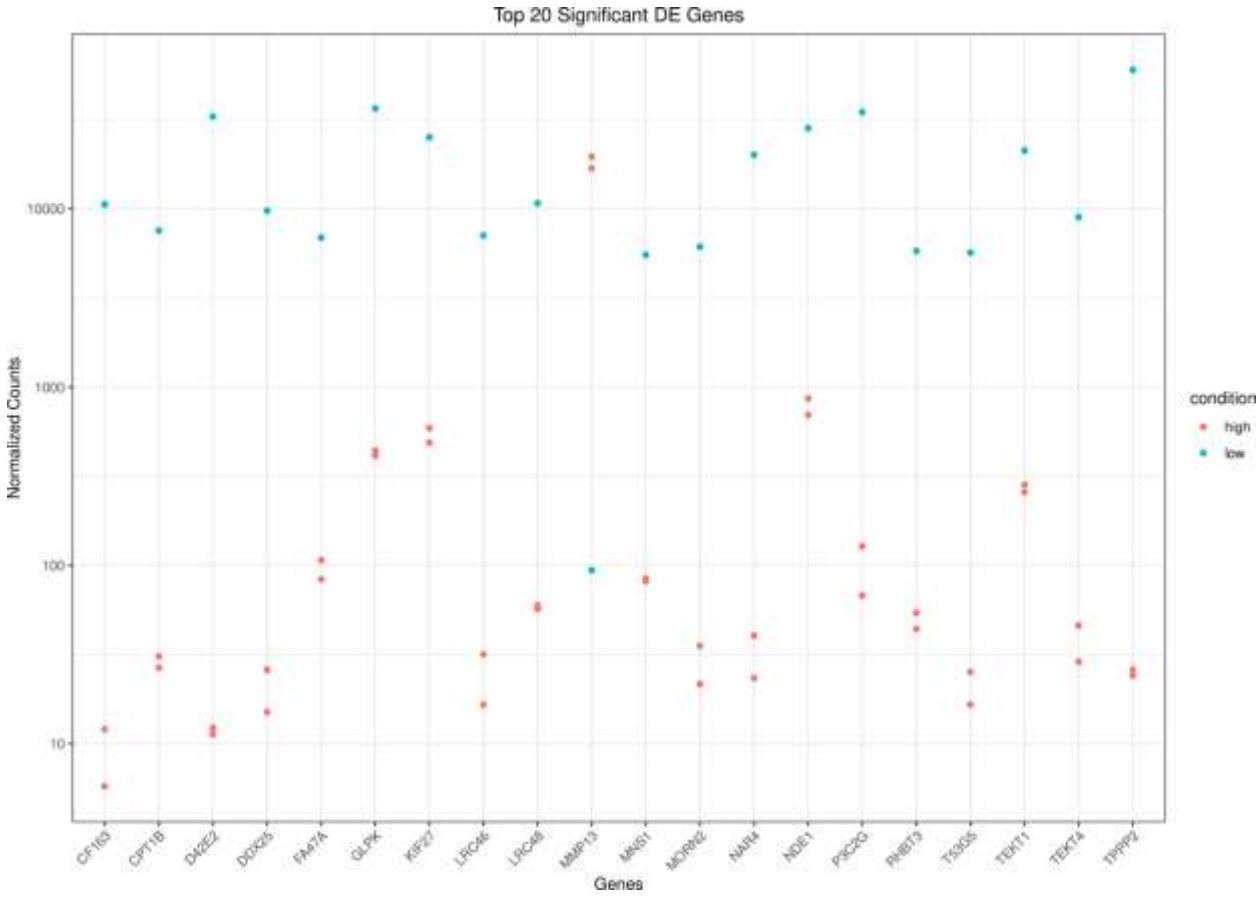

Figure 4.20: Expression plot showing the top 20 DE genes of testes tissue in heavy-weight (high) and low-weight (low) domestic Japanese quails.

Functional enrichment analysis of the identified differentially expressed genes (DEGs) revealed several biological processes, molecular pathways, and cellular functions such as spermatogenesis (GO:0007283), muscle structure development (GO:0061061), mitotic cell cycle phase transition (GO:0044772), mitotic cell cycle phase transition (GO:0044772), connective tissue development (GO:0061448), among others (Figure 4.21).

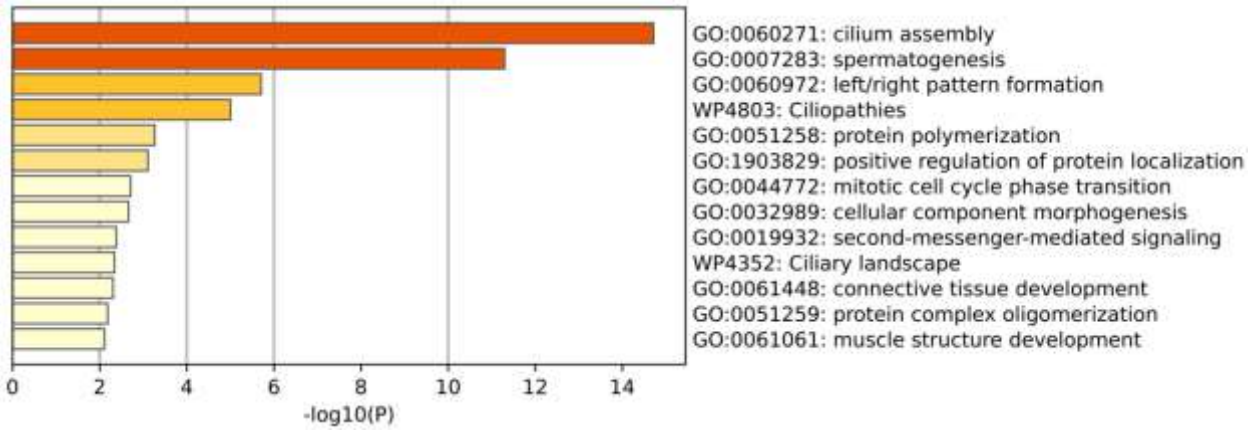

Figure 4.21: Bar graph of the top enriched terms across domestic Japanese quail significant DE genes, colored by p-values.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

This Chapter discusses the findings of this study to provide an interpretation of the genetic background and signatures of selection assessment of wild African harlequin quails and domestic Japanese quails. The genetic characterization and demographic history results from the first and second objectives involving the mtDNA marker and GBS data, respectively, are compared to present a more comprehensive discussion. Signatures of selection detection results from wild African harlequin and domestic Japanese quails from the second and third objectives have also been discussed together to allow for a comparative and inclusive discussion. This Chapter also contains conclusions and recommendations that are drawn from the study.

