Modelling environmental suitability and estimating effective population size of South African indigenous chickens implication of conservation.

MODELLING ENVIRONMENTAL SUITABILITY AND ESTIMATING EFFECTIVE POPULATION SIZE OF SOUTH AFRICAN INDIGENOUS CHICKENS: IMPLICATION OF CONSERVATION

by

RENEILWE ROSE MOGANO

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Supervisor: Prof BJ Mtileni

Co-Supervisor: Dr TJ Mpofu

Co-Supervisor: Dr K Hadebe

DECLARATION

"I hereby declare that the dissertation submitted for the degree Master of Agricultural Science at the Tshwane University of Technology, is my own work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references".

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Reneilwe Rose Mogano Signature

DEDICATIONS

This study is dedicated to

My parents Mr MJ Mogano and Mrs S Mogano

My brother OJ Mogano, my sisters TE and BA Mogano, and my best friend SK Seapela

I am forever grateful for the support and encouragement you've given me.

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ABSTRACT

Indigenous chickens are an important Farm Animal Genetic Resource (AnGR) for poverty alleviation in rural communities across South Africa. Locally adapted populations thrive despite production and climate challenges, as their extensive production system lacks protection from agro-ecological environments, resulting in their diversity. A lack of standardized descriptions, low investment in determining their production potential, and the rise of new markets all contribute to genetic erosion and dilution. Delays in conservation and improvement, limited market access, and low government investments have resulted in only a well-known conservation program for four indigenous chicken breeds in South Africa at the Agricultural Research Council-animal production. This research was therefore carried out to investigate the environmental suitability of indigenous chickens and estimate the risk status in different regions of KwaZulu-Natal and Limpopo provinces, South Africa. A total of 244 village-based indigenous chickens were collected from rural areas in South Africa, including the Harry Gwala (n=21) and uMzinyathi (n=25) districts of KwaZulu-Natal province and Capricorn (n=85), and Sekhukhune (n=113) districts of Limpopo province. A total of 100 conservation flocks: Ovambo (OV; n=10), Potchefstroom Koekoek (PK) (n=20), Naked Neck (NN; n=20), Venda (VD; n =20), White Leghorn (WL; n=10), White Plymouth Rock (WR; n=10), and New Hampshire (NH; n=10) from the Agricultural Research Council-Animal Production (ARC-AP) were used as reference population. All chickens were genotyped using Illumina chicken iSelect SNP60k Bead chip. The raster package in RStudio was used to extract values from 28 environmental variables which included annual trends (e.g., mean annual temperature and annual precipitation), seasonality (e.g., annual range in temperature and precipitation), and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters), soil variable, solar radiation and elevation. GQIS software was utilized to align layers, resulting in a 109.47 X 126.24mm layer size, and convert them to ASC files. The GGally r package was used to create a correlation matrix, identifying 10 out of 28 environmental variables as key drivers of environmental adaptation, including minimum temperatures, elevation, soil cation exchange capacity, soil clay content, organic carbon content, soil pH, soil silt content, and solar radiation.

ENMeval was used for model tuning and evaluation, which identified linear and quadratic (LQ) features with a regularization-multiple = 0.5 as the best parameter combinations to optimise Maxent and develop suitability maps. Maxent software was used to create suitability maps for chickens in Limpopo and KwaZulu-Natal, with a test gain applied for important variables. Chickens from Dipakakeng, Mgababa, and Podu villages had localized predicted suitability probabilities, while those from Nhlonga had a broader distribution with elevation and BIO6 being important variables. PLINK software was used for genetic diversity calculation. The genetic diversity of indigenous chickens ranged from 0.341 to 0.338 for observed heterozygosity and from 0.381 to 0.387 for expected heterozygosity, whereas the conservation flocks ranged from 0.240 to 0.339 and 0.377 to 1 for both observed and expected heterozygosity. The admixture analysis was conducted using a LEA r package in RStudio, the lowest cross-validation error (0.87) was detected at K = 5. Population structure was done using LEA r package in RStudio, where PC1 and PC2 explained 5.64% of the total variation and resulted in 5 clusters with the Venda, Naked Neck, and White Leghorn being separated from village chickens. SNeP software was used to calculate effective population size, both village chickens and conservation flocks showed a steady decrease of effective population size at 12 generations ago. Redundancy analysis (RDA) was done to examine genotype-environment associations using a vegan r package, the first three RDA axes capture 46.8% of total genetic variation, and 386 outlier SNPs associated with all 10 environmental variables were identified. The results of this study provide insight into the risk status, geographic suitability, and contributing environmental factors of indigenous chickens which can be used to influence conservation and improvement decisions

Keywords: indigenous chickens, genetic diversity, local adaptation, effective population, signatures of selection.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AEZs	Agro-Ecological Zones
AFLP	Amplified Fragment Length Polymorphism
AnGR	Animal Genetic Resources
cM	CentiMorgan
DNA	Deoxyribonucleic Acid
DAD-IS	Domestic Animal Diversity Information System
ENM	Ecological Niche Modelling
EHH	Extended Haplotype Homozygosity
GISs	Geographical information systems
IBD	Identity by descent
iHS	Integrated Haplotype Score
LD	Linkage disequilibrium
SBS	Sequencing by Synthesis
SNP	Single Nucleotide polymorphisms
SFS	Site Frequency Spectrum
Ne	Effective population size
NN	Naked Neck
mtDNA	Mitochondrial Deoxyribonucleic Acid
OV	Ovambo
PK	Potchefstroom Koekoek
Hp	Pooled Heterozygosity

VD	Venda
WL	White Leghorn
WR	White Plymouth Rock
NH	New Hampshire
TMRCA	Time to Most Recent Common Ancestor
NGS	Next Generation Sequencing
PCA	Principle component Analysis
CLR	Composite Likelihood Ratio
ROH	Run of Homozygosity
SOLiD	Sequencing by Oligo Ligation Detection
SAPA	South Africa Poultry Association
SASAS	South African Society for Animal Science
ISAG	International Society for Animal Genetics
AACAA	All Africa Conference on Animal Agriculture

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Indigenous chickens are one of the most widely distributed domestic animal species worldwide (Malomane *et al*., 2021). They have evolved to survive in harsh environmental conditions, including small-scale villages, free-range, and organic production systems, acquiring diverse genetic traits (Bobbo and Baba, 2013; Lawal *et al.*, 2018; Manyelo *et al.*, 2020; Walugembe *et al.*, 2019). In Southern Africa, indigenous chickens are mainly kept in low-output production systems in rural communities (Muchadeyi, *et al,* 2007; Mtileni *et al.*, 2010; Gunya *et al.*, 2020). These chickens enhance household food security by providing eggs and meat, alleviating poverty by employing rural youth, and improving their socioeconomic situation (Kumar *et al.*, 2021). They also contribute to gender equality by empowering women economically (Gunya, *et al.*, 2020; Desta, 2021; Islam *et al.*, 2021). Despite their contribution, indigenous chickens are characterized by low productivity making them difficult to compete with commercial breeds (Desta, 2021b). Therefore, they are often negatively selected or ignored even though they produce high-quality eggs and meat and are disease-resistant (Abebe *et al*, 2015). Hence, farmers are gradually introducing, selecting, and breeding exotic chickens to enhance indigenous chicken production (Gebremariam, and Mazengia, 2017; Lozano-Jaramillo *et al.*, 2019).

This will eventually lead to a decline in the genetic diversity of indigenous chicken populations. The loss of important genetic material will further burden animal production and make it vulnerable to future challenges (Malomane *et al.*, 2019). According to the Domestic Animal Diversity Information System (DAD-IS), in Africa, only 12.0 % of local breeds are not at extinction risk, while 5.5 % of local breeds are at risk and 81.84 % status remains unknown (FAO, 2021). Extinction can be caused by abiotic or biotic factors like disease, predation, habitat loss due to human activities, natural disasters, and climatic and geological changes (Khan *et al.*, 2016). Notably, genetic diversity is essential for animal evolution and conservation genetics, as it enables them to adapt to changing environments (Toro and Caballero, 2005). Therefore, conserving distinct populations will contribute more to the total within-species genetic variation (Fernandez-Fournier, Lewthwaite and Mooers, 2021). The

Identification of adaptive variation driven by extreme environments has emerged as a major focus of evolutionary biology (Shi *et al.*, 2022). Adaptive variation in populations determines their long-term survival, population size, and extinction likelihood, influenced by factors like climate change and future predicted threats (Hohenlohe, Funk and Rajora, 2021). Adaptive variants can be identified using a complementary technique that screens numerous genetic markers for environmental or adaptive traits in any species (Flanagan *et al.*, 2017). Hence, modern genetic sequencing techniques have made this possible.

Advances in next-generation sequencing (NGS) technologies, which are DNA sequencing techniques that have revolutionized genomic research (Behjati and Tarpey, 2013). The development of NGS enables the use of whole-genome loci for conservation purposes, enabling analytical methods to identify an animal's adaptation capacity to environmental change (Flanagan *et al.*, 2017; Hoffmann *et al.*, 2015). Moreover, the landscape genomics approach investigates the adaptive evolution of species in response to spatial environmental heterogeneity (Vincent *et al.*, 2013). Identifying genomic selections of populations evolved through natural and artificial selection can aid in understanding their ability to withstand diseases and harsh environmental conditions (Fleming *et al.*, 2016; Pluess *et al.*, 2016).

1.2 Problem statement

The poultry industry remains South Africa's largest agricultural sector, accounting for 16.6% of total agricultural production and 39.9% of total animal products according to South African Poultry Association (SAPA) (2021). South Africa is the most substantial commercial chicken producer on the African continent, followed by Egypt, Morocco, Nigeria, and Algeria, in that order (Nkukwana, 2019). They can efficiently convert feed types into protein, including residuals from households, food processing, and foraging (Nkukwana, 2019; Kumar *et al.*, 2021). Their meat and eggs are affordable, nutritious protein sources with no religious restrictions, making them a superior choice over other animal products like pork (Belay and Oljira, 2019; Daghir *et al.,* 2021)**.** In addition, for religious holidays like New Year's, Christmas, and Easter, chicken meat is the only special meal that can be afforded by the impoverished (Belay and Oljira, 2019). Economically, farmers rely on chicken sales as a source of income because they require little capital, making them an affordable start-up business for almost every

household (Manyelo *et al.*, 2020). In South Africa, a farmer can charge an average of R80 (USD 7.22) per chicken, according to Gunya *et al.*, (2020). Single bird sales or sales of few numbers characterize most rural marketplaces, with many sellers competing (Oljira, 2019). For that reason, such marketing structures are often overlooked or criticized since they are not sustainable (Asresie and Mitiku, 2015).

Chickens have various roles that they hold in different social, economic, and cultural customs (Mishra *et al.,* 2020). Indigenous chickens contribute to cultural beliefs, it is believed that white or black feathered chickens can shield off bad spirits that target family members (Mishra *et al*., 2020). In other cultures, chickens are commonly exchanged as a gift in rural households (Assefa, 2019), or as a provision of a special meal for an important visitor or a relative (Assefa, 2019). Indigenous chicken farmers face environmental and management challenges such as disease, predation, insufficient nutrition, climate change, lack of veterinary services, lack of proper housing, lack of feeding, and inbreeding (Malatji *et al.*, 2016; Desta, 2021a). Despite constraints, Indigenous chickens offer potential resilience genes and traits that should be preserved for future use in response to the evolving environment (Markos *et al*., 2016). Hence, the diversity within indigenous chickens is large and this can provide the breeding base for animals that are adapted to a variety of local environments (Okumu *et al.*, 2017).

Chickens have gradually evolved to form specific environmental adaptations such as arid tolerance, desert tolerance, cold tolerance, and plateau tolerance (Luo *et al.*, 2020; Tian *et al.*, 2020). Shi *et al.* (2022) found that SNPs in two genes (*SLC3A1* and *TSHR*) appeared to be strongly related to adaptation to tropical temperatures in African local chicken ecotypes. According to a study by Zhang *et al.* (2016) highland chicken populations expressed genes related to the development of cardiovascular and respiratory systems, DNA repair, immune response, and radiation response. Indicating a strong adaptation to oxygen scarcity and high-intensity solar radiation. In addition, indigenous chickens have developed resistance to parasite infection and can endure shortages of both food and water (Bettridge *et al.*, 2018; Ibeagha-Awemu *et al.*, 2019; Malatji *et al.*, 2016). Two Ethiopian chicken populations showed a solid proof of co-adaption with ascarids and lice parasite populations as well as genetic resistance heritable traits to both parasitism (Bettridge *et al.*, 2018).

Farmers' preferred traits can aid in designing and implementing indigenous stock genetic improvement projects that are agro-ecologically friendly and sustainable (Brown *et al.*, 2017; Yakubu *et al.*, 2020). A study by Tilahun *et al*. (2022) indicated that farmers prioritize adaptive traits like predator escape, disease resistance, stress tolerance, and scavenging over economic traits. In some studies, both economic and adaptive traits were preferred by farmers (Okeno *et al.*, 2012; Gebremariam *et al.*, 2017). However, it is emphasized that the characterization of animal genetic resources is a critical effort for the long-term usage of animal genetic resources (AnGR) (Samaraweera *et al.*, 2021). The AnGR is a term that encompasses all animal species, breeds, and strains of economic, scientific, and cultural value to humanity, utilized in agriculture and food production (Rege and Gibson, 2003).

Unfortunately in most Southern African countries, there is limited data on the characteristics of village-based indigenous chickens making it difficult to capture the diversity that could be used in conservation strategies (Muchadeyi and Dzomba, 2017). In addition, the diversity of these chicken populations is threatened by the use of exotic breeds leading to the extinction of indigenous chickens (Haftu, 2016; Okumu *et al.*, 2017), and an uncontrolled mating system that led to high levels of inbreeding (Zulu *et al*., 2015). Even so, the genetic mechanism underlying the adaptation of indigenous chickens is unclear. Hence, the environmental factor that triggers adaptive response has not been analysed thoroughly (Muchadeyi and Dzomba, 2017). To achieve desired production goals, sustainability, conservation, and utilization of the indigenous chicken, knowledge of genetic mechanisms underlying the adaptation is an entry point. This research was therefore carried out to investigate the environmental suitability of indigenous chickens and estimate the risk status in different regions of KwaZulu-Natal and Limpopo provinces, South Africa.

1.3 Significance of study

The study introduces a novel method for preventing the genetic degradation of indigenous chickens using genomic population and distribution modelling. The study can aid conservation efforts by assessing indigenous chicken genetic variability, identifying SNPs linked to specific environmental condition, and predicting their future distribution under climate change. Therefore, the study's implication can aid in future planning and management to preserve genetic adaptive variation within individuals

and between populations and improve knowledge on the structure of indigenous chicken populations.

1.4 Study aim

To investigate the environmental suitability of indigenous chickens and estimate the risk status in different regions of KwaZulu-Natal and Limpopo provinces of South Africa.

The specific objectives of the study were:

i. To investigate the environmental suitability of indigenous chickens from KwaZulu-Natal and Limpopo provinces using a species distribution modelling.

ii. To estimate the genetic diversity, effective population size, and population structure as well as genomic signatures of adaptation of the indigenous chicken population at the village level from KwaZulu-Natal and Limpopo provinces.

1.5 Hypotheses

The hypotheses tested were:

i. The environmental suitability of indigenous chicken from regions under study in KwaZulu-Natal and Limpopo provinces are significantly different and cannot be predicted**.**

ii. The estimated effective population sizes of indigenous chicken from Kwazulu-Natal and Limpopo provinces are not significantly different.

1.6 Research outputs and author contributions

The following research outputs were generated during the study:

1.7 Peer-reviewed publications

Mogano RR, Mpofu TJ, Mtileni BJ and Hadebe K. 2024. The risk status, signatures of selection, and environmental suitability of village-based indigenous chickens from certain regions of Limpopo and KwaZulu-Natal provinces of South African, section Livestock Genomics. The manuscript has been submitted in a peer-reviewed journal,

Frontiers in Genetics (Manuscript ID: 1450939). This paper forms part of this dissertation and it's entirely the work of **Miss. Mogano.**

Mogano RR, Mpofu TJ, Mtileni B and Hadebe K. 2024. South African indigenous chickens' genetic diversity, and the adoption of ecological niche modelling and landscape genomics as strategic conservation techniques. The manuscript has been submitted to *Poultry Science's* peer-reviewed journal (Manuscript ID: PSJ-D-24-01355). This paper forms part of this dissertation and it's entirely the work of **Miss. Mogano.**

1.8 Conference proceedings

Mogano RR, Mpofu TJ, Mtileni BJ, and Hadebe K., Modelling environmental suitability and estimating effective population size of South African indigenous chickens: Implication for conservation. Proceedings of the 53rd Annual Congress of the South African Society for Animal Science, 26-28 September 2022, Pietermaritzburg. This paper forms part of this dissertation and it's entirely the work of **Miss. Mogano**.

Mogano RR, Mpofu TJ, Mtileni BJ, Hadebe K, Matelele T, and Chokoe T., Landscape genomic approach to estimate the environmental suitability of village-based indigenous chickens in South African major production regions. Proceedings of the 39th International Society for Animal Genetics conference, 2-7 July 2023, Cape Town. P45. This paper forms part of this dissertation and it's entirely the work of **Miss. Mogano.**

Mogano RR, Mpofu TJ, Mtileni BJ, and Hadebe K. Characterization of the indigenous chicken small-scale production systems in different regions KwaZulu-Natal and Gauteng provinces of South Africa. Proceedings of the 8th All Africa Conference on Animal Agriculture (AACAA8). Travelodge, Gaborone, Botswana, 26 -29 September 2023. This paper forms part of this dissertation and it's entirely the work of **Miss. Mogano.**

1.9 Dissertation Overview and Layout

The main aim of the study is to investigate the environmental suitability of indigenous chickens and estimate the risk status in different regions of KwaZulu-Natal and Limpopo provinces of South Africa. Ecological niche modelling and population

structure analysis were applied to achieve the objectives of the study. The dissertation contains 5 chapters, consisting of a general introduction, a review of the literature, two chapters of the research and, a general discussion and conclusion. In the two research chapters, each chapter is structured as a manuscript with its abstract, introduction, materials and methods, results, discussion, and conclusion.

In chapter 1, the background and justification, problem, purpose, hypotheses of the study and research outputs generated from the study were highlighted.

Chapter 2 covers historical and current literature on the domestication of indigenous chickens in Southern Africa, and their sustainable use. Characterization of indigenous chicken production systems, conservation of indigenous chickens, modelling environmental suitability and adaptation, next generation sequencing technologies, signatures of selection and their applied methods, effective population size and its estimation methods and lastly future prospects and opportunities of the study.

Chapter 3 covers the first objective of study that investigated the environmental suitability of indigenous chickens from KwaZulu-Natal and Limpopo provinces using a species distribution modelling

In Chapter 4, we explore the second objective, in which the genetic diversity, effective population size, and unrevealed adaptive patterns of indigenous chickens were evaluated.

Chapter 5 presents a summary, conclusion, and recommendations derived from the study.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The use of AnGRs is essential to the daily lives of many developing and underdeveloped countries (Eusebi *et al*., 2020). Human population growth, changing markets, and climate change impact the availability of these AnGRs (Paiv *et al.*, 2016). These animals live in human-defined and human-influenced environments and are exposed to selection from both human and environmental influences (Sponenberg, 2020). For that reason, efforts should be made to prevent the loss of AnGRs and to protect them (Hasan, 2017). Notably, genetic diversity is a force that drives population resilience; its loss will eventually lead to the extinction of these AnGRs (Ovaska *et al.*, 2021). Above all, indigenous chickens are one of the important AnGRs among limited communities of Southern Africa (Mtileni *et al.*, 2012).

Indigenous chickens are preferred due to their scavenging and mothering abilities behaviours (Mahoro *et al.*, 2018), adaptation to varied production, and low management requirements (Bobbo and Baba, 2013; Lawal *et al.*, 2018; Walugembe *et al.*, 2019). However, the unrestrained crossbreeding of indigenous breeds with exotic breeds to create highly productive breeds poses a threat to the genetic diversity of indigenous chicken populations (Agarwal *et al.*, 2020). At present, genetic diversity parameters can be estimated directly from DNA, providing accurate estimates of genetic variances between and within breeds, populations, and individuals (Eusebi *et al*., 2020). Informative molecular markers, such as microsatellites and single nucleotide polymorphisms (SNPs) are a type of genomic variation that are robust and present in all genomes (Liu *et al.*, 2019), have greatly simplified the assessment of the genetic diversity of indigenous chickens (Mekchay *et al.*, 2014; Flanagan and Jones, 2019). Microsatellites, often found in non-coding genomes, are not linked to phenotype-affecting genes, making them infrequently used in studying local breeds' adaptive genetic diversity (Chokoe *et al.*, 2022). Therefore, SNP markers have revolutionized the evaluation of genetic diversity and improved animal prioritization for genetic diversity conservation (Koopaee and Koshkoiyeh, 2014). The chicken genome was sequenced and more than 2.8 million SNPs were published in 2004 (Hiller *et al.*, 2004; Muir *et al.*, 2008). Through continuous development, a 60K bead chip for chicken was developed and it covers the complete genome evenly (Groenen *et al.*,

2011). At present, the Affymetrix® Axiom® 600 K SNP Array is the first SNP genotyping array for chickens that has been made commercially available as a product to the public (Kranis *et al.*, 2013). SNP arrays have low mutation and false genotyping rates and are abundant in the genome, making them ideal for automation and standardization in high-throughput technologies (Gärke *et al.*, 2012). SNP arrays are crucial in studying species evolution, domestication, breed formation, complex trait genetic mechanisms, and improving selection methods for animal production genetic improvement (Fan *et al.*, 2010).

Locally adapted African chicken populations are highly genetically diverse and heterogeneous (Akbarian *et al.*, 2016; Malomane *et al.*, 2019), highlighting their value in genetic conservation (Wong *et al.*, 2017). Most indigenous chickens have not been subjected to artificial selection but have been subjected to natural selection (Guèye, 2000; Muchadeyi and Dzomba, 2017). This resulted in a variety of morphologies, productivity levels, and disease resistance, which ensures that chickens can survive in diverse ecological zones (Muchadeyi and Dzomba, 2017). Therefore, ecotype variation accounts for the majority of the genetic variation in local African chickens (Mpenda *et al.*, 2019). Malomane *et al.* (2019) reported a high genetic diversity maintained in the wild and less selected African chicken populations. High genetic diversity persists due to a larger mating pool, lower inbreeding rates, and genetic material exchange through breed inter-crossing, reducing artificial selection (Dharmayanthi *et al.*, 2017; Mtileni *et al.*, 2016). This is also supported by Nxumalo *et al.* (2020) who revealed that there is a significant genetic variety within and between ecotypes, with distinct sub-structuring between them. The potential of these species necessitates further investigation into improvement strategies based on their genetic differences and responses to different management settings and environmental conditions (Mujyambere *et al.*, 2022). Therefore, this chapter critically reviews the historical and current literature on the domestication of indigenous chickens, production systems, environmental adaptation, next-generation technologies, signatures of selections, and the effective population size of indigenous chicken populations.

2.2 History of indigenous chickens in Southern Africa

Domestic chickens are believed to have resulted from multiple domestication events over the last 8000 years (Hata *et al.*, 2021; Sawai *et al.*, 2010). Domestic chickens belong to the genus *Gallus,* which includes four morphological species: the Red junglefowl (*Gallus gallus)*, the Grey junglefowl (*Gallus sonneratii*), the Ceylon junglefowl (Gallus *lafayetti),* and the Green junglefowl (Gallus *varius)* (Sawai *et al.*, 2010). The red junglefowl is considered the ancestor of all indigenous chickens due to its morphological similarities associated with adaptation to hot and dry environments, such as the naked neck phenotype, small body size, and frizzled plumage (Dessie *et al.*, 2011; Dahloum *et al*., 2016). The natural habitat of red junglefowl is the sub-humid and humid tropical areas in South and Southeast Asia (Lawal *et al.*, 2018). In contrast, indigenous chickens are effectively adapted to diverse environments, including arid and semi-arid areas (Al-jumaili *et al.*, 2020). It is revealed that domestic chicken and red junglefowl share two sweep regions, with distinct roles in temperature gradient adaptation, production, reproduction, and immunity for each chicken population (Lawal *et al.*, 2018). Hence, evolution is a continuous process, starting with the initial animal domestication events, it allows us to exploit all the potential of livestock and poultry species (Darji *et al.*, 2022).

The analysis of mitochondrial DNA (mtDNA) allows the identification of wild ancestors and the maternal lines that have contributed to a breed or population (Di Lorenzo *et al.*, 2015). The introduction of indigenous chickens in Africa remains relatively little documented (Mwacharo *et al*., 2013). The earliest evidence of chicken on the African continent is from Egypt and it dates from around 2000 BC (Hassaballah *et al.*, 2015). Molecular evidence supports two arrival waves for domestic chickens in Africa (Mwacharo *et al*., 2013). The first wave of African arrivals, around 3000 years ago, originated from the Indian subcontinent via maritime and terrestrial routes, followed by the second wave in the mid-first millennium AD along Africa's eastern coast (Gheyas *et al.*, 2021). Domestic chickens were introduced in Southern Africa during the 1600s by early settlers and traders from Europe and sub-Saharan Africa (van Marle-Koster *et al.*, 2008). In contrast, Mtileni *et al.* (2011a) reported the maternal lineage of South African indigenous chickens assumed to be of Chinese, Southeast Asian, and Indian sub-continental origin. The introduction and spread of domesticated chickens into Africa and their subsequent spread within and between countries significantly

influenced the genetic makeup of indigenous African chickens (Osman *et al*., 2016). Since their introduction and domestication, African chickens have played an important role in people's livelihoods, making them one of the valuable genetic resources.

2.3 Sustainable use of indigenous chickens in Southern African

In the varied agro-ecological zones (AEZs) of South Africa, indigenous chickens are reared to fulfil multiple purposes, meeting nutritional, economic, and socio-cultural needs, such as gifts payments (Assan *et al*., 2017; Belay and Oljira, 2019; Tamir *et al.*, 2019). Their use and value differ from region or community (Table 2.1) (Padhi, in press). For example, in Rwanda, 47% of chickens are reared for egg production and 39% for home consumption (Mahoro *et al.*, 2017).

In Ethiopia, 27 to 51%, 19 to 44 %, and 25 and 20 % are reared for cash income, home consumption, cultural use, and replacement of flock, respectively (Moges *et al.,* 2010; Adduisu *et al*., 2013). In Zimbabwe, 65% of indigenous chickens are used to generate income (Mlambo *et al.*, 2011). In South Africa, 28.4% are used to generate income, 43.2% for home consumption, and some were reserved for incubation and 13.6% for cultural use (Nyoni and Masika, 2012). Indigenous chickens are also relevant in promoting gender equality in developing countries (Abebe and Tesfaye, 2017). Women are commonly the ones who manage and make investment decisions regarding chicken farming. However, this may differ from region to region (Campbell *et al.*, 2018; Belay and Oljira, 2019). In Rwanda, 30.8% of children owned the flock compared to women with only 16.9% (Hirwa *et al.*, 2019). In South Africa and Zimbabwe, 73.1% and 58% of women own the flock, respectively (Mlambo *et al.*, 2011; Idowu *et al*., 2018), and in Ethiopia, only 67% of women-owned the flock (Islam *et al.*, 2021). It is believed that chicken management is a female occupation because they are known to be at home to take care of the family and manage chickens (Issa *et al.*, 2013; Idowu *et al*., 2018).

Table 2.1: Role of indigenous chicken in rural communities in Southern Africa.

Role/Purpose of indigenous chicken	**Country (Reference)**
Nutritional needs	
Egg production	South Africa (Mtileni *et al.*, 2009; Yusuf *et al.*, 2014), Zimbabwe (Muchadeyi *et al.*, 2004), Botswana (Moreki *et al.,* 2010), Zambia (Simainga *et al.*, 2011)
Meat production	Zimbabwe (Muchadeyi *et al.*, 2004), Zambia (Simainga *et al.*, 2011)
Home Consumption	Zambia (Simainga *et al.*, 2011), Malawi (Gondwe and Wollny, 2007), South Africa (Yusuf *et al*., 2014; Idowu *et al*., 2018), Namibia (Masaire *et al.*, 2018), Mozambique (Tomo, 2009), Lesotho (Lebajoa, 2001)
Economic needs	
Income generation (sales)	Botswana (Gabanakgosi *et al.*, 2013; Moreki *et al.,* 2016), Madagascar (Ursule *et al.*, 2020), Swaziland (Bongani and Masuku, 2013), Zimbabwe (Mlambo *et al.*, 2011)
Investment	South Africa (Tarwireyi and Fanadzo, 2013)
Socio-cultural needs	Zimbabwe (Mapiye *et al.*, 2008), South Africa (Nyoni and Masika, 2012)
Environment	
Biological pest control	South Africa (Malatji *et al.*, 2016), Namibia (Petrus, 2011), Zimbabwe (Muchadeyi *et al.*, 2005)

Indigenous chickens can also help in cleaning the environment during scavenging and contributing to nutrient recycling (Desta, 2021a) and biological pest control (Malatji *et al.*, 2016), also their excreta and litter materials are used to convert ecologically degraded land to cultivatable lands (Malatji *et al.*, 2016). However, local chicken farming in Southern African countries remains in the developing stage (Liswaniso *et al.*, 2020). Despite their roles, characterization of indigenous chickens is crucial for understanding their types, breeds, and risk status, enabling conservation decisions,

and determining what to conserve. The first step is to characterize the production system to identify threats and opportunities for overall improvement (Mtileni *et al.*, 2009; Dana *et al.*, 2010).