5.1 Discussion

For generations, harvesting wild African harlequin quails has been ongoing in many parts of Western Kenya. However, little attention has been paid to their sustainable use and conservation status. Several genetic diversity studies have been conducted on other quail species, such as the domestic Japanese and wild common quails, using mtDNA and microsatellite markers (Amaral et al., 2007; Barilani et al., 2005; Chazara et al., 2010; Puigcerver et al., 2014; Sanchez-Donoso et al., 2012). However, genetic information on the wild African harlequin quail is lacking. This study examined the genetic diversity and demographic history of wild African harlequin quail populations of Siaya County using mtDNA D-loop data and SNP information generated through genotyping by sequencing approach. The wild African harlequin quail genome was also examined to assess how selection has affected it compared to the domestic Japanese quail. This is the first study to produce molecular information on the wild African harlequin quails of Kenya, thereby creating a foundation that will lead to further studies and possible development of domestic harlequin quail breeds.

5.1.1 Genetic background of wild African harlequin using mtDNA

The mtDNA haplotype and phylogenetic relationship analyses showed the genetic variation among the wild African harlequin individuals in relation to other reference quail species. The wild African harlequin quail haplotypes clustered in one clade of the maximum likelihood phylogenetic tree showing their genetic diversity, similar ancestry, and distinction from the other quail species. In contrast, only a single mtDNA haplotype was detected among sampled domestic Japanese quail individuals, consistent with low genetic diversity within the domesticated population. MtDNA haplotype sharing between wild and domestic quails has previously been used to detect and evaluate hybridization (Barilani et al., 2005). However, there was no mtDNA haplotype sharing between the sampled wild African harlequin quails and Japanese quail individuals, separating the two species into different haplogroups. This observation was further supported by the median-joining network analysis where the wild African harlequin quail haplotypes were grouped in their own haplogroup (haplogroup 1) separately from the Japanese and common quails. The differences between the related quail species could be attributed to the mutations that have occurred since their molecular divergence time estimated between 5.13-17.60 MYA (Stein et al., 2015; Jetz et al., 2012; Seabrook-Davison et al., 2009). Haplotype 1 was the most recent haplotype with a star-like phylogeny at eight clades. The wild African harlequin quail samples were acquired from three different populations within the same county; this could explain the slight genetic variation characterized by the low nucleotide diversity and F_{ST} value.

The negative Tajima's D and Fu's Fs values all indicated possible demographic large-scale expansion of the wild African harlequin quails. The demographic expansion estimates θ_0 and θ_1 also showed that the wild African harlequin quails expanded from a small population size (near zero) to a very large one. This could be through a range expansion with high levels of migration between neighboring demes that is commonly observed in wild quails that migrate seasonally (Excoffier et al., 2004; Ray et al., 2003). This observation was further supported by the unimodal mismatch distribution and the small and insignificant SSD and raggedness index values. The demographic expansion

parameter τ was used to infer time since population expansion, and that estimate was placed around the quaternary glaciation period. Vegetation shift from closed canopy to open savannah and grassland during the quaternary glaciation period developed habitats suitable for many wild bird species, such as the wild African harlequin quail (deMenocal, 1995, 2004). Climate change and oscillations between wetter and drier climates have repeatedly promoted cycles of range contraction and expansion (Lorenzen et al., 2010). This may explain the seasonal and varied presence of wild African harlequin quails in Siaya and other neighboring counties (Wamuyu et al., 2017). Human population increase and urbanization have also contributed to migratory behavior changes and the decline of wild African harlequin quail numbers in some regions of Western Kenya. Additionally, habitat loss and change continue to influence migratory behavior and threaten migratory bird species (Hewson et al., 2016).

Detecting hybridization is crucial as it has been linked to reduced or absent migratory behavior in wild quail populations (Barilani et al., 2005). Barilani et al. (2005) also reported the presence of *C. japonica* mtDNA haplotypes and admixed microsatellite genotypes in the sampled wild common quail populations hence suggesting the possibility of hybridization. However, in this study, no Japanese or common quail mtDNA haplotypes were found in the sampled wild African harlequin quail populations of Siaya, thereby suggesting no hybridization. Therefore, there is no reason to suspect the artificial release of domesticated Japanese quail into the wild, as was the concern in Kenya due to the sudden increase of exotic Japanese quails experienced from 2013-2015. However, using more than one genetic marker (mtDNA alongside genomic DNA) is highly encouraged as this will advance admixture testing (Barilani et al., 2005; Chazara et al., 2010).

5.1.2 Diversity and demographic history of the wild African harlequin quail

Molecular characterization studies using DNA fragment molecular markers such as the mtDNA have provided insightful information on the genetic origin, diversity, and variation in poultry and wild birds (Murunga et al., 2018). This objective used SNP

information from GBS data to examine the wild African harlequin quail diversity, population structure, and demographic history. In addition, mtDNA analysis results were compared to determine whether DNA fragment molecular markers are as suitable and effective as genome-wide SNP information.

The seasonal migration of wild birds, such as the wild African harlequin quail, affects their population size and structure (Newton, 2006). Since there is little information about the seasonal migration patterns of wild African harlequin quails, sampling individuals from a similar deme or multiple demes of a single population is highly likely. This could explain the slight genetic variation characterized by the low F_{ST} value from SNP analysis, a similar observation from mtDNA analysis. The sampled populations originated from the same county and could also explain why the genetic variation of wild African harlequin quails was predominantly among individuals and less among sampled populations; hence, no well-defined population structure was observed through PCA analysis. Even though individuals from one deme of migrating birds can interbreed, they still share genetic similarities with individuals from the same population (Harpending, 2002). PCA analysis separated the wild African harlequin quails from the domestic Japanese quails into two distinct clusters. This difference is expected, as the two species have an estimated divergence time between 5.13-17.60 Ma (Seabrook-Davison et al., 2009; Jetz et al., 2012; Stein et al., 2015).

Even though no mtDNA haplotype sharing was observed, the wild African harlequin quail individual that showed evidence of low levels of Japanese quail ancestry in ADMIXTURE analysis conducted on SNP data still lacks strong evidence to suggest hybridization in the wild. The observed apparent admixed nature of the individual was not noted in other values of *K* and could be an artifact of the analysis. In addition, there are no known hybrids of *C. d. delegorguei* and *C. c. japonica* that exist in the wild; hence further investigation involving a larger sample size and more populations is required. To reaffirm the mtDNA analysis results on hybridization detection, the GBS analysis findings further alleviate concerns of possible extreme hybridization and introgression present in the local wild

African harlequin quail populations of Siaya County since the excessive introduction of domestic Japanese quail breeds into the country. Detecting hybridization is crucial in cases of suspected interaction between domestic and wild quail species to monitor the genetic integrity of the local populations. Currently, there is no reason to suspect the artificial release of domestic Japanese quail breeds into the wild, as was the concern.

The demographic history of the wild African harlequin quails of Siaya was outlined on a stairway plot from 560,000 years ago. The population bottleneck event observed around 180 ka is consistent with climate change during the Pliocene–Pleistocene boundary, where climate oscillations resulted in range contraction of species during dry arid climate periods (deMenocal, 1995; Hewitt, 2004). The observed bottleneck is also consistent with the emergence of *Homo sapiens* in East Africa some 200,000 years ago (White et al., 2003; McDougall et al., 2005), which was associated with updated and improved technology (Bradfield et al., 2020, Hallet et al., 2021). African hominins were known for hunting terrestrial prey and the development of advanced tools facilitated the incorporation of animal protein into their diets (Faith, 2014). However, the time period when early hominins became skilled hunters is still debatable (Marean & Assefa, 1999; Ferraro et al., 2013).

Both mtDNA and SNP data analyses suggested that wild African harlequin quail populations of Siaya experienced a population expansion soon after the bottleneck. The negative mtDNA Tajima's D and Fu's Fs test values, and the SNP stairway plot demographic expansion estimates all signaled a large-scale demographic expansion of the wild African harlequin quail populations of Siaya County. The unimodal mismatch distribution that was also detected when the observed nucleotide-site differences between pairs of mtDNA haplotypes were computed is characteristic of a population that has experienced demographic expansion (Rogers & Harpending, 1992). This could be through a range expansion with high levels of migration between neighboring demes that is commonly observed in wild quails that migrate seasonally (Ray et al., 2003; Excoffier, 2004). In addition, the mtDNA demographic expansion parameter τ and the SNP stairway

plot analysis were both used to infer time since population expansion. That estimate was placed around the quaternary glaciation period. Glacial changes during this period promoted the general cooling of African climates. East African vegetation shifted from closed canopy to open savannah and grassland vegetation starting in the mid-Pliocene (deMenocal, 1995, 2004). Grasslands, bushy areas, and croplands serve as habitats for many wild quail species, including the African harlequin quail, and this may explain their expansion during that period. In East Africa, vegetation changes and the development of periodical cooler and drier climatic conditions after 2.8 Ma greatly influenced species distribution and genetic isolation that further intensified after 1.7 and 1.0 Ma (deMenocal, 1995). Oscillations between wetter and drier climates have repeatedly promoted cycles of range contraction and expansion (Lorenzen et al., 2010). This may also explain the seasonal presence of wild African harlequin quails following the long and short rainy seasons (Wamuyu et al., 2017). Their sudden availability and increased numbers are also encouraged by food availability, especially after the rainy season, when grain farms create suitable habitats.

The effective population size of wild African harlequin quails of Siaya has been declining over the last 20,000 years, which coincides with climate change in East Africa, the massive late Pleistocene changes in range and abundance of smaller vertebrates (Steele, 2007), and the extinction of megafauna (50 ka -10 ka) (Stuart, 1999; Van Der Kaars et al., 2017). Climate change during the last glacial maximum (~20 ka) has been linked to a decline in the effective population size of several species in Southern and tropical Africa (Sithaldeen et al., 2015). Changes in temperature and precipitation were found to have a profound effect on food supply, reproduction, and the migration patterns of birds (Carey, 2009). In addition, this decline began after hydroclimatic changes that favored *Homo sapiens* demographic expansion and dispersal to other parts of the world, approximately 70–60 ka (Rito et al., 2019; Schaebitz et al., 2021). It is hypothesized that during this period, human hunters were responsible for the massive extinction events and habitat destruction as they spread from Africa to other continents (Martin, 1967; Faith, 2014). However, the role

played by humans in the late Pleistocene extinction events is still debated (Nagaoka et al., 2018).

Over the past 200,000 years, human occupation in East Africa has contributed to regional vegetation and habitat change (Schaebitz et al., 2021). This change was further facilitated by the transition from hunting and gathering to agriculture where rainforests and woody vegetation in the savanna were slowly converted into farms and pasturelands (Patin et al., 2014). Wild quails survive away from protected areas and end up in farms and pasturelands, where they are commonly hunted, thus also contributing to their population changes over time (Gatesire et al., 2014). Traditional hunting of wild African harlequin quails involves using captured females to attract males (Wamuyu et al., 2017). This leads to the capture of more males than females, thereby affecting the breeding sex ratio, which impacts the effective population size. This could also explain the disappearance of wild African harlequin quails from certain neighboring regions and their reduction in number within Siaya County, where they were usually present in very high numbers compared to other regions.

Wild African harlequin quails were initially present in most parts of Western Kenya. However, due to climatic change and intensive human activities such as overhunting and destruction of their habitats, parts of Siaya, Kisumu, and Homa Bay counties have become potential refugial areas. These activities influence and have contributed to migratory behavior changes and the population decline of wild African harlequin quails and other migratory bird species (Hewson et al., 2016). The wild African harlequin quails of Siaya County seemed to be concentrated in regions near the lake due to the presence of untouched natural vegetation, unlike the mainland. So far, no comprehensive phylogeographic and climatic oscillation studies have been done on wild African harlequin quails or other wild bird species in the Lake Victoria basin. Therefore, the geographical impact of Lake Victoria on species diversity still needs to be studied. Even though this study concentrated on the wild African harlequin quail populations of Siaya,

additional sampling sites could reveal if there is any range-wide population structure and how interconnected different populations are.