2.4 Characterization of indigenous chicken production systems

Production systems can be categorized based on the resources available, flock size, housing structure, vaccination program, and feeding system (Conan *et al.*, 2012). Indigenous chickens in South Africa are commonly reared under a traditional scavenging system (extensive system) by smallholder and village farmers (Mtileni *et al.*, 2009; Gunya *et al.*, 2020); however, they can also be reared under a semi-intensive and intensive production system (Mtileni *et al.*, 2009). Noteworthy, the intensive production system is rare due to the low productivity of indigenous chickens, the cost of feed, and poor management (Desta, 2021a). Indigenous chickens are mostly reared as part of mixed farming in the extensive system, to a lesser degree, in semi-intensive systems (Mtileni *et al.*, 2009; Magothe *et al.*, 2012). In the extensive chicken production system, the indigenous chickens are exposed to predators, harsh environmental conditions, parasites, and disease outbreaks that co-exist in the environment (Muchadeyi *et al*, 2007; Mahoro *et al.*, 2017). This production system is characterized by poor housing infrastructure, low-quality feed, and limited healthcare input (Mtileni *et al.*, 2012). In this system, there are no proper records kept, thereby the farmers depend upon recall to trace the performance of their flock (Abdelqader *et al*., 2007; Desta, 2021). However, this is opposed, as some farmers do note a period of inactivity in their local chicken production due to avian diseases (Moussa *et al.*, 2019).

2.4.1 Flock size and structure

The flock size and management among indigenous chickens in African countries vary (Sambo *et al.*, 2015; Manyelo *et al.*, 2020). Birhanu *et al.* (2021) analyzed data from 3,555 indigenous chicken keepers in Nigeria, Ethiopia, and Tanzania, finding an average flock size of 20.3 chickens per family, with Ethiopia having 8.0 chicks and Nigeria having 26.5. Therefore, variability in flock size is associated with the production system, management, and availability of feed under subsistence chicken farming.

Table 2.2: Household flock sizes in several countries across Africa as described in different studies.

Country	Locations	Flock size (range)	Flock size (Mean)	Reference
Zimbabwe	Zhombe	2-75	20.7	(Mlambo *et al.*, 2011)
	Rushinga	1-50	17.0±10.5	(Muchadeyi *et al.*, 2004)
South Africa	Eastern Cape	3-45	17±2	(Nyoni and Masika, 2012)
	KwaZulu-Natal	1-52	17.2 ± 1.4	(Omonijo and Mukaratirwa, 2022)
	Limpopo, KwaZulu-Natal	10-30	22.03±2.85 28.40±2.57	(Malatji *et al.*, 2016)
Botswana	Serowe-palapyt sub-district	1-20	18	(Moreki, 2010)
	Kgatleng district	11-34	22.6±6.85	(Mushi *et al.*, 2006)
Kenya	Komolorume and Kawere region	1-64	19	(Olwande *et al.*, 2010)
	Siaya, Turkana, West Pokot	1-81	22.40±0.8	(Okeno *et al.*, 2012)
Uganda	Kamuli district	1-14	6.7 ± 0.2	(Natukunda *et al*., 2011)
	Northern-Western regions	1-20	19.80 ± 1.21	(Yussif *et al.*, 2022)

The difference in flock size is mainly affected by predation, diseases, season, and theft (Moges *et al*., 2010; Yusuf *et al*., 2014). These flocks are reared as a mixed flock combing chickens of different age groups (Muchadeyi and Dzomba, 2017). Studies on flock structure have shown an average sex ratio of 1 cock: 5 hens in most African countries (Gondwe, 2004; Muchadeyi *et al*., 2007; Abdelqader *et al.*, 2008).

Mujyambere *et al.* (2022) highlighted that the flock structure in these countries varies according to ratios of cock: hens: pullets: cockerels: chicks, ranging from 1:3:3:1:5 (in Ethiopia) to 1:6:2:1:5 (in Zimbabwe), 1:4:4:3:6 (in Kenya), and 1:4:5:4:8 (in Uganda). Small flock size reduces effective population size due to restricted gene flow, increased inbreeding, and genetic drift. For example, local farmers reported that no other breeds were introduced into their chicken flocks (Pham *et al.*, 2013). Therefore, the flock had no diversity, resulting in a less fit population and fewer reproducing adults to contribute to the next generation.

Table 2.3: Flock structure in several African countries.

Flock structure (overall mean)						
Country	**Hen**	**Cocks**	**Pullets**	**Cockerel**	**Chicks**	**Reference**
South Africa	12..52	4.86	4.45 (growers)		8.16	(Yusuf *et al.*, 2014)
Zimbabwe	23.2 ± 2.62	1.5 ± 0.27	4.2 ± 1.35	1.9 ± 0.43	10.2 ± 1.94	(Muchadeyi *et al.*, 2004)
Rwanda	7.1	2.2	4.7	3.9	8.3	(Hirwa *et al.*, 2019)
Kenya	6.03±0.1	2.01±0.1	4.01±0.3	2.32±0.2	8.02±0.1	(Okeno *et al*., 2011)
Ethiopia	4.3±0.8	1.7±0,03	1.03±0.06	0.4±0.7	1.26±0.11	(Tadele *et al*., 2019)
Uganda	14.52±0.8	3.90±0.32	-	-	10.08±1.39	(Natukunda *et al*., 2011)
Tanzania	56.98	3.13	2.887	2.528	62.547	(Ngongolo and Chota, 2021)

2.4.2 Feed and water provision

In an extensive chicken production system, indigenous chickens roam around freely without restriction and scavenge for feed to meet their nutritional requirements (Mtileni *et al.*, 2009; Melesse, 2014). Therefore, their feed resources vary depending on local conditions (Mtileni *et al.*, 2009; Kingori *et al*., 2010). However, some farmers may provide supplementary feeds in the form of cereal grains such as maize, sorghum, and household waste which are often sprinkled on the ground (Masaire *et al.*, 2018; Desta, 2021). Feed supplements are often offered early in the morning (Waktole *et al.*, 2018). However, this disagrees with other studies, Gebremariam *et al.* (2017) highlighted that 36.7% of the farmers supplemented their chickens three times a day, 28.8% twice a day, and 35.6% once a day. Bekele *et al*. (2015) indicated that 92.9% of farmers supplemented their chicken during the rainy season when feed is scarce, and 5.2% provided supplements during the dry season. Chickens of different age classes are fed together (Larbi *et al*., 2013; Islam *et al.*, 2021).

In contrast, studies indicated that farmers fed separately to different age classes preferably young chicks and hens (Yemane *et al.*, 2013; Bekele *et al*., 2015). Concerning water provision, drinking water is irregularly provided (Larbi., *et al* 2013; Mazimpaka *et al.*, 2018; Islam *et al.*, 2021). Studies indicated that farmers provided drinking water in different containers such as plastic containers, cut vehicle tyres, plastic bowls, broken clay pots, and tins (Kingori *et al*., 2010; Yusuf *et al*., 2014).

2.4.3 Housing

Housing under the extensive chicken production systems is not developed, and where it exists, low-grade materials such as half drums without air inlets are used to provide shelter (Mtileni *et al.*, 2013). In most households, chicken housing is provided for night sheltering only (Yusuf *et al*, 2014; Zulu *et al.*, 2015; Waktole *et al.*, 2018; Islam *et al.*, 2021). Material such as wire mesh and corrugated metal sheets, thatched bricks, and mud are used to shelter chickens (Masaire *et al.*, 2018). The lack of proper housing facilities for indigenous chickens could be due to a lack of awareness, cost, and availability of housing materials (Islam *et al.*, 2021). Some farmers shared the same house with the chickens at night but in separate rooms (Mengesha, 2012; Assefa *et al*., 2019). Woven baskets and perch in the kitchen and sheep yard are used to shelter chickens at night (Assefa *et al*.,2019). As a result, this can compromise the health of

both owners and chickens through the transmission of diseases and parasites.

2.4.4 Diseases and predators

In an extensive chicken production system, indigenous chickens are constrained by a high mortality rate due to infectious diseases and parasites (Okeno *et al*., 2012; Marwa *et al.*, 2016). A study conducted by Malatji *et al.* (2016) indicated that over 71.80% of farmers in Limpopo and KwaZulu-Natal provinces reported Newcastle disease as the leading cause of mortality, alongside fowl pox and infectious bursal diseases. The disease challenge is aggravated by the absence of epidemiology records leading to the reoccurring of diseases with no proper treatment and managerial facilities to prevent such infections (Sahin *et al.*, 2015). There are no health control management practices that include isolating sick birds from the rest of the flock as well proper carcass removal practices compromising the health of healthy animals (Mohammed, 2018). Indigenous chickens face early life pathogen and disease risks but have developed defense mechanisms against certain diseases (Bettridge *et al.*, 2014). Therefore, they possess valuable genes controlling specific behavioural, physiological, disease, and parasite resistance traits (Minga *et al*., 2004).

Control of viral infection by vaccination remains a challenge under traditional systems due to the lack of vaccination programs, limited veterinary services in rural areas, and being expensive (Blackie, 2014; Ekunseitan *et al.*, 2016). Noteworthy, in this production system, farmers use traditional medicines such as aloe vera, aloe ferox, salt, garlic, buttercup, ginger, and red paper to treat various chicken diseases and control parasites (Idowu *et al*., 2018; Kejela *et al.*, 2019; Ndlovu *et al.*, 2021).

Low-input and low-output free-range production systems accommodate poorly resource farmers but expose the flock to predators, especially chicks (Anyona *et al*., 2023). Otiang *et al*. (2020) reported that the mortality of chicks accounted for 60% due to predation. Common predators were dogs, cats, rats, snakes, hawks, and eagles (Muchadeyi *et al*., 2005; Asresie *et al*., 2015). Traits such as mothering abilities have been reported to reduce chick mortality due to predation and breeds such as Venda and Potchefstroom Koekoek are known for their mothering abilities (Manyelo et al., 2020). The ability to hatch the optimum proportion of eggs set for hatching and to look after the chicks, is the most important trait in chicken profile choice among rural farmers (Terfa *et al*., 2019). This ensures the inheritance and transmission of genetic

resources from generation to generation, thereby contributing to the sustainability and diversity of indigenous chickens.

2.5 Conservation of Indigenous Chickens in South Africa

2.5.1 Conservation strategies

There are two major conservation strategies used to ensure the safeguarding of endangered species, *in-situ* and *ex-situ* strategies (Floris *et al.*, 2020; Hanson and Ellis, 2020). *In-situ* conservation is defined by breeds that are kept in their natural habitats or farms, which are traditional production methods (Manyelo *et al.*, 2020), with no changes made to how they are managed, and fed (Hamid, 2019). Jawor *et al.* (2020) found that genetic variability measurements had no statistically significant differences between expected and observed heterozygosity, indicating that the structure of these local chicken populations has not changed after ten years of *in-situ* conservation. Zeng *et al.* (2022) demonstrated that there is a higher genetic diversity and differentiation that occurred during in-situ conservation, while reduced genetic diversity occurred during the *ex-situ* conservation program of three indigenous chicken breeds. *Ex-situ* conservation involves maintaining breeds outside of their traditional production settings through technological advancements like cryopreservation (Hanson and Ellis, 2020) and has a role as a backup to *in-situ* conservation in case genetic problems occur (Asmara *et al.*, 2017). Even so, this *ex-situ* has inherent issues, including costly genotype preservations, and can spread disease or lead to a loss of association between genotypes and the environment (Hamid, 2019).

In the year 1994, South African indigenous chickens were recognized as a genetic resource that needs to be conserved (van Marle-Koster and Casey, 2001). The Agricultural Research Council Animal Production (ARC-AP) commenced a breeding program in 1994 for four indigenous chickens as a base for conservation and genetic improvement (van Marle-Koster and Casey, 2001; Mtileni *et al.*, 2016). These breeds were represented by the Naked Neck, Lebowa-Venda, Ovambo, and Potchefstroom Koekoek and have been managed under conservation to date. These animals originated from rural areas in the Northern and Southern parts of South Africa and Ovamboland in Namibia (van Marle-Koster and Nel, 2000). The program was structured to avoid inbreeding and to conserve observed phenotypic differences and genetic variation within breeds (van Marle-Koster *et al.*, 2008).

Studies have proven that there are differences in genetic variation, genetic relation, and effective population size between conserved indigenous chickens and village chicken populations (van Marle-Koster *et al.*, 2008; Mtileni *et al.*, 2011b; Khanyile *et al.*, 2015). Noteworthy, a previous study indicated a decrease in genetic variation in both conserved and village population flocks (Khanyile *et al.*, 2015), which could be due to poor management and inbreeding as well as bottlenecks experienced by chickens (Mtileni *et al.*, 2016). Therefore, the characterization of the production systems is the first step to take if genetic improvement is to be achieved (Okeno *et al*., 2012). It helps us understand production, management, farmer's breeding practices, markets, consumer needs, and related factors that are essential to developing an overall improvement strategy (Okeno *et al.*, 2012; Musa, 2022).

2.5.2 Breeds under conservation

Naked Neck

The naked neck is from a heterogeneous pool of village chickens in the Eastern Cape province of South Africa and the phenotype of this breed is caused by a single gene (*Na* gene) that is dominantly expressed (Khanyile *et al.*, 2015; van Marle-Koster *et al.*, 2008). Naked neck chickens can thrive by scavenging for food and are well-adapted to harsh environments (Manyelo *et al.*, 2020). They are also dual-purpose breeds, they are reared for both meat and egg production (Abdi - Soojeede, 2022). The naked neck is considered to be the only breed with a distinct phenotype (Khanyile *et al.*, 2015; van Marle-Koster *et al.*, 2008), and two types exist the complete naked neck and the not purebred tassel neck (Manyelo *et al.,* 2020) since these animals have less plumage, they tolerate higher temperatures (Fernandes *et al.*, 2023). Naked neck birds are very colourful-black brownish, multicolour, red-brownish, and black feather combinations (Faruque *et al.*, 2010). They reach sexual maturity at 155 days, with males weighing about 3.5 kg and females weighing 3 kg at maturity (Gunya *et al.*, 2020; Hlokoe *et al.*, 2022). The naked neck breed can produce up to 138.9 eggs per year, with an average egg weight of 55.5 g (Glenneis, 2023).

Figure 2.1: Naked Neck chicken

Ovambo

Ovambo chickens are dark-coloured breeds native to Namibia's Ovamboland. Their colour protects them from predators by functioning as camouflage (Mutibvu *et al.,* 2019). Their small size body allows them to fly and roost on top of the trees to escape predators (Hlokoe *et al.,* 2022). Ovambo chickens are known to be aggressive and vigorous due to their habit of catching and eating mice and young rats (Manyelo *et al.*, 2020). It is a dual-purpose breed reared for meat and egg production (Manyelo *et al.*, 2020). Males reach sexual maturity at 2.2 kg body weight and 1.5 kg for females at about 140 days old (Motsepe *et al.*, 2016). The average weight of the Ovambo egg can reach up to 52.5 g, and their hens can produce 129 eggs per year (Abudabos *et al.*, 2017).

Figure 2.2: Ovambo chicken

Venda

The Lebowa-Venda chicken is distinguished by particular multi-colors, white, black, and red and, they were named after their origin and they were discovered by veterinarian Dr. Naas Coetzee while conducting research in Venda, Limpopo province of South Africa (van Marle-Koster *et al.*, 2008). The breed is used for egg and meat production (Hlokoe *et al*., 2022). The Venda chicken genotype is disease-resistant, has high survivability, and is capable of surviving extreme environmental conditions with minimum additional feed (Ng'ambi *et al.*, 2013). They reach sexual maturity at around five months of age (Manyelo *et al.*, 2020). Males weigh 2.9 kg to 3.6 kg when they reach maturity, and hens weigh about 2.4 kg to 3 kg (Ng'ambi *et al.*, 2013). Hens produce large, slightly pink-tinted eggs with an average egg weight of 53g (Manyelo *et al.*, 2020).

Figure 2.3: Venda chicken

Potchefstroom Koekoek

The Potchefstroom Koekoek breed is a native composite chicken breed, which was developed in the 1950s at South Africa's Potchefstroom Agricultural College. Koekoek is a cross between the Black Australorp and the White Leghorn, with a dash of Barred Plymouth Rock (van Marle-Koster *et al.*, 2008; Khanyile *et al.*,2015). The name Koekoek refers to the barred colour pattern of the birds (Grobbelaar *et al.*, 2010). Koekoek colour pattern is a sex-linked gene that is useful for colour sexing in cross-breeding for egg-producing chickens in medium-input production systems (Grobbelaar *et al.*, 2010). The average egg production of a Koekoek hen is 196 eggs per year, with an average egg weight of 55.7kg (Heit, 2017).

Figure 2.4 Potchefstroom Koekoek

They are dual-purpose breeds and are adaptable to smallholder production systems because they are productive foragers, they are perfect for both extensive and intensive management systems (Idowu *et al.*, 2021). They reach early sexual maturity at 130 days (Dessie and Getachew, 2016; Manyelo *et al.*, 2020). The average weight is around 3–4 kg for males and 2.1 kg for females (Dessie and Getachew, 2016). The chicken genotype is self-sustaining, disease-resistant, and has a good temperament. It was developed for traits such as egg production and carcass with appealing yellow skin colour (Mutibvu *et al*., 2019). Hence, the importance of conservation between and within breed genetic diversity is widely recognized (Hamid, 2019; Zeng *et al.*, 2022). It is critical to define and comprehend the processes involved in conservation efforts to apply the most effective conservation methods (Mestanza-Ramón *et al.*, 2020).

2.6 Maintenance of the genetic diversity and structure of indigenous chicken in South Africa as an indicator of conservation hotspot and or risk

Genetic diversity is one of the important foundation factors that predict whether a breed survives and reproduces or eventually faces extinction (Al-Qamashoui *et al.*, 2014; Liu, Zhao, *et al.*, 2019; Des Roches *et al.*, 2021). Hence, all species require genetic diversity to adapt; without it, species cannot survive changing environments, climate change, and new pest and disease risks (Hoban *et al.*, 2021). Genetic diversity is defined as the variety of genetic variations that evolved within and among animals throughout domestication (Mtileni *et al.*, 2012; Hoban *et al.*, 2021). It causes changes in DNA sequence, epigenetic profiles, protein structure or isoenzymes, physiological

traits, and morphological traits (Salgotra and Chauhan, 2023).

The genetic material present in reproducing individuals of a population determines the diversity of the population (Salgotra and Chauhan, 2023). However, a variety of factors influence genetic diversity over time. The primary causes of changes in genetic diversity are mutation, selection, genetic drift, and gene flow (Salgotra and Chauhan, 2023). Hence, genetic diversity is the primary driving force behind population selection and evolution. Individual selection can be natural or artificial, depending on the variance present (Salgotra and Chauhan, 2023).

Specifically, low genetic diversity is often interpreted as an indicator of inbreeding depression and increased genetic drift, which is related to reduced individual survival along with a reduced capacity for population growth (Spielman *et al*., 2004; Teixeira and Huber, 2021). In contrast, high levels of genetic diversity are seen as a key to promoting population survival and guaranteeing the adaptive potential of the natural population in the face of rapidly changing environmental pressures (Markert *et al.*, 2010; Salgotra and Chauhan, 2023). Hence, genetic diversity acts as a buffer against the uncertainty of environmental conditions, particularly in medium and long-term strategies (Verma, 2017).

Genetic diversity analysis is crucial for reconstructing genetic events that shaped the diversity parameters of current populations, such as ancestry, prehistoric and historical migrations, admixture events, and genetic isolation (Caballero and Toro, 2000). In this regard, molecular markers such as microsatellites, mtDNA sequences, SNPs available in commercial chips, and complete metagenome sequences are commonly used to measure genetic diversity (Dave *et al.*, 2021). They provide a basis for assessing genetic diversity between or within populations, studying population interactions, and providing information on population history (Mtileni *et al.*, 2011a). In this regard, molecular diversity studies in local chicken breeds have been conducted to assess these genetic changes (van Marle-Koster *et al.*, 2008; Hassen *et al.,* 2009; Mtileni *et al.*, 2011a; Khanyile *et al.*, 2015; Bortoluzzi *et al.*, 2018; Lasagna *et al.*, 2020).

Table 2.4: Estimated genetic diversity of indigenous chicken in several countries

Country	**Populations**	**Markers**	**MAF**	**In ranges**		**References**
				H_O	H_E	
Tanzania	Tanzanian Ecotypes	Microsatellite	5.70 ± 2.61 197 to 569	0.62 0.8975	0.62 0.9388	(Lyimo *et al.*, 2013; Mwambene *et al.*, 2019)
South Africa, Malawi, and Zimbabwe	South African Malawi Zimbabwe ecotypes	Microsatellite	-	0.6 to 0.8 0.2 0.3 to 0.5	0.9 0.8 0.8 to 0.9	(Ncube *et al.*, 2014)
Zimbabwe Malawi	Ecotypes population	Microsatellite	6.1 to 6.7 5.9	0.590 to 0.605 0.607	0.642 to 0.661 0.625	(Muchadeyi *et al.*, 2007)
South Africa Malawi Zimbabwe	Southern African and Conserved chickens	SNP Markers	0.27 to 0.29 0.24 to 0.28	0.68 0.62	0.65 to 0.68 0.71 to 0.65	(Khanyile *et al.*, 2015)
South Africa	Village, Conserved exotic chickens	Microsatellites	4.98 ± 1.48 3.67 ± 1.12 3.42 ± 1.50	0.67 ± 0.02 0.53 ± 0.03 0.47 ± 0.03	0.68 ± 0.02 0.59 ± 0.03 0.53 ± 0.05	(Nxumalo *et al.*, 2020)
Botswana	Tswana chickens broiler breed	SNP markers	0.266- 0.241 0.259 ± 0.0007	0.610 - 0.613 0.347 ± 0.023	613 - 0.608 0.577 ± 0.0002	(Machete, James *et al.*, 2021)
Rwanda	Indigenous chicken	Microsatellites	0.437	0.616	688	(Habimana *et al.*, 2020)
Cameroon	Indigenous chicken ecotypes	Microsatellites	3.34 to 8.80	0.50 to 0.60 0.626 to 0.664	0.51 to 0.68 0.617 to 0.64	(Fotsa *et al.*, 2011; Keambou *et al.*, 2014)

2.7 Modelling environmental suitability and adaptation of indigenous chickens

The fluctuation of climate change causes environmental challenges worldwide (Mdoda *et al.*, 2020). Climate variability poses a significant strain on smallholder production systems which are already working under limited resources (Popoola *et al.*, 2020). The impact of environmental changes on animals affects their health, growth, and fertility as well as the diseases or pathogen dynamics (Passamonti *et al.*, 2021). Despite these challenges, adaptation may lessen the impact of climate change (Oduniyi *et al*., 2020). In this regard, populations respond to environmental changes through migration, phenotypic adjustments, or genetic adaptation (Lorant *et al*., 2020). Genetic adaptation is the modification of allele frequencies by natural or artificial selection (Lorant *et al.,* 2020). Adaptability revolves around fitness, which describes an individual's relative ability to live and reproduce the next generation to ensure the population's continued survival (Naskar *et al.*, 2012). Fitness and adaptation are influenced by genetic makeup and it determines an animal's tolerance to adverse conditions such as high temperature, drought, pests, and diseases (Chaidanya and Sejian, 2015). Genetic changes in animals due to environmental constraints can result in morphological, behavioural, and physiological changes that maintain or reduce the mismatch between phenotype and environment (Gaughan *et al.*, 2019; Radchuk *et al.*, 2019).

Morphological adaptations include short and thin hair, light hair colour, lightly pigmented skin, higher density of sweat glands, slender legs, and less subcutaneous fat (Gaughan *et al.*, 2019). Several studies have been done on various chicken phenotypes to assess their performance in hot environments (Moraa *et al.*, 2015; Ngeno *et al.*, 2015; Habimana *et al.*, 2020). For instance, the naked neck mutation and other related genetic variations, such as frizzle, silky, and dwarfism, promote thermoregulation in hot and humid locations by reducing feather mass (Cahaner *et al.*, 2008; Vandana *et al.*, 2021). In contrast, highland ecotypes, which include the crested, bearded, and feathered shank phenotypes, have thick plumage as a response to the cold environment (Moraa *et al.*, 2015). Moreover, various plumage colours are observed in indigenous chickens (Bibi *et al.*, 2021), which also helps in camouflaging against predators.

Behavioural responses, help in the adaptation or acclimatization process when animals are subjected to a high heat load (Nyoni *et al.*, 2018; Gaughan *et al.*, 2019). Shade-seeking is one of the most rapid and significant behavioural changes observed in heat-challenged animals (Sejian *et al.*, 2018). In chickens, some of the responses observed are panting, closing their eyes, lying down, and dust bathing (Vandana *et al.*, 2021), also reduced feed intake, increased water intake, decreased lying time, and reduced defecation (Nyoni *et al.*, 2018; Gaughan *et al.*, 2019; Vandana *et al.*, 2021). Lastly, physiological adaptation is the biological reaction of an individual organism to environmental stress (Neethirajan, 2020). Environmental stressors cause physiological responses in chickens such as increased rectal temperature, respiration rate, and heartbeat (Vandana *et al.*, 2021). Notwithstanding, the genetic process underlying local adaptation in natural populations is still poorly understood mainly due to the traits driving local adaptation being mostly quantitative traits (Lorant *et al.*, 2020). This is in agreement with Merilä and Hendry (2014), who also concluded that phenotypic adaptability, rather than genetic adaptation, appears to be significantly more common in mediating natural population responses to climatic change. Therefore, natural selection acts on phenotypic features by altering the frequency of underlying alleles and moving population phenotypes toward local optima (Lorant *et al.*, 2020). These optima are dependent on local conditions, and ecologically significant genes frequently differ amongst subpopulations in varied habitats, resulting in allele frequency divergence over time (Lorant *et al.*, 2020). Hence, the abundance of phenotypic variation in indigenous chicken ecotypes encourages the selection and development of climate-resilience ecotypes that correspond to different agro-ecological zones (Kennedy *et al.*, 2022). Bioclimatic indicators, characterized by annual patterns of temperature and precipitation, are used to explain the geographic distribution of natural populations along climate gradients (Noce *et al.*, 2020). They consolidate and condense growing climate datasets, enabling the creation of ecological niche models (Bioclimatic envelope) for species based on their favorable environmental suitability (Pearson and Dawson, 2004; Araújo and Luoto, 2007). The datasets are accessible to both experts and non-experts, and various options are available from WorldClim (Hijmans *et al.*, 2005), CHELSA (Karger *et al.*, 2017), CliMond (Kriticos *et al.*, 2011), ecoClimate (Lima-Ribeiro *et al.*, 2015), CMCC-Bioclimlnd (Noce *et al*.,2020) and KGClim (Cui *et al.*, 2021).

2.8 Ecological mapping and landscape genomics approach to study environmental suitability and adaptation

The Next Generation Sequencing (NGS) technologies made it possible to study the genomic basis of local adaptation for any organism. The genomic approach provides insights into demographic parameters, phylogenetic issues, inbreeding effects, introgression detection, and genetic disease understanding (Johnson and Koepfli, 2014). The approach considers fitness, gene expression, and adaptation at the genomic level to manage threatened populations under changing climates (Harrisson *et al.*, 2014; Khan *et al.*, 2016), through access to genome-wide data and annotated wild species (Khan *et al.*, 2016; Segelbacher *et al.*, 2021). Genomics has been matched up by advancements in spatial analyses that when combined with increased computational capacity will allow for the quantification of the spatial distribution of the biotic and abiotic factors across the landscape (Larsen *et al*., 2022). Spatial data are available due to the increase of Geographical Information Systems (GIS) which is a digital technology that integrates hardware and software to analyse, store and map spatial data technologies (Storfer *et al*., 2018; Teixeira, 2018).