5.1.3 Signatures of selection detection in wild and domestic quails

Signatures of selection detection in both wild and domesticated species is considered a key step toward understanding the molecular basis of adaptive evolution. This information can be used to identify genomic regions and underlying biological mechanisms affecting their important traits (Saravanan et al., 2020). The application of CLR and iHS in detecting selective sweeps in wild African harlequin and domestic Japanese quail genomes was successful. The use of several complementary methods for signatures of selection detection allows for improved testing as each statistical test captures patterns in data differently (Fariello et al., 2013). The linkage disequilibrium (LD)-based iHS approach, which requires haplotypes per individual of one population to detect selection, was anticipated to be more reliable for this study due to the single Siaya and Kajiado County populations that were sampled and the lack of a good reference population in relation to quail (Voight et al., 2006). In addition, the CLR method was also adopted for this study as it uses allele frequency data to compare a neutral and a selective sweep model, and it is not highly sensitive to assumptions about the underlying recombination rate or recombination hotspots (Williamson et al., 2007). Furthermore, the CLR method can detect a beneficial mutation that spread in the entire population. In contrast, methods based on extended haplotype length and high linkage disequilibrium only detect the beneficial mutation that has yet to spread throughout the whole population (Voight et al., 2006; E. T. Wang et al., 2006; Williamson et al., 2007).

Both methods identified candidate genes associated with crucial biological, molecular, and cellular processes such as immune response, growth, reproduction, and morphological and behavioral traits. Some of the key candidate genes involved in various immune response processes and pathways include MAPK1, MAPK13, MAPK14, CREB1, DYNLL2, RAC1, and ITGB3. The MAPK signaling pathway was enriched in the wild African harlequin and domestic Japanese quail. Mitogen-activated protein kinases

(MAPKs) are a group of serine/threonine protein kinases that are highly conserved and have essential roles in cellular processes, such as proliferation, stress responses, apoptosis, and immune response regulation (Y. Liu et al., 2007). The MAPK13 and MAPK14 (p38 MAPK pathway) genes, which play an essential role in inflammatory responses, were positively selected in the wild African harlequin quail. The p38 pathway participates in the innate and adaptive immune response process by controlling the production of inflammatory cytokines (TNFα, interleukin (IL)-1, IL-10, and IL-12) by specialized dendritic and macrophage cells, CD40-induced gene expression, and proliferation of B cells, antigen processing in $CD8^+$ conventional dendritic cells, and T cell homeostasis and function (Arthur & Ley, 2013; Soares-Silva et al., 2016; Han et al., 2020).

In addition to the p38 pathway, the extracellular signal-regulated kinase (ERK1/2) is also a MAPK pathway involved in positive selection and the differentiation of DP thymocytes to either CD4 or CD8 T cells (Fischer et al., 2005). The MAPK1, a key component of the ERK1/2 pathway, was shown to participate in adaptive immune responses during bacterial infection in Nile tilapia (Wei et al., 2020). In this study, the MAPK1 functional analysis revealed its association with bacterial invasion (*Salmonella*, *Shigella*, *Yersinia*, and *Escherichia* species) and virus infection in domestic Japanese quails. MAPK1, through the MAPK/ERK pathway, facilitates the activation and proliferation of immune cells, including T cells, B cells, and macrophages (Sun et al., 2015). Another candidate gene associated with *Salmonella* infection in the domestic Japanese quail was the DYNLL2 gene which is predicted to be involved in cytoskeletal motor activity and cytoskeletal protein binding. An observed increase in DYNLL2 protein in gamma delta T-lymphocytes (γδ T-lymphocytes) of chickens infected with *Salmonella* Enteritidis showed its role in regulating immune response (Sekelova et al., 2017). Like commercial chicken, domestic Japanese quails are reared on farms under different housing systems such as battery cages, free-range and floor-raised systems, among others. Choice of the housing system in connection to other production factors such as infrastructure, stocking density (farm and flock size), manure collection, disease status of the flock, and rodent and insect load does determine the risk of *Salmonella* infection (Van Hoorebeke et al., 2011).

The ITGB3 gene is involved in the blood coagulation pathway through adrenergic signaling in platelet activation. ITGB3 encodes for glycoprotein IIIa (GPIIIa) and, along with the alpha IIb chain, forms the platelet adhesive protein receptor complex glycoprotein IIb/IIIa (GP IIb/IIIa), which mediates platelet aggregation by acting as a receptor for fibrinogen (Cerhan et al., 2007). It plays a critical role in many cellular processes, such as cytoskeletal organization, cell adhesion, migration, proliferation, and survival (Ridley et al., 1992; W. T. Arthur et al., 2002; Guo et al., 2008). Its immune roles in the T cell development (Luo et al., 2013), B cell development and signaling (Walmsley et al., 2003), epidermal homeostasis resulting in wound healing (Winge & Marinkovich, 2019), and phagocytosis (Lee et al., 2000; Han et al., 2019) are continuously being studied. Phagocytosis biological process, observed in both quail species, is a necessary component of the innate immune response. It plays an essential role in host-defense mechanisms by enabling the uptake and destruction of infectious pathogens through specialized cell types like macrophages, neutrophils, and monocytes. In an infectious condition, the Fc gamma R-mediated phagocytosis opsonizes and clears pathogen particles or microbes (Cox & Greenberg, 2001; Dillon et al., 2015). Similarly, the RAC1 candidate gene detected in domestic Japanese quail was also associated with wound healing by regulating the innate immune response in keratinocytes (Bustelo et al., 2007; Pedersen et al., 2012; Winge & Marinkovich, 2019).

Candidate genes associated with autoimmune thyroid disease (LOC107321549, PRF1, LOC107321563) were detected by both tests in domestic Japanese quail. Obesity has been associated with an increased risk of several autoimmune diseases, including autoimmune thyroiditis in obese strain chicken, mice models, and humans (Kofler et al., 1983; McLachlan et al., 2019; Song et al., 2019). This could be a result of domestic Japanese quails being heavier, sometimes obese, than wild quails due to intense selection pressure through modern breeding techniques aimed at improved meat production (Mills et al., 1997; Lukanov & Pavlova, 2020). LOC107321549 (HLA class II histocompatibility antigen, DM beta chain) and LOC107321563 (Class I histocompatibility antigen, F10

alpha chain) are linked to adaptive immune response, MHC class II protein complex assembly, positive regulation of T cell activation and proliferation biological processes.

PPP1CA, WNT5A, GRIA1, CREB1, ADCY8, and ALK candidate genes, associated with adaptation and behavior, were detected in the wild African harlequin quail. Melanogenesis and ear morphogenesis biological processes are crucial for wild African harlequin quail breeding behavior, social interactions, and survival. Melanogenesis involves melanin production, which plays a significant role in structural plumage in birds (Jeon et al., 2021). Plumage color and patterns are helpful in camouflage, mating, and differentiating between male and female wild African harlequin quails (Mason & Bowie, 2020). Melanin-based patterns are influenced by melanocyte migration, differentiation, cell death, and/or interaction with neighboring skin cells at the cellular level (Inaba & Chuong, 2020). The wild African harlequin quail songs and calls are also essential to their communication and behavior; hence ear morphogenesis is also a vital biological process for their survival and reproduction. The WNT5A gene, among other genes, was implicated in melanogenesis, ear morphogenesis, sex differentiation, and muscle development. Through activating multiple intracellular signaling cascades, the WNT gene family controls cell proliferation, differentiation, apoptosis, survival, migration, and polarity (Kikuchi et al., 2012). According to Kikuchi et al. (2012), the regulation of cellular functions, including migration and differentiation, makes the WNT5A gene a target for selection.

The GluA1 subunit has been implicated in the regulation of circadian rhythms and behavior (Ang et al., 2021). The Glutamate receptor 1 (GluA1), encoded by GRIA1 gene, forms part of the AMPA receptor that mediates fast glutamate signaling in the central nervous system (synaptic plasticity). Positive selection of genes associated with behavioral response to stress and circadian entrainment (GRIA1, CREB1, ADCY8, ALK) could explain the timely seasonal migration and behavioral patterns of wild African harlequin quails. The PPP1CA gene encodes for a protein, one of the three catalytic subunits of protein phosphatase 1 (PP1). The PP1 protein was found to be a key regulator of period and light-induced resetting of the circadian clock in the common fruit fly (Fang

et al., 2007) and different mammals (Eide et al., 2005; Gallego et al., 2006; Schmutz et al., 2011). It regulates the circadian period length, in counterbalance with casein kinase 1δ and ε (CK1δ/ε), through the regulation of the speed and rhythmicity of period circadian regulator 1 and 2 (PER1 and 2) phosphorylation (Lee et al., 2011; Schmutz et al., 2011).

Functional analysis of the candidate gene ACE showed an association with the regulation of secretion and glucose homeostasis. ACE catalyzes the proteolytic conversion of angiotensin I to angiotensin II, which maintains blood pressure stability, fluid balance and electrolytes in the body (Nakai et al., 1995; Danilov et al., 2016). Angiotensin II stimulates sodium and water reabsorption in the kidneys, leading to increased blood volume and blood pressure. In addition, the inhibition of ACE has been shown to improve glucose homeostasis (Danilov et al., 2016). The positive selection of the ACE gene favors wild African harlequin quail migration and its survival in harsh environments with limited water and food resources.

Candidate genes associated with growth and reproduction were detected in the wild African harlequin and domestic Japanese quail. The enriched Hippo signaling pathway plays a crucial role in cellular differentiation, tissue, and organ development by controlling organ size through the regulation of cell proliferation and apoptosis (Justice et al., 1995; Xu et al., 1995; Heallen et al., 2011; Wu & Guan, 2021). It has also been implicated in other diverse roles, such as tissue homeostasis, wound healing and regeneration, immunity, tumorigenesis, and embryogenesis (Wu & Guan, 2021). The Wnt–Hippo signaling pathway-related genes identified in wild African harlequin quails include SOX2, FZD7, WNT3, NKD1, and WNT4. The transcription factor SOX2 is involved in osteoblast differentiation (E. Seo et al., 2013), inner ear development (Kiernan et al., 2005), neurogenesis, and the proliferation and/or maintenance of stem cells (Oesterle et al., 2008), among others. The positive selection of genes associated with tissue and organ size could explain how the wild African harlequin quails have managed to maintain their small body size, supporting their growth and survival in the wild. As a result, the wild

African harlequin quail can fly longer distances during migration and at faster speeds (Wamuyu et al., 2017).

RPS6KB2 and CREB1 genes were involved in the follicle-stimulating hormone (FSH) signaling pathway and growth hormone synthesis, secretion, and action. The RPS6KB2 gene is involved in cell growth and regulation (Slattery et al., 2011), whereas CREB1 encodes a phosphorylation-dependent transcription factor that mediates the response to a variety of cellular processes, including regulation of transcription, signal transduction, glucose homeostasis, and growth-factor-dependent cell survival, proliferation, and memory (Kinjo et al., 2005). CREB1 stimulates transcription upon binding to the DNA cAMP response element (CRE) located in the promoter region of target genes, leading to the recruitment of transcriptional coactivators and the initiation of gene transcription (Shankar et al., 2005). In humans, the CREB/CREB1 gene is involved in immune function (Cerhan et al., 2007) as it promotes proliferation and survival and differentially regulates Th1, Th2, and Th17 responses (Wen et al., 2010). It has also been shown to be a critical driver of vaccine efficacy in non-human primates (Tomalka et al., 2021).