2.8.1 Habitat suitability

Ecological niche models (ENMs) also known as species distribution models (SDMs) are a type of method that uses occurrence data along with environmental data to create a correlative model of the environmental conditions that match a species' ecological requirements and predicts the relative suitability of habitat (Warren and Seifert, 2011). This method explains the natural occurrence of animal distribution patterns or examines the impact of climate change on their distribution (Lozano-Jaramillo *et al.*, 2019). Popular modelling methods include Bioclimate Analysis and Prediction System (BIOCLIM), Genetic Algorithm for Rule-set Production (GARP), Generalised Linear Models (GLM), and Maximum Entropy Modelling (Maxent) (Srinivasulu *et al.,* 2021).

The most popular one is the machine learning algorithm maximum entropy modelling in the Maxent program (Phillips *et al*., 2006). Maxent uses presence-only data to calculate a set of functions that relate environmental factors and habitat suitability to estimate the species niche and probable global range (Phillips *et al*., 2006). It is les

susceptible to small sample size and can manage the complicated interactions between predictor variables and response variables (Elith *et al.*, 2011). Several studies applied ENM to estimate the habitat suitability of indigenous chickens (Gebru *et al.*, 2022; Kebede *et al.*, 2021; Lozano-Jaramillo *et al.*, 2019). Lozano-Jaramillo *et al.* (2019) used a predictive distribution model on two exotic breeds, the Koekoek and Kuroiler to estimate areas of potential suitability for these breeds in Ethiopia. Gheyas *et al.* (2021) using ENM identified six environmental factors that contributed to the dispersal of African chicken populations in the environmental space based on the Principal Component Analysis (PCA), demonstrating high heterogeneity in agro-climatic conditions. Researchers identified selection signatures influenced by environmental variables like land use, soil organic carbon, minimum temperature, driest quarter precipitation, pre-seasonality, and wettest quarter precipitation Kebede *et al.* (2021) using ENMs classified chicken populations into three ecotypes that integrated phenotypic and environmental information, these ecotypes demonstrated significant phenotypic differentiations.

These technological developments have led to a rapid increase in studies that combine geographic and genomic data to test for spatial patterns of genes under selection, altogether called landscape genomics (Joost *et al.*, 2007; Manel *et al.*, 2010). Therefore, the landscape genomic approach can be used to screen for loci under selection and their association with production variables simultaneously through the use of GISs (Mdladla *et al*., 2018). Landscape genomic studies have been conducted on both animals and plants (Vincent *et al.*, 2013; Leamy *et al.*, 2016; Vangestel *et al.*, 2016; Mdladla *et al*., 2018; Vajana *et al.*, 2018). The use of landscape genomic approaches will aid in the overlaying of chicken genetic profiles over environmental coordinates, as well as the identification and characterization of genes conferring adaptive qualities to various environmental conditions (Muchadeyi and Dzomba, 2017). Merging landscape genomics, geo-referencing of breed distributions, spatial genetic diversity, and climatic, ecological, epidemiological, and production system information (Hanotte and Jianlin, 2005), will assist in making priority decisions for local breed conservation and aid in understanding the genetic basis of animal adaptation to the environment and the co-evolution of livestock and their production system (Joost, 2010). Several studies have used the landscape genomics approach to study the adaptive variances in local livestock ecotypes (Mdladla *et al.*, 2018;

Vajana *et al.*, 2018; Lozano-Jaramillo *et al.*, 2019; Vallejo-Trujillo *et al.*, 2022). Gheyas *et al.* (2021) conducted the first study that adopted a powerful integrative approach that combined ENM with genomic analysis to dissect environmental drivers of local adaptation and to determine their impact on the genome.

2.8 Next generation sequencing technologies used in landscape genomics

In the 1970s, sanger sequencing was developed and was taken as the main technology in the first generation of laboratory and commercial sequencing applications by Frederick Sanger and colleagues (Liu *et al.*, 2012). Since its invention, Sanger sequencing has been the main technology for DNA sequencing. Sanger sequencing is still regarded as the standard of excellence for sequencing by the scientific community, but it has limitations (Fakruddin and Chowdhury, 2012). This method suffers from errors in sequence sensitivity (about 10-20%) and is insufficient for detecting clinically relevant low-level mutant alleles or organisms (Fakruddin and Chowdhury, 2012; Altimari *et al.*, 2013; Jennings *et al*., 2016). Reading Sanger sequences is difficult and takes great ability and concentration (Jennings *et al*., 2016). Because of these limitations, it was obvious that faster, better throughput and less expensive technologies were required. As a result, a succession of novel massive parallel sequencing technologies known as NGS technologies were developed (Arsenic *et al.*, 2015). NGS technologies are different from the Sanger sequencing method in aspects of extensive parallel analysis, high throughput, and reduced cost (Liu *et al.*, 2012; Salk *et al*., 2018). The application of these technologies has played a key role in several research areas which include expression analysis by gene expression profiling, metagenomics, single-cell genomics, epigenetics, and clinical real-time diagnostics (Buermans and den Dunnen, 2014; Wuyts and Segata, 2019).

The first NGS technology to be released in 2005 was the Roche 454 sequencing (Margulies *et al.*, 2005), which generated about 200,000 reads of 110 base pairs (bp) (van Dijk *et al.*, 2014). This technology utilized luminescent detection of pyrophosphate released upon incorporation of correct nucleotides during sequencing by synthesis (SBS) and produced long sequences (reads) (Alekseyev *et al.*, 2018). This technology had a few drawbacks, including a short length of nucleotide sequences, a higher error rate, and cost per run (Akaçin *et al.*, 2022). As a result, Roche-454's acceptance declined due to advancements in Illumina sequencing and higher cost, in 2013 Roche

announced the discontinuation of the Roche-454 sequencing platform (Segerman, 2020). Followed by Illumina (Solexa) sequencing which is the most popular platform amongst available NGS platforms (Buermans and den Dunnen, 2014). Illumina utilizes sequencing by synthesis (SBS) technology developed by Solexa which uses reversibly terminated fluorescently labelled nucleotides (Alekseyev *et al.*, 2018). One disadvantage is that it has additional needs for sample load control, with an abundance in these clusters that overlap and cause inaccurate sequencing (Kekeç *et al.,* 2022). The overall error rate is around 1% (Callahan *et al.*, 2016; Schirmer *et al.*, 2016).

The third to be released was Sequencing by Oligo Ligation Detection (SOLiD) in 2007 (Metzker, 2010). This methodology is based on the sequential ligation of fluorescent probes (Valouev *et al.*, 2008). This method's main drawbacks are its inaccurate results and long read times in SOLiD chemistry (Kekeç *et al.*, 2022). However, in recent editions, this has been improved (Ambardar *et al.*, 2016).

Ion Torrent is the first method for achieving semiconductor sequencing without optical sensing (Goodwin *et al.,* 2016). The technology is well-suited for gene-panel sequencing as well as point-of-care clinical applications, such as transcriptome profiling and splice site identification (Slatko *et al.*, 2018). However, the difficulty in sequencing across homopolymer sections is an inherent limitation of Ion Torrent technology (Malapelle *et al.*, 2015). NGS can be used with the combination of molecular marker methods such as microsatellites, genome mapping, amplified fragment length polymorphism (AFLP), and SNP for animal breeding strategies (Sabir *et al.*, 2014). They are DNA-based oligonucleotide sequences facilitating the detection of variations or polymorphisms in the population for specific regions of DNA (Singh *et al.*, 2014; Chugh *et al.*, 2022; Weiying *et al.*, 2022).

SNP markers have proven their importance in carrying out different genetic and breeding studies in a large number of organisms including plants (Hong *et al.*, 2015). SNPs are the result of mutations that produce base pair differences among chromosome sequences. SNPs are the common form of variation in the genome and they are greatly used to study genetic differences between individuals and populations (Vallejos-vidal *et al.,*2020). They are widely available in both coding and non-coding regions of the genome and may contribute to changes in these regions (Sabir *et al.*, 2014; van Dijk *et al.*, 2014).

SNP chips have already been developed and used in some domestic animals (Fan *et al.*, 2010), such as cattle which include low and high-density chips (Matukumalli *et al.*, 2009; Boichard *et al.*, 2012), sheep and pigs (Ramos *et al.*, 2009) as well for chickens (Groenen *et al.*, 2011) and goats (Tosser-Klopp *et al.*, 2014). SNP arrays play an important role in studying species evolution, domestication, breed formation, exploring genetic mechanisms of complex traits, and lastly, improving selection methods for genetic improvement of animal production (Fan *et al.*, 2010).

2.9 Signatures of selection and adaptation

Selection refers to a process in which genetic and phenotypic characteristics are fixed within a population (Cho *et al.*, 2021). It can be characterized by selection coefficient (s) which is calculated at a specific locus as the relative difference in fitness given out by two alleles (Rousseau *et al.*, 2017). Selection pressures have been influential in enabling chickens to adapt to their environment and may leave signatures of selection in the chicken population genome (Walugembe *et al.*, 2019). Signatures of selection are defined as the reduction, elimination, or change of genetic variation in genomic regions that are adjacent to causative variants in response to both natural and artificial pressures (Bertolini *et al.*, 2018).

2.9.1 Selection pressures

2.9.1.1 Natural selection pressures

In natural selection, three types of selection are distinguished based on the dynamics of the selected allele (Barroso *et al*., 2020; Enard, 2021), which include: positive, negative (purifying), and balancing selection (Gouveia *et al.*, 2014). Positive selection is defined as a process through which a new mutation or previously rare allele increases an individual's fitness and reaches fixation (López *et al*., 2015). When positive selection intervenes in the newly arisen selective advantageous allele or mutation it increases in frequency in the population and carries linked neutral alleles that surround the selected site along with it and co-increase in frequency, this occurrence is known as selective sweep (Gouveia *et al.*, 2014). Identification of positive selection or selective sweeps has been studied in both commercial breeds and indigenous chickens (Rubin *et al.*, 2010; Qanbari *et al.*, 2019) and sheep (Molotsi *et al.*, 2018; Eydivandi *et al.*, 2021) and cattle (Ramey *et al.*, 2013).

Lawal *et al.* (2018) used pooled heterozygosity (Hp) to unravel signatures of positive selection in Red Junglefowl and indigenous chicken from Ethiopia, Saudi Arabia, and Sri Lanka populations. Their study found two candidates sweep regions from the Red Junglefowl shared with all indigenous populations, and four unique regions among indigenous chicken populations, with one region containing annotated genes. The signature of positive selection specific to domestic chicken may come from the domestication process itself or after the domestication of the species following geographical dispersal or in response to human and natural selection (Lawal *et al.*, 2018), analysing chicken populations from different parts of the world required.

Selective sweeps can result in a hard sweep which occurs when the new mutation is positively selected and is so strongly associated with the genetic background where it arose (Barroso *et al.*, 2020). Conversely, a soft sweep occurs when a mutation is already separating in the population at the onset of selection (Barroso *et al.*, 2020). As a result of a selective sweep, different genetic variations, and various haplotype structures are fixed over time within a separated subpopulation, resulting in huge, well-defined ranges of animal breeds and genetic populations (Ma *et al.*, 2015).

Negative selection is the removal of deleterious alleles from a population these deleterious alleles reduce the reproductive success of individuals and can lead to a reduction in genetic diversity in regions of the genome because neutral polymorphisms at the site linked to deleterious alleles are also removed from the population (Barroso *et al*., 2020; Enard, 2021). For example, a small effective population size combined with artificial selection might result in a high probability of inbreeding depression, resulting in the accumulation of deleterious alleles that rise in frequency due to genetic drift (Charlesworth and Willis, 2009). Derks *et al.* (2018) identified potential deleterious variants from three purebred white-egg layers, 152 mutations were identified that likely induce embryonic lethality in the homozygous state. In addition, in a homozygous state, these recessive deleterious mutations have the potential to reduce an individual's fitness (Bosse *et al.*, 2019). Bortoluzzi *et al.* (2020) demonstrated that traditional fancy chicken populations that have recently been bottlenecked have a higher proportion of deleterious mutations than populations that are kept at a small population size since domestication. Hence, the timing and nature of population bottlenecks can substantially shape the deleterious variation landscape in small populations (Bortoluzzi *et al.*, 2020). Therefore, identifying deleterious variants can

improve genomic breeding procedures to reduce their high frequency in the population (Derks *et al.*, 2018).

Another form of selection is balancing selection which is the maintenance of multiple alleles at the selected site (Fijarczyk and Babik, 2015; Barroso *et al*., 2020). A long-term balancing selection can alter the skew site frequency spectrum (SFS) at linked neutral sites causing an excess of intermediate frequency-derived variants (Zeng *et al*., 2021). This selection can eventually increase the time to the most recent common ancestor (TMRCA) due to maintaining multiple alleles for a long time (Charlesworth, 2006). Notwithstanding, variants under balancing selection are susceptible to a complicated interplay between heterozygotes' advantage and homozygotes' disadvantage (Derks and Steensma, 2021). Ommeh *et al.* (2010) proposed a balancing selection process as an evolutionary factor for the maintenance of the A/G SNP of the *Mx* gene, implying that both alleles may be important for innate immunity in chickens. Therefore, finding novel balancing selection targets can aid in understanding evolution, uncover conserved features over extended periods, and reveal previously linked regions with phenotypes (Siewert and Voight, 2017). Natural selection impacts both selected variant frequency and linked neutral genetic variation, allowing for the measurement of its expression in the genome by detecting its impact on linked neutral variation (Enard, 2021).

2.9.1.2 Artificial selection pressure

In contrast to natural selection, artificial selection is a process where humans select traits to develop a particular phenotype, in which the gene pool of the next generation does not depend necessarily on fitness components but also on traits chosen by a farmer (Gouveia *et al.*, 2014). Signals shaped by artificial selection are consistently associated with important economic traits during the process of breed formation (Ma *et al.*, 2018). The detection of artificial selection signals can aid in mapping the causal mechanisms underlying economic traits in the genome and provide a clear understanding of how diversity evolved in different chicken breeds (Wong *et al.*, 2017; Ma *et al.*, 2018). Guo *et al.* (2016) identified many genes under putative selection in chicken fighting are related to immunity and disease resistance, indicating artificial selection for individuals with improved innate immunity and disease resistance. Hence, selection events, both natural and artificial, leave footprints on the genome, which can

be identified using statistical techniques like DNA sequence or SNP genotyping data in livestock populations (Qanbari and Simianer, 2014).

2.9.2 Detection methods of signature of selection

There are various statistics used to detect the signature of selection, including runs of homozygosity (ROH) (Qanbari and Simianer, 2014), pooled heterozygosity (Hp) statistic (Rubin *et al.*, 2010), composite likelihood ratio (CLR), extended haplotype homozygosity (EHH) (Sabeti *et al.*, 2002), integrated Haplotype Score (iHS), Wright's fixation index F_{st} (Wright, 1943) and hapFLK (Bonhomme *et al.*, 2010).

There are statistics used based on within-population to detect the signature of selection, which includes ROHs, which search for continuous parts of the genome without heterozygosity in the diploid state and are used on a genome-wide scale to detect signals of recent or past selection (Qanbari and Simianer, 2014). Rubin *et al.* (2010) introduced pooled heterozygosity (Hp) statistic, a variability estimator based on allelic counts across sliding windows of adjacent loci to look for areas that deviate from the norm. Another method is the composite likelihood ratio (CLR), which uses coalescent simulations to derive a distribution of the test statistic under the null hypothesis of no selection (Qanbari and Simianer, 2014). Sabeti *et al.* (2002) developed extended haplotype homozygosity (EHH), which depends on the discrepancy between extended haplotype homozygosity in a region and the frequency of that haplotype in the population, it identifies long-range haplotypes and also detects genomic regions that are candidates for having undergone recent selection. Voight *et al.* (2006) developed the integrated Haplotype Score (iHS) which is an extension of EHH based on a comparison of EHH between derived and ancestor alleles within a population. Extreme positive or negative iHS reflects excessive haplotype homozygosity on the ancestral and derived allelic background (Qanbari and Simianer, 2014). The iHS is the most widely used haplotype-based statistic and has higher power to detect selection when the selected allele has swept to intermediate frequencies, but the derived allele must have existed only on a distinct haplotype before selection and must not have reached fixation (Voight *et al.*, 2006; Sabeti *et al.*, 2002).

Also, different methods can be applied between populations to identify the signature of selection which includes, Wright's fixation index F_{st} (Wright, 1943) which has been

developed in three-set parameters (F_{ST}, F_{IS}, F_{IT}). The general application of F_{ST}-based scans for selection is to identify outliers in the empirical distribution of the statistics computed genome-wide (Fariello *et al.*, 2013). The F_{ST} has an advantage over multi-locus testing such as sit frequency spectrum (SFS) or linkage disequilibrium (LD) based methods, in that it is SNP-specific and can reveal the actual genetic variants under selection (Qanbari and Simianer, 2014). Another method is hapFLK which is built upon the original FLK statistic (Bonhomme *et al.*, 2010). These hapFLK statistics account for the haplotype structure in the sample, and it uses multipoint-LD (Scheet and Stephens, 2006) that regroups chromosomes into local haplotype clusters, aiming to exploit this clustering model to compute haplotype frequencies which can be used to measure the difference between populations (Fariello *et al.*, 2013). A cross-population extended haplotype homozygosity was developed to detect selective sweeps in which the selected alleles have achieved fixation in one population but remain polymorphic in another population (Sabeti *et al.*, 2007). Previous studies have been conducted using these statistical methods to identify signatures of selection (Walugembe *et al.*, 2019; Wang *et al.*, 2019).

2.9.3 Studies of selection signatures in indigenous chicken

The identification of selection signatures can efficiently identify selected genes and genomic regions, aiding in understanding the relationship between genotype and phenotype in chicken populations (Liu *et al.*, 2016). Mahdabi *et al.* (2021) conducted a study to identify signatures of selection in Iranian indigenous chicken ecotypes (i.e., Lari and Khazak) using four statistical methods F_{ST}, hapFLK, ROH and CRL, where 90 genes were detected by at least two of the four applied methods which revealed candidate genes associated with growth (*DCN, MEOX2*, and *CACNB1*), reproduction (*ESR1* and *CALCR*), disease resistance (*S1PR1, ALPK1,* and *MHC-B*), behaviour pattern (*AGMO, GNAO1*, and *PSEN1*) and morphological traits (*IHH* and *NHEJ1*). Njau *et al.* (2022) detected major genes within the major histocompatibility complex (MHC) region in local chicken ecotypes in Kenya using F_{ST}. Selection pressure within these local chicken ecotypes contained a few genes namely *TRIM 7.2*, *TRIM 39.2*, *ZNF 692*, *TRIM 7*, *KIFC1*, *BLEC 3*, and *YLEC* which are linked to immune response and tolerance/resistance to diseases. This highlights selection signatures within the MHC region of local chickens may be important for survival during disease outbreaks. In support, Walugembe *et al.* (2019) suggested that these regions would be essential

to improving ecotype survival throughout the regions despite exposure to prevalent diseases and harsh climatic conditions.

2.10 Effective population size

Effective population size (N_e) is defined as the size of an idealized population that would produce the same genetic variation as the population under study (Wright, 1931). N_e plays a major role in conservation; it relates to the rate at which genetic drift occurs, the rate of inbreeding, and the loss of genetic diversity, which is of concern in conservation (Ryman *et al*., 2019). The purpose of N_e in conservation is to evaluate the threat status of populations and to design breeding programs for both wild/captive populations and local breeds (Jiménez-Mena *et al*., 2016). Preserving high levels of genetic diversity and keeping inbreeding low are important factors in the management of threatened populations (Ryman *et al.*, 2019). Minimizing the loss of genetic variability is the main concern of conservation and can be achieved through maximization of the N_e, hence it decreases the occurrence of genetic drift (Palinkas-bodzsar *et al.*, 2020). Estimating *Ne* and monitoring its changes are crucial for understanding threatened species' genetic health, evaluating risks of inbreeding, depression, and extinction, assessing genetic management effectiveness, and projecting future population demographics (Wang *et al*., 2016).

The N_e determines how fast allele frequencies change by genetic drift, the success of natural selection, and expected genetic diversity levels for neutral loci (Waples *et al.*, 2013). Under Hardy-Weinberg equilibrium, allele and genotype frequencies remain constant in large populations, but they consistently change due to mutation, selection, migration, and genetic drift (Wang *et al*., 2016). Effectively neutral mutations have a fitness effect to reach frequency zero (Fixed) or be lost therefore, their fate is dominated by genetic drift and largely unaffected by selection (Lanfear *et al*., 2014). When genetic drift is the only force acting on the population, the number of alleles at a given locus decreases over time (Barroso *et al*., 2020). Hence, as N_e increases, genetic drift becomes weaker due to the larger population (Lan fear *et al*., 2014). Mutation and drift can also act in the opposite direction and mutation-drift equilibrium is reached when the rate of allele creation by mutation equals the rate of allele loss by drift (Barroso *et al.*, 2020). The variance of genetic drift experienced in a population can be quantified under the Wright-Fisher Model, the variance of genetic drift in a

generation population is p(1- p)/(2*Ne*) for a diploid population (Hui and Burt, 2015). Hence, it is possible to estimate the N_e of a closed population by exploring the extent of temporal changes in allele frequencies (Hui and Burt, 2015). On that account, a summary statistic for demographic inference can be derived from the genome-wide distribution of allele frequencies, which is a function of average genealogies (Lapierre *et al.*, 2017). This distribution is called the Site frequency spectrum (SFS), which describes the distribution of allele frequencies across separating sites of given loci in a population or sample (Barroso *et al.*, 2020).

The shape of the SFS is affected by the population's genetic processes and therefore, serves as the input of population genetics methods (Schraiber and Akey, 2015). Since it captures the dependency between the effective size of the population and the speed at which new mutations drift to a higher frequency (Palamara *et al.*, 2012). Due to the randomness of the genetic drift, one initial random-mating population can evolve into one of a small group of sub-populations with different allele frequencies at two loci and with different linkage disequilibrium (LD) (Qanbari, 2020). In isolated populations, individuals often share identical by descent (IBD) genetic material, known as co-ancestry at a single site, despite appearing unrelated (Carmi *et al.*, 2013).

A sharing of long IBD genetic segments is an indication of populations that have experienced recent genetic drift (Carmi *et al.*, 2013). The observation of shared segments has resulted in many applications such as demographic inference (Palamara *et al.*, 2012), characterization of the population (Gauvin *et al.*, 2014), detection of selection (Han and Abney, 2012), mapping haplotypes associated with traits (Browning and Thompson, 2012), and pedigree reconstruction (Huff *et al.*, 2011), and phasing and imputation (Kong *et al.*, 2008). The availability of genome-wide SNP methods to identify IBD sharing between individuals has been developed (Palamara *et al.*, 2012). GERMLINE is a method that uses a length threshold to create a dictionary with chucks haplotypes and IBD segments, allowing for minimal length and flexibility to account for genotyping errors (Gusev *et al.*, 2009). Another flexible method is the hidden Markov Model (HMM), which provides basic support to probabilities of genotyping errors and the LD model can be added (Han and Abney, 2012).

2.10.1 Estimating effective population size

The N_e cannot be measured directly but can be estimated using two methods; demographic methods based on life-history traits and genetic methods based on genetic markers (Marandel *et al.*, 2020). Demographic methods estimate N_e as mathematical functions of demographic parameters such as mean and variance in offspring numbers, survival at age, and birth rates (Marandel *et al.*, 2020). This method is valuable because beyond providing a point estimate of N_e, they directly identify key demographic processes that shape the N_e (Trask *et al.*, 2017). Advancements in genotyping and sequencing technologies, coupled with computer processing speed, have significantly boosted the popularity and effectiveness of genetic methods that extract allele frequencies influenced by population demography mainly N_e (Marandel *et al.*, 2020). The development of high-density SNP chips and high-throughput genotyping provides substantial data for a more accurate estimate of LD and N_e (Liu, He, *et al.*, 2017).

2.10.2 Linkage disequilibrium

Linkage disequilibrium (LD) is the non-random association of alleles at different loci at a rate that is inversely proportional to N_e (Waples *et al.*, 2016*;* Liu *et al.*, 2017). The LD method is the commonly used genetic estimator of N_e; it can make more powerful estimates from single samples than temporal methods that require two or more samples from a population separated by time (Jones *et al.*, 2016). Some studies estimated and compared N_e in chickens (Khanyile *et al.*, 2015; Zulu, *et al*., 2015; Mtileni *et al.*, 2016).

The LD method is also accessible through software packages such as LDNe (Waples and Do, 2008), and NeEstimator (Do *et al.*, 2014). To estimate recent N_e with the LD method, it is required to measure LD between inherited independently loci with recombination rates equal to 0.5 and to infer past Ne by measuring LD between physically related loci with recombination fractions less than 0.5 (Hill, 1974; Waples *et al*., 2016). Understanding LD in a specific genomic region provides insights into the history of natural and artificial selection, gene conversion, mutations, and other factors causing gene frequency evolution (Gurgul *et al.*, 2014; O'Brien *et al.*, 2014). Quantifying LD levels is crucial for determining the number of markers needed for genome association. Higher LD over longer segments requires fewer markers, while faster decay of LD necessitates denser panels (Abdullah *et al.*, 2015; Qanbari, 2020).

The squared correlation coefficient r^2 is the popular measure of LD alongside D' (Sved and Hill, 2018), they both range from 0 to 1, when D' is <1 it indicates the occurrence of an ancient recombination between loci while =1 indicates no recombination, r^2 represents the correlation between genotypes and molecular marker pairs (Barria *et al.*, 2019). In association studies, r^2 is preferred due to the inverse relationship between its value and the sample size required to detect a significant association between the causative variant and molecular marker (Barria *et al.*, 2019). In general, it is expected that LD strength decreases with the increased distance between markers located on the same chromosome (Berihulay *et al.*, 2019; Fabbri *et al*, 2020). LD is suggested to be greater in domestic animals due to the forces that generate LD being more common in many breeds (Gurgul *et al.*, 2014). Even so, LD only extended over sub-centiMorgan (cM) levels in most natural populations including humans (Harmegnies *et al.*, 2006). Chickens are species that have a unique genetic architecture that is made up of macro, intermediate, and micro-chromosomes, and each chromosome set varies in genetic structure and composition and is characterized by various LD patterns (Khanyile *et al.*,2015b).

A higher recombination rate on micro-chromosomes is expected to reduce LD compared to macro-chromosomes (Megens *et al.*, 2009; Khanyile *et al.*, 2015). However, the degree of haplotype sharing is expected to decrease with an increasing recombination rate, which should result in lower transferability of genetic parameters for micro-chromosomes (Megens *et al.*, 2009). Haplotype blocks are two or more alleles in loci that are closely linked and are transferred together (Khanyile *et al.*, 2015b). The extent of LD has been studied in both commercial chicken breeds and indigenous chickens (Rao *et al.*, 2008; Megens *et al.*, 2009; Wragg *et al.*, 2012). Khanyile *et al.* (2015) studied LD between village chicken populations and conserved flocks, LD was higher in conserved flocks and ranged from 0.29 to 0.36 between markers that were less than 10kb apart than village chicken populations, but LD was consistently high on chromosome 16 and low on chromosome 25 for both village chicken population and conserved flock, this chromosomal difference in LD is supported by previous studies (Aerts *et al.*, 2007; Megens *et al.*, 2009; Qanbari *et al.*, 2010). However, the study of Megens *et al.* (2009) focused on selected regions of 1 cM on macro-chromosomes (GGA1 and 2) and 1,5 -2 cM regions on micro-

chromosomes (GGA26 and 27).

Both LD and N_e are crucial parameters for evaluations of population genetic diversity and can provide a powerful method to characterize and understand the genetic architecture underlying complex traits (Liu *et al.*, 2017). Monitoring *Ne* has increased due to its impact on genetic stochasticity population viability, providing valuable insights into demographic and genetic risks to extinction through a comprehensive understanding of census and *Ne* (Harris *et al.*, 2017).

Table 2.5: Different methods of estimating effective population size

Methods	**Estimation tools applied**	**Breed**	**Country**	**Reference**
Demographic methods	Wright model estimates based on the sex ratio	Kokok balenggek chicken populations	Indonesia	(Rusfidra *et al.*, 2015)
	Wright model estimates based on the sex ratio	Indigenous chicken breeds	France	(Restoux *et al.*, 2022)
	Wright model estimate based on the sex ratio	Southern African village population	South Africa	(Zulu *et al*., 2015)
Genetic methods	Linkage-disequilibrium based estimate using SNeP software	Southern African village populations	South African	(Khanyile *et al.*, 2015)
	Pair-wise sequential Markovian coalescent (PSMC) and Multiple sequential Markovian coalescent (MSMC)	Indigenous chickens and red Jungle-flows	South, Southeast, and East Asia as well as Europe	(Wang *et al.*, 2021)
	Heterozygous excess test and linkage disequilibrium test	Indigenous chickens and conservation flock	South Africa	(Mtileni *et al.*, 2016)

The present study aims to investigate the environmental suitability of indigenous chickens and estimate the risk status in different regions of KwaZulu-Natal and Limpopo provinces of South Africa. To achieve the study aim, the Illumina iSelect chicken SNP60K Beadchip was used to identify genomic regions associated with adaptability traits, and a landscape genomic approach was employed to identify environmental factors influencing genetic adaptation and genes influencing local adaptation.