Several candidate genes associated with lipid and cholesterol transport and metabolism in domestic Japanese quails, such as the APOA (APOA1, APOA4, APOA5) and ABCA (ABCA2, ABCA5, ABCA7) gene families, were identified (Albrecht & Viturro, 2007; Dominiczak & Caslake, 2011). Additionally, several muscle cell differentiation, tissue, and structure development genes (CAV2, COL6A3, SLC9A1, SMARCD3, MSX2, PRF1, COL11A1) were identified. The DYNLL2 gene, apart from its role in immune response, was also found to be a regulator of chicken myogenesis, providing insights into breast muscle development in chickens and other birds (Li et al., 2022).

Thyroid-stimulating hormone (TSH) signaling pathway genes (GNA12, GNAO1, MAP2K6, SRC, GNA13) were also identified in domestic Japanese quails. The thyroid hormone signaling pathway has a wide range of functions in terms of individual development, maintenance of homeostasis, cell proliferation and differentiation, and glucose metabolism (Liu et al., 2019). In addition, the cAMP signaling pathway might

elicit a vital role in forming egg-laying traits by influencing ovarian follicle development (X. Chen et al., 2021). Out of the several cAMP signaling pathway genes (VIPR2, MAPK1, RAC1, SLC9A1) identified in domestic Japanese quails, the VIPR2 gene has been implicated in the regulation of egg production as it has been associated with brooding behavior in chicken and geese (Luan et al., 2014; Huang et al., 2022).

In this study, only two genes (CBFB and RET) overlapped between the CLR and iHS tests in the domestic Japanese quail, in contrast to the wild African harlequin quail, where no overlapped genes were observed. CBFB protein is mainly associated with definitive hemopoiesis (Speck et al., 1999). In contrast, RET is known for its protein kinase activity leading to the activation of signaling pathways involved in cell growth, differentiation, and survival, such as MAPK and AKT (Schuchardt et al., 1994; Taraviras et al., 1999).

As indicated by other studies, the CLR test detected old sweeps associated with an adaptation that occurred on a broad time scale (Wang et al., 2018). The iHS test complements CLR by detecting signatures of recent selection in the study populations (Mohd-Assaad et al., 2018).

5.1.4 Transcriptomic landscape of the domestic Japanese quail

Commercial poultry growth and reproduction are affected by various factors such as genetics, feeding regimen, diseases, and poultry production system, among others (King'ori, 2011; Muchadeyi & Dzomba, 2017). Domestic Japanese quails, also reared in intensive poultry production systems, tend to experience similar conditions. Genetic selection for improved feed efficiency and higher meat yield has shown the importance of the role played by genes in influencing growth and reproduction (Baéza et al., 2022). This study identified several differential expressed genes associated with crucial growth and reproduction traits in the domestic Japanese quail. Hence the investigation of its transcriptomic landscape was successful.

The top significant differentially expressed genes were involved in various reproduction processes such as spermatogenesis (TPPP2, DDX25, MNS1, TPPP2, MORN2) and cilium assembly (KIF27, TEKT1, TEKT4, DDX4, MNS1). Spermatogenesis is a critical process

in male reproduction; hence indicators like sperm count and motility are often used to determine the male fertility (Zhu et al., 2019). Kinesin Family Member 27 (KIF27) gene encodes for the Kinesin-like protein KIF27 which belongs to kinesins, a superfamily of the microtubule-dependent motor proteins expressed in the testis and are responsible for spermatogenesis and germ cell transport (Yao et al., 2022). Another gene predominantly expressed in the testis is the TEKT1 gene, a filament-forming protein linked to the development of the sperm tail basal body and axoneme (Larsson et al., 2000). Proper sperm function ensures the fertilization of an egg, and the TPPP2 gene encodes a protein linked to the human sperm motility (Zhu et al., 2019).

Most of the top significant differentially expressed genes had functions associated with spermatogenesis, highlighting how vital the process is in male reproduction. The expression of these genes was higher in low-weight domestic Japanese quails than in heavy-weight quails, showing a relationship between spermatogenesis and the weight of an individual. There has been emerging evidence linking obesity in males to male reproductive potential. Male obesity has been shown to negatively impact reproduction in males by reducing sperm quality by altering their physical and molecular structure (Håkonsen et al., 2011; Palmer et al., 2012). In another study by Sermondade et al. (2012), overweight men were at significantly increased odds of presenting with oligozoospermia (low sperm count) or azoospermia (complete absence of sperm) compared with normal-weight men. Even though artificial selection favors the development of heavy-weight domestic Japanese quails, its effects, in the long run, could prove detrimental to its breeding and production process.

5.2 Conclusion

This study aimed to utilize genomic approaches to assess the genetic background and signatures of selection in wild African harlequin quails. Additionally, a transcriptomic approach was also to be used to investigate differential gene expression in domestic Japanese quails. Mitochondrial DNA molecular marker and SNP information from genotyping by sequencing data were utilized under the genomic approach, whereas RNA-

Seq analysis was used under the transcriptomic approach. Mitochondrial DNA, a small DNA fragment molecular marker, was compared to genome-wide SNP information to check its suitability and effectiveness in examining genetic diversity and variation in animal studies. The findings of this study were then meant to provide crucial information to key stakeholders like Siaya County and other county governments with indigenous wild African harlequin quail populations, Kenya Agricultural and Livestock Research Organization (KALRO), National Environment Management Authority of Kenya (NEMA), Kenya Wildlife Service (KWS), and the Ministry of Agriculture, Livestock, Fisheries, and Co-operatives to allow for biodiversity conservation and proper utilization of its genetic resources.

5.2.1 Genetic diversity and demographic history

Mitochondrial DNA and genotyping by sequencing approaches successfully examined the genetic diversity and demographic history of wild African harlequin quail populations of Siaya County. Therefore, the null hypotheses that mitochondrial DNA marker and SNP data from GBS will not reveal the genetic diversity in wild African harlequin quails were rejected. No population structure was observed in the study regions; however, more study sites from different Counties are recommended to fully capture individuals that follow different migratory routes before any final population structure conclusions are made. There was no evidence of hybridization between wild African harlequin and domestic Japanese quails in the Siaya region, alleviating concerns about the existence of hybrids. A noticeable decline in the number of wild African harlequin quails in the region that was previously observed was also confirmed through the demographic history analysis, raising concerns in line with their conservation status. This study has provided the necessary information required for decision-making regarding their preservation and utilization as a source of protein. Even though the harlequin quail subspecies (*C. d. delegorguei*, *C. d. histrionica* and *C. d. arabica*) conservation status are listed collectively under the “least concern” category according to the International Union for Conservation of Nature (IUCN), more studies aimed at monitoring their distribution and effective population size

changes need to be conducted, especially in areas where the harlequin quail is considered as a complementary poultry protein source.

The mtDNA marker provided insightful information confirmed with genome-wide SNP data, proving that molecular markers based on short DNA fragments are still helpful for genetic characterization studies, especially for researchers in the developing world who might need more financial resources for whole genome sequencing and computational resources required for whole genome analyses. However, it is essential to note that such markers contain less information than genome-wide SNP data. In this study, the mtDNA marker could detect the signal of rapid demographic expansion and tell its approximate time. On the other hand, genome-wide SNP data gave more information, i.e., its whole demographic history from past to present spanning 560,000 years. Admixture analyses using genome-wide data are also superior to mtDNA haplotype-sharing tests done using haplotype data acquired from short DNA fragments.

5.2.2 Signatures of selection detection

Signatures of selection were detected in the wild African harlequin quail. Their differences from domestic Japanese quail selection signatures were also identified using SNP data from GBS; hence, the null hypotheses were rejected.

The findings of this study revealed putative genomic regions and candidate genes associated with immune response, growth, reproductive, morphological, and behavioral traits. The wild African harlequin candidate genes were primarily associated with morphological and behavioral traits, whereas the domestic Japanese quail candidate genes were mainly associated with growth and production traits. Detecting genes associated with morphological and behavioral traits in wild African harlequin quails showed how evolution still favors adaptation traits for wild species. This study also confirmed the effects of years of artificial selection to improve production in the domestic Japanese quail, as the key genes identified were associated with production traits.

Information on the effects of artificial selection on domestic Japanese quails may inform conservation policies for wild quails, especially in regions where domestic quails have

been used to restock wild quail populations. Loss of alleles associated with such adaptation traits may negatively influence the wild African harlequin quail's survival. In addition, this information could also be used to aid the development of domestic wild African harlequin quails, which may help reduce the hunting of the wild species, hence their conservation.

5.2.3 Transcriptomic landscape of the domestic Japanese quail

Differential gene analysis of the testes, associated with growth and reproduction in the domestic Japanese quail, was successful; hence, the null hypothesis was rejected. As expected, most of the significant differentially expressed genes in the testes of the domestic Japanese quail were associated with male reproduction processes such as spermatogenesis, a definitive and characteristic biological process in males. Furthermore, low-weight domestic Japanese quails were found to have higher levels of differentially expressed genes linked to spermatogenesis when compared to heavy-weight quails, a critical effect of years of artificial selection aimed at improved meat production.

Even though the study highlighted differential gene expression between the two conditions, more biological replicates are required for a more definitive investigation of the differential expressed genes behind these traits. Transcriptome analysis of other organs associated with growth and reproduction is also highly recommended, and the validation of the RNA-Seq results using RT-qPCR is also highly recommended.

5.3 Recommendations

The findings of this study recommend the implementation of the following strategies and policies:

i. Encourage the Ministry of Agriculture, Livestock, Fisheries, and Co-operatives, and national agricultural institutions such as the Kenya Agricultural and Livestock Research Organization (KALRO) to develop domestic African harlequin quail breeds using generated molecular information to discourage unlawful harvesting of wild African harlequin quail species.

ii. DNA fragment markers such as mtDNA D-loop are still informative and useful in origin and diversity studies despite providing less information when compared to genome-wide characterization markers and methods.
iii. The development of conservation strategies that will manage hunting and allow for the protection of the wild African harlequin quail diversity and proper utilization of its genetic resources by rural smallholder farmers.
iv. Breeding between wild and domestic quail species should be monitored to prevent the introduction of hybrids in the wild, which may lead to the loss of key adaptation traits.
v. Intensive modern breeding technologies using artificial selection may result in poor reproductive ability in domestic poultry species and should hence be monitored to avoid the loss of alleles associated with reproduction.

The study findings recommend the following approaches for further investigations to ensure a more comprehensive understanding of the wild African harlequin quail population structure, demographic history, and evolution.

1. Sampling wild African harlequin quail populations found in other counties and countries could help develop a clear population structure due to their migratory behavior that hasn't been fully understood yet.
2. The wild African harlequin quail migratory routes should be mapped appropriately to monitor their migration and population size changes effectively.
3. Phylogeographic and climatic oscillation studies should be done on wild African harlequin quails or other wild bird species in the Lake Victoria basin to properly understand the role played by climate change in wild avian species distribution.
4. Sampling more wild African harlequin quail populations will allow more signatures of selection tests, hence improving the identification of valid signatures in its genome.
5. Whole-genome sequencing is recommended, especially for non-model species like the wild African harlequin quail, to build up the findings from this study.

6. Gene expression studies are recommended for comparative analysis with candidate genes identified through signatures of selection analyses.

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APPENDICES

Appendix I: Results of gene ontology analysis for key terms identified by CLR method in wild African harlequin quail

Enrichment term		***P-Value***	**Genes**
cjo04916	Melanogenesis	0.01528632	FZD7, WNT5A, WNT8B, LOC107320948, WNT3, WNT4
cjo04310	Wnt signaling pathway	0.01065333	PPP3R1, FZD7, WNT5A, WNT8B, DKK1, WNT3, NKD1, WNT4
GO:0031532	Actin cytoskeleton reorganization	0.0365151	FGF7, INSRR, PLEK, PARVA, PLEK2
GO:1902724	Positive regulation of skeletal muscle satellite cell proliferation	0.0477268	HGF, GPC1
GO:0042699	Follicle-stimulating hormone signaling pathway	0.0477268	FSHB, FSHR
GO:0002088	Lens development in camera-type eye	0.04467469	CRYGN, WNT5A, PROX1, CDON
GO:0048839	Inner ear development	0.02927857	SOX2, PTPN11, PROX1, ROBO1
GO:0007626	Locomotory behavior	0.03223471	RASD2, FSHR, PTEN, ADCY8, PEX13
GO:0019229	Regulation of vasoconstriction	0.01871609	EDN3, ADRA1B, ADRA1A
GO:0046887	Positive regulation of hormone secretion	0.00818115	ADCYAP1, EDN3, PTPN11
GO:0045670	Regulation of osteoclast differentiation	0.01127175	CEBPB, FSHB, FSHR