2.11 Future prospects and opportunities

As reviewed, indigenous chickens are neglected and face many constraints within their production systems, which threatens their survival. Consequently, quantifying the genetic diversity of vulnerable populations is crucial for their conservation, as species' ability to withstand climate change mostly relies on genetic diversity. Environmental heterogeneity plays a key role in shaping diversity within and among populations (Nikzat *et al*, 2021). However, the use of ENM in livestock is still at its outset (Vallejo-Trujillo *et al.*, in press). Integrating landscape genomics and ecological modelling allows researchers to fully examine the underlying genetic adaptation that drives species to be locally adapted to their habitats. Genome-wide SNP arrays enable the detection of adaptive genetic variation in livestock genomes and their correlation with environmental conditions (Mdladla *et al.*, 2017). Future use of landscape genomics and ecological niche modelling could help pair genotypes with suitable environments and predict species distribution due to changing climatic conditions. This could apply to animals and diseases, defining ecotypes and characterizing based on environmental habitability using models like phenotypic distribution modelling (PDM), this could also predict animal performance in response to climatic conditions. This information may aid farmers in selecting the breed to utilize in their own agro-ecology or breeding companies in deciding which ecology they should introduce a breed into (Lozano-Jaramillo *et al.*, 2019).

2.12 Conclusion

The review emphasized the importance of locally raised chickens in maintaining sustainable livelihoods in underdeveloped countries. Studies have been conducted to characterize indigenous chicken production systems, indicating the need for the protection of indigenous chickens. Indigenous chickens have distinct genotypes that enable them to thrive in difficult environments. The environment plays a role in the evolution of domesticated animals because it affects species survival and diversity within and among populations. The advancement of genomic tools allows for the right design of conservation strategies capable of preserving the genetic material possessed by indigenous chickens for future climate changes.

CHAPTER 3: INVESTIGATE THE ENVIRONMENTAL SUITABILITY OF INDIGENOUS CHICKENS FROM KWAZULU-NATAL AND LIMPOPO USING A LANDSCAPE GENOMICS APPROACH

Abstract

Climate change causes many selection pressures such as drought, pathogen spread, and high temperatures which affect the sustainability of livestock production. Indigenous chickens are genetic resources that are well known to be diverse and thrive well in harsh climatic conditions. Understanding the genetic basis of local adaptation will benefit the breeding of climate-resilient livestock as well as the preservation of their distinctive adaptive diversity. The study applied ecological niche modelling to explore the key environmental variables and determine potential suitability habitats for indigenous chickens in the provinces of KwaZulu-Natal and Limpopo in South Africa. A total of 244 village-based indigenous chickens were collected from rural areas in South Africa's Capricorn (n=85), Sekhukhune (n=113) Districts of Limpopo province, Harry Gwala (n=21), and uMzinyathi (n=25) Districts of KwaZulu-Natal province. The raster package in RStudio was used to extract values from 28 environmental variables which included annual trends (e.g., mean annual temperature and annual precipitation), seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters), soil variable, solar radiation and elevation. GQIS software was utilized to align layers, resulting in a 109.47 X 126.24mm layer size, and convert them to ASC files. The GGally r package was used to create a correlation matrix, identifying 10 out of 28 environmental variables as key drivers of environmental adaptation, including minimum temperatures, elevation, soil cation exchange capacity, soil clay content, organic carbon content, soil pH, soil silt content, and solar radiation. ENMeval was used for model tuning and evaluation, which identified linear and quadratic (LQ) features with a regularization-multiple = 0.5 as the best parameter combinations to optimise Maxent and develop suitability maps. Maxent software was used to create suitability maps for chickens in Limpopo and KwaZulu-Natal, with a test gain applied for important variables. Chickens from Dipakakeng, Mgababa, and Podu villages had localized predicted suitability probabilities, while those from Nhlonga had a broader distribution

with elevation and BIO6 being important variables. The study's overall findings emphasize the potential of using ecological niche modelling in conservation strategies to find unexplored regions for breed reintroductions and translocations and extend market access.

Keywords: Maxent**,** habitat suitability, Climate change,

3.1 Introduction

Global warming has an impact on livestock sustainability and production, creating environmental changes that lead to biodiversity loss (Archer *et al.*, 2021). Rapid climate change can cause species range shifts, a decrease in populations, and even extinction if the changes surpass organisms' physiological tolerance (Urban, 2015). On the contrary, as the human population grows, so does the need for animal products (Rashamol and Sejian, 2018). Sustainable improvement of livestock output to meet rising global demand is therefore critically dependent on the ability to use/develop climate-tolerant breeds (Gheyas *et al.*, 2021). Indigenous chickens have evolved over the years, and selection pressures exerted by the habitats in which they are nurtured have created particular modifications in their genomes, leading to local adaptations (Muchadeyi and Dzomba, 2017). Different ecotypes, with their inherent genetic and adaptive diversity, could offer genetic solutions to many current and future challenges (Vallejo-Trujillo *et al.*, 2022).

Recent developments in remote sensing and geographical information systems (GIS) technologies, increased the availability of environmental data (Kebede *et al.*, 2021), while, next-generation sequencing (NGS) made it possible to study the genomic basis of local adaptation (Storfer et al., 2018). The advancement of these technologies enabled the combination of geographic and genomic data in the search for geographical patterns of genes under selection (Manel *et al.*, 2010; Joost *et al.*, 2013). The integrated ecological niche modelling and genomics will have major implications for defining and conserving livestock adaptation diversity, as well as for providing genetic solutions for producing "climate resilient" animal breeds (Vallejo-Trujillo *et al.*, 2022).

Ecological niche models (ENMs) also known as species distribution models (SDMs) are used to investigate the relationship between species distribution ranges and

corresponding environmental variables that meet a species' ecological requirements and predict the relative suitability of habitat (Li, Gao, *et al.*, 2022; Warren and Seifert, 2011). They were established initially for ecological and conservation purposes, are increasingly being used to estimate the spatial distribution and potential risk of occurrence of a variety of diseases and vector species (Sindato *et al.*, 2016).

The most commonly used ENM/SDMs technique is implemented in the Maxent program (Phillips *et al*., 2006). Maxent uses the entropy principle to estimate species niches, primary contributing environmental variables, and geographic range using presence-only data (Phillips *et al*.,2006). Applies to both continuous and categorical data simultaneously, predicts optimal probability distributions efficiently, is analysable, and provides continuous outputs, enabling precise distinctions between the modelled suitability of different areas (Phillips *et al*., 2006). ENM has an impact on conserving biodiversity and has been applied for many purposes such as estimating the potential distribution of invasive species (Gormley *et al.*, 2011; de Andrade *et al*., 2019) and vector-borne diseases (Alkhamis and VanderWaal, 2016; Okely and Al-Khalaf, 2022). There is no information available in Southern African regions about the utility of ENM for village chickens or other livestock. However, ENM has been used in various African regions with just a few studies available (Vajana *et al.*, 2018; Lozano-Jaramillo *et al.*, 2019; Gheyas *et al.*, 2021; Kebede *et al.*, 2021; Vallejo-Trujillo *et al.*, 2021; Chaiyarat *et al.*, 2023). The combination of ENM and landscape genomics will be fundamental conservation strategies. It will help researchers and farmers better understand genotype-environment interactions and improve breeding strategies by introducing the right genotypes to their intended environments. This will also aid in ensuring the retention of quantitative and qualitative traits acquired by village chickens. The study's goal is to estimate the environmental suitability of village-based non-descript indigenous chickens from KwaZulu-Natal and Limpopo provinces.

3.2 Materials and Methods

3.2.1 Ethics

The study was approved by the research committee at Tshwane University of Technology (approval reference: AREC2021/11/004) and Agricultural Research Council (approval reference APIEC22/10).

3.2.2 Sampling site

A total of 244 village-based indigenous chickens were collected from rural areas in South Africa's Capricorn (n=85), Sekhukhune (n=113) Districts of Limpopo province, Harry Gwala (n=21), and uMzinyathi (n=25) Districts of KwaZulu-Natal province. Sampled areas were chosen for the study based on their unique difference in climatic characteristics and farming challenges and practices, as well as farmers involved in the community-based breeding program.

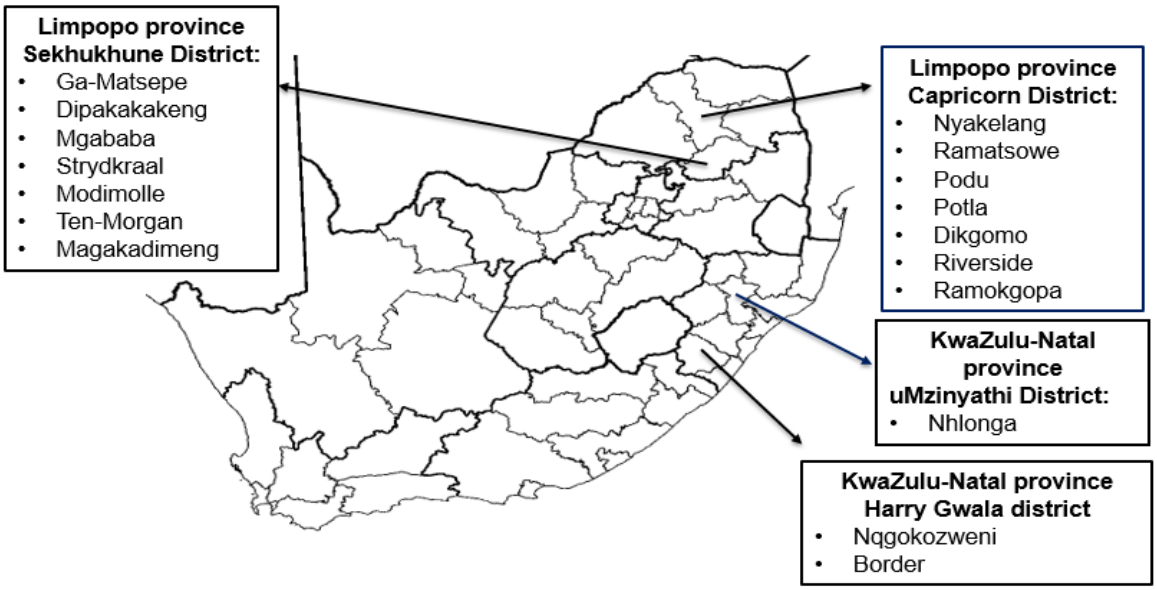


Figure 3.1: Sampling site of village chicken population in KwaZulu-Natal and Limpopo provinces.

Sampling site of village chicken populations in KwaZulu-Natal and Limpopo provinces.

The uMzinyathi District Municipality of KwaZulu-Natal province is characterized by a temperate climate with warm to hot summers and mild to cool winters, with a mean annual temperature of 17ºC and mean daily maximum temperature of 24ºC and minimum of 9, 6ºC. The rainfall levels are between 836mm and 400mm depending on the season (https://www.unisdr.org/preventionweb/files/16503_gccsouthafrica1.pdf). The Harry Gwala District experiences summer rainfall, with average annual rainfall ranging from 900 to 1,400mm. Temperatures in the District vary based on height, ranging from 3500m along the Drakensberg to 600m in the south-east (Ninela and Elliott, 2014). The Sekhukhune District varies from flat to undulating plains with temperatures ranging from 29 to 30 ºC in summer, and in winter they are between 3 and 23ºC. The rainfall is estimated at between 350mm to 420mm falling mainly

between October and March and summer is only 70 to 80mm (Tefera *et al.*, 2004). Capricorn District is a semi-arid climate region with hot, wet summers and cool, dry winters (Mpandeli *et al.*, 2019). Average annual rainfall varies between 300mm in the north and 1000mm in the south in the district (Mpandeli *et al.*, 2019).

3.2.3 Environmental data

Single geographic coordinates (latitude and longitude) were recorded for each sampled village, during sampling of the chicken populations to be used for ENM. A total of 28 environmental variables were extracted using the raster R package, these variables have biological relevance for indigenous chickens (Table 3.1). Environmental data included bioclimatic data (n=19), solar radiation, elevation from the WorldClim database at a spatial resolution of 30-arc seconds ($1km^2$) based on mean values of 30 years (1970-2000) (Fick and Hijmans, 2017), and soil variables (n=7) from Soil Grid system at 250m resolution (Hengl *et al.*, 2017). Using GQIS software version 2.28.3, file layers were aligned and made to have the same extent and resolution before being transformed into acceptable ASCII raster files for species distribution modelling. Due to a lack of data on recorded GPs from sampling sites, no vegetation data was extracted, and hence they are not included in the study.

3.2.4 Environmental correlation

To prevent overestimation and reduce model complexity, correlation analysis was performed using ggcorr in the GGally package (Schloerke *et al.,* 2013) in R (R Development Core Team, 2019) with a correlation value threshold of ≥ 0.6.

3.2.5 Ecological niche modelling

To automate the tuning and evaluation of ecological niche models and species distribution models, the ENMeval r package was used. Maxent version v3.3.3 (Phillips *et al.*, 2006) was used for ecological niche modelling (ENM), and the predictive performance of the most important environmental variables was also measured using test gain in Maxent v3.3.3 (Phillips *et al*., 2006). Suitability maps were generated using Maxent's cumulative output.

Table 3.1 Description units of environmental variable

Variables	**Description**	**Code**
Soil Variables	Soil pH (×10i n H_2O)	Soil pH
	Soil sand content (Weight %)	Soil sand content
	Soil silt content (Weight %)	Soil silt content
	Soil clay content (Weight %)	Soil clay content
	Soil water availability capacity (Volume %)	Soil water availability capacity
	Soil bulk density (Kg /m3)	Soil bulk density
	Soil organic carbon (g/kg)	Soil organic carbon
Temperature	Annual mean temperature (^{0}C)	BIO1
	Mean Diurnal range(^{0}C)	BIO2
	Isothermility [(BIO2/BIO7)*100)] (^{0}C)	BIO3
	Temperature seasonality (standard deviation^100)(^{0}C)	BIO4
	Max Temperature of warmest month (^{0}C)	BIO5
	Min Temperature of coldest month (^{0}C)	BIO6
	Temperature annual range (BIO5-BIO6)(^{0}C)	BIO7
	Mean Temperature of wettest quarter (^{0}C)	BIO8
	Mean Temperature of driest quarter(^{0}C)	BIO9
	Mean Temperature of warmest quarter(^{0}C)	BIO10
	Mean Temperature of coldest quarter(^{0}C)	BIO11
Solar radiation	Solar radiation of the seventh month	-
Precipitation	Annual precipitation(mm)	BIO12
	Precipitation of wettest month(mm)	BIO13
	Precipitation driest month(mm)	BIO14
	Precipitation of seasonality (mm)	BIO15
	Precipitation of wettest quarter (mm)	BIO16
	Precipitation of Driest Quarter(mm)	BIO17
	Precipitation of Warmest Quarter(mm)	BIO18
	Precipitation of Coldest Quarter(mm)	BIO19

3.3 Results

3.3.1 Selection of environmental variables

Before ecological niche modelling (ENM), the environmental variables were shortlisted by removing highly correlated variables by using a threshold of ≥ 0.6 (Figure 3A, B, and C). After the correlation test, environmental variables that were selected for the study were the minimum temperatures of the coldest month (BIO6), minimum temperature of the wettest quarter (BIO8), mean temperature of the coldest quarter (BIO11), elevation, soil cation exchange capacity, soil clay content, soil organic carbon content, soil pH, soil silt content and solar radiation of seventh month were used for suitability mapping. Due to the failure of GIS software to align the soil pH layer with the other nine extracted environmental layers, soil pH was only included in the RDA and not in the environmental suitability prediction. As a result, the study was limited in its ability to fully investigate the potential influences of soil pH on the prediction of suitable habitats.

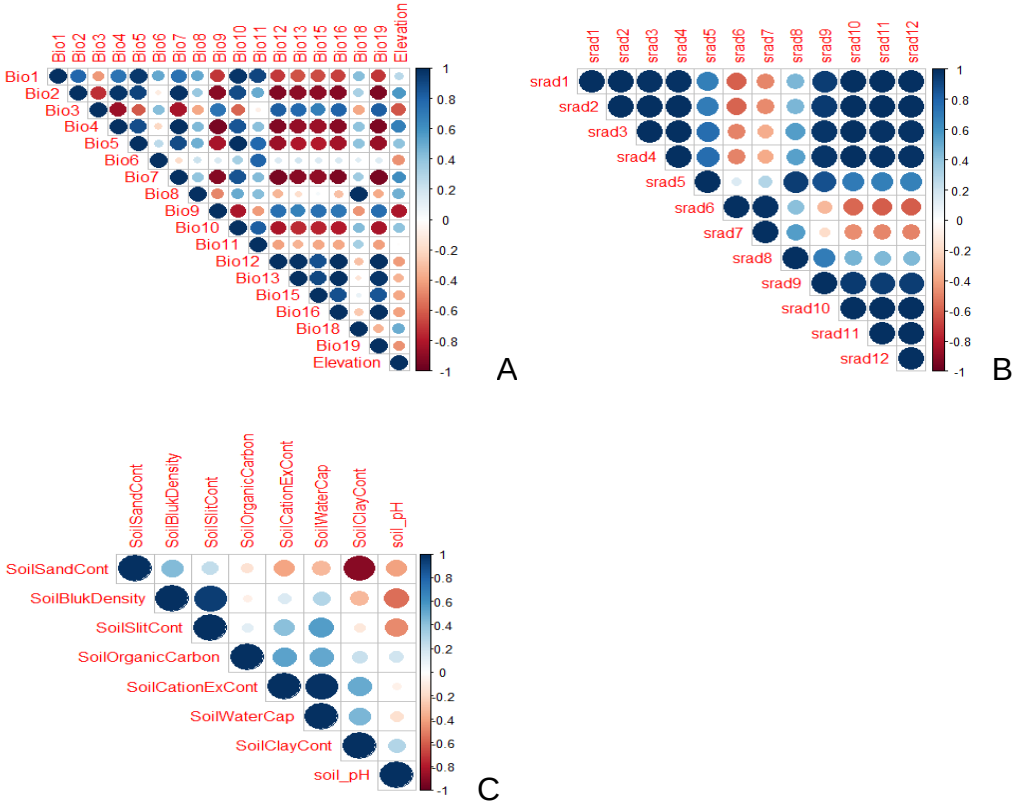


Figure 3.2: Correlation test of climatic variables (A) solar radiation (B) and Soil variables (C).

3.3.2 Ecological niche modelling

Feature classes (FC) allow complex relationships to be modelled by mathematically transforming the different covariate variables (Elith *et al.*, 2010). The ENMeval identified LQ (linear and quadratic products) FC with regularization multiplier (rm) =0.5 as the best parameter (Figure 3.3). regularization multiplier is a parameter that introduces new constraints, meaning it imposes penalties on the model (Morales *et al.*, 2017). Akaike information criterion corrected for small samples (AICc) is used to measure the accuracy of the model (Conner *et al.*, 2004). The lowest AICc delta value was considered to be a point of optimal FC and was selected to produce suitability maps by Maxent.

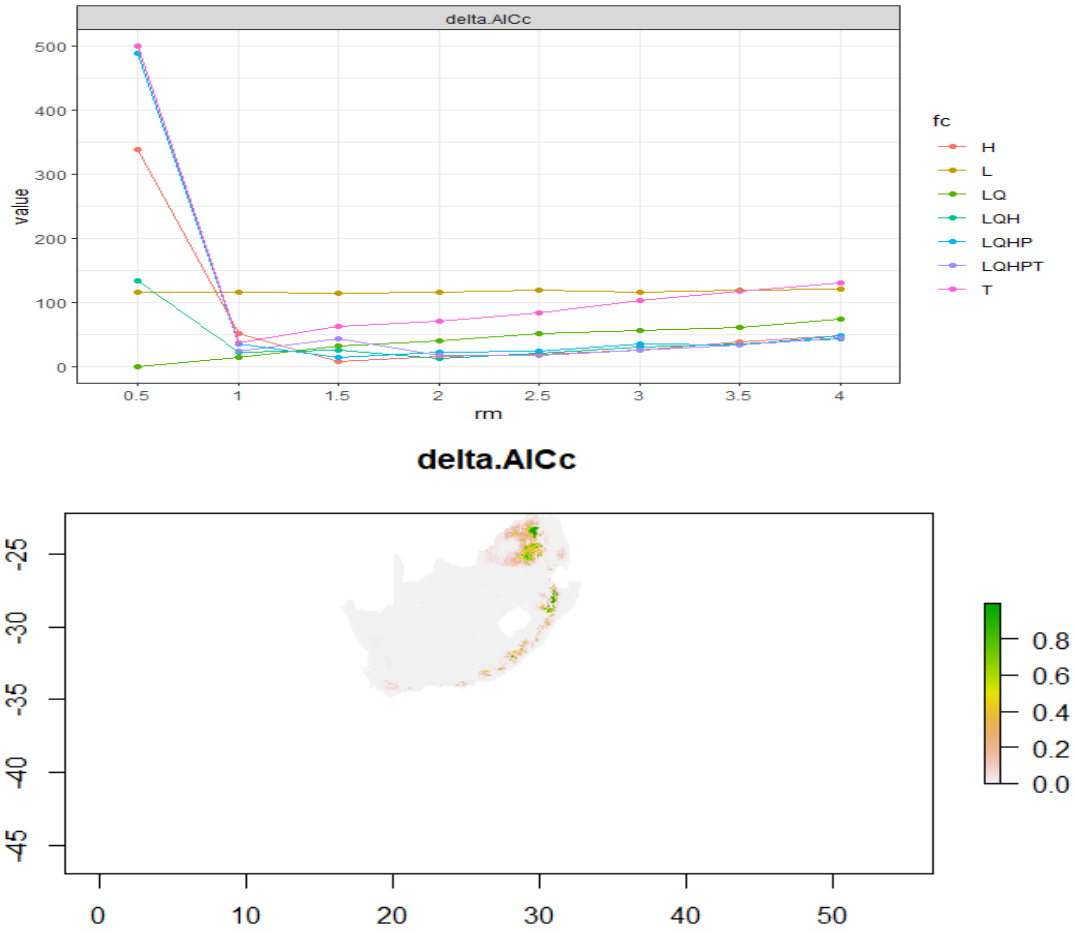
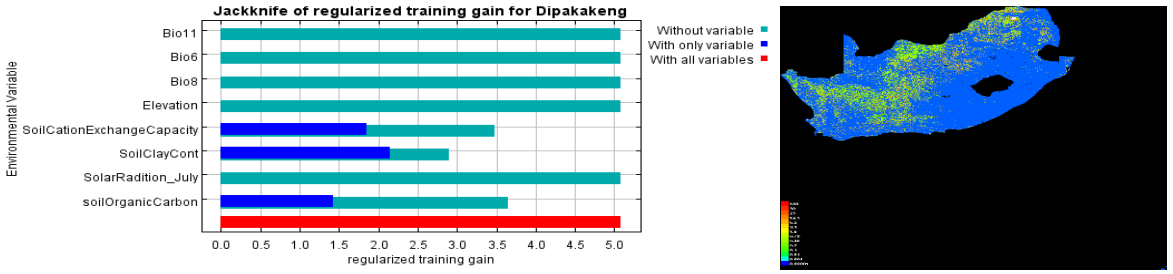


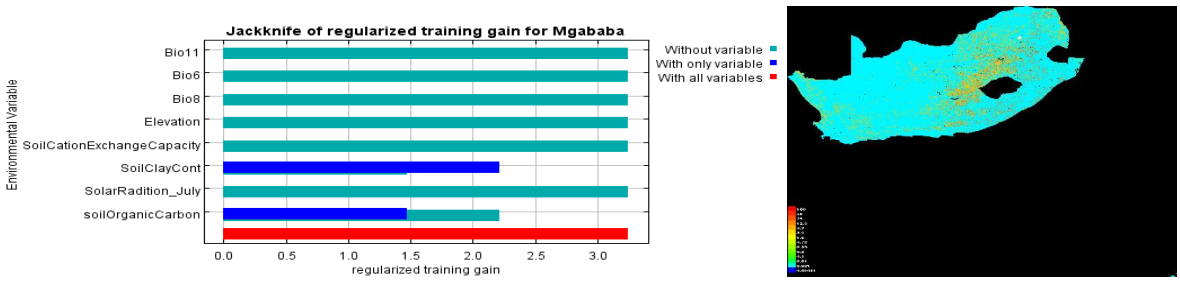
Figure 3.3: AICc values for analysed feature combination, with a map produced using a best optimum parameter (LQ and RM= 0.5).

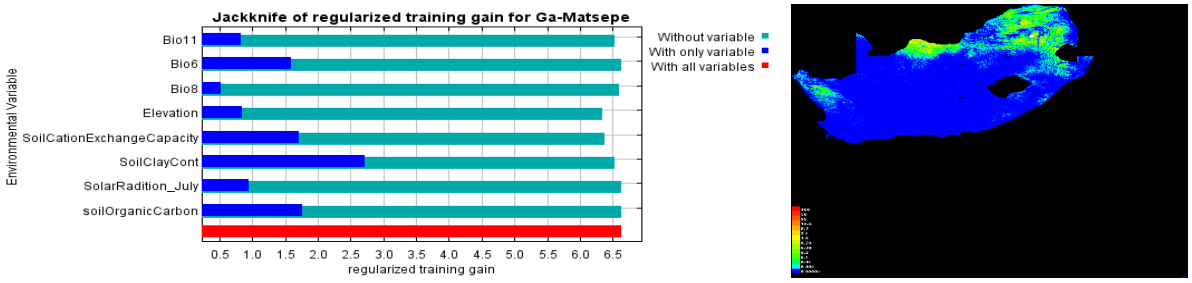
3.3.3 Habitat Suitability

The variable's relevance was determined using the jack-knife test and suitable areas were predicted (Figure 3.4-3.7). For Limpopo province, this indicated that the soil clay

content had the most importance when used in isolation and also decreased gain when excluded, for the following villages Dipakakeng, Mgababa, Ten-Morgan, Ramokgopa, Riverside, Podu, and Dikgomo. On the contrary, Strydkraal village, BIO6 had the most importance when used in isolation and also decreased gain when excluded, for Modimolle village solar radiation decreased gain when excluded and Magakadimeng village BIO11 decreased gain when excluded. For KwaZulu-Natal province, BIO6 had the most importance and elevation decreased gain when excluded for Nhlonga village. For Nqgokozweni village, soil clay content had the most importance when used in isolation and solar radiation decreased gain when excluded, and soil organic carbon had the most importance when used in isolation and BIO6 decreased gain for Border village.







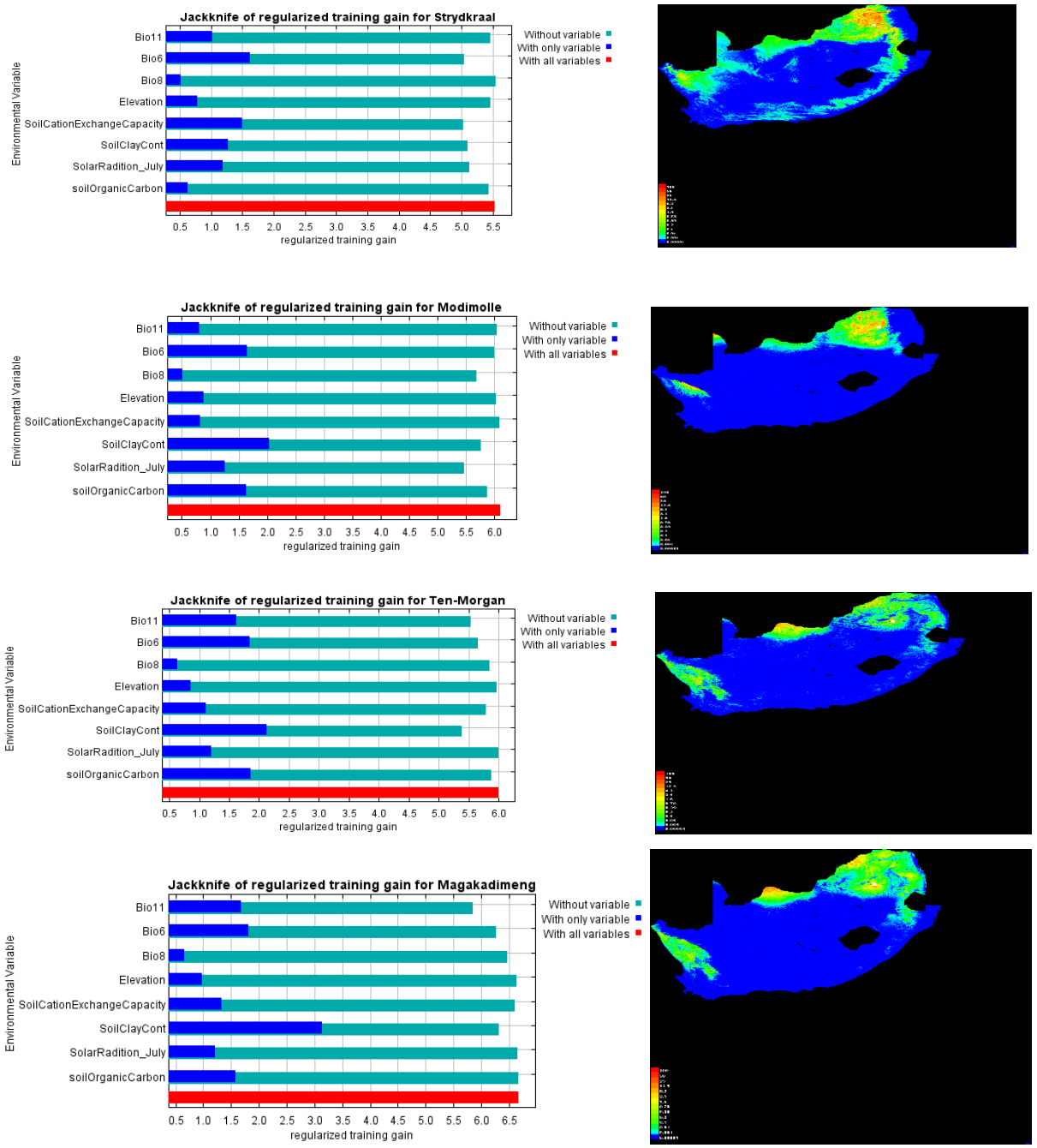
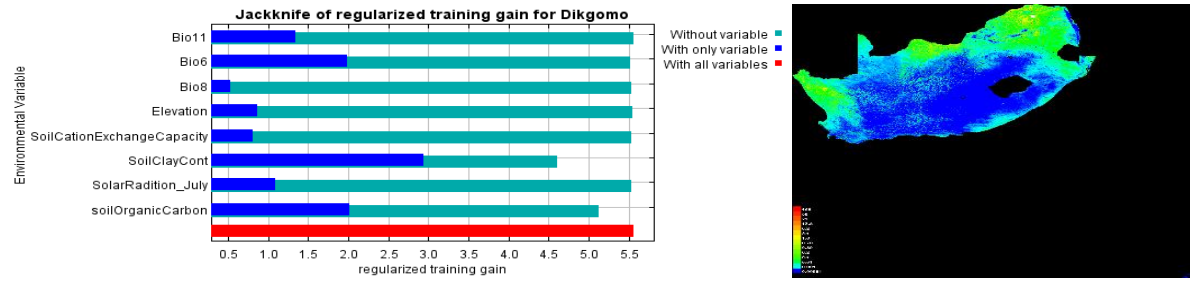


Figure 3.4: Jack-knife of regularized training gain tests and suitability maps of villages from Sekhukhune District in Limpopo province.



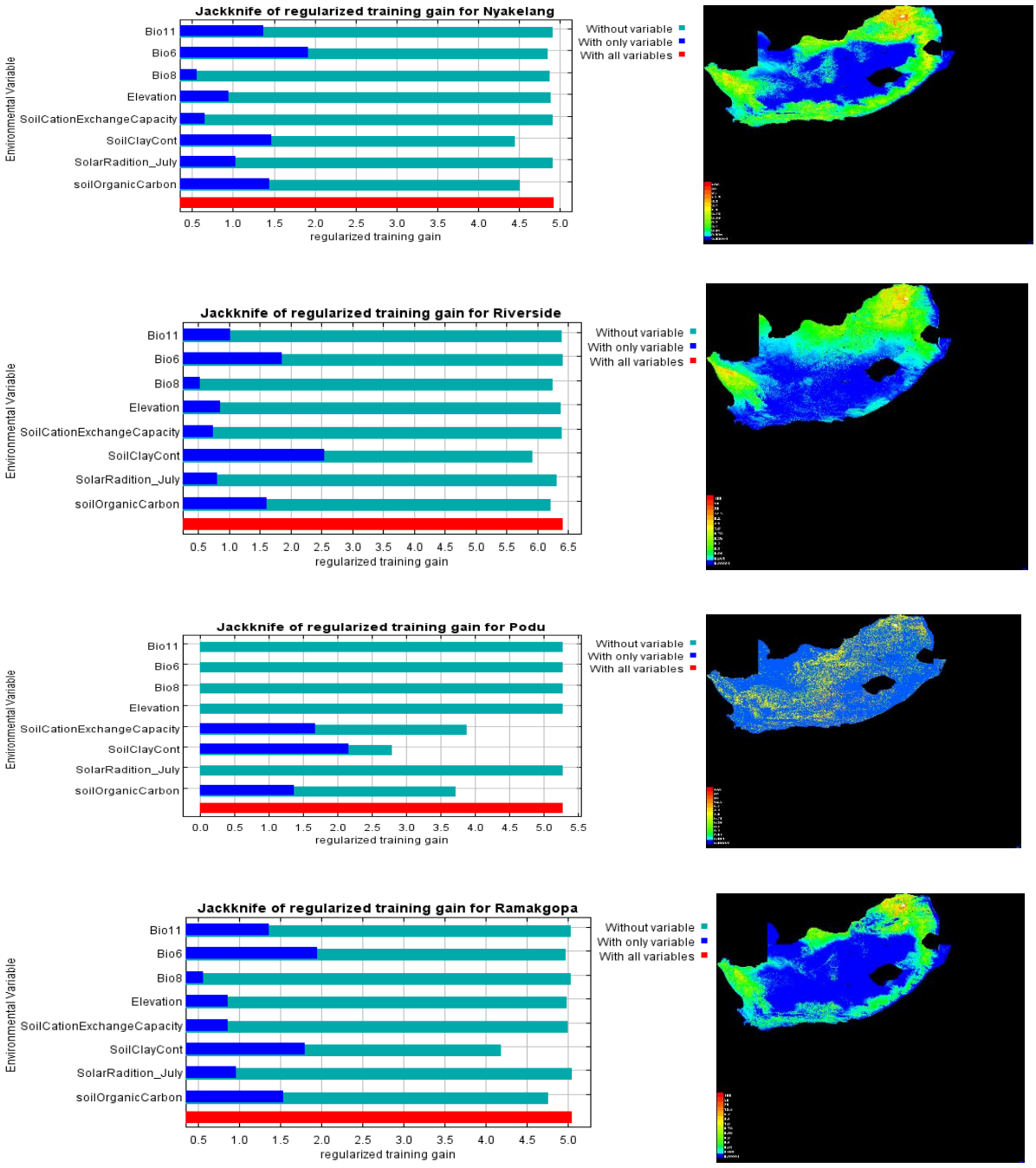


Figure 3.5: Jack-knife of regularized training gain tests and suitability maps of villages in Capricorn district, Limpopo province.

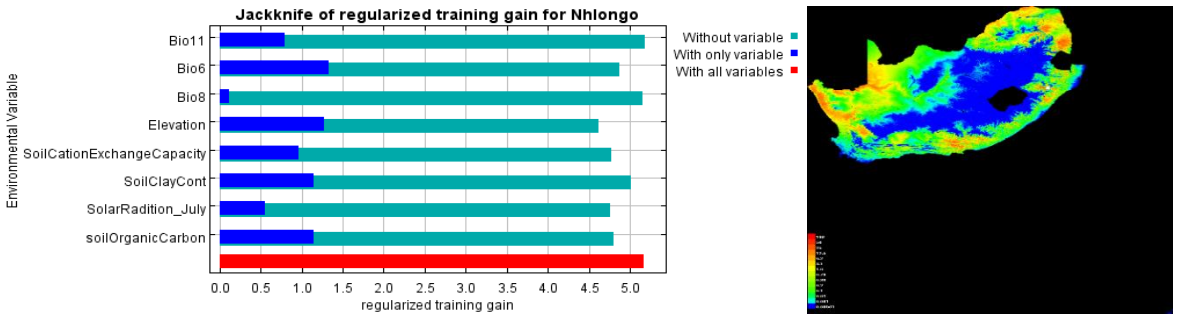


Figure 3.6: Jack-knife of regularized training gain tests and suitability maps of villages in uMzinyathi district, KwaZulu-Natal province.

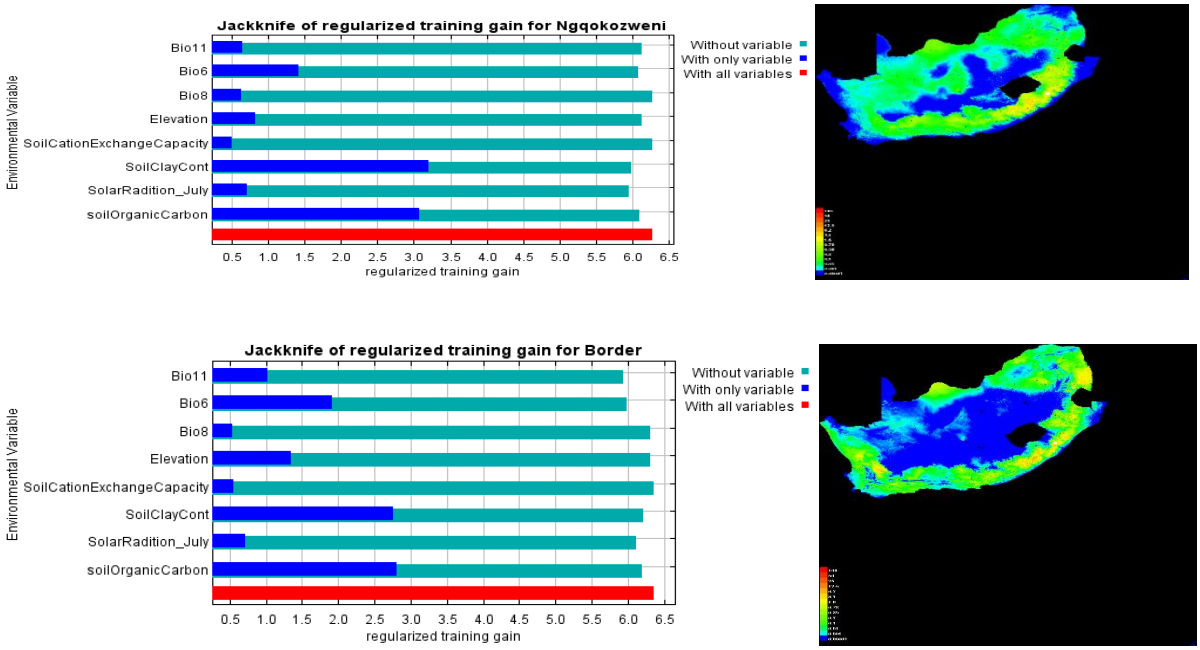


Figure 3.7: Jack-knife regularization training test of villages in Harry Gwala district, of KwaZulu-Natal province.

3.4 Discussion

Understanding the processes that produce adaptive genetic variation across species niches has become critical in conservation strategies for adaptive diversity and breeding improvements. In this way, adaptability to various habitats can be explained by looking at breed origins, where environmental and human-caused environmental selection pressures have influenced their adaptation to various production constraints (Lozano-Jaramillo *et al.*, 2019).

Several studies have integrated ENM and landscape genomics as a novel approach for conservation, however, there is no evidence of its utility in Southern African regions with only a few available studies in Africa (Vajana *et al.*, 2018; Lozano-Jaramillo *et al.*, 2019; Gheyas *et al.*, 2021; Kebede *et al.*, 2021; Vallejo-Trujillo *et al.*, 2021). The ENM method studies environmental heterogeneity in livestock environments using multiple environmental factors, displaying significant environmental pressures driving adaptive evolution (Vallejo-Trujillo *et al.*, 2022). The ENM method was applied in this study to identify unexplored geographical regions where these species may occur in future

climate change scenarios. Only ten variables were taken into consideration for ENM out of the 28 environmental components that were correlated.The process of using ENM for defining potential ecotypes and predicting suitable habitats includes choosing the right variables to improve the predictive capacity of the model (Gebru *et al.*, 2022). A jack-knife test is a vital metric for assessing variables that have the greatest contribution to the model for suitability mapping (Vallejo-Trujillo *et al.*, 2022).

The research found that BIO6 (min temperature of the coldest month) and elevation are crucial factors in determining the ideal habitats for Nhlonga village chickens through the jack-knife test. In relation to future climate change, Nhlonga village chickens were distributed more widely, as shown by hotter colors in potentially beneficial habitats extending to the provinces of the Northern Cape, Limpopo, and Eastern Cape. Demonstrating their potential to live and prosper in these regions due to climate conditions resemblances such as increasing temperature and drought. The province of KwaZulu-Natal experiences temperature increases, mild winters, and extreme weather occurrences, most notably droughts and flooding (Connolly-Boutin and Smit, 2016; Leonard, 2022). Northern Cape province has hot summers and is prone to drought, whereas Limpopo is considered the hottest province, with frequent drought and high temperatures (Maponya, 2012; Maposa *et al.*, 2021; Bahta and Musara, 2023).

The suitability maps indicate that chickens from Nqgokozweni and Border villages are most suitable for their current locations, with some regions showing intermediate suitability. The suitability of mapping for chickens in Nqgokozweni village was influenced by solar radiation and soil clay content, while in Border village, soil organic carbon content and BIO11 were significant factors through the jack-knife tests.

The study revealed that soil clay content significantly impacts the mapping of suitable habitats for chickens in Limpopo villages notably Dipakakeng, Mgababa, and Podu both when included in the model and omitted based on test gain. Maxent uses only present data to relate environmental variables and occurrence points to create a probability of potential geographical suitability (Phillips *et al.*, 2006). The clay-rich soil in these village areas makes it challenging to compare its suitability to other parts of South Africa. Soil is a categorical variable, so this alone indicates that soil types differ across the country, resulting in poor predictions. The overall study revealed the

importance of the environment in influencing the adaptation of village chickens. The complex nature of climatic stressors acting as selection pressures makes the study of the genetic basis of environmental adaptation difficult. However, our study illustrates the use of ENM as a powerful predictive tool for adaptation analysis in livestock. The findings of our study highlight the impact of environmental factors on village chickens' ability to adapt to their anticipated habitats.

Our study revealed that soil characteristics like soil organic carbon and clay content, which affect the type and amount of food accessible to chickens, are related to the local adaption. Clay is an important part of the soil, which consists of alum inosilicate minerals (Abdeldjouad *et al.*, 2019), through their physical and chemical properties, clay minerals can be expected to have more nutrient reserves (Kome *et al.*, 2019). As well as soil organic, which have shown importance in mapping suitability maps. A high soil organic carbon environment will be distinguished by the presence of numerous earth-dwelling animals such as insects and worms, which are good sources of protein-rich food for chickens (Gheyas *et al.*, 2021).

The study also revealed that temperature plays a biological role in the local adaption of South African village chickens and that limiting environmental variables like BIO6, BIO11, and solar radiation of the seventh month were important in mapping suitable habitats. In terms of limiting environmental variables, it implies that village chickens can survive cold and hot conditions, as well as food shortages, higher infection risk, and predators during that specific timeframe. For instance, multiple factors determine the chicken's thermo-neutral zone (Fernandes *et al.*, 2023). The quantity, shape, and distribution of feathers. Feathers have significant insulating qualities at low temperatures, but they also block heat dissipation at low temperatures (Rufino *et al.*, 2021), indicating that chickens can survive cool and cold months or quarters. Chickens have specific phenotypes that enable them to survive in specific regions or temperatures, as mentioned in references to high temperature or hot quarters in a year. For instance, the naked neck phenotype, as well as the Frizzle, Silky, and Dwarfism, promote thermoregulation in hot and humid regions by reducing feather mass (Cahaner *et al.*, 2008; Vandana *et al.*, 2021). In general, the ideal thermoneutral zone temperature for broilers is between 18 - 22 °C, (Kumari and Nath, 2018) and laying 18-25°C (Khatibi *et al.*, 2021). Temperatures exceeding 30°C often have a negative influence on chickens. However, village chickens are often exposed to air

temperatures exceeding 30°C while scavenging outside, with increased radiant heat loads from direct sun (Nyoni *et al.*, 2022). Therefore. the extent and variation of temperature tolerance thresholds in rural poultry remains unknown (Nyoni *et al.*, 2019).

3.5 Conclusion

The ecological niche modelling is a method that can be used to conserve indigenous chickens without causing further loss of their genetic resources. Understanding how animals adapt to their environments and the factors influencing breed-specific performance variations is crucial due to varying environmental conditions in different locations. The study's findings can aid in climate change conservation by guiding future planning and the introduction or translocation of breeds to suitable habitats that meet their fitness and guarantee optimal production performance. In this way, the study encourages the use of genetic resources to develop climate-resilient farming that support sustainable farming and contributes to the successful breeding program.

3.6 Limitations and recommendation

The study did not include vegetation data because GPs from sampled sites do not contain any vegetation data. Vegetation provides nutrient-dense feed and protection to indigenous chickens. The failure of GIS software to align the soil pH layer with the other nine extracted environmental layers has resulted in soil pH being excluded to the environmental suitability prediction. It is highly recommended that future studies incorporate vegetation data and soil pH variables to fully understand the mechanism underlying village chicken adaptation.

CHAPTER 4: ESTIMATION OF THE GENETIC DIVERSITY, EFFECTIVE POPULATION SIZE AND POPULATION STRUCTURE OF VILLAGE-BASED INDIGENOUS CHICKEN POPULATION FROM KWAZULU-NATAL AND LIMPOPO PROVINCES

Abstract

Sustainable animal production requires the improvement and conservation of genetic resources. Indigenous chickens are a significant genetic resource because they help alleviate poverty in South Africa's less fortunate rural communities. However, they are under threat due to the rising human population and demand for animal products. The adoption of commercial breeds endangers their gene pool, potentially leading to the risk of extinction. Understanding the level of genetic diversity in these breeds is an essential component of conservation. The study explored the genetic diversity, population structure, and effective population size of indigenous chickens at the village level in KwaZulu-Natal and Limpopo provinces. A total of 244 village-based indigenous chickens from rural villages of Capricorn (n=85), and Sekhukhune Districts (n=113) were sampled from Limpopo province. In KwaZulu-Natal province, Harry Gwala (n=21), and uMzinyathi (n=25) Districts were sampled. A total of 100 conservation flocks were collected as references from the Agricultural Research Council-Animal Production (ARC-AP). Namely, Ovambo (OV; n=10), Potchefstroom Koekoek (PK) (n=20), Naked Neck (NN; n=20), Venda (VD; n =20), White Leghorn (WL; n=10), White Plymouth Rock (WR; n=10), and New Hampshire (NH; n=10). Genotyping was done using Illumina iSelect chicken SNP60k Beadchip. PLINK software was used for genetic diversity calculation. The genetic diversity of indigenous chickens ranged from 0.341 to 0.338 for observed heterozygosity and from 0.381 to 0.387 for expected heterozygosity, whereas the conservation flocks ranged from 0.240 to 0.339 and 0.377 to 1 for both observed and expected heterozygosity. The admixture analysis was conducted using a LEA r package in RStudio, the lowest cross-validation error (0.87) was detected at K = 5. Population structure was done using LEA r package in RStudio, where PC1 and PC2 explained 5.64% of the total variation and resulted in 5 clusters with the Venda, Naked Neck, and White Leghorn being separated from village chickens. SNeP software was used to calculate effective population size, both village chickens and conservation flock showed a steady decrease of effective population

size at 12 generations ago. Redundancy analysis (RDA) was done to examine genotype-environment associations using a vegan r package, the first three RDA axes capture 46.8% of total genetic variation and 386 outlier SNPs associated with all 10 environmental variables were identified. The study highlights the use of SNP marker panels to investigate the diversity and population structure of village chickens in South Africa. These results highlight the importance of village chickens as genetic reservoirs for future challenges.

Keywords: Effective population size, genetic diversity, indigenous chickens

4.1 Introduction

The poultry sector is a crucial and rapidly growing area of agricultural production that serves as a significant source of food (Cendron *et al.*, 2020). The demand for animal products stimulates the intensity of production systems**.** Indigenous chickens are threatened due to exotic breeds that are becoming more common (Abebe *et al.*, 2015; Fathi *et al.*, 2018). The commercialization of chicken breeding favoured the use of highly productive chicken breeds, resulting in smaller population size of low-performing local breeds (Granevitze *et al.*, 2007). Indigenous chickens are valuable genetic resources that are found in many households in developing countries. In South African rural communities, they provide various socioeconomic functions such as animal protein, income, gift payments, and cultural, religious, and other traditions (Mtileni *et al.*, 2012; Tarwireyi and Fanadzo, 2013; Malatji *et al.*, 2016). Indigenous chicken breeds, which are the result of centuries of adaption, domestication, and breeding, are a significant source of genes for future breeding and are regarded as a vast collection of different genotypes (Rashid *et al.*, 2020; Soltan *et al.*, 2018; Tolone *et al.*, 2023). Genome analyses can reveal the genetic diversity of indigenous chicken populations, providing the foundation for genetic improvements that boost performance and productivity (Gheyas *et al.*, 2022).

Molecular technologies enable geneticists to evaluate the genetic structure of diverse populations using strategies such as genome-wide studies, which have already been used (Lawal *et al.*, 2018; Malomane *et al.*, 2019; Nie *et al.*, 2019). The genetic diversity of indigenous chickens has been examined using molecular markers such as microsatellites in various parts of the world (Mtileni *et al.*, 2011; Sartore *et al.*, 2016;

Samaraweera *et al.*, 2021). However, the development of high-density single nucleotide polymorphism (SNP) arrays has enabled the analyses of a population's genetic structure using a large number of markers with uniform distribution across all chromosomes (Strillacci *et al.*, 2017; Mastrangelo *et al.,* 2020*;* Malomane *et al.*, 2021). Which also, facilitates the collection of massive amounts of genome-wide linkage data suitable for measuring effective population size (N_e) in livestock (Uimari and Tapio, 2011; Khanyile *et al.*, 2015).

4.2 Material and Methods

4.2.1 Chicken population and blood samples

A total of 244 village-based indigenous chickens from rural villages of Capricorn (n=85), and Sekhukhune Districts (n=113) were sampled from Limpopo province. In KwaZulu-Natal province, Harry Gwala (n=21), and uMzinyathi (n=25) Districts were sampled (Table 4.1) Sampled areas were chosen for the study based on their unique difference in climatic characteristics and farming challenges and practices, as well as farmers involved in the community-based breeding program. The sample size was determined using Cochran's sample size formulae:

$$N = \frac{n = \mathbb{Z}^2 \times P\,(1-P) \div e^2}{1 + (\mathbb{Z}^2\ x\ P(1-P) \div e^2 N} \qquad \text{Equation 1}$$

A total of 100 conservation flocks as reference flocks were collected from the Agricultural Research Council-Animal Production (ARC-AP). Namely, Ovambo (n=10), Potchefstroom Koekoek (n=20), Naked Neck (n=20), Venda (n =20), White Leghorn (n=10), White Plymouth Rock (n=10), and New Hampshire (n=10). The sampling location for both conserved and village chicken is illustrated in Figure 4.1.

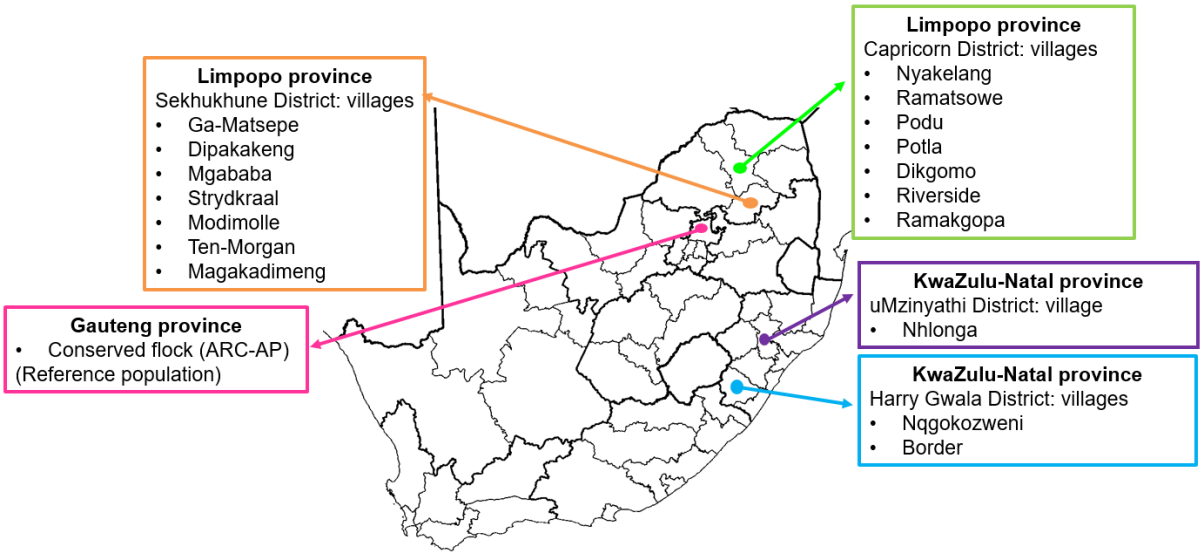


Figure 4.1: Sampling areas of village chicken in KwaZulu-Natal and Limpopo provinces and reference population from ARC-AP in Gauteng province.

Table 4.1 Sampling size per village

Districts	Villages	Sample size
Capricorn District	Dikgomo	23
	Nyakelang	13
	Podu	5
	Ramokgopa	23
	Ramatsowe	8
	Riverside	10
Sekhukhune District	Mgababa	25
	Magakadimeng	5
	Modimolle	6
	Dipakakeng	15
	Ga-Matsepe	9
	Strydkraal	45
	Ten Morgan	11
Harry Gwala District	Nqgokozweni	11
	Border	10
uMzinyathi District	Nhlonga	25

Blood samples were collected for both village-based chickens and conservation flocks using FTA-cards, from the wing vein and blood was applied on the circles outlined on the Whatman FTA card (Whatman BioScience, UK) then the FTA card was left to dry. The nucleic acids in FTA cards are automatically stabilized no need for require refrigeration, the cards were transported and stored at room temperature. The FTA cards were transported and stored at room temperature, for the nucleic acids are automatically stabilized without the need for refrigeration (DE-FTA-Whatman, Carl Roth®). DNA was extracted from the FTA cards using the NucleoSpin Tissue, min toolkit for DNA (Macherey-Nagel GmbH and Co. KG, Germany) according to the manufacturer's protocol. DNA quality was checked on a 1% agarose gel and quantity of DNA was evaluated using Qubit@ 4.0 Fluorometer (Invitrogen, Thermo Scientific), range of 50-350ng/ul.

4.2.2 SNP genotyping and quality control

A total of 344 chickens were genotyped using Illumina chicken iSelect SNP60K Bead chip using the Infinium assay compatible with the Illumina HiScan SQ genotyping platform at the Agricultural Research Council-Biotechnology Platform in South Africa. Infinium whole genome genotyping assay is designed to interrogate a large number of SNPs at unlimited levels of loci multiplexing (www.illumina.com). The genotype input files were converted into PLINK v1.07 (Purcell *et al.*, 2007) format using the Genome Studio program.

PLINK software (Purcell et al., 2007) was used to prune individuals for inclusion criteria based on the following criteria of quality control: individuals with a genotyping rate less than 95%, SNPs with a call rate less than 95%, SNPs with lower minor allele frequency (MAF) ≤ 5% and those that deviated from Hardy Weinberg equilibrium (HWE) $p < 0.0001$ were filtered out. Before quality control, 57636 SNP markers were available, none of the individuals were removed for low genotyping (mind > 0.95), and 16500 markers were excluded based on the Hardy-Weinberg test ($p > 0.0001$). Therefore, the total genotyping rate for all remaining individuals was 0.951768, with 22 SNPs failing the missingness test (Geno > 0.95) and 4684 SNPs failing the frequency test (MAF < 0.05). After genotyping pruning, there were 37845 SNPs available for further downstream data analysis.

4.2.3 Genetic diversity

PLINK (v1.07) (Purcell *et al.*, 2007) software was used to estimate observed and expected heterozygosity, inbreeding coefficient, and minor allele frequency distribution per population.