Appendix II: Results of gene ontology analysis for key terms identified by CLR method in domestic Japanese quail

Enrichment term		***P-Value***	**Genes**
cjo04672	Intestinal immune network for IgA production	0.003132	LOC107321549, CXCL12, MADCAM1
GO:0001755	Neural crest cell migration	0.001395	RET, SEMA3A, SEMA3B, SEMA3E, RAC1
GO:0010634	Positive regulation of epithelial cell migration	0.046182	TGFB2, RAB25, PPM1F
GO:0006887	Exocytosis	0.048664	RAB25, SNAP23, EXOC4, RAB3IL1, STXBP6
GO:0010634	Positive regulation of epithelial cell migration	0.048785	DOCK5, TGFB2, RAB25, PPM1F
GO:0016477	Cell migration	0.033119	TGFB2, BAMBI, GPC1, SIX2, SDC3, FN1
gga04510	Focal adhesion	0.029392	CAV2, HGF, FN1, TNC, VEGFA, CCND1, COL6A3
GO:0050839	Cell adhesion molecule binding	0.026494	KIRREL3, TJP1, TENM2, NRXN1, MSN
GO:1903427	Negative regulation of reactive oxygen species biosynthetic process	0.006323	PARK7, SLC30A10, RHOA

Appendix III: Results of gene ontology analysis for key terms identified by iHS method in wild African harlequin quail

Enrichment term		***P-Value***	**Genes**
cjo04550	Signaling pathways regulating pluripotency of stem cells	0.00967374	FZD5, LHX5, MAPK14, MAPK13
gga04510	Focal adhesion	0.03757789	PARVG, EGF, ITGB3, PARVB, PPP1CA
cjo04640	Hematopoietic cell lineage	0.02234318	LOC107324620, ITGB3, LOC107317826
IPR009030	Insulin-like growth factor binding protein, N-terminal	0.02524663	FBN3, EGF, CRIM1, VWCE
cjo04658	Th1 and Th2 cell differentiation	0.02605396	MAPK14, LOC107317826, MAPK13
GO:0003779	Actin binding	0.02710011	ANKRD13D, PARVG, MICAL1
cjo04659	Th17 cell differentiation	0.03780637	MAPK14, LOC107317826, MAPK13
gga04261	Adrenergic signaling in cardiomyocytes	0.0510549	CREB1, MAPK14, MAPK13, PPP1CA
cjo04380	Osteoclast differentiation	0.04158861	ITGB3, MAPK14, MAPK13
cjo04611	Platelet activation	0.05718005	ITGB3, MAPK14, MAPK13

Appendix IV: Results of gene ontology analysis for key terms identified by iHS method in domestic Japanese quail

Enrichment term		***P-Value***	**Genes**
GO:0007283	Spermatogenesis	0.041935705	DAZAP1, TMEM203, GPX4, LIMK2
GO:0015485	Cholesterol binding	0.040183512	STAR, APOA1, APOA4, ERLIN2
GO:0008283	Cell proliferation	0.049248554	OCA2, DAZAP1, OGFOD1, ECD, TGFBI
GO:0031016	Pancreas development	0.047218286	ALDH1A2, HNF1B, HNF1A
GO:0060067	Cervix development	0.038627878	LHX1, WNT5A
GO:0030300	Regulation of intestinal cholesterol absorption	0.038627878	APOA1, APOA4
GO:0008211	Glucocorticoid metabolic process	0.038627878	STAR, APOA1
GO:0008203	Cholesterol metabolic process	0.028124765	LIPC, ABCA5, STAR, HNF1A
GO:0042632	Cholesterol homeostasis	0.006641128	ABCA2, LIPC, ABCA5, APOA1, APOA4
GO:0051726	Regulation of cell cycle	0.014287092	STK11, SRC, ZNF703, RACK1, APOA1
GO:0046889	Positive regulation of lipid biosynthetic process	0.015378697	SIRT4, APOA1, APOA4

Appendix V: Questionnaire used for data collection

Sample ID_____ Date_____

Farmer's name_____

Sex _____ Age _____

County _____ Sub County_____

Village_____ GPS_____

Poultry species reared?

Chicken ☐ Guinea fowl ☐ Quail ☐ Duck ☐ Pigeon ☐ Other_____

What type of management system do you practice?

Extensive ☐ semi-intensive ☐ intensive ☐ others, specify_____

Condition of the poultry structures, if any_____

Are there wild harlequin quails on your farm?

Yes ☐ No ☐

How many wild harlequin quails do you capture in a day? _____

Why do you capture wild harlequin quails?

Consumption ☐ Sale ☐ Other_____

Have you noticed if there is a decline in wild harlequin quail numbers on your farm per season?

Yes ☐ No ☐

Do you prefer wild or domesticated quails? _____

Do you have a license to harvest wild harlequin quails?

Yes ☐ No ☐

Appendix VI: Publications from this research work

1. **Ogada, S.,** Otecko, N. O., Moraa Kennedy, G., Musina, J., Agwanda, B., Obanda, V., Lichoti, J., Peng, M.-S., & Ommeh, S. (2021). Demographic history and genetic diversity of wild African harlequin quail (*Coturnix delegorguei delegorguei*) populations of Kenya. *Ecology and Evolution*, 11, 18562– 18574. https://doi.org/10.1002/ece3.8458

2. **Ogada, S.**, G., Musina, J., Lichoti, J., & Ommeh, S. (2022). A review of the distribution, nutritional value, and conservation status of wild harlequin quails (*Coturnix delegorguei delegorguei*) in Kenya. *Journal of Agriculture, Science and Technology*, 21(1).

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Appendix VII: Conferences where this work was presented

1. International Society of Animal Genetics (ISAG) Virtual Conference (July 26-30, 2021).
2. 5th National Museums of Kenya Biennial Science Conference (November 7-9, 2018).