4.2.4 Population Structure Analyses

In preparation for population structure analysis, SNP data with all chickens were filtered to remove linked SNPs and closely related samples. Closely related individuals were removed based on a threshold of IBD ≥ 0.5 and only 1 individual was removed from the data set for high relatedness to appropriately illustrate genome-wide structure rather than local linkage disequilibrium in population structure analysis. Linkage disequilibrium pruning of the SNP set was carried out using a threshold of r^2 >0.2 to remove correlated pairs of SNPs. Therefore, 27258 SNPs from the remaining sampled chickens were available for downstream analysis.

Admixture analysis estimates individual ancestries by efficiently computing maximum likelihood estimates in a parametric model (Alexander and Lange, 2011). R package LEA (Frichot and Francois, 2015) was used to assess the most probable number of ancestral populations based on the SNP genotype data. The optimal number of clusters (K-value) was determined as the lowest cross-entropy validation (CV error). The cross-entropy criterion was used to determine the number of ancestral populations.

4.2.5 Effective population size

Effective population size was estimated on the known relationship between r^2, N_e, and the recombination rate (c) between two loci. LD makes it possible to estimate effective population size (N_e). Using the following equation 1 of Sved (1971)**.**

$$E(\mathrm{r}^2) = \frac{1+1}{(\alpha+\mathrm{KNeC})n} \qquad \text{Equation 2}$$

r^2 is the LD between different makers, N_e is the effective population size, c is the genetic distance between various markers measured, n is the individual experimental sample size, α = 1 in the absence of mutation, and α = 2 if the mutation is present, k = 4 for auto chromosomes and k = 2 for x chromosome and this was implemented in SNeP software, which primarily deals with estimating historical N_e trends (Barbato *et al.*, 2015)**.**

4.2.6 Environmental-association analysis

Redundancy analysis (RDA) was done using a vegan R package (Oksanen *et al.*, 2022). RDA is a multivariate linear regression technique capable of analysing several loci at the same time to identify weak multi-locus signatures of selection (Legendre and Legendre, 2012). Genetic data and climatic variables were analysed in RDA to identify covarying sets of genotypes and their association with environmental variables. The outlier SNPs from RDA were investigated with chicken genes from Ensemble (release 110; Accessed August 2023) and the UniProt database tool was used to identify genes' biological functions.

4.3 Results

4.3.1 Genetic diversity of village chickens and conserved flock

The mean number of observed (H_O) and (H_E) heterozygosity and inbreeding coefficient per village chickens and conserved flock are presented (Table 4.1). The genetic diversity of indigenous chickens ranged from 0.341 to 0.338 for observed heterozygosity and from 0.381 to 0.387 for expected heterozygosity, whereas the conservation flocks ranged from 0.240 to 0.339 and 0.377 to 1. White leghorn had the lowest observed heterogeneity compared to other conservation flocks and village chickens. Inbreeding was low in both village chickens and conservation flocks.

Table 4.2 Estimates of the genetic diversity (He/Ho, FIS) of village-based indigenous chickens used in the study as well as ARC conservation flock representatives.

Provinces	**Districts**	**Municipality/Breed**	**No. of animals**	**Mean (H_O)±SD**	**Mean (H_E)±SD**	**Mean (F)±SD**
Limpopo	Capricorn	Mole mole	82	0.346±0.11	0.387±0.11	0.11±0.13
	Sekhukhune	Elias Motsoaledi	49	0.361±0.12	0.383±0.12	0.05±0.11
		Fetakgomo	67	0.341±0.12	0.383±0.12	0.10±0.12
KwaZulu-Natal	Harry Gwala	uMzimkhulu	21	0.373±0.15	0.387±0.11	0.005±0.05
	uMzinyathi	uMsinga	25	0.338±0.15	0.381±0.14	0.08±0.12
Gauteng (Reference population)	City of Tshwane	Ovambo	10	0.399±0.23	0.415±0.19	-0.09±0.04
		Venda	20	0.334±0.24	0.434±0.27	-0.11±0.24
		Naked Neck	20	0.349±0.23	0.422±0.24	-0.06±0.04
		Potchefstroom Koekoek	20	0.370±0.20	0.377±0.17	-0.02±0.15
		New Hampshire	10	0.306±0.25	0.520±0.30	-0.160±0
		White Leghorn	10	0.240±0.23	0.575±0.33	-0.09±0.07
		White Plymouth Rock	10	0.325±0.22	1±0.26	0.092±0.07

MAF, minor allele frequency; H_E, expected heterozygosity; Ho, observed heterozygosity; F, Inbreeding coefficient

4.3.2 Population structure analysis

The first Principal component explained 3.08% of the total variation and separated th e NN, VD and WL conserved flock from village chickens. The second principal comp onent explained 2.56% of variations, which clustered three conserved flocks, OV and NH and WR with village chickens. There is a high level of divergence in NN, VD and WL conserved flocks from village chickens.

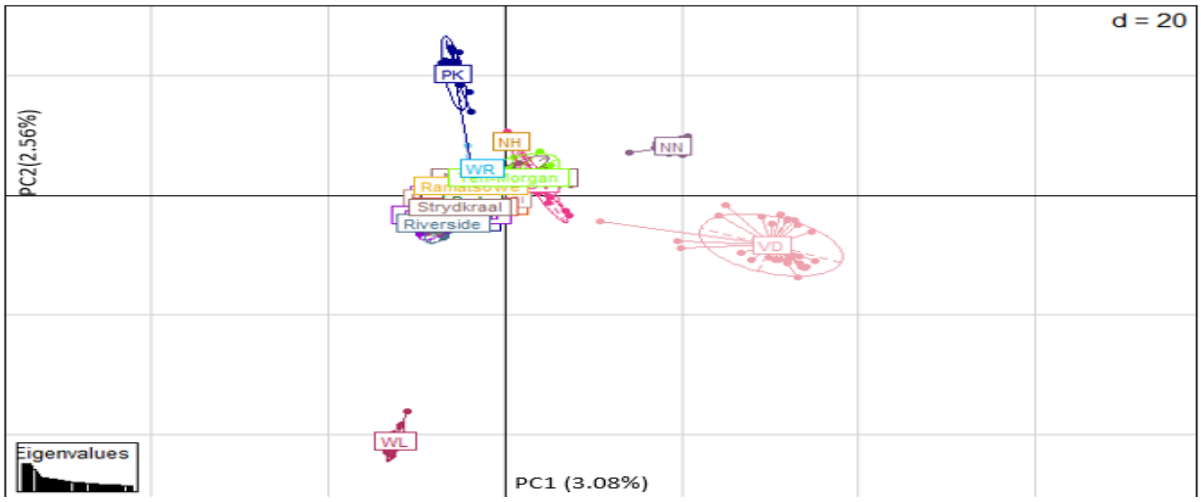


Figure 4.2: Principal component analysis of indigenous chicken and conservation flock. Venda (VD), Naked Neck (NN), Potchefstroom Koekoek (PK), Ovambo (OV), White Leghorn (WL), New Hampshire (NH) and White Plymouth Rock (WR).

4.3.3 Admixture clustering analysis

Admixture analysis is illustrated in Figure 4.3 and it was run from K=2 to K=8. In K= 2 there is a noticeable partial admixture in VD conserved flock. At K=3, the NN and VD formed distinct clusters from village chickens and two conserved flocks, OV and PK. At K=4, PK and OV still kept a slightly similar allele frequency as village chickens. K= 5, being the optimal K value of the lowest cross-validation error (0.87), where there is further separation from PK from village chickens. Increasing to K=6, the three conserved flocks NN, VD, and PK, exhibited distinct clusters from village chickens up until K = 8 with OV having slightly similar allele frequency.

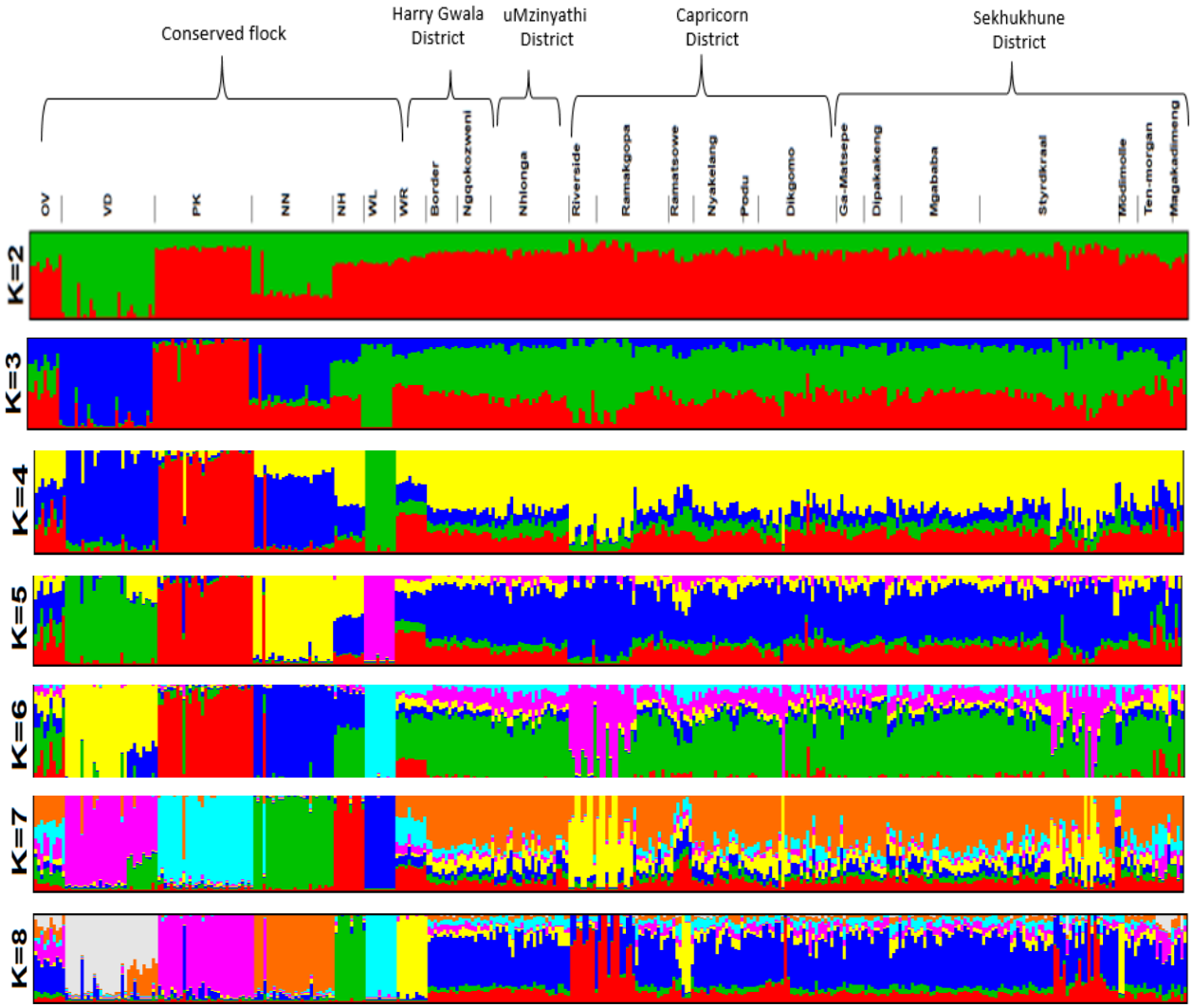
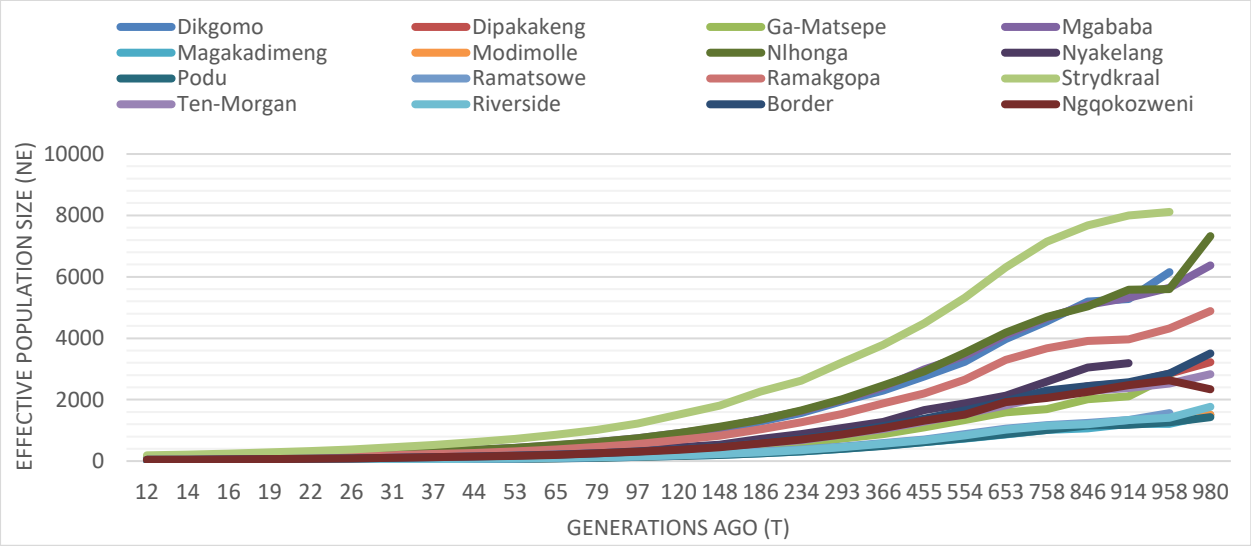


Figure 4.3 Admixture clustering of conservation flock and village chickens

4.3.4 Estimated effective population size (N_e)

Figure 4.4 illustrates the estimated N_e at the village level generations ago based on the generated graph. Compared to other village chickens, indigenous chickens from Nhlonga and Strydkraal had the highest estimated N_e that is above 4000 at 983, and there was a constant decrease of N_e for village birds 149 generations ago.



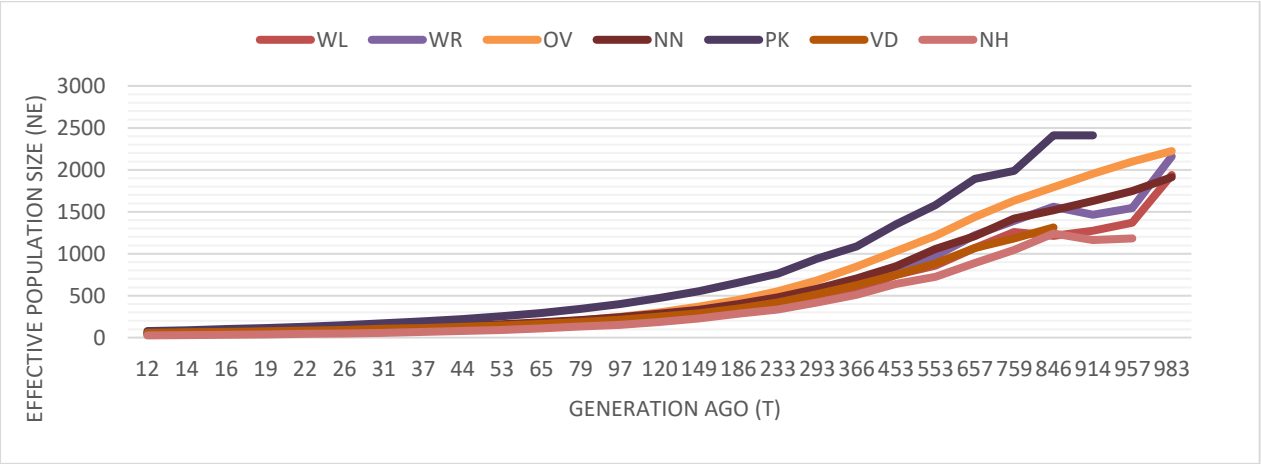


Figure 4.4: Trends of N_e of village chicken and conservation flock at the Agricultural Research Council- Animal production.

4.3.5 Signatures of adaptation

Redundancy analysis

All village chickens were distributed in RDA space with no clear clustering, reflecting their relationship with the ordination axes, which are linear combinations of the predictor variables. The first three of the RDA axes were significant and these together accounted for 46.36% of the total captured variance (Figure 4.5).

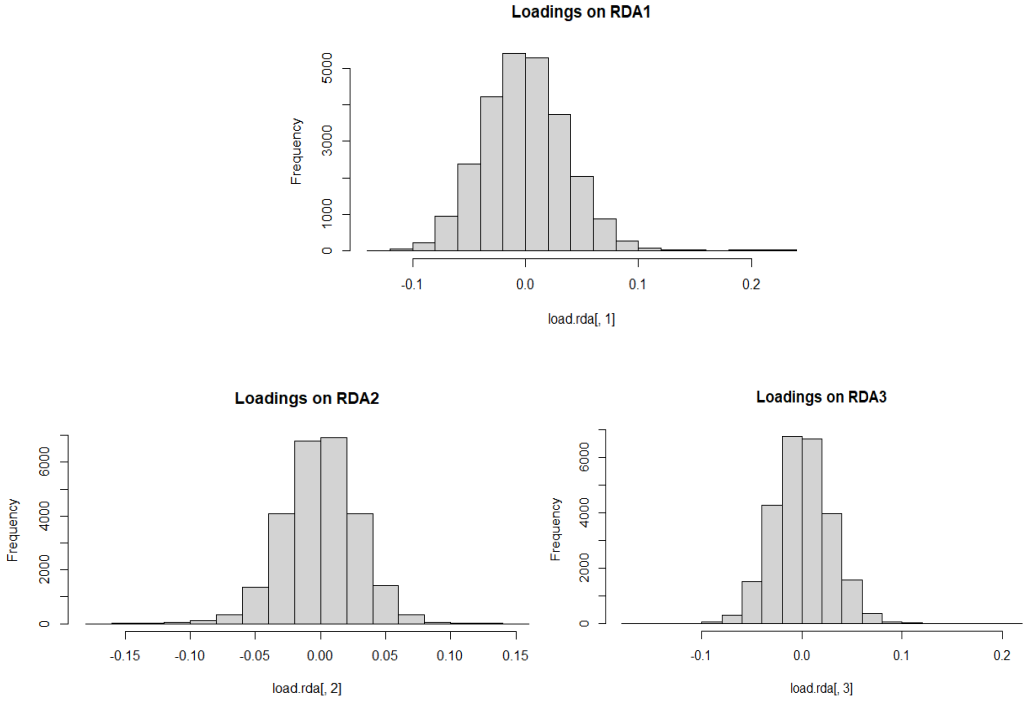
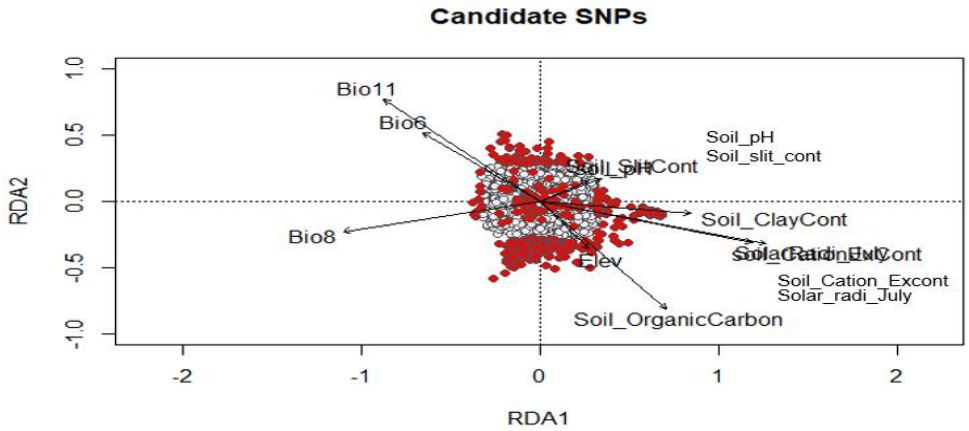


Figure 4.5: RDA loadings

Significant candidate SNPs on RDA 1 axis represent multi-locus sets of SNP genotypes associated with solar radiation and soil cation exchange content. SNPs on RDA 2 axis represent genotypes associated with BIO11 and Soil organic carbon content and SNPs on RDA 3 axis represent genotypes associated with Elevation and soil slit content.



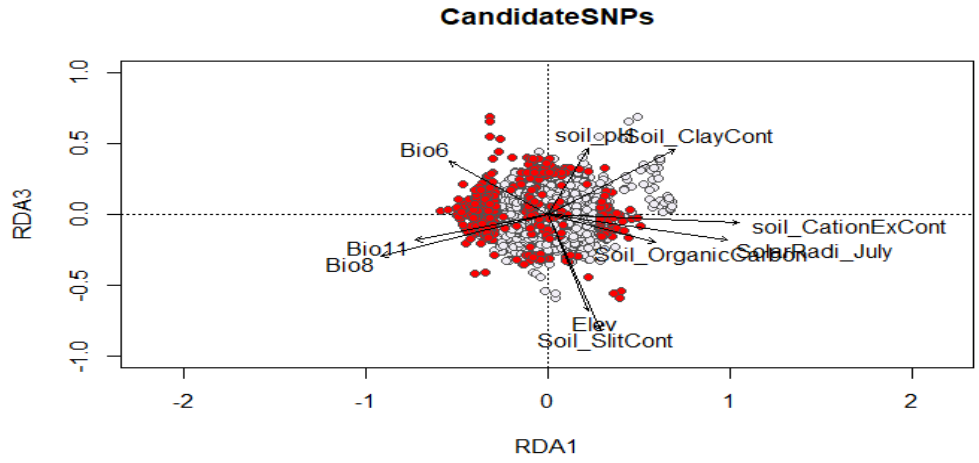


Figure 4.6: Significant candidate SNPs related with environmental variables.

A total of 386 outlier SNPs were identified and differed with their association with environmental predictors (as shown in Appendix A; Table 4.3) The genes with a high correlation (r=0.6 to 0.4) were investigated and were found to be located as follows: *MAPT* (r=0.60) gene located on chromosome 27 is involved in the MAPK signalling pathway and has been linked to solar radiation in the seventh month. The *ND2* gene (r= 0.48) located on chromosome 10 is involved in metabolic pathways and has been linked to soil cation exchange. The *UXS1* gene (r=0.44) located on chromosome 1 is involved in the biosynthesis of nucleotide sugars pathways and has been linked to BIO6 (Ref). The *ERBB4* gene (r=0.44) located on chromosome 7 is involved in the MAPK signalling pathway and has been linked to BIO11. The *NRG2* gene (r=0.48) located on chromosome 13 is involved in the ErbB signalling pathway and has been linked to elevation. *MYO18B* gene (r=0.43) located on chromosome 15 and is involved in motor proteins pathways and has been linked to soil slit content. *AGRN* gene (r=0.41) located on chromosome 21 is involved in ECM-receptor interaction pathways and has been linked to BIO6. The following are some of the high correlation genes identified the *TLCD4, ADAMTS2, MUC2, SLC9A9, LMTK2, LRBA, ADGRL, KHDRBS3, WDR89, EPHA4, NPM1, and GJBS*.

4.4 Discussion

Population structure research is essential for sustainable management and genetic advancement (van Marle-Koster *et al.*, 2008; Mtileni *et al.*, 2011; Khanyile *et al.*, 2015; Psifidi *et al.*, 2016; Nxumalo *et al.*, 2020). The importance of genetic diversity for ensuring resilient population selection cannot be overstated. One of the fundamental indicators of genetic variety is heterozygosity, this study found that the H_O in the village chicken populations ranged from 0.338 to 0.373, while in the conserved flock, it ranged from 0.240 to 0.399. The HE in village chicken populations ranged from 0.381 to 0.387, while in the conserved flock, it ranged from 0.377 to 1. Overall both village chickens and conserved flocks had a low observed heterogeneity. According to these findings, the heterozygosity values were greater than those of native Indonesian chickens (Ho=0. 241, H_E= 0.247) reported by Riztyan *et al.* (2011). In contrast, there were lower than those of Tswana native chickens reported by Machete *et al.*, (2021). The F value was positive for village chicken populations, indicating a heterozygosity deficiency, indicating population sub-structural brings the Wahlund effect (Samaraweera *et al.*, 2021). Many chicken production systems have few breeding males, leading to inbreeding and heterozygote deficit due to their lack of control over mating (Kossoga *et al.*, 2023). The results align with Indonesian chicken populations experiencing heterogeneity deficiency, with conservation flocks exhibiting excess heterogeneity (Riztyan *et al.*, 2011). A prior study indicated an excess heterogeneity in the South African conservation flock compared to village chicken populations (Mtileni, *et al.*, 2011). Through proper breeding and selection practices, good management was evident in the conservation flock.

The population structure clustered the conserved flocks into 5 distinct clusters, with VD, WL, and NN having a divergence from the village chicken population. This study and that of (Mtileni *et al.*, 2011; Khanyile *et al.*, 2015) are in agreement as they showed that conserved flocks diverged from their founder village chicken populations. This could be because the conserved flocks are formed from a small number of individuals, causing individuals to diverge through time (Mtileni *et al.*, 2011). Both the PCA and admixture analysis indicated a clear sub-structuring between village chickens and conserved flocks. Admixture analysis exhibited a low genetic variance in NN, VD and, WL. In contrast, NH, OV, WR, and, PK retained the same genetic variation as village

chickens. Trends in N_e indicated a decrease in genetic diversity in both conservation flocks and village chickens. Franklin (1980) introduced the 50/500 rule, stating that populations with of less than *Ne* of 50 are at risk of extinction. Conservation flocks, the analysis of N_e revealed that at least four of the conserved flocks may have been under threat with the *Ne* of 26, 34, 37 and 38 at 12 generations for WL, OV, WR, and NH, respectively due to their N_e being less than the critical minimum value ($Ne \approx 50$). This could be due to that conservation flocks are maintained as a close population making them to be more susceptible to genetic drift and inbreeding. For village chickens, it implies, poor management practices, such as keeping breeding stock for extended periods, leading to overlapping generations (Khanyile *et al.*, 2015). In addition, small breeding stock and high mortality rates, especially in chicks, can lead to inbreeding depression, failing to enhance genetic diversity within a population. Zhang *et al.* (2020) reported that *Ne* of all naïve chicken populations decreased over time and was higher compared to commercial chickens. Johnson *et al.* (2004) reported over the past 50 years, there has been a rise in genetic isolation and habitat fragmentation, indicating low Ne estimates for prairie chickens. Highlighting the sustainable genetic diversity of native chicken populations is needed to make sure they can meet future challenges.

The study utilized redundancy analysis, a common method in landscape genomics, to comprehend the adaptation mechanisms of indigenous chickens by examining linear correlations between genotypes and environments (Rellstab *et al.*, 2015). The study investigated the link between environmental variation and genetic polymorphisms to understand evolutionary responses to geographical heterogeneity by fitting all selected variables (as indicated in Chapter 3) simultaneously. As a result, RDA revealed 386 outliers related to environmental variables with high to moderate levels of correlation.

Given that RDA detected so many genes, the focus was on a few genes with a high correlation. The *MAPT* gene (r=0.60) is associated with solar radiation (of the seventh month), which promotes microtubule assembly and stability and might be involved in the establishment and maintenance of neuronal polarity (Cha *et al.*, 2015). This gene is significant because it enables chickens to respond to changes both inside and outside their bodies through their nervous system. The *ND2* (r=0.48) gene participates in the mitochondrial respiratory chain and oxidative phosphorylation and is involved in energy metabolism (Barker *et al.*, 2012; Yang *et al.*, 2020). The *JARID2* gene (r=0.47)

is crucial to embryonic development, including heart and liver development, neural tube fusion, and haematopoiesis (UniProt; accessed on 28 August 2023). Kharrati-Koopaee *et al.* (2021) reported that the *JARID2* gene can help with hypoxia adaptation by binding to chromatin. There may be significance in the gene for indigenous chickens in KwaZulu-Natal, as it is South Africa's highest-altitude province at 3.451 meters.

The *ADAMTS2* gene (r=0.46) processes the procollagens I, II, and III's amino-propeptides; and may be necessary for the normal deposition of collagen fibrils in animals (Bekhouche and Colige, 2015). This gene was also found to be associated with genetic resistance to Marek's diseases and functions as tumour suppression in chickens (Dong *et al.*, 2019). The *CNTN5* gene (r=0.49) mediates cell surface interactions during nervous system development (UniProt; accessed on 28 August 2023). The *ADGRAL3* gene (r=0.40) was found to be linked to elevation and is involved in brain development and neuronal functions (Gheyas *et al.*, 2021). This gene was found to be associated with soil organic carbon in Ethiopian local chickens (Gheyas *et al.*, 2021). The *MUC2* gene (r=0.51) was found to be linked with BIO6, which is responsible for lubrication and protection of the external surface of the internal epithelium tissue (UniProt; Accessed 28 August 2023). The gene is important as the chicken small intestine epithelium is composed of hormones, digesting enzymes, nutrient transporters, and glycoproteins that act as a physiological barrier against pathogenic pathogens (Peterson and Artis, 2014; Zhang, Li, *et al.*, 2019). The *SLC9A9* gene (r=0.43) was found to be linked to solar radiation of the seventh month and is responsible for insulin-induced glucose uptake by muscle, liver, and adipose tissue (Zhuang *et al.*, 2022), so these organs can use glucose as a source of energy so they can function properly. The *LMTK2* gene (r=0.40) was found to be linked with soil pH and is involved in neuronal outgrowth and development, axonal transport, intracellular vesicle trafficking, and apoptosis (Chibalina *et al.*, 2007; Manser *et al.*, 2012; Conti *et al.*, 2017). The *LRBA* gene (r=0.50) was found to be linked with BIO11, is an important gene that is involved in a syndrome of immune deficiency and autoimmunity (Li *et al.*, 2019), and was also identified as the most promising gene in *salmonella pullorum* resistance (Li *et al.*, 2019). The *KHDRBS3* gene (r=0.52) was found to be linked with BIO11 and found to be associated with diaphyseal total bone content, as well as cortical traits in chickens (Johnsson *et al.*, 2015). The *WDR89* gene

(r=0.48) in humans is associated with a response to lamotrigine, maculopapular eruption, and epilepsy (Sutera *et al.*, 2019). The *EPHA4* gene (r=0.43) was found to be linked with BIO11 and plays a role in the development of the nervous system controlling different steps of axonal guidance (UniProt). *NPM1* gene (r=0.44) was linked with BIO11 and involved in ribosome biogenesis, regulation of proliferation, and growth suppression pathways (Zarka *et al.*, 2020). The *GJB5* gene (r=0.53) is associated with cell adhesion (Kiser *et al.*, 2019). The *TLCD4* gene (r=0.40) is involved in lipid trafficking and metabolism in humans and mice (Attwood and Schiöth, 2021). This gene was also found to be associated with fat to protein rate in Murrah buffaloes (Lázaro *et al.*, 2023). The *ERBB4* gene (r= 0.44) is involved in the calcium signalling pathway, regulators of neural and muscle development (Ren *et al.*, 2018), and was also found to be involved in fear response (Chen *et al.*, 2017). RDA can also be used to create an adaptive measure that predicts individual performance under various environmental conditions (Capblancq *et al.*, 2018). RDA was used to identify loci involved in climate adaptation in *Arabidopsis thaliana* (Lasky *et al.*, 2012). Vallejo-Trujillo *et al.* (2022) performed an RDA analysis which correlated 33% of sweep regions with major environmental drivers that correspond to Ethiopian chickens with environmental parameters.

4.5 Conclusion

Overall the study examined the genetic diversity, population structure, and effective population size of village chickens and conservation flocks. It found high diversity in village chickens as well in Ovambo and Potchefstroom Koekoek. The principal component revealed population divergence indicating a distinct division between village chickens and conservation flocks. Trends of effective population size were low in both populations, particularly the conservation flocks. The RDA identified 386 SNP outliers associated with ten environmental variables located on different chromosomes and with varying correlations. However, all genes had biological relevance in the local adaptation of indigenous chickens. The findings of this study emphasize the importance of conserving indigenous chicken as a unique genetic resource, as well as how the environment contributes to indigenous chicken diversity through exposure to various natural pressures.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSION

5.1 Introduction

In this chapter, general conclusions are made answering the three objectives identified in this study and the limitations discovered throughout the research are discussed. Lastly, the recommendations based on the findings and analysis are outlined and future investigations.

5.2 General conclusion

The escalating human population, climate change, and expanding market demands, all affect the current diversity of indigenous chickens. Over the decades, there has been a significant genetic selection in poultry products to meet consumer preferences and demand for specific breeds and lines (Ben Larbi *et al.*, 2018). The introduction of exotic breeds to enhance indigenous chicken productivity has proven unsuccessful due to their inability to adapt to production systems, leading to genetic erosion by indiscriminate crossbreeding (Besbes, 2009; Desta, 2021b). The invention of NGS and spatial technologies has enabled researchers to comprehend the evolutionary changes in livestock. This supports successful conservation initiatives that are crucial for the long-term sustainability of rare genetic resources, especially in the face of climate change challenges. Our study emphasizes the significance of genetic diversity, which is related to population size, and largely determines whether populations can adapt or face extinction. This study offers guidelines for preserving genetic variety and understanding environmental factors driving local adaptation. The first objective examined key environmental factors that affect the distribution of village chickens in the event of future climate changes. This can support breeding initiatives and future planning to create climate-resilient breeds, increasing the market surplus of appropriate genotypes that fit their intended environments. The second objective examined the diversity of village chickens and identified the genes that promote local adaption. Such knowledge can aid in conservation efforts by knowing what needs to be preserved and managing population size to secure these traits. Overall, the study demonstrates that identifying the environmental drivers of adaptation before exploring the relevant genetic pathways is a powerful new way to understand natural selection

in domestic animals. This approach effectively captures intricate interactions between various environmental variables, including those between genotype and environment. (Vallejo-Trujillo *et al.*, 2022).

5.3 Recommendations and limitations

The data used in the study have been drawn from a wide range of environmental sources such as climatic and soil factors. However, the study did not include other crucial drivers of adaptation, such as vegetation and pathogenic or parasitic infestation data. However, our methodology presents an intriguing prospect for such incorporation in future studies. In addition, other models such as the phenotypic distribution model, can also be used to study variation within quantitative traits in response to the most significant set of environmental variables identified by ENM.

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APPENDIX A: Identified RDA outliers in relation to environmental variables

Chr	Outlier SNPs	SNP position	Axis	Gene	function	Predicator variable	correlation	Reference
0	GGaluGA000438_A	0	1	-		Solar radiation of 7^{th} month	0.570932775	
0	GGaluGA000689_G	0	1	-		Solar radiation of 7^{th} month	0.259755885	
0	GGaluGA001016_G	0	1	-		Soil pH	0.668014831	
0	GGaluGA001283_A	0	1	-		Solar radiation of 7^{th} month	0.298274267	

0	GGaluGA001691_G	0	1	-		Soil pH	0.612309645	
0	GGaluGA001719_G	0	1	-		Soil pH	0.577008153	
0	GGaluGA333626_A	0	1	-		Soil pH	0.668014831	
0	GGaluGA345101_G	0	1	-		Soil cation exchange	0.339741136	
0	GGaluGA345127_A	0	1			Soil pH	0.668014831	
0	GGaluGA345140_A	0	1	-		Solar radiation of 7^{th} month	0.411297083	

0	GGaluGA345167_G	0	1	-		Soil pH	0.583394715	
0	GGaluGA345246_A	0	1	-		Solar radiation of 7^{th} month	0.612114102	
0	GGaluGA345253_A	0	1	-		Solar radiation of 7^{th} month	0.556320967	
0	GGaluGA345258_A	0	1	-		Soil pH	0.603772813	
0	GGaluGA345360_A	0	1	-		Solar radiation of 7^{th} month	0.532212232	
0	GGaluGA345366_A	0	1			Soil cation	0.363076928	

						exchange		
0	GGaluGA345372_G	0	1	-		Soil pH	0.668014831	
0	GGaluGA345380_A	0	1	-		Solar radiation of 7^{th} month	0.492100334	
0	GGaluGA345461_A	0	1	-		Soil pH	0.522567433	
0	GGaluGA345485_G	0	1	-		Soil pH	0.665484501	
0	GGaluGA345489_G	0	1	-		BIO11	0.566896075	
0	GGaluGA345495_C	0	1	-		Solar radiation	0.574177515	

						of 7th month		
0	GGaluGA345518_A	0	1	-		Soil cation exchange	0.398343585	
0	GGaluGA345540_G	0	1	-		Soil organic carbon content	0.540597403	
0	GGaluGA345588_A	0	1	-		Solar radiation of 7th month	0.607095436	
0	GGaluGA345589_G	0	1	-		Solar radiation of 7th month	0.609720876	
0	GGaluGA345757_G	0	1	-		Soil pH	0.532921944	

0	GGaluGA354605_A	0	1	-		Solar radiation of 7th month	0.609462436	
0	GGaluGA354675_C	0	1	-		Solar radiation of 7th month	0.601339376	
0	GGaluGA360289_A	0	1	-		Solar radiation of 7th month	0.61125605	
0	GGaluGA360310_G	0	1	-		Solar radiation of 7th month	0.601339376	
0	GGaluGA360405_A	0	1	-		Soil pH	0.581089594	
0	GGaluGA360443_A	0	1	-		Solar radiation	0.538015863	

						of 7th month		
0	GGaluGA360458_A	0	1	-		Solar radiation of 7th month	0.606385828	
0	GGaluGA360482_A	0	1	-		Solar radiation of 7th month	0.53588369	
0	Gga_rs16027111_C	222183	1	-		Soil pH	0.532921944	
0	GGaluGA344885_G	448990	1	-		Solar radiation of 7th month	0.583812358	
27	Gga_rs14302623_A	2610339	1	*MAPT*	It is located in the nervous system and is involved in the process of microtubule	Solar radiation of 7th month	0.609462436	UniProt,(Cha *et al.*, 2015)

					stabilization as well as the formation of the cytoskeleton.			
0	GGaluGA347263_G	7690473	1	-		Soil pH	0.59855582	
1	Gga_rs13861394_G	48676526	1	*GRIN2B*	Function as a ligand-gated ion channel in the central nervous system, has a role in excitatory synaptic transmission.	BIO8	0.305255438	UniProt
1	Gga_rs15366328_A	106836558	1	-		Solar radiation of 7th month	0.604503473	
1	Gga_rs13924905_G	117316055	1	*ENSGALG00010003521*		Solar radiation of 7th month	0.343526703	
1	GGaluGA042731_A	128521686	1	-		Soil cation exchange	0.460931816	

1	GGaluGA044867_G	136321669	1	-		Solar radiation of 7th month	0.301383	
1	GGaluGA044867_G	136321669	1	*GCC2*	Encodes a membrane protein localized to the trans-Golgi network and is related to the vesicular transport between the endosomes and the Golgi.	Solar radiation of 7th month	0.301382609	(Wu *et al.*, 2022)
1	Gga_rs13947014_A	142307262	1	*ENSGALG00010006221*		Solar radiation of 7th month	0.466738322	
1	Gga_rs15462937_A	154513024	1	-		BIO8	0.376918616	
1	Gga_rs15523624_C	183681253	1	*CNTN5*	Mediate cell surface interactions during nervous system development.	Soil pH	0.490807038	UniProt
2	GGaluGA167394_A	130300569	1	-		BIO8	0.305351506	

2	GGaluGA170562_A	142400711	1	-		Soil cation exchange	0.390399739	
3	GGaluGA206756_G	9514328	1	*EHBP1*	Required for perinuclear sorting and insulin-regulated recycling of SLC2A4/GLUT4 in adipocytes.	BIO8	0.326132522	(Guilherme *et al.*, 2004)
3	GGaluGA216016_A	35506417	1	*OPN3*	Is associated with light-independent functions such as the regulation of melanogenesis and apoptosis in epidermal melanocytes, may be involved in photo relaxation of airway smooth muscle cells.	Solar radiation of 7th month	0.315956633	(Ozdeslik *et al.*, 2019)
3	Gga_rs14347250_G	45039910	1	-		BIO8	0.38854121	

3	Gga_rs14356816_C	50829540	1	-		BIO11	0.430724931	
3	Gga_rs15359681_A	55542623	1	*ENSGALG00010006629*		Soil cation exchange	0.332269176	
3	GGaluGA229903_G	79969785	1	*ENSGALG00010004882*		Soil pH	0.300978597	
4	Gga_rs15597184_G	62785313	1	*FGF20*	A novel central player in the development of vertebrate skin appendages, including hair follicles and exocrine glands.	Solar radiation of 7th month	0.347410908	(Biggs *et al.*, 2018; Wells *et al.*, 2012)
4	GGaluGA268598_A	86602861	1	-		Soil pH	0.663042228	
5	Gga_rs16468580_G	14556016	1	*MUC2*	Responsible for lubrication and protection of the external surface of the internal epithelium tissue.	BIO11	0.515664	UniProt

5	GGaluGA283462_A	39569745	1	*NRXN3*	Involved in cell recognition and cell adhesion, plays a role in angiogenesis	Solar radiation of 7th month	0.351916	(Bottos *et al.*, 2009)
5	GGaluGA284084_A	40771771	1	-		Soil pH	0.568704	
5	Gga_rs15736049_A	56204029	1	*ENSGALG00010020239*		Solar radiation of 7th month	0.394916	
5	Gga_rs14552339_G	57143305	1	*ENSGALG00010020247*		Soil cation exchange	0.338525	
6	GGaluGA304457_A	29878437	1	*ENSGALG00010023121*		BIO8	0.456257	
6	GGaluGA305836_A	33039706	1	*CTBP2*	Function in brown adipose tissue (BAT) differentiation.	BIO8	0.347642	UniProt

8	Gga_rs15911039_A	13112398	1	*TLCD4*	Is involved in lipid trafficking and metabolism.	Solar radiation of 7th month	0.402395	(Attwood and Schiöth, 2021)
8	Gga_rs14652414_G	24095217	1	*TUT4*	Essential for both oocyte maturation and fertility.	Soil pH	0.348098	UniProt
9	Gga_rs14663375_C	10399040	1	*SLC9A9*	Is responsible for insulin-induced glucose uptake by muscle and adipose tissue.	Solar radiation of 7th month	0.431287	(Zhuang *et al.*, 2022)
9	GGaluGA344028_G	24218624	1	-		BIO8	0.344037	
10	Gga_rs15559396_C	1153367	1	-		BIO11	0.464416	
10	GGaluGA070801_G	15680150	1	*ND2*	Participates in mitochondrial respiratory chain and oxidative phosphorylation, involved in energy metabolism.	Soil cation exchange	0.483112	(Barker *et al.*, 2012; Yang *et al.*, 2020)
11	Gga_rs14022503_G	9324097	1	*ENSGALG00010025540*		Soil pH	0.45396	

11	Gga_rs14964520_A	12002435	1	*ENSGALG00010010192*		Solar radiation of 7th month	0.601339	
12	Gga_rs14692320_A	14147	1	-		Solar radiation of 7th month	0.611979	
12	Gga_rs14029638_G	35026	1	*ENSGAG00010028209*		BIO11	0.486083	
12	GGaluGA080925_G	1040195	1	-		Solar radiation of 7th month	0.39556	
12	Gga_rs15656424_A	12454240	1	*FHIT*	Is associated with body mass index, and may be associated with carcinogenesis.	BIO8	0.339138	(Ahmad *et al.*, 2016; Joannes *et al.*, 2014)

12	GGaluGA087997_G	16378623	1	*FOXP1*	Regulates gene transcription, controls adipocyte differentiation, and plays a tissue-specific role.	Solar radiation of 7th month	0.357006	UniProt,(Liu, Huang, *et al.*, 2019)
13	Gga_rs14060730_A	12317148	1	-		Solar radiation of 7th month	0.485896	
13	GGaluGA096104_A	13280239	1	*ADAMTS2*	It process the procollagens I, II, and III's amino-propeptides; and may be necessary for the normal deposition of collagen fibrils.	Soil cation exchange	0.46034	(Bekhouche and Colige, 2015)
13	Gga_rs29007783_G	18860595	1	-		Solar radiation of 7th month	0.59197	
14	Gga_rs15003166_G	1082003	1	*LMTK2*	Involved in neuronal outgrowth and development, axonal transport, intracellular vesicle trafficking, and apoptosis.	Soil pH	0.401391	(Chibalina *et al.*, 2007; Conti *et al.*,

								2017; Manser *et al.*, 2012)
14	GGaluGA104361_A	12967732	1	-		Solar radiation of 7th month	0.348991	
15	GGaluGA106699_A	2030299	1	-		Soil pH	0.275557	
15	Gga_rs15020396_G	5302531	1			Soil pH	0.322798	
17	GGaluGA114611_G	5399508	1	-		Soil cation exchange	0.417505	
18	GGaluGA119634_A	3815304	1	*B3GNTL1*	Is a putative glycosyltransferase.	Solar radiation of 7th month	0.32445	(Wu *et al.*, 2022)
20	Gga_rs15170561_G	2747608	1	*TOX2*	Putative transcriptional activator involved in the	Solar radiation	0.287132	UniProt

					hypothalamus-pituitary-gonadal system.	of 7th month		
20	Gga_rs16172230_A	10301225	1	-		Soil cation exchange	0.311473	
20	GGaluGA180443_A	10438342	1	*XKR7*	The biochemical function and tissue distribution of members of the Xkr family are not well understood.	Solar radiation of 7th month	0.498692	(Suzuki *et al.*, 2014)
23	Gga_rs14291225_G	4804301	1	-		Solar radiation of 7th month	0.601339	
23	Gga_rs13623028_A	6005041	1	*MYOM3*	Involved in binding of myosin to other proteins such as titin, stabilizing the thick filament lattice and the sarcomere structure (found in striated muscles).	Solar radiation of 7th month	0.336885	(Schoenauer *et al.*, 2008)

26	Gga_rs16346673_A	8011	1	-		Soil pH	0.668015	
26	GGaluGA196819_G	2987508	1	*PLXNA2*	Play a role in axon guidance invasive growth and cell migration	Solar radiation of 7th month	0.357965	UniProt
	RDA2							
0	GGaluGA000051_G	0	2	-		BIO11	0.358782	
0	GGaluGA000319_A	0	2	-		BIO11	0.302977	
0	GGaluGA000580_A	0	2	-		BIO11	0.268772	
0	GGaluGA000840_A	0	2	-		BIO11	0.190498	
0	GGaluGA000872_A	0	2	-		BIO6	0.409833	
0	GGaluGA001396_G	0	2	-		BIO11	0.342572	

0	GGaluGA001797_G	0	2	-		Soil organic carbon	0.608252	
0	GGaluGA271401_A	0	2	-		Soil organic carbon	0.644419	
0	GGaluGA345283_A	0	2	-		BIO6	0.431741	
0	GGaluGA345365_A	0	2	-		BIO11	0.275914	
0	GGaluGA345416_A	0	2	-		Soil organic carbon	0.644419	
0	GGaluGA360195_A	0	2	-		BIO11	0.383779	
0	GGaluGA360247_G	0	2	-		BIO11	0.319337	
0	GGaluGA360297_G	0	2	-		Soil organic carbon	0.598915	

0	GGaluGA360452_A	0	2			Soil organic carbon	0.425539	
0	Gga_rs14725137_G	292354	2	-		BIO6	0.264881	
0	Gga_rs14689052_A	389095	2	-		BIO11	0.284844	
0	Gga_rs13772846_G	579201	2	-		BIO6	0.375454	
28	Gga_rs16209716_G	684728	2	-		Elevation	0.297659	
28	GGaluGA201504_G	1474215	2	*MFSD12*	It regulates skin and hair pigmentation.	BIO11	0.329363	(Hédan *et al.*, 2019)
27	Gga_rs16719252_C	2956025	2	-		Soil clay content	0.141902	
27	Gga_rs14302011_A	2012987	2	*TLK2*	Involved in DNA replication, DNA repair, transcription, chromatin structure, chromosome segregation, RNA interference, viral	Elevation	0.238691	UniProt

					latency, and cell cycle checkpoint.			
27	GGaluGA200100_G	3731373	2	-		BIO6	0.352326	
27	GGaluGA200497_A	4458047	2	-		Soil organic carbon	0.481089	
0	Gga_rs16765066_G	26304442	2	-		BIO6	0.472365	
0	Gga_rs16123132_A	27929968	2	-		BIO11	0.542869	
0	Gga_rs14778854_C		2	-		BIO11	0.605303	
1	GGaluGA003981_G	6583362	2	-		BIO6	0.369874	
1	Gga_rs15200439_G	13158546	2	-		BIO6	0.34756	

1	Gga_rs13842165_A	28800862	2	*NRCAM*	Involved in the development of optic fibres in the retina.	BIO11	0.251643	UniProt
1	Gga_rs14803801_G	29831809	2	*PRICKLE1*	Involved in the planar cell polarity pathway.	BIO6	0.384737	(McNeill and Woodgett, 2010)
1	GGaluGA010542_G	31864765	2	*LRIG3*	Plays a role in embryo development, including cranio-facial morphogenesis and neural crest formation.	BIO11	0.210361	(Abraira *et al.*, 2008)
1	Gga_rs13849591_G	36348081	2	*LRIG3*	Plays a role in embryo development, including cranio-facial morphogenesis and neural crest formation.	BIO11	0.483575	(Abraira *et al.*, 2008)
1	Gga_rs13853671_A	40142197	2	*ENSGALG00010014881*	-	BIO6	0.364983	
1	Gga_rs13855683_G	41875670	2	*ENSGALG00010011199*	-	Soil clay content	0.158313	

1	GGaluGA023549_G	69261786	2	*PARVG*	Plays a role in the regulation of cell adhesion and cytoskeleton organization.	BIO6	0.241006	UniProt
1	Gga_rs13883961_G	71754673	2	*LRP6*	Involved in numerous biological activities such as cell proliferation, specification, migration, metastatic cancer, and embryonic development.	Soil clay content	0.243693	(MacDonald and He, 2012)
1	GGaluGA026119_A	79592879	2	*STXBP5L*	Plays a role in vesicle trafficking and exocytosis inhibition, neurotransmitter release by inhibiting basal.	Soil clay content	0.246296	UniProt
1	Gga_rs13887360_G	80600766	2	*CHD1L*	Promote cell proliferation, enhance cell migration, and inhibit apoptosis by regulating various complex networks.	BIO6	0.335955	(Xu *et al.*, 2016)
1	Gga_rs15332915_A	88873985	2	*C1H3ORF52*	Involved in membrane structure.	BIO6	0.346587	(Cheng *et al.*, 2019)
1	GGaluGA032419_A	95433715	2	*GBE1*	Required for normal glycogen accumulation.	BIO11	0.258916	(Chen *et al.*, 2022),UniProt

1	Gga_rs13924926_G	117411374	2	-		BIO6	0.369914	
1	Gga_rs13924967_G	117487641	2			BIO11	0.422062	
1	GGaluGA041232_A	124377338	2	*ARHGAP6*	Regulate actin polymerization during many cellular activities, including tumour cellular.	BIO11	0.226669	(Tribioli *et al.*, 1996; Schaefer *et al.*, 1997)
1	Gga_rs13937342_A	132997809	2	*AFF3*	Mediating transcriptional activity, revealing an association with the positive transcription elongation factor b (P-TEFb).	BIO6	0.350997	(Bitoun *et al.*, 2007)
1	Gga_rs13940030_G	135573467	2	*UXS1*	Is unique among the enzymes involved in nucleotide sugar biosynthesis in that it is found within the secretory pathway lumen.	BIO6	0.442417	(Xiao *et al.*, 2020)

1	Gga_rs13942782_G	138216261	2	*MYO16*	Serve in intracellular movements, involved in targeting of the catalytic subunit of protein phosphatase 1 during brain development.	BIO11	0.314843	UniProt
1	Gga_rs13712468_C	149395062	2	-				
1	GGaluGA050403_A	154425848	2	*FBXL3*	Role in the maintenance of both the speed and the robustness of the circadian clock oscillation.	BIO11	0.463569	UniProt
1	GGaluGA051950_G	160862971	2	-		BIO6	0.287257	
1	Gga_rs15497877_A	173593810	2	-		BIO11	0.337634	
1	GGaluGA057568_A	180632601	2	-		Soil clay content	0.304238	
1	Gga_rs13981373_G	181735314	2	-		BIO6	0.3463	

1	Gga_rs13992644_G	192077117	2	*TENM4*	Involved in neural development, regulating the establishment of proper connectivity within the nervous system.	BIO11	0.350606	(Hong *et al.*, 2012)
1	GGaluGA062629_G	195409234	2	-		BIO11	0.29851	
1	GGaluGA140145_A	32267173	2	-		BIO11	0.37032	
2	Gga_rs10729700_A	32929834	2	*JAZF1*	Regulates cell growth and proliferation through different physiological pathways, thereby acting as a transcriptional growth factor.	BIO6	0.344331	(A Abuderman *et al.*, 2020)
2	Gga_rs15084136_A	36031561	2	-		BIO11	0.213304	
	GGaluGA141341_A	36593043	2	-		BIO11	0.304566	

2	GGaluGA142667_A	40162929	2	*OSBPL10*	Involved in lipid counter transport between the endoplasmic reticulum and the plasma membrane.	BIO11	0.294823	UniProt
2	Gga_rs15088767_A	41004774	2	-		BIO11	0.348476	
2	Gga_rs14188183_G	59222173	2	-		BIO6	0.392187	
2	Gga_rs14189950_G	60439301	2	-		BIO11	0.34779	
2	Gga_rs14190499_G	60987066	2	*JARID2*	It is crucial to embryonic development, including heart and liver development, neural tube fusion, and haematopoiesis.	BIO6	0.475138	UniProt
2	Gga_rs14195082_A	64526132	2	-		Soil_pH	0.202072	
2	Gga_rs15118483_G	79104255	2	-		BIO6	0.386874	

2	Gga_rs14220432_A	94753701	2	-		BIO11	0.287786	
2	Gga_rs13640047_C	98545603	2	-		BIO6	0.34181	
2	GGaluGA159003_G	98620682	2	*PTPRM*	Plays a role in adipogenic differentiation.	BIO11	0.259757	UniProt
2	Gga_rs14225325_A	103152561	2	-		BIO11	0.382529	
2	GGaluGA161488_A	106631880	2	-		Soil clay content	0.282233	
2	Gga_rs14232790_A	112617669	2	*CHD7*	Probable transcription regulator, involved in the in 45S precursor rRNA production.	BIO11	0.36609	UniProt
2	Gga_rs14238834_A	119948911	2	*PKIA*	Interacts with the catalytic subunit of the enzyme after the cAMP-induced dissociation of its regulatory chains.	BIO11	0.28614	UniProt

2	Gga_rs14241422_C	123037752	2	-		BIO6	0.330965	
2	Gga_rs14249696_G	131239733	2	*EMC2*	Associated with embryonic development.	BIO11	0.336711	(Fang *et al.*, 2022)
2	Gga_rs14251874_A	136204598	2	-		BIO11	0.263556	
2	Gga_rs15158544_G	139849672	2	-		BIO6	0.401384	
2	GGaluGA170215_G	141447770	2	-		BIO6	0.386766	
2	GGaluGA170471_G	142116401	2	*KHDRBS3*	Found to be associated with diaphyseal total bone content, as well as the cortical traits.	BIO11	0.539117	(Johnsson *et al.*, 2015)
2	GGaluGA171676_C	145445023	2	-		BIO6	0.26	
3	Gga_rs14315833_G	8645980	2	*ALK*	Associated with parasite resistance traits.	BIO11	0.234763	(Arzik *et al.*, 2022)

3	Gga_rs13503629_A	9528980	2	*EHBP1*	Required for perinuclear sorting and insulin-regulated recycling of SLC2A4/GLUT4 in adipocytes.	BIO6	0.262727	(Guilherme *et al.*, 2004)
3	Gga_rs13733710_A		2	*PLCB4*	Play role in Immune response and heat stress.	Bio6	0.393056	(Jin *et al.*, 2017; Li *et al.*, 2020)
3	Gga_rs16729741_C		2	-		BIO11	0.492655	
3	Gga_rs13723135_A	34716803	2	*AKT3*	Function in controlling fat and cholesterol composition, contributes to immunity processes.	BIO11	0.219451	(Farmanullah *et al.*, 2020; Tsiperson *et al.*, 2013)
3	GGaluGA216266_G	36175337	2	*CHRM3*	Mediates cellular responses, inhibiting adenylate cyclase, breaking phosphoinositide's, and modulating potassium channels through G proteins, with Pi turnover being the primary transducing effect.	BIO6	0.382008	UniProt

3	Gga_rs13724003_A	36675339	2	-		BIO11	0.339663	
3	Gga_rs14341210_G	37571945	2	-		BIO11	0.541957	
3	Gga_rs14343948_C	42212061	2	*RNASET2*	Regulates antioxidant tone and is required for physiological response to reactive oxygen.	Elevation	0.278347	(Gondret *et al.*, 2017)
3	GGaluGA218188_G	42342712	2	*RPS6KA2*	Is associated with reproduction.	BIO6	0.314453	(Sun *et al.*, 2023)
3	Gga_rs14367309_A	65331622	2	-		BIO11	0.295454	
3	Gga_rs14373013_A	70766828	2	-		BIO11	0.336752	
3	Gga_rs14379070_G	78522143	2	-		BIO11	0.34352	
3	Gga_rs14389831_G	89439162	2	*CSMD1*	Acts as a tumour suppressor.	BIO11	0.30332	(Kamal *et al.*, 2017)

3	Gga_rs14405234_A	104442900	2	-		BIO11	0.172121	
3	GGaluGA236963_G	104509695	2	-		BIO11	0.330605	
3	GGaluGA237309_A	105844475	2	-		Soil silt content	0.188737	
3	GGaluGA237957_G	107670367	2	-		BIO11	0.348504	
3	Gga_rs16348764_G	1285816	2	-		BIO6	0.287543	
4	Gga_rs15476289_C	3020921	2	-		BIO11	0.316492	
4	GGaluGA242707_C	3161005	2	*KLHL9*	Involved in distal myopathy.	BIO6	0.308012	(Cirak *et al.*, 2010)
4	Gga_rs14430470_A	12983537	2	-		BIO6	0.361048	
4	Gga_rs14435465_G	17485479	2	-		Soil clay content	0.216688	

4	Gga_rs13576865_A	25230815	2	-		BIO6	0.421547	
4	Gga_rs14445405_G	31886929	2	*NR3C2*	Showing features for DNA binding.	BIO6	0.350457	UniProt
4	GGaluGA251938_G	32448004	2	*LRBA*	Coordinates immune receptor signalling to increase effector function and so plays an important role in immune regulation.	BIO11	0.501649	(Wang *et al.*, 2001)
4	GGaluGA254118_A	38194284	2	*GSTCD*	Is associated with lung functionality and respiratory system.	BIO6	0.238441	(Aboulnaga *et al.*, 2021; Liu, Azimi, *et al.*, 2017)
4	Gga_rs14455367_G	40713404	2	-		BIO11	0.510365	
4	Gga_rs14462628_A	47754794	2	*ADGRL3*	Involvement in the brain and the neuronal development and processes.	Elevation	0.400325	(Gheyas *et al.*, 2021)

4	GGaluGA261434_G	59995702	2	*PPP3CA*	Involved in the calcineurin signalling pathway, which initiates skeletal muscle differentiation.	BIO6	0.352845	(Friday *et al.*, 2003)
4	Gga_rs14477470_A	60513755	2	*CENPE*	Plays an important role in chromosome congress ion, microtubule-kinetochore conjugation and spindle assembly checkpoint activation.	BIO11	0.289628	UniProt
4	Gga_rs15601457_G	65227298	2	*LNX1*	Ubiquitin_ligases that interact with Numb a key regulator of neurogenesis and neuronal differentiation.	BIO11	0.322165	(Lenihan *et al.*, 2014)
4	Gga_rs13550100_G	68601761	2	*PDS5A*	Regulates muscle cell proliferation and muscle fiber transformation.	BIO11	0.255703	(Wang *et al.*, 2020)
4	GGaluGA264376_A	70308348	2	-		Soil clay content	0.218179	

4	Gga_rs16448899_A	89076844	2	-		Elevation	0.520781	
5	Gga_rs16736715_G	239332	2	*TMEM132A*	Has a role in both embryonic and postnatal brain development Increased resistance to cell death caused by serum deprivation in cultured cells.	BIO11	0.283864	UniProt
5	GGaluGA272203_G	1553354	2	-		BIO11	0.303566	
5	Gga_rs14352346_G	4989663	2	-		Soil clay content	0.206971	
5	GGaluGA274762_G	11330406	2	-		BIO11	0.252395	
5	GGaluGA274797_G	11421851	2	*PIK3C2A*	Involved with insulin secretion, which is regulated by the insulin receptor and it also participates in the exocytosis of insulin granules.	BIO11	0.381737	(Marchesi *et al.*, 2021)

5	GGaluGA274944_A	12139609	2	*GTF2H1*	Involved in DNA repair factor IIH (TFIIH) core complex.	BIO8	0.177678	UniProt
5	GGaluGA275823_C	14612505	2	-		Elevation	0.285755	
5	GGaluGA280970_G	29805529	2	*RYR3*	Play a role in triggering muscle contraction.	BIO11	0.340215	UniProt (Sporer *et al.*, 2012)
5	GGaluGA282400_G	35263968	2	-		BIO11	0.364315	
5	Gga_rs14547172_G	52581455	2	*SYNE2*	Involved in cardiomyopathy networks.	BIO11	0.387993	(Kharrati-Koopaee *et al.*, 2021)
5	Gga_rs13590914_A	52629954	2	SYNE2	Involved in cardiomyopathy networks.	BIO11	0.337106	(Kharrati-Koopaee *et al.*, 2021)
5	Gga_rs14547414_C	52775726	2	*WDR89*	It is essential for the elongation step of protein synthesis.	BIO11	0.486733	(Chen, Stanbouly, *et al.*, 2021)

6	GGaluGA294542_G	4710384	2	*ENSGALG00010011328*		Elevation	0.669031	
6	Gga_rs16537473_G	8712763	2	*ENSGALG00010011380*		BIO11	0.45859	
6	Gga_rs14578424_A	17983333	2	*ENSGALG00010023741*		BIO6	0.433617	
6	Gga_rs10727195_A	27267941	2	*ENSGALG00010023085*		BIO6	0.445292	
6	Gga_rs16558943_A	28062984	2	*ENSGALG00010023092*		BIO11	0.323245	
6	Gga_rs14590924_G	31003444	2	*RGS10*	Function as GTPase activating protein (GAP) for Gαi family of G-proteins, highly expressed in the central nervous system (CNS) and immune system.	BIO11	0.376108	(Waugh *et al.*, 2005)

7	Gga_rs16571933_G	1609228	2	*ENSGALG00010015519*		BIO11	0.435926	
7	Gga_rs14600805_G	3229998	2	*ERBB4*	Involved in the calcium signalling pathway, regulators of neural and muscle development, also involved in fear response.	BIO11	0.447538	(Chen *et al.*, 2017; Ren *et al.*, 2018)
7	Gga_rs14608371_A	13219459	2	*ABI2*	Is associated with muscle development.	Soil Organic carbon	0.201431	(Ma *et al.*, 2022)
7	GGaluGA312831_A	14595204	2	-		BIO11	0.233066	
7	Gga_rs14610948_G	17223472	2	-		BIO11	0.530392	
7	Gga_rs14613256_A	20726831	2	*FAP*	Associated with proliferation and function of fibroblasts.	BIO8	0.194261	(Papah *et al.*, 2018)

7	Gga_rs16594933_A	22446374	2	-		BIO11	0.308584	
7	GGaluGA316024_G	24505528	2	*ENSGALG00010022080*		BIO11	0.32516	
7	GGaluGA318577_A	30047727	2	*ACMSD*	Encodes for a critical enzyme of the kynurenine pathway of the tryptophan metabolism.	BIO11	0.293359	UniProt, (Martí-Massó *et al.*, 2013)
7	GGaluGA320978_C	36515245	2	-		BIO11	0.313398	
7	GGaluGA321196_A	36958722	2	-		Soil clay content	0.314136	
7	GGaluGA321212_A	36979435	2	-		Soil Organic carbon	0.169888	
8	Gga_rs14633891_C	2430283	2	*DENND1B*	It regulate cell inflammation and apoptosis by sponging miR-30e-3p.	BIO11	0.528472	(Chen *et al.*, 2021)

8	GGaluGA322786_A	3591953	2	*RGS4*	Is associated with myometrium relaxation.	BIO11	0.294681	(Zwane *et al.*, 2021)
8	GGaluGA324393_G	6475929	2	*RASAL2*	Metabolic regulator involved in energy homeostasis and adipogenesis.	BIO11	0.291841	(Ding *et al.*, 2021)
8	GGaluGA325049_G	7385552	2	-		BIO11	0.238475	
8	GGaluGA325424_G	8018804	2	*FAM129A*	Is found to be associated with abdominal fat weight.	BIO11	0.322506	(Zhang, Shen, *et al.*, 2020)
8	Gga_rs16632921_G	16860915	2	*ENSGALG00010024405*		BIO11	0.41579	
8	Gga_rs14645777_C	17356752	2	*ENSGALG00010024443*		BIO11	0.192217	

9	Gga_rs14669338_A	5293361	2	*PIK3CB*	Is associated with white/red earlobe colour formation.	BIO6	0.217912	(Luo *et al.*, 2018)
9	Gga_rs14667573_A	6842640	2	*ENSGALG00010026146*		Elevation	0.276946	
9	GGaluGA336941_A	7380996	2	*EPHA4*	Plays a role in the development of the nervous system controlling different steps of axonal guidance.	BIO11	0.435702	UniProt
9	Gga_rs13765098_G	9064184	2	*COL4A3*	Play an important role in the structural maintenance and normal development of skeletal muscle.	BIO11	0.394519	(Baumert *et al.*, 2018; Mohammadkhah *et al.*, 2018)
9	GGaluGA338995_G	12812899	2	*ATP13A5*	Associated with biological processes important for lipid metabolism and the development of skeletal muscle.	BIO6	0.273273	(Zhang *et al.*, 2017)

9	Gga_rs13736133_G	22577221	2	*TIPARP*	Is associated with a favourable prognosis.	Soil clay content	0.239674	(Zhang, Cao, *et al.*, 2020)
10	GGaluGA065293_A	543396	2	-		BIO11	0.427638	
10	GGaluGA073231_A	21780573	2	-		BIO11	0.343209	
10	GGaluGA073413_A	22058298	2	-		BIO6	0.356646	
11	Gga_rs14020878_A	6827222	2	-		BIO11	0.414842	
11	Gga_rs14021051_A	7330533	2	*TENM2*	Involved in neural development, regulating the establishment of proper	BIO6	0.365335	(Fleming *et al.*, 2016)

					connectivity within the nervous system.			

11	Gga_rs14028860_A	20340084	2	-		BIO6	0.234665	
12	GGaluGA083711_G	6897734	2	*FGD3*	Is associated with birth weight.	BIO11	0.330315	(Stronen *et al.*, 2019; Takasuga *et al.*, 2015)
12	GGaluGA087987_G	16325233	2	*FOXP1*	Has a role in adipocyte differentiation, regulation of tissue and cell type-specific gene transcription during development and adulthood.	BIO6	0.277092	(Liu, Huang, *et al.*, 2019)
12	Gga_rs14046703_G	17502782	2	-		BIO11	0.2185	

13	GGaluGA090168_G	521806	2	*NRG2*	Involved primarily in the development of the nervous and cardiovascular systems.	Elevation	0.483567	(Mei and Nave, 2014)
13	Gga_rs15690310_A	2914053	2	*NPM1*	Involved in ribosome biogenesis, regulation of proliferation and growth suppression pathways.	BIO11	0.446624	(Zarka *et al.*, 2020)
13	Gga_rs16697936_A	8614002	2	-		BIO11	0.374222	
13	Gga_rs15697934_G	11349617	2	*SGCD*	Involved in cardiomyopathy and muscular dystrophy.	BIO11	0.299999	(Li, Abdel-Moneim, *et al.*, 2022)
13	Gga_rs14061794_A	13265234	2	-		Soil clay content	0.201973	
13	GGaluGA096906_C	15134755	2	*ENSGALG00010017293*		BIO11	0.28469	

13	GGaluGA097376_A	16276058	2	*ENSGALG00010016127*		BIO11	0.26367	
14	GGaluGA100538_A	4148009	2			BIO11	0.323332	
14	Gga_rs14075775_C	7452309	2	*UNKL*	Has a role in protein, Zink ion, and metal ion binding.	Soil clay content	0.185774	(Iso-Touru *et al.*, 2016)
14	GGaluGA102883_G	8403962	2	-		BIO11	0.213389	
15	Gga_rs15762378_A	1219433	2	*ARVCF*	Has functions related to adherents junctions, which are cadherin-based adhesive structures that connect actin filaments between neighbouring cells.	Soil clay content	0.197845	(Carnahan *et al.*, 2010)
15	GGaluGA108657_G	7014165	2	*MYO18B*	Associated with biological processes important for lipid	Soil slit content	0.436453	(Zhang *et al.*, 2017)

					metabolism and the development of skeletal muscle.			
15	Gga_rs14091453_A	7151427	2	-		BIO11	0.186947	
15	Gga_rs14092446_C	8127457	2	*DDTL*	DDTL protein is scare, DDTL transcripts were found in many different types of tissues, with the highest levels seen in the liver, kidney, and adipose tissue.	BIO11	0.31617	(Illescas *et al.*, 2020)
15	Gga_rs15026369_G	12318502	2	-		BIO11	0.418278	
17	Gga_rs14104760_A	1164757	2	-		Elevation	0.251052	

17	GGaluGA112977_G	2210126	2	*LRSAM1*	Is associated with muscle disorder.	BIO11	0.331961	(Allais *et al.*, 2019; Pampouille *et al.*, 2018)
17	GGaluGA113385_A	2791572	2	*CACNA1B*	Is involved in calcium channel activity.	BIO6	0.25633	(Hu *et al.*, 2021)
17	Gga_rs14102617_A	3288244	2	*TNFSF8*	Contributes significantly to the initiation of apoptosis and also play critical roles in the immune response to Salmonella enteritis's inoculation.	Soil clay content	0.285502	(Tohidi *et al.*, 2018; Wu *et al.*, 2015)
17	Gga_rs14101785_C	4822703	2	-		BIO11	0.47639	
17	Gga_rs13744398_A	5933197	2	*ZER1*	Is found to be associated with body mass index.	BIO11	0.222147	(Iranzo-Tatay *et al.*, 2022)

17	GGaluGA115485_A	6861230	2	*RAPGEF1*	It plays a significant function in reproduction.	BIO6	0.29716	(Azmal *et al.*, 2019)
17	Gga_rs13661747_G	8386852	2	-		BIO11	0.411003	
17	GGaluGA117147_A	9307583	2	*NDUFA8*	Plays a critical role in the transfer of electrons from NADH to the respiratory chain.	BIO6	0.381171	(Lindholm *et al.*, 2014)
17	Gga_rs15789928_G	9928790	2	-		BIO11	0.381238	
18	Gga_rs15810909_G	1051164	2	*MYH1B*	Regulates muscle contraction, play important roles in skeletal muscle development.	BIO11	0.296162	UniProt (Tajsharghi *et al.*, 2008)
18	GGaluGA118240_G	1485545	2	*MYOCD*	Associated with muscle development and muscle fibre	BIO6	0.371973	UniProt, (Gu *et al.*, 2022)

					regeneration and repair mechanisms.			
18	Gga_rs15039004_A	4155582	2	*OGFOD3*	Identified to be associated with dyslipidaemia.	BIO11	0.377136	(England *et al.*, 2017)
19	GGaluGA125678_A	3310846	2	*CLIP2*	Involved in neuronal migration/maturation.	BIO6	0.331196	(Jabbi *et al.*, 2012)
19	Gga_rs15046418_A	3565397				BIO11	0.358099	
20	GGaluGA174700_G	637585				BIO8	0.219637	
20	GGaluGA178527_A	7512082	2	*CDH4*	Involved in diverse cell type sorting and potentially playing a crucial role in retinal development.	BIO11	0.216208	UniProt
20	GGaluGA178919_G	8102870	2	*MTG2*	Plays a role in the regulation of the mitochondrial ribosome	BIO11	0.369318	UniProt

					assembly and of translational activity.			
20	Gga_rs14278862_A	10874807	2	-		BIO11	0.467704	
21	Gga_rs14281159_C	365994		*CAMTA1*	Involved in neuromuscular process controlling balance, heterocycle metabolic process and positive regulation of transcription.	BIO6	0.335486	(Marchesi *et al.*, 2021)
21	Gga_rs14281575_G	873221	2	-		Elevation	0.260824	
21	Gga_rs15181561_A	2128180	2	*SSU72*	Involved in the C-terminal domain of RNA polymerase II dephosphorylation, RNA processing and termination.	Elevation	0.208739	UniProt
21	Gga_rs16178648_A	2135314	2	-		BIO11	0.3405	

21	GGaluGA183387_G	2736349	2	*AGRN*	Function in neurons is highly regulated by alternative splicing, glycan binding and proteolytic processing. Modulates calcium ion homeostasis in neurons, specifically by inducing an increase in cytoplasmic calcium ions.	BIO6	0.417864	UniProt
22	GGaluGA186811_G	3783822	2	*LRRTM4*	Involved in synapse development and maintenance of the nervous system.	BIO11	0.282366	(Laurén *et al.*, 2003)
23	Gga_rs14289153_G	2596404		*YTHDF2*	Modulate mRNA metabolism, including mRNA maturation, translation, and degradation.	BIO6	0.347778	(Zhao *et al.*, 2017)
23	GGaluGA188346_A	2889994	2	-		BIO6	0.437524	

23	GGaluGA189562_A	4841599	2	*GJB5*	May aid in the transmission of signalling molecules within tissues.	BIO11	0.530405	(Frankenberg *et al.*, 2007)
24	GGaluGA190726_G	1174308	2	-		BIO6	0.41518	
24	GGaluGA191239_G	1969360		*NTM*	It's associated to the control of oviduct development and differentiation in chickens.	BIO11	0.219049	(Cho *et al.*, 2021)
24	Gga_rs14294033_G	2317886	2	*MED12*	An important regulator of hematopoietic stem cell homeostasis.	BIO6	0.382408	(Zhang, Wang, *et al.*, 2019)
24	Gga_rs15215725_A	2374076	2	*OPCML*	Found to be associated with body weight and growth rate.	BIO11	0.477885	(Gu *et al.*, 2011)
24	GGaluGA191552_G	2434624	2	-		Soil clay content	0.16693	

24	Gga_rs15216173_A	2499086	2	*IGSF9B*	Is involved in cell adhesion at synapses.	BIO11	0.383857	UniProt
24	Gga_rs16196837_A	3705029	2	*GRIK4*	Involved in the function of central nervous system and has a role in excitatory synaptic transmission.	BIO8	0.176183	UniProt
24	GGaluGA193073_A	4724363	2	-		BIO11	0.357711	
24	GGaluGA193703_G	5878930	2	*NCAM1*	Is a cell adhesion molecule involved in neuron-neuron adhesion, neurite fasciculation, and outgrowth of neurites.	BIO11	0.328713	UniProt
24	GGaluGA193743_G	5939657	2	*ENSGALG00010026134*		BIO6	0.353195	

26	GGaluGA195343_A	983689	2	-		BIO11	0.381825	
26	Gga_rs14298585_A	2077663	2	*DSTYK*	Involved in induction of apoptosis.	BIO6	0.380363	(Becic *et al.*, 2018)
26	GGaluGA196968_G	3192081	2	*TRAF3IP3*	Is expressed in the immune system, where it aids in cell maturation, tissue development, and immune response.	BIO6	0.39797	(Li *et al.*, 2018)
0	GGaluGA345091_C	0	3			Soil organic carbon	0.336306	

0	GGaluGA345118_A	0	3	-		Soil organic carbon	0.349682	
0	GGaluGA345264_G	0	3	-		Soil organic carbon	0.335538	
0	GGaluGA345313_G	0	3	-		Elevation	0.347621	
0	GGaluGA345442_G	0	3	-		Soil organic carbon	0.477765	
0	GGaluGA345484_G	0	3	-		Soil organic carbon	0.350636	
0	GGaluGA345569_A	0	3	-		Soil organic carbon	0.301835	

0	GGaluGA345572_G	0	3	-		Soil organic carbon	0.350636	
0	Gga_rs14689014_A	316690	3	-		Soil pH	0.327948	
28	Gga_rs14306237_G	1419359	3	*PIP5K1C*	Is required for the dynamics of focal adhesions to control adipocyte homeostasis.	Soil Organic Carbon	0.209451	(Huang *et al.*, 2022)
28	GGaluGA202715_C	4013248	3	*NR2C2AP*	Is found to be associated with cognitive ability.	Soil Organic Carbon	0.328242	(Kornilov *et al.*, 2019)
28	GGaluGA202719_G	4018601	3	*BORCS8*	Facilitates ARL8-dependent coupling of the motor proteins kinesin-1 and -3 to lysosomes to mediate lysosome movement.	Soil Organic Carbon	0.234082	(Guardia *et al.*, 2016)
1	GGaluGA016761_A	51087457	3	-		Soil Organic Carbon	0.239353	

1	Gga_rs14844728_G	73166885	3	*CCDC91*	Associated significantly with carcass depth.	Soil Organic Carbon	0.339373	(Bordbar *et al.*, 2022)
1	Gga_rs15317145_A	81249634	3	*ENSGALG00010006964*		Soil Organic Carbon	0.350636	
1	Gga_rs13935261_A	128906624	3	*DHRSX*	Involved in the positive regulation of starvation induced autophagy.	Soil Organic Carbon	0.268846	(Zhang *et al.*, 2014)
1	Gga_rs13966366_A	168986142	3	*FNDC3A*	Mediation of spermatid-sertili adhesion during spermatogenesis.	Elevation	0.203972	(Chikoko *et al.*, 2022)
1	GGaluGA062810_A	196793737	3	-		BIO8	0.13424	
1	Gga_rs14937110_G	197385068	3	-		Soil organic carbon	0.244067	

1	Gga_rs13787697_A	198170553	3	-		Soil organic carbon	0.311529	
1	Gga_rs16739932_G	200652089	3	-		Elevation	0.266156	
2	Gga_rs14130583_G	4150518	3	-		Soil organic carbon	0.215793	
2	Gga_rs15859839_G	21113368	3	-		BIO8	0.257181	
2	Gga_rs14151391_A	22837783	3	*HEPACAM2*	Functions as a mitotic kinetics regulator and, most likely, as a tumour suppressor gene.	Soil organic carbon	0.290825	(de las Heras-Saldana *et al.*, 2020)

2	GGaluGA139600_G	31035726	3	*GPNMB*	Has a significant role as a negative regulator of inflammation.	Soil organic carbon	0.320874	(Ripoll *et al.*, 2007; Zhou *et al.*, 2017)
2	Gga_rs14209923_A	83121122	3	*ENSGALG00010008962*		Soil pH	0.303905	
2	Gga_rs13636115_C	84076282	3	*EPB41L4B*	Involved in regulation of the tight junction of epithelial cells and plays a role in wound healing.	Soil pH	0.279996	(Fan *et al.*, 2021)
2	Gga_rs14222707_G	98699615	3	*PTPRM*	Involved in mediating cell–cell interactions and adhesion.	Elevation	0.288863	(Khrunin *et al.*, 2020)
2	GGaluGA167331_A	130084559	3	*ZFPM2*	Plays a central role in heart morphogenesis and development of coronary vessels from epicardium, by regulating genes that are essential during cardio genesis.	Soil organic carbon	0.239626	UniProt

3	Gga_rs14314155_G	9912294	3	-		Soil organic carbon	0.267378	

3	Gga_rs15750093_A	15914185	3	*MRPS5*	Is associated with feed intake.	Soil pH	0.245292	(Olivieri *et al.*, 2016)
3	GGaluGA214455_G	31380271	3	*ENSGALG00010010631*		Soil organic carbon	0.221472	
3	GGaluGA216929_A	38930068	3	*SIPA1L2*	Function is unknown.	Soil organic carbon	0.298709	(Tao *et al.*, 2019)
3	GGaluGA217528_G	40876635	3	*WDR27*	Is associate with inhibitory control and memory. Is also associated with feed efficiency.	Soil organic carbon	0.31449	(Gnanadesikan *et al.*, 2020; Taussat *et al.*, 2020)

3	GGaluGA217949_G	41868186	3	*AFDN*	Has essential roles in the organization of cell-cell junctions of polarized epithelia.	Soil organic carbon	0.288324	(Ohama *et al.*, 2018)
3	Gga_rs15352385_A	50517592	3	-		Soil pH	0.2796	
3	GGaluGA223503_G	57025844	3	*ENSGALG00010006683*		Elevation	0.25319	
3	Gga_rs14365721_G	62426814	3	*MCM9*	Involved in homologous recombination repair following DNA inter-strand cross-links and has a role during gametogenesis.	Soil organic carbon	0.381239	UniProt
4	Gga_rs16350958_G	2754672	3	*HTR2C*	Behavioural fear response, regulation of appetite, regulation of corticotrophin-releasing hormone secretion, and nervous system processes.	Soil pH	0.202013	UniProt, (Brummett *et al.*, 2014)

4	Gga_rs14426371_C	9242317	3	*ENSGALG00010014250*		Soil pH	0.283847	
4	GGaluGA254643_A	39970355	3	*TENM3*	Involved in the development and processes of brain, eye, ear, and nervous system	Soil pH	0.242427	(Gheyas *et al.*, 2021)
4	GGaluGA256770_G	47047103	3	*ENSGALG00010004923*		Soil pH	0.252261	
4	Gga_rs14482973_A	66850995	3	*ENSGALG00010017397*		Soil organic carbon	0.246824	
7	Gga_rs14622937_A	30155133	3	*ZRANB3*	Known to affect feed efficiency and mammalian mesoderm segmentation.	Soil pH	0.295227	(Bovine HapMap Consortium *et al.*, 2009)

7	Gga_rs14623062_G	30327824	3	*DARS*	Catalyses the specific attachment of an amino acid to its cognate tRNA in a 2 step reaction: the amino acid (AA) is first activated by ATP to form AA-AMP and then transferred to the acceptor end of the tRNA.	BIO8	0.22999	UniProt
7	GGaluGA319259_A	31949316	3	*ENSGALG00010009269*		Soil Organic carbon	0.265235	
8	Gga_rs14651416_G	23114671	3	*BEND5*	Acts as a transcriptional repressor.	BIO8	0.306375	UniProt
9	Gga_rs14662119_G	11416774	3	*PLSCR5*	Is connected to metabolism and body homeostasis.	Soil Organic carbon	0.242476	(Szmatoła *et al.*, 2020)
10	Gga_rs15565103_A	4893026	3	*RORA*	Plays a critical function in the regulation of circadian	Soil Organic carbon	0.238924	(Hams *et al.*, 2020)

					rhythm, inflammation, metabolism, and cellular development.			
10	Gga_rs14951894_G	18251295	3	*MEGF11*	Regulate the mosaic spacing of specific neuron subtypes in the retina.	Soil pH	0.257477	UniProt
11	GGaluGA075400_A	5429570	3	*ENSGALG00010025630*		Soil Organic carbon	0.327866	
11	GGaluGA077323_A	11039507	3	*CMTM4*	Regulate the functions of immune cells through stabilizing PD-L1 protein.	Soil Organic carbon	0.416241	(Mezzadra *et al.*, 2017; Zhang *et al.*, 2022)
12	Gga_rs14981618_G	13298021	3	*ENSGALG00010022338*		Soil clay content	0.222855	
12	Gga_rs14982898_A	14579285	3	*SLC25A26*	Encodes the mitochondrial S-adenosylmethionine carrier (SAMC) and belongs to the mitochondrial carrier family.	Soil Organic carbon	0.292216	(Ji *et al.*, 2021)

12	Gga_rs14046170_G	17020111	3	-		Soil Organic carbon	0.174635	
13	GGaluGA091633_A	3310071	3	*KCNIP1*	Mediates the regulation of potassium channel by calcium in the mammalian atrium at high rate.	Soil slit content	0.227301	(Tsai *et al.*, 2016)
13	Gga_rs14051956_G	6708624	3	*GABRG2*	An inhibitory neurotransmitter in vertebrates that mediates neuronal inhibition by binding to *GABA* receptors and opening an integral chloride channel.	Elevation	0.253439	(Fleming *et al.*, 2016) ,UniProt
14	GGaluGA105653_A	15054041	3	*LYRM1*	Enhance proliferation and inhibit apoptosis of preadipocytes.	Soil Organic soil	0.350636	(Qiu *et al.*, 2009; Zhu *et al.*, 2010)

17	Gga_rs15027206_G	10125489	3	*OLFML2A*	Function is unknown	Soil pH	0.269314	(Ma *et al.*, 2021; Pérez-Ibave *et al.*, 2016)
19	Gga_rs14119011_G	3542635	3	*SPNS2*	Involved in hearing ability.	Soil silt content	0.272351	(Chen *et al.*, 2014; Pearson *et al.*, 2019)
19	Gga_rs15843023_G	3684271	3	*ANKFY1*	Involved in intracellular endosome/vesicle trafficking, PDGF signalling pathway.	Elevation	0.376313	(Chang *et al.*, 2021)
21	GGaluGA183243_G	2490473	3	*ENSGALG00010021959*		Soil Organic carbon	0.214748	
23	GGaluGA186983_G	16867	3	-		Elevation	0.426225	
23	Gga_rs10727406_G	2561948	3	*ASAP3*	Promotes cell proliferation.	BIO8	0.260815	UniProt

24	Gga_rs14295304_A	3409698	3	*ENSGALG00010026219*		Soil organic carbon	0.258217	
26	GGaluGA196978_G	3206224	3	*IRF6*	Participates in several functional roles in the immune system's regulation.	Soil pH	0.194759	(Liu *et al.*, 2011)
26	Gga_rs10731282_G	3591015	3	-		Soil silt content	0.224778